# 2.6.4 薬物動態試験の概要文

# 目　次

目　次 ..... 2
表 一 覧 ..... 3
図 一 覧 ..... 4
略号一覧 ..... 5
用語の定義一覧 ..... 6
1 まとめ ..... 7
1.1 吸収 ..... 9
1.2 分布 ..... 10
1.3 代謝 ..... 11
1.4 排泄 ..... 11
1.5 薬物間相互作用 ..... 11
2 分析法 ..... 12
2.1 放射性標識 ..... 12
2.2 放射能の測定 ..... 12
2.3 Arm，DHA 及び Lmf の定量 ..... 12
2.4 代謝物の同定及び構造解析 ..... 13
3 吸収 ..... 14
3.1 吸収及びバイオアベイラビリティ ..... 14
3.2 トキシコキネティクス ..... 16
4 分布 ..... 19
4.1 *In vitro* 血漿・血清蛋白結合率及び血球への移行性 ..... 19
4.2 組織及び臓器分布 ..... 20
4.3 胎盤通過性 ..... 28
5 代謝（動物種間の比較） ..... 31
5.1 代謝物の推定 ..... 31
5.2 *In vitro* 代謝 ..... 33
5.3 *In vivo* 代謝 ..... 34
5.3.1 血漿中代謝物 ..... 34
5.3.2 尿中代謝物 ..... 35
5.3.3 糞中及び胆汁中代謝物 ..... 35
6 排泄 ..... 36
6.1 尿，糞及び胆汁中への排泄 ..... 36
6.2 乳汁中移行 ..... 38
7 薬物間相互作用 ..... 38
7.1 酵素阻害 ..... 38

7.2 酵素誘導 ..... 38
8 その他の薬物動態試験 ..... 39
9 考察及び結論 ..... 39
10 参考文献 ..... 40

## 表 一 覧

Table 1-1 Arm/Lmf の ADME 試験及び TK 試験 ..... 7
Table 1-2 Arm/Lmf の *in vitro* 試験 ..... 9
Table 2-1 Arm，DHA，Lmf，及び desbutyl-Lmf の濃度測定法 ..... 13
Table 3-1 ラット 2 週間毒性試験における Arm 及び DHA の TK パラメータ ..... 17
Table 3-2 雄性イヌ神経毒性試験における Arm 及び DHA の TK パラメータ ..... 18
Table 3-3 ラット 4 週間毒性試験における Lmf の TK パラメータ ..... 19
Table 4-1 雄性アルビノラットに Arm/Lmf 10 mg/kg（[$^{3}$H]-Arm として 1.43 mg/kg）を単回静脈内投与したときの臓器・組織内放射能濃度 ..... 21
Table 4-2 雄性アルビノラットに Arm/Lmf 20 mg/kg（[$^{3}$H]-Arm として 2.86 mg/kg）を単回又は反復経口投与したときの臓器・組織内放射能濃度 ..... 22
Table 4-3 雄性有色ラットに Arm/Lmf 10 mg/kg（[$^{14}$C]-Arm として 1.43 mg/kg）を単回静脈内投与したときの臓器・組織内放射能濃度 ..... 24
Table 4-4 雄性アルビノラットに Arm/Lmf 1 mg/kg（[$^{14}$C]-Lmf として 0.86 mg/kg）を単回静脈内投与したときの臓器・組織内放射能濃度 ..... 25
Table 4-5 雄性アルビノラットに Arm/Lmf 20 mg/kg（[$^{14}$C]-Lmf として 17.1 mg/kg）を単回又は反復経口投与したときの臓器・組織内放射能濃度 ..... 26
Table 4-6 Arm/Lmf 1 mg/kg （[$^{14}$C]-Lmf として 0.86 mg/kg）を雄性有色ラットに単回静脈内投与したときの臓器・組織内放射能濃度 ..... 28
Table 4-7 Arm/Lmf 30 mg/kg （[$^{3}$H]-Arm として 4.29 mg/kg）を妊娠ラットに単回経口投与したときの臓器・組織内放射能濃度 ..... 29
Table 4-8 Arm/Lmf 30 mg/kg （[$^{14}$C]-Lmf として 25.7 mg/kg）を妊娠ラットに単回経口投与したときの臓器・組織内放射能濃度 ..... 30
Table 5-1 単回投与後の血漿中 Arm 及び主代謝物の血漿中放射能に対する割合 ..... 34
Table 5-2 単回投与後の尿中 Arm 及び主代謝物の尿中放射能に対する割合 ..... 35
Table 5-3 単回投与後の糞中 Arm 及び主代謝物の糞中放射能に対する割合 ..... 36
Table 6-1 雄性ラット，雌性ウサギ，雄性イヌに Arm/Lmf を単回投与したときの Arm の放射能排泄率 ..... 37
Table 6-2 雄性ラット，雌性ウサギ，雄性イヌに Arm/Lmf を単回投与したときの Lmf の放射能排泄率 ..... 37
Table 7-1 CYP の各分子種に対する阻害能 ..... 38

# 図 一 覧

Figure 2-1 Arm 及び Lmf の化学構造式及び標識部位 ..... 12
Figure 3-1 雄性ラットに Arm/Lmf（[$^{3}$H]-Arm）を単回投与したときの血液中及び血漿中放射能濃度-時間推移 ..... 15
Figure 3-2 雄性ラットに [$^{14}$C]-Lmf を単回投与したときの血液中及び血漿中放射能の濃度-時間推移 ..... 16
Figure 4-1 Arm/Lmf 10 mg/kg （ [$^{14}$C]-Arm として 1.43 mg/kg）を雄性有色ラットに単回静脈内投与したときの投与後 5 分及び 8 時間の組織中放射能分布 ..... 23
Figure 4-2 Arm/Lmf 1 mg/kg（ [$^{14}$C]-Lmf として 0.86 mg/kg）を雄性有色ラットに単回静脈内投与したときの投与後 5 分及び 24 時間の組織中放射能分布 ..... 27
Figure 5-1 Arm の推定代謝部位 ..... 32
Figure 5-2 DHA の構造式 ..... 32
Figure 5-3 Lmf の推定代謝部位 ..... 33
Figure 5-4 N- desbutyl-lumefantrine ..... 33

# 略号一覧

| 略号 | 省略していない表現（英） | 省略していない表現（日） |
|---|---|---|
| ADME | Absorption, Distribution, Metabolism and Excretion | 吸収，分布，代謝，排泄 |
| AUC | area under the drug plasma (serum/blood) concentration-time curve | 血漿（血清/血液）中薬物濃度-時間曲線下面積 |
| BA | bioavailability | バイオアベイラビリティ |
| CL | clearance (unit: L/h or mL/min/kg) | クリアランス |
| Cmax | maximumplasma (serum/blood) drug concentration (unit: µmol/L or µg/mL) | 最高血漿(血清/全血)薬物濃度 |
| CYP | cytochrome P450 | チトクローム P450 |
| DHA | dihydroartemisinin | ― |
| desbutyl-Lmf | desbutyl-lumefantrine | desbutyl ールメファントリン |
| HPLC | High performance liquid chromatography | 高速液体クロマトグラフィー |
| HPLC-ECD | high performance liquid chromatography coupled to electrochemical detection | 電気化学検出器を接続した高速液体クロマトグラフィー |
| HPLC-UV | high performance liquid chromatography coupled to ultraviolet absorbance detection | 紫外吸光度検出器を接続した高速液体クロマトグラフィー |
| i.v. | intravenous | 静脈内 |
| Ki | inhibition constant | 阻害定数 |
| LC-MS | liquid chromatography coupled to mass spectrometry | 液体クロマトグラフィー-質量分析法 |
| LC-MS-MS | liquid chromatography coupled to tandem mass spectrometry | 液体クロマトグラフィー-タンデム質量分析法 |
| LLOQ | lower limit of quantification | 定量下限 |
| LSC | liquid scintillation counter | 液体シンチレーションカウンター |
| MDR1 | multidrug resistance 1 | 多剤耐性蛋白質 1 |
| od | omni die | 1 日 1 回 |
| PK | pharmacokinetics | 薬物動態（学） |
| p.o. | per os | 経口 |
| QWBA | quantitative whole body autoradiography | 定量的全身オートラジオグラフィー |
| S12 | post-mitochondrial liver fraction obtained from the supernatant at 12'000×*g* centrifugal force | S12 画分 |
| T1/2 | elimination half life | 消失半減期 |
| TK | toxicokinetics | トキシコキネティクス |
| Tmax | time to the maximum observed plasma (serum/blood) concentration | 最高血漿（血清/血液）中薬物濃度到達時間 |

## 用語の定義一覧

| 用語 | 定義 |
|---|---|
| 薬剤名の表記方法 | JAN を取得している薬剤はカタカナ表記，未取得の薬剤は英語表記とした。 |
| 配合錠の表記方法 | 配合錠の場合，含有する薬剤を「／」で示した。<br>例）アルテメテルとルメファントリンの配合錠→Arm/Lmf |
| 薬剤名の略号 | 以下の薬剤（配合錠）については略号を用いた。<br>アルテメテル，CGP 56696：Arm<br>ルメファントリン，CGP 56695，benflumetol：Lmf |
| Arm/Lmf の表記方法 | 用量及び製剤を問わず Arm/Lmf が投与された場合は Arm/Lmf と示す。ただし，以下については特定して表記した。<br>Coartem®/Riamet®，COA566，co-artemether，CGP 56695/CGP 56696：Arm/Lmf |
| Arm/Lmf の投与量 | 配合剤も含めて，Arm と Lmf は 1:6 の比率で投与した。<br>Arm/Lmf の投与量は Arm 及び Lmf の投与量の和で示す。<br>標識体と非標識体を混合して投与した場合は，標識体の投与量のみ（ ）内に示す。<br>例）Arm/Lmf 20 mg/kg（[$^3$H]-Arm として 2.86 mg/kg）：[$^3$H]-Arm として 2.86 mg/kg，非標識の Lmf として 17.14 mg/kg，計 20 mg/kg の Arm/Lmf を投与 |

# 1 まとめ

本剤は有効成分としてアルテメテル（Arm）20 mg 及びルメファントリン（Lmf）120 mg を含有する配合錠（Arm/Lmf 20/120 mg）である。Arm は活性代謝物の Dihydroartemisinin（DHA）に代謝される。Arm/Lmf 並びに Arm，DHA 及び Lmf のラット，ウサギ，イヌ，及びサルにおける吸収，分布，代謝並びに排泄（ADME）を，*in vivo* 及び *in vitro* 試験で検討した。標識あるいは非標識 Arm 又は Lmf を投与した ADME 試験及びトキシコキネティクス（TK）試験における用法・用量の一覧をTable 1-1に，*in vitro* 試験をTable 1-2に示す。

本項では，本化合物の名称として Arm/Lmf を用いる。ただし，Arm/Lmf は社内報告書では Coartem®/Riamet®，COA566，co-artemether，CGP 56695/CGP 56696 と記載されており，本項の一部の図表でもこれらの名称で記述している。なお， Lmf は benflumetol とも表記される。

本化合物の薬物動態試験には毒性試験と同一の動物種及び系統を用いた。

**Table 1-1 Arm/Lmf の ADME 試験及び TK 試験**

| 試験の種類 | 種，性<br>*系統* | 放射性標識 | 投与薬剤 | 投与量*<br>[mg/kg] | 投与剤型 | 投与経路 | 投与期間 | 報告書番号 |
|---|---|---|---|---|---|---|---|---|
| ADME | ラット，雄<br>*Tif:RAIf LE/Mol* | $^{3}$H-Arm<br>or<br>$^{14}$C-Arm | Arm/Lmf | 10<br>20, 100, 1000 | 溶液<br>懸濁液 | 静脈内<br>経口 | 単回<br>単回 | 4.2.2.2-1-DMPK(CH) 1997/241,<br>4.2.2.4-1-DMPK(CH) 1997/534 |
| PK | ラット，雄 | – | Lmf | 1 | 溶液 | 静脈内 | 単回 | 4.2.2.2-6-BPK(CH) 1996/026 |
| キラル安定性 | ラット，雄<br>*Tif:RAIf* | – | Lmf | 100 | 懸濁液 | 経口 | 単回 | 4.2.2.1-14-BPK(CH) 1996/026 |
| ADME | ラット，雄<br>*Tif:RAIf LE/Mol* | $^{14}$C-Lmf | Arm/Lmf | 1<br>20, 100, 1000 | 溶液<br>懸濁液 | 静脈内<br>経口 | 単回<br>単回 | 4.2.2.2-4-DMPK(CH) 1997/240<br>4.2.2.4-2-DMPK(CH) 1997/209 |
| | イヌ，雄<br>*ビーグル* | $^{14}$C-Lmf | Arm/Lmf | 1<br>20 | 溶液<br>カプセル | 静脈内<br>経口 | 単回<br>単回 | |
| ADME | イヌ，雄<br>*ビーグル* | $^{14}$C-Arm | Arm/Lmf | 10<br>20<br>200 | 溶液<br>懸濁液 | 静脈内<br>経口 | 単回<br>単回 | 4.2.2.2-2-DMPK(CH) 1997/003<br>4.2.2.4-1-DMPK(CH) 1997/534 |
| PK | サル，雌，<br>*カニクイザル* | – | Arm/Lmf | 350 | カプセル | 経口 | 単回 | 4.2.2.2-5-BPK(CH) 1996/095 |
| TK, 2 週間毒性試験 | ラット，雌雄，<br>*HanWistar* | – | Arm/Lmf | 0, 20, 200 | 懸濁液 | 経口 | 2 週間<br>od | 4.2.3.7.7-5-DMPK R0570030 |

| 試験の種類 | 種，性<br>*系統* | 放射性標識 | 投与薬剤 | 投与量*<br>[mg/kg] | 投与剤型 | 投与経路 | 投与期間 | 報告書番号 |
|---|---|---|---|---|---|---|---|---|
| TK, 幼若ラット | ラット，雌雄,<br>*Han Wisster* | – | Arm | 0,10,30,100 | 懸濁液 | 経口 | 2 週間<br>od | 4.2.3.5.4-3-DMPK R0570013 |
| TK, 神経毒性 | イヌ，雄<br>*ビーグル* | – | Arm/Lmf<br>Arm | 1000<br>600, 300 | 懸濁液<br>懸濁液 | 経口 | 3 日間, 8 日間<br>od | 4.2.3.7.3-2-DMPK R0510009B |
| TK, 神経毒性 | イヌ，雄<br>*ビーグル* | – | Arm | 0, 20 | 溶液 | 筋注 | 5 日間,<br>30 日間<br>od | 4.2.3.7.3-4-BPK(F) 1997/004 |
| TK, 神経毒性 | イヌ，雄<br>*ビーグル* | – | Arm | 40 | 溶液 | 筋注 | 7 日間<br>od | 4.2.3.7.3-5-DMPK R0410073 |
| TK, 神経毒性 | イヌ，雄<br>*ビーグル* | – | Arm | 0, 10, 40 | 溶液 | 筋注 | 3 日間, 8 日間<br>od | 4.2.3.7.3-6-DMPK R0510001 |
| TK,神経毒性 | イヌ，雌雄<br>*ビーグル* | – | Arm | 0,20,40,80<br>0,50,150,600 | 溶液<br>カプセル | 筋注<br>経口 | 8 日間<br>od | 4.2.3.7.3-3-DMPK(F) 1998/014 |
| TK, 4 週間毒性試験 | ラット，雌雄,<br>*Tif:RAIf* | – | Arm/Lmf | 0, 200, 600, 1000 | 懸濁液 | 経口 | 4 週間<br>od | 4.2.3.2-1-BPK(CH) 1995/079 |
| TK, 13 週間毒性試験 | ラット，雌雄,<br>*Tif:RAIf* | – | Arm/Lmf | 0, 100, 300, 1000 | 懸濁液 | 経口 | 13 週間<br>od | 4.2.3.2-2-BPK(CH) 1996/020 |
| TK, 幼若 13 週間毒性試験 | ラット，雌雄,<br>*Tif:RAIf* | – | Arm/Lmf | 0, 100, 300, 1000 | 懸濁液 | 経口 | 13 週間<br>od | 4.2.3.5.4-1-DMPK(CH) 1997/177 |
| TK, 13 週間毒性試験 | ラット，雌雄,<br>*Tif:RAIf* | – | Lmf | 0, 100, 300, 1000 | 懸濁液 | 経口 | 13 週間<br>od | 4.2.3.2-7-DMPK(CH) 1997/178 |
| TK, 13 週間毒性試験 | イヌ，雌雄<br>*ビーグル* | – | Lmf | 0, 60, 200, 600 | 懸濁液 | 経口 | 13 週間<br>od | 4.2.3.2.8-DMPK(CH) 1997/006 |
| TK, 神経毒性 | イヌ，雄<br>*ビーグル* | – | Arm/Lmf | 1000 | 懸濁液 | 経口 | 3 日間, 8 日間<br>od | 4.2.3.7.3-2-DMPK R0510009A |
| TK, 4 週間毒性試験 | イヌ，雌雄<br>*ビーグル* | – | Arm/Lmf | 0, 60, 200, 600 | カプセル | 経口 | 4 週間<br>od | 4.2.3.2-3-BPK(CH) 1995/080 |
| TK, 13 週間毒性試験 | イヌ，雌雄<br>*ビーグル* | – | Arm/Lmf | 0, 20, 60, 200 | カプセル | 経口 | 13 週間<br>od | 4.2.3.2-4-BPK(CH) 1996/024 |
| TK, 13 週間毒性試験 | イヌ，雌雄<br>*ビーグル* | – | Arm/Lmf | 0, 20, 60, 200 | カプセル | 経口 | 13 週間<br>od | 4.2.3.2-4-BPK(F) 1996/005 |
| 胚・胎児発生に関する試験 | ラット，<br>雌,<br>*Tif:RAIf* | $^{3}$H-Arm | Arm/Lmf | 30 | 懸濁液 | 経口 | 単回 | 4.2.2.3-4-DMPK(CH) 1997/099 |

| 試験の種類 | 種，性<br>*系統* | 放射性標識 | 投与薬剤 | 投与量*<br>[mg/kg] | 投与剤型 | 投与経路 | 投与期間 | 報告書番号 |
|---|---|---|---|---|---|---|---|---|
| 胚・胎児発生に関する試験 | ウサギ，雌<br>*NZW* | | Arm/Lmf | 0, 210, 700, 2100 | 懸濁液 | 経口 | 13 日間 od | 4.2.3.5.2-4-BPK(F) 1996/022 |
| 胚・胎児発生に関する試験 | ラット，雌，<br>*Tif:RAIf* | $^{14}$C-Lmf | Arm/Lmf | 30 | 懸濁液 | 経口 | 単回 | 4.2.2.3-5-DMPK(CH) 1997/139 |
| 胚・胎児発生に関する試験 | ウサギ，雌<br>*NZW* | $^{14}$C-Lmf | Arm/Lmf | 175 | 懸濁液 | 経口 | 単回 | 4.2.2.3-6-DMET(EU) 29/1996 |
| 胚・胎児発生に関する試験 | ウサギ，雌<br>*NZW* | $^{14}$C-Lmf | Arm/Lmf | 175 | 懸濁液 | 経口 | 単回 | 4.2.2.3-7-DMPK(CH) 1997/079 |
| 胚・胎児発生に関する試験 | ウサギ，雌<br>*NZW* | $^{14}$C-Arm | Arm/Lmf | 175 | 懸濁液 | 経口 | 単回 | 4.2.2.3-8-DMPK(CH) 1997/004 |
| 胚・胎児発生に関する試験 | ウサギ，雌<br>*NZW* | – | Arm/Lmf | 0, 210, 700, 2100 | 懸濁液 | 経口 | 13 日間 od | 4.2.3.5.2-4-BPK(CH) 1996/085 |
| 胚・胎児発生に関する試験 | ウサギ，雌<br>*NZW* | – | Lmf | 0, 1000 | 懸濁液 | 経口 | 13 日間 od | 4.2.3.5.2-10-BPK(CH) 1996/084 |

4.2.2.2-3-BPK(F) 1996/029, 4.2.3.2-4-BPK(F) 1996/005, 4.2.3.2-2- BPK(F) 1996/007, 4.2.3.5.2-4-BPK(F) 1996/022 における濃度データはほぼ定量下限未満であり，薬物動態を評価できなかった。
*：Arm 及び Lmf の投与量を合算した総投与量で示す。配合剤も含めて，Arm と Lmf は 1:6 の比率で投与した。

**Table 1-2　　Arm/Lmf の *in vitro* 試験**

| 試験の種類 | 報告書番号 |
|---|---|
| 血清・血漿蛋白結合率並びに血球移行性 | 4.2.2.3-1-DMPK(F) 1998/004<br>4.2.2.3-2-BPK(F) 1996/044 |
| 血液及びヘモグロビンを用いた代謝試験 | 4.2.2.4-3-DMPK(CH) 1997/242 |
| 肝 S12 分画を用いた代謝試験 | 4.2.2.4-4-DMPK(CH) 1997/181<br>4.2.2.4-6-DMPK(CH) 1997/035<br>4.2.2.4-7-DMPK(CH) 1997/155 |
| 肝ミクロソーム並びに CYP 発現系を用いた代謝酵素の同定及び酵素活性阻害試験 | 4.2.2.4-5-DMPK(CH) 1997/156 |
| 肝ミクロソームを用いた CYP の発現誘導／阻害試験 | 4.2.2.4-8-DMPK(CH) 1997/072<br>4.2.2.4-9-DMPK(CH) 1997/073 |
| 肝細胞を用いた酵素誘導試験 | 4.2.2.4-10-DMPK R0900123 |

## 1.1　　吸収

雄性ラットに Arm を経口投与したときの絶対的バイオアベイラビリティ（BA）は 19.7%であると報告されている（Li et al. 1998）。雄性ラットに Arm/Lmf を経口投与したとき Lmf の吸収率

は 0.9%～19%であった。雄性イヌに Arm/Lmf を経口投与したときの Lmf の絶対的 BA は 8%～24%であった。

非絶食時の雄性ラットに Arm/Lmf 20 mg/kg（[$^{3}$H]-Arm として 2.86 mg/kg，非標識の Lmf として 17.14 mg/kg，Arm:Lmf は 1:6 の比率で投与した。以下標識体の用量のみを記載）を単回経口投与したときの血漿中放射能の，AUC0-144h は 59.42 µmol· h/L，T1/2 は約 50 時間であり，Tmax（中央値）は 0.5 時間であった。Arm/Lmf 20 mg/kg（ [$^{14}$C]-Lmf として 17.14 mg/kg）を単回経口投与したときの血漿中放射能の AUC0-144h は 68.9 µmol· h/L ，T1/2 は約 50 時間であった。Arm/Lmf をラット及びイヌに反復投与したとき，Arm 及び DHA の血漿中濃度は経時的に減少した。雄性イヌに Arm/Lmf 20 mg/kg（[$^{14}$C]-Arm として 2.86 mg/kg）を単回経口投与したときの血漿中放射能の AUC0-168h は 151.26 µmol· h/L であった。

Arm/Lmf 20 mg/kg（[$^{14}$C]-Lmf として 17.14 mg/kg）を単回経口投与したときの血漿中放射能の AUC0-168h は，絶食時で 109 µmol· h/L，非絶食時で 155 µmol· h/L であった。イヌ及びラットの Lmf の累積性については，初回投与に比べ反復投与後に曝露量が増加したものの，一定の傾向はみとめられなかった。

また，個体間変動を含めたすべてのデータから判断すると，検討したいずれの動物種でも Arm, DHA 及び Lmf の曝露量に明らかな性差はないと考える。

## 1.2 分布

Arm の血清中蛋白結合率は，マウス，ラット，ウサギ，イヌ，及びカニクイザルで，それぞれ 98.3%～98.4%，97.3%，97.1%～97.2%，97.1%～97.3%，及び 96.0%～96.2%であり，濃度依存性は認められなかった（最終濃度：0.323～10 µg/mL）。Lmf の血清中蛋白結合率は，マウス，ラット，ウサギ，イヌ，及びカニクイザルで，それぞれ 99.8%，99.9%，99.93%，99.92%，及び 99.94%であった（最終濃度：10 µg/mL）。

ヒト血漿及び赤血球において，Arm は主に α1-酸性糖蛋白質（33%），次に血清アルブミン（17%），高，低及び超低比重リポ蛋白質（それぞれ 12%，9.3%及び 12%）並びに γ グロブリン（0.4%）に結合した。Arm の 11%が血球に移行し，4.6%は非結合型 Arm であった。Lmf は 1 µg/mL（1.89 µmol/L）の濃度で主にリポ蛋白質，特に高比重リポ蛋白質（99.6%）に結合した。約 62%がヒト血清アルブミンに，約 8%が血球に移行した。

[$^{3}$H]-Arm，[$^{14}$C]-Arm，又は[$^{14}$C]-Lmf 有色及びアルビノラットに，単回経口投与並びに単回静脈内投与したとき，Arm，Lmf，及びその代謝物は全身に分布した。静脈内投与後 5 分で全身に広く分布し，Arm は副腎，褐色脂肪，甲状腺，及び脳・脊髄等の神経組織に，Lmf は肝臓と血漿に高い放射能が認められた。経口投与では，Arm は消化管及び肝臓以外，Lmf は消化管及び腸間膜リンパ以外への分布は静脈内投与後の放射能分布パターンと類似していた。有色ラットの組織内放射能分布はアルビノラットと類似しており，メラニン含有組織である眼から放射能が比較的早く消失することから，メラニンへの親和性は強くないと考える。

妊娠 13 日目のラット及び妊娠 17 日目のウサギに経口投与したとき，Arm 及び Lmf ともに胎児への移行が認められた。投与後 24 時間の胎児放射能の母体血中放射能に対する比は，ラットの Arm 及び Lmf はそれぞれ 0.34～0.74 及び 0.004～0.262，ウサギではそれぞれ 0.50～0.99 及び 0.02～0.03 であった。胎児は Arm，Lmf，及びそれらの代謝物に曝露されていることが示唆された。

## 1.3 代謝

各種動物及びヒト肝ミクロソームを用いた *in vitro* 試験において，Arm 及び Lmf の主な代謝酵素は CYP3A4/5 であった。Arm の主な代謝経路は，1）水酸化，2）グルクロン酸抱合，3）O-脱メチル化（薬理的に活性のある代謝物 DHA）と推定された。Lmf の主な代謝経路は，1）N-脱ブチル化（薬理的に活性のある代謝物 desbutyl-Lmf），2）C-水酸化，3）グルクロン酸抱合と推定された。

## 1.4 排泄

Arm を経口投与したとき，Arm 及びその代謝物は尿及び糞中にほぼ同じ割合で排泄され，投与後 7 日までにほとんど排泄された。Lmf を経口投与したとき，未変化体として約 90%が糞中から回収された。desbutyl-Lmf はわずかであり，投与後 7 日までにほぼ排泄された。

妊娠ラット及び妊娠ウサギの乳腺に放射能が定量されたことから，Arm 及び Lmf とその代謝物は乳汁中に移行すると考える。

## 1.5 薬物間相互作用

Arm は *in vitro* 試験において，CYP1A2 に対して弱い阻害作用を示したが，臨床的に問題となる程度ではなかった。Lmf は CYP2D6 を阻害し，Ki 値は 0.997 μmol/L（0.527 μg/mL）であった。non-immune の患者での Lmf の最高血漿中濃度（平均値）は約 11 μmol/L（5.72 μg/mL，A2401 試験）であり， $K_i$ 値に比べ高値であることから，治療域の狭い化合物では薬物間相互作用が臨床的に問題となる可能性が示唆された。

ヒト肝細胞を用いた *in vitro* 試験では， Arm，DHA 及び Lmf（それぞれ 2.5，7 及び 200 μM の濃度で検討）は CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19 及び CYP3A を誘導しなかった。一方，Arm 及び DHA 等のアルテミシニン誘導体は，ヒト初代肝細胞及びヒト腸細胞株 LS174T において， CYP2B6，CYP3A4 及び MDR1 を誘導することが報告されている。

以上のことから， *in vitro* 試験では分子種で結論が一致していないが，Arm 及び DHA の CYP 誘導については否定できないと考える。したがって，CYP3A4 の誘導により，CYP3A4 によって代謝される薬剤の薬物動態に影響が生じる可能性が考えられる。

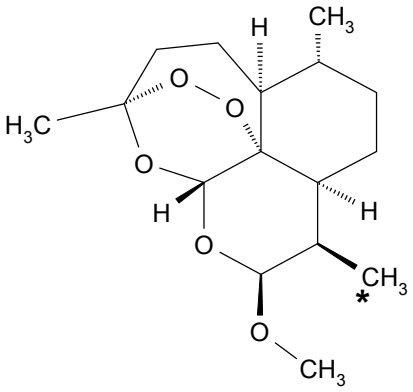
# 2 分析法

## 2.1 放射性標識

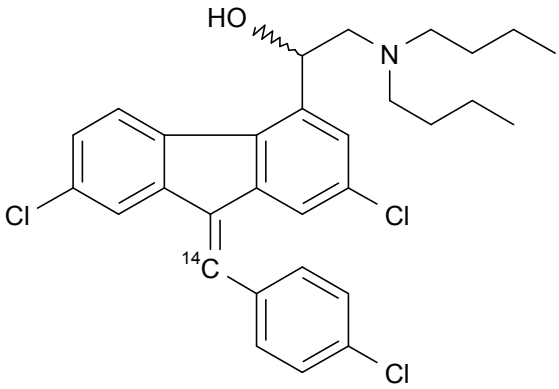
Arm 及び Lmf の ADME 試験（Table 1-1）では，[$^{14}$C]-Arm，[$^{3}$H]-Arm，[$^{14}$C]-Lmf を用いた。それぞれの化学構造式を標識部位と併せてFigure 2-1に示す。

**Figure 2-1 Arm 及び Lmf の化学構造式及び標識部位**

**Arm**




**Lmf**




*：[$^{14}$C] 又は[ $^{3}$H]-標識部位　　$^{14}$C ：[$^{14}$C]-標識部位

HPLC-放射能検出により測定した放射化学的純度は概して 98%以上であった。

## 2.2 放射能の測定

血液，血漿，血清，尿，胆汁，及びクロマト画分中の放射能は，適切なシンチレータを加え，液体シンチレーションカウンター（LSC）により直接測定した。糞中の放射能濃度は，ホモジネート試料を可溶化した後，LSC によって測定した。臓器及び組織中の放射能濃度は，定量的全身性オートラジオグラフィー（QWBA）又は臓器及び組織を摘出後可溶化し LSC によって測定した[2.6.5.5A-DMPK(CH)1997/241]，[2.6.5.5B-DMPK(CH)1997/240]，[2.6.5.7A-DMPK(CH)1997/099]，[2.6.5.7D-DMPK(CH)1997/139]。

## 2.3 Arm，DHA 及び Lmf の定量

非臨床試験における主な Arm，DHA，Lmf，及び desbutyl-Lmf の濃度測定法をTable 2-1に示す。血漿中の定量は HPLC-ECD 法，HPLC-UV 法，LC-MS 法及び LC-MS/MS 法により行った。内部標準物質として arteether，artemisinin，安定同位体標識 Arm[2.6.5.2F-R0500720-01]，halofantrine 又は安定同位体標識 Lmf[2.6.5.2N-R0301253-01]を使用した。血漿中における測定対象物質の安定性は，ラット血漿中 Arm 及び DHA では-75±10°C で少なくとも 5 ヵ月まで[2.6.5.2D-DMPK R0500059]，イヌ血漿中 Arm 及び DHA では-75±10°C で少なくとも 20 週間まで[2.6.5.2C-DMPK R0400692]，ヒト血漿中 Arm 及び DHA では-75°C ±10°C で少なくとも 12 ヵ月まで（5.3.1.4-4-

DMPK R0700513）確認されている。イヌ血漿中 Lmf では-70°C 以下で少なくとも 23 週間まで [2.6.5.2 I-DMPK R0300924C]，ヒト血漿中 Lmf では-70°C 以下で少なくとも 21 ヵ月まで（5.3.1.4-9- DMPK R0300924A-02），ヒト血漿中 desbutyl Lmf では-70°C 以下で少なくとも 4.5 ヵ月まで [2.6.5.2H-R0300924B]確認されている。

**Table 2-1　　Arm，DHA，Lmf，及び desbutyl-Lmf の濃度測定法**

| 測定対象物質 | 検出方法 | 試料 | 定量下限 | 定量上限 | 報告書番号 |
|---|---|---|---|---|---|
| Arm, DHA | LC-MS/MS | ヒト血漿 | 5 ng/mL | 200 ng/mL | BAPK(EU) R0301212, BAPK(EU) R0301212-01 |
| | LC-MS/MS | イヌ血漿 | 5 ng/mL | 200 ng/mL | DMPK R0400692 |
| | LC-MS/MS | ラット血漿 | 20 ng/mL | 200 ng/mL | DMPK R0500059 |
| | LC-MS | ヒト血漿 | 5 ng/mL | 200 ng/mL | BAPK(F) R00-1840 |
| | HPLC-ECD | ヒト血漿 a) | 10 ng/mL | 200 ng/mL | BPK(F) 1996/001 |
| Lmf | LC-MS/MS | ヒト血漿 | 0.05 µg/mL | 20 µg/mL | DMPK R0300924A |
| | LC-MS/MS | イヌ血漿 | 0.1 µg/mL | 50 µg/mL | DMPK R0300924C |
| | HPLC-UV | ヒト血漿 | 0.05 µg/mL | 50 µg/mL | DMPK(F) R00-2105 |
| | HPLC-UV | ヒト血漿 a) | 35 nmol/L (18.5 µg/mL) | 1554 nmol/L | BPK(CH) 1994/038 |
| | HPLC-UV エナンチオマー分離 | ヒト血漿，イヌ血漿，ラット血漿 | S(+) enantiomer: 1.1 µg/mL<br>R(-) enantiomer: 0.5 µg/mL | S(+) enantiomer: 2.06 µg/mL<br>R(-) enantiomer: 2.37 µg/mL | BPK(CH) 1996/147 |
| desbutyl-Lmf | LC-MS/MS | ヒト血漿 | 0.005 µg/mL | 1 µg/mL | DMPK R0300924B |
| Lmf, desbutyl-Lmf | HPLC-UV | ヒト血漿 | Lmf: 0.05 µg/mL<br>desbutyl-Lmf: 5 ng/mL | Lmf: 20 µg/mL<br>desbutyl-Lmf: 1 µg/mL | BAPK(F) R00-2105-02, BAPK(F) R00-2105-02-01 |

Source：2.6.5.2A-BAPK(EU) R0301212, 2.6.5.2B-BAPK(EU) R0301212-01, 2.6.5.2C-DMPK R0400692, 2.6.5.2D-DMPK R0500059, 2.6.5.2E-BAPK(F) R00-1840, 2.6.5.2G-DMPK R0300924A, 2.6.5.2H-DMPK R0300924B, 2.6.5.2 I-DMPK R0300924C, 2.6.5.2J-DMPK(F) R00-2105, 2.6.5.2K- BAPK(F) R00-2105-02, BAPK(F) R00-2105-02-01, 2.6.5.2 L-BPK(CH) 1994/038, 2.6.5.2 M-BPK(CH) 1996/147, 5.3.1.4-1-BPK(F) 1996/001

ラット，イヌ及びヒト血漿中の Lmf ラセミ体の *S*- 及び *R*-エナンチオマーである CGP 64455 *S(+)*及び CGP 64456 *R(-)*を，キラル分離カラムを用いて HPLC-UV で測定した [Table 2.6.5.2 M-試験 1996/147]。*S*-及び *R*-エナンチオマーの LLOQ は，血漿試料 0.5 mL を用いた場合，それぞれ 1.1 及び 0.5 µg/mL であった。

a) ヒト血漿試料を用いてバリデートした濃度測定法を，ラット，ウサギ，イヌ，及びサルの血漿試料に適用した。

## 2.4　　代謝物の同定及び構造解析

代謝物の組成分析は，放射能検出器を接続した HPLC を用いた。*In vitro* 代謝試験はマウス，ラット及びヒト肝ミクロソーム及び組換え型ヒトチトクローム P450 を用いて実施した（Table 1-2）。血漿（ラット及びイヌ），尿及び糞（ラット，ウサギ，イヌ，及びヒト），ラット胆汁中の *in vivo* 代謝物の組成を検討した。構造既知代謝物との保持時間の比較，あるいは単離後の質量分析及び核磁気共鳴分光分析により，代謝物の構造を同定した。

# 3 吸収

[$^{3}$H] - 標識，又は [$^{14}$C] - 標識した Arm 及び Lmf を，動物（ラット，ウサギ，イヌ，及びサル）に，静脈内，経口，皮下，又は筋肉内投与して検討した。

## 3.1 吸収及びバイオアベイラビリティ

### Arm

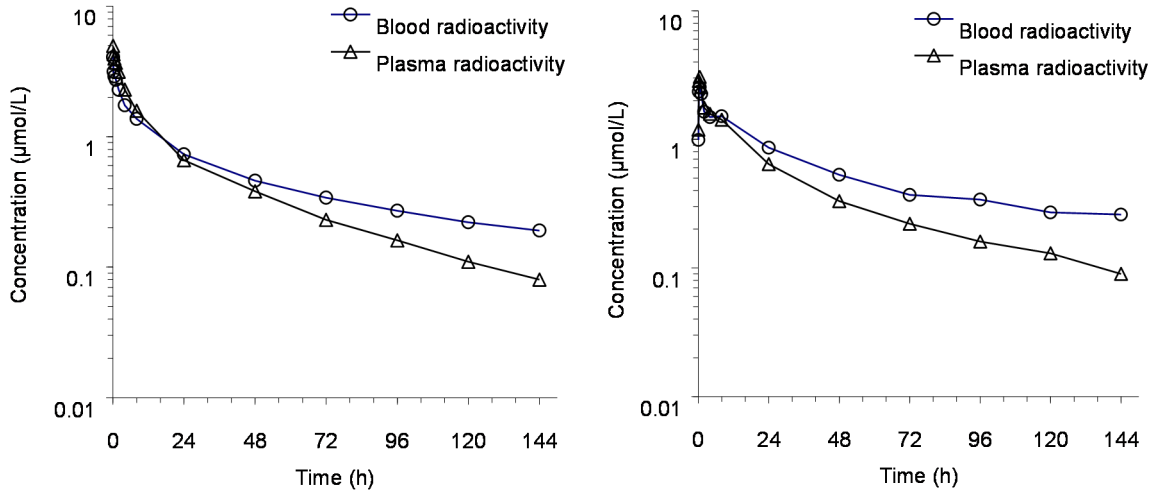
雄性ラットに 10 mg/kg の Arm を経口投与したときの絶対的バイオアベイラビリティ（BA）は 19.7%であると報告されている（Li et al. 1998）。

自由摂餌の雄性ラットに Arm/Lmf 10 mg/kg（[$^{3}$H]-Arm として 1.43 mg/kg）を単回静脈内投与したときの血漿中放射能の AUC0-144h は 68.22 µmol· h/L，T1/2 は約 40 時間であった。Arm/Lmf 20 mg/kg（[$^{3}$H]-Arm として 2.86 mg/kg）を単回経口投与したときの Arm の血漿中放射能の，AUC0-144h は 59.42 µmol· h/L ，T1/2 は約 50 時間であり，Tmax（中央値）は 0.5 時間であった（Figure 3-1）。Arm/Lmf 20 mg/kg（[$^{3}$H]-Arm として 2.86 mg/kg）を 1 日 1 回 10 日間反復経口投与したとき，初回投与後 24 時間の血漿中放射能濃度は 0.49 µmol/L，最終投与後 24 時間では 1.56 µmol/L であり，累積比は 3.2 であった[2.6.5.3A-1997/241]。

雄性イヌに Arm/Lmf 10 mg/kg（[$^{14}$C]-Arm として 1.43 mg/kg）を静脈内投与したとき，Arm の血漿中放射能の AUC0-168h は 137.8 µmol· h/L であった。Arm 及び DHA は速やかに消失し，投与後 4 時間以降の未変化体は定量下限未満（0.034 µmol/L）であった。 Arm/Lmf 20 mg/kg（[$^{14}$C]-Arm として 2.86 mg/kg）を単回経口投与したときの血漿中放射能の AUC0-168h は 151.26 µmol· h/L であった[2.6.5.3B− 1997/003]。

**Figure 3-1　　雄性ラットに Arm/Lmf（[$^3$H]-Arm）を単回投与したときの血液中及び血漿中放射能濃度-時間推移**

**10 mg/kg, i.v.**　　**20 mg/kg, p.o.**




Source 4.2.2.2-1− 1997/241-PT- Figure 1
○：血液中放射能，Δ：血漿中放射能，1 µmol/L = 298.38 µg Eq/L

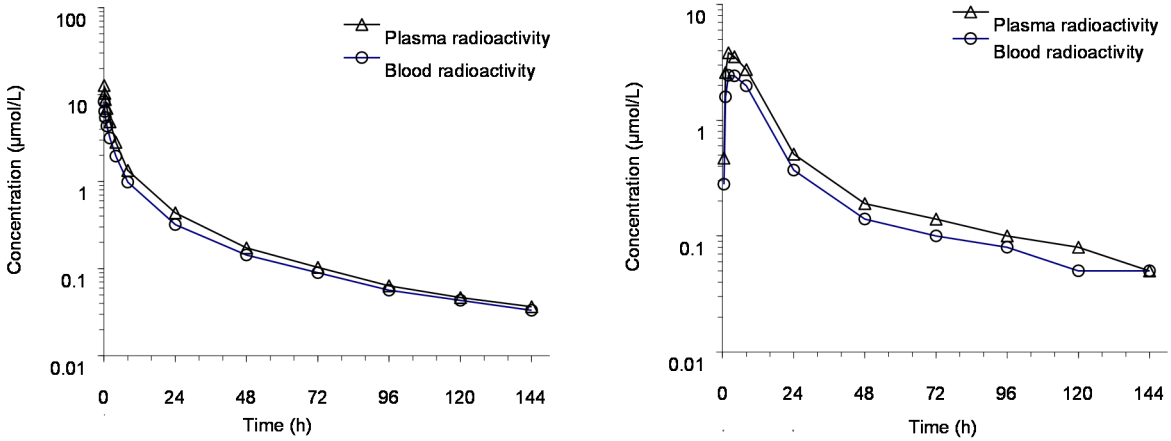
**Lmf**

雄性ラットに 20～1000 mg/kg の Arm/Lmf を経口投与したときの Lmf の吸収率は 0.9%～19%であり，絶対的 BA は算出できなかった。雄性イヌに 20 mg/kg の Arm/Lmf を経口投与したときの Lmf の絶対的 BA は 8%～24%であった[2.6.5.3D-1997/240]。

雄性ラットに Lmf 1 mg/kg を単回静脈内投与したときの AUC0-24h は約 40 µmol· h/L（21.36 µg · h/mL），血漿クリアランスは 0.8 mL/min/kg であった[2.6.5.3F− 1996/026]。雄性ラットに Arm/Lmf 1 mg/kg （[$^{14}$C]-Lmf として 0.86 mg/kg）を単回静脈内投与したときの Lmf の血漿中放射能の AUC0-144h は 59.29 µmol· h/L ， T1/2 は約 50 時間であった。絶食時の雄性ラットに Arm/Lmf 20 mg/kg（[$^{14}$C]-Lmf として 17.14 mg/kg）を単回経口投与したときの血漿中放射能の AUC0-144h は 68.9 µmol· h/L であり，T1/2 は約 50 時間，Tmax は 2 時間であった。非絶食時の雄性ラットに Arm/Lmf 20 mg/kg を単回経口投与したときの血漿中放射能の AUC0-48h は 170 µmol· h/L，Tmax は 2 時間であった。[2.6.5.3D-1997/240]。

**Figure 3-2　　雄性ラットに [$^{14}$C]-Lmf を単回投与したときの血液中及び血漿中放射能の濃度-時間推移**

**1 mg/kg, i.v.**　　**20 mg/kg, p.o.**




Source: 4.2.2.2-4− 1997/240-PT-Figure 2
○：血液中放射能，Δ：血漿中放射能，1 µmol/L = 528.95 µg Eq/L

雄性イヌに Arm/Lmf 1 mg/kg（[$^{14}$C]-Lmf として 0.86 mg/kg）を静脈内投与したとき，Lmf の血漿中放射能の AUC0-168h は 50.8 µmol· h/L であった。Arm/Lmf 20 mg/kg（[$^{14}$C]-Lmf として 17.14 mg/kg）を単回経口投与したときの血漿中放射能の AUC0-168h は，絶食時で 109 µmol· h/L，非絶食時で 155 µmol· h/L であった。未変化体（Lmf）の AUC0-168h は，絶食時で 103 µmol· h/L，非絶食時で 115 µmol· h/L であった [2.6.5.3D-1997/240]。非絶食時の方が絶食時とくらべ，放射能及び Lmf の曝露量は高かった。

雌性カニクイザルに Arm/Lmf 50/300 mg/kg を単回経口投与したときの Lmf の AUC0-120h は 123.8 ～ 237.3 µmol · h/L （ 65.5 ～ 125.5 µg · h/mL ） ， T1/2 は約 12.3 ～ 39.8 時間であった [2.6.5.3E− 1996/095]。

## 3.2　　トキシコキネティクス

ラット及びイヌにおける毒性試験での Arm，DHA，並びに Lmf のトキシコキネティクス（TK）を評価した。また，ラット及びにウサギに経口投与したときの生殖発生毒性試験，ラット及びイヌに経口並びに筋肉内投与したときの神経毒性試験の TK についても評価した。

### Arm

雌雄ラットに Arm/Lmf 20 及び 200 mg/kg/日を 2 週間反復経口投与した。投与初日では，雌雄ともに 20 及び 200 mg/kg/日群間の Arm 並びに DHA の曝露量は用量比を超えて増加した（Table 3-1）。また，投与初日と比べ投与後 14 日の曝露量が減少したことから，Arm 及び DHA の代謝の自己誘導の可能性が考えられる[2.6.5.4A- R0570030]。

雌雄ラットに Arm/Lmf 100，300 及び 1000 mg/kg/日を 13 週間反復経口投与したとき，ほとんどの血漿中 Arm 及び DHA 濃度は定量下限未満（10.0 ng/mL）であった[2.6.5.4B-BPK(F)1996/007]。

雌雄幼若ラットに Arm 10，30 及び 100 mg/kg/日を 2 週間（生後 7～21 日間）反復経口投与したとき， 10 mg/kg/日群の血漿中 Arm 及び DHA 濃度はいずれも定量下限未満（20.0 ng/mL）であった。30 mg/kg/日群では投与後 1～4 時間の時点のみ定量可能であった。100 mg/kg/日群は TK 用検体を採取する前に試験を中止した[2.6.5.4C-DMPK R0570013]。

**Table 3-1　　ラット 2 週間毒性試験における Arm 及び DHA の TK パラメータ**

| Atm/lmf 投与量 (mg/kg) | 成分 | 採血日 (日) | AUC0-24h (ng· h/mL) | | Cmax (ng/mL) | | Tmax* (h) | |
|---|---|---|---|---|---|---|---|---|
| | | | M | F | M | F | M | F |
| 20 (Arm 投与量：2.86) | Arm | 1 | 104 | 309 | 107 | 130 | 0.5 | 0.5 |
| | | 3 | 203 | 240 | 132 | 174 | 0.5 | 1 |
| | | 14 | 30 | 177 | 38.5 | 122 | 0.5 | 0.5 |
| | DHA | 1 | 234 | 206 | 194 | 83.5 | 0.5 | 1 |
| | | 3 | 174 | 115 | 87.3 | 96.7 | 1 | 1 |
| | | 14 | 45 | 87 | 57.5 | 82.3 | 0.5 | 1 |
| 200 (Arm 投与量：28.6) | Arm | 1 | 3417 | 16990 | 815 | 3220 | 1 | 1 |
| | | 3 | 700 | 2982 | 635 | 1250 | 0.5 | 1 |
| | | 14 | 198 | 1243 | 65.2 | 402 | 0.5 | 1 |
| | DHA | 1 | 12103 | 7160 | 2630 | 1390 | 2 | 2 |
| | | 3 | 1080 | 4384 | 435 | 1400 | 0.5 | 1 |
| | | 14 | 365 | 2038 | 141 | 592 | 1 | 1 |

Source：4.2.3.7.7-5- R0570030，平均値，*：中央値，M :雄，F :雌

雌雄イヌに Arm/Lmf 20，60 及び 200 mg/kg/日を 13 週間反復経口投与したとき，ほとんどの血漿中 Arm 及び DHA 濃度は定量下限未満（10.0 ng/mL）であった[2.6.5.4E-BPK(F)1996/005]。

雄性イヌに Arm 300 mg/kg/日（初日のみ Arm 600 mg/kg/日）又は Arm/Lmf 1000 mg/kg（Arm として 143 mg/kg）を 7 日間反復経口投与した。ラットの結果と同様に，投与後 3 日と 7 日における用量補正した Arm 及び DHA の曝露量は，投与初日に比べて減少した（Table 3-2）[2.6.5.4D-DMPK R0510009B]。

**Table 3-2 雄性イヌ神経毒性試験における Arm 及び DHA の TK パラメータ**

| Arm/Lmf 投与量 (mg/kg) | 成分 | 採血日 (日) | AUC0-24h (ng· h/mL) | Cmax (ng/mL) | Tmax (h) |
|---|---|---|---|---|---|
| **Arm** | | | | | |
| 600 | Arm | 1 | 22479 | 3358 | 2.33 |
| 300 | | 3 | 602 | 130 | 1.75 |
| 300 | | 7 | 73 | 49 | 0.83 |
| 600 | DHA | 1 | 13375 | 2572 | 1.67 |
| 300 | | 3 | 1389 | 609 | 1.67 |
| 300 | | 7 | 186 | 144 | 1 |
| **Arm/lmf** | | | | | |
| 1000 (Arm 投与量：143) | Arm | 1 | 1294 | 486 | 1.17 |
| | | 3 | 52 | 29 | 1.04 |
| | | 7 | 16 | 18 | 0.33 |
| | DHA | 1 | 2253 | 1027 | 1.17 |
| | | 3 | 363 | 187 | 1.25 |
| | | 7 | 7 | 9 | 0.5 |

Source：4.2.3.7.3-2-DMPK R0510009B，（Arm 投与量），平均値

イヌの神経毒性試験では，雄性イヌに Arm 20 mg/kg/日を 4 週間反復筋肉内投与したとき，反復経口投与試験（2.6.5.4E-BPK(F)1996/005）と異なり， 血漿中 Arm 濃度は定量可能であった（10.0 ng/mL）。血漿中トラフ濃度は投与後 5 日目まで上昇したが，投与後 8 日目以降低下した。Arm は血液脳関門を通過し，脳脊髄液中 Arm 濃度は血漿中 Arm 濃度の約 10%未満であった。血漿中 DHA 濃度は定量下限未満（10.0 ng/mL）であり，定量できなかった [2.6.5.4F-BPK (F)1997/004] 。雄性イヌに Arm を 8 日間反復筋肉内投与したとき，反復投与最終日の Arm の曝露量は投与初日と比べ約 2 倍に増加した[2.6.5.4G-DMPK R0410073]，[2.6.5.4H-DMPK R0510001]，[2.6.5.4 I– 1998/014]。

ウサギにおける胚・胎児発生に関する試験では，Arm/Lmf 210（Arm/Lmf 30/180），700（Arm/Lmf 100/600），2100（Arm/Lmf 300/1800） mg/kg を 13 日間反復経口投与したとき，母動物の血液中 Arm 濃度は 2100 mg/kg 投与群で，DHA 濃度は 700 mg/kg 及び 2100 mg/kg 投与群で検出されたが，薬物動態パラメータを算出できなかった[2.6.5.7.C-BPK(F)1996/022]。

### Lmf

3～5 週齢の雌雄幼若ラットに Arm/Lmf 100，300 及び 1000 mg/kg/日を 13 週間反復経口投与したとき，投与後 8 時間の Lmf 濃度は用量比を下回った[2.6.5.4 L-1997/177]。

雌雄ラットに Arm/Lmf 200，600 及び 1000 mg/kg/日を 4 週間反復経口投与したとき，1000 mg/kg/日群の最終投与日の Lmf の曝露量は，投与初日に比べ高かった（Table 3-3）[2.6.5.4J-BPK(CH)1995/079]。雌雄ラットに Arm/Lmf 100，300 及び 1000 mg/kg/日を 13 週間反復経口投与したとき，Lmf のトラフ値並びに曝露量はばらつきが大きく，明らかな累積性は認められなかった [2.6.5.4K-BPK(F)1996/020]，[2.6.5.4 L-DMPK(CH)1997/177]，[2.6.5.4 M-DMPK(CH)1997/178]。

**Table 3-3　　ラット 4 週間毒性試験における Lmf の TK パラメータ**

| Atm/lmf 投与量 (mg/kg) | 成分 | 採血日 (日) | AUC0-24h (μg· h/mL) | | Cmax (μg/mL) | | Tmax (h) | |
|---|---|---|---|---|---|---|---|---|
| | | | M | F | M | F | M | F |
| 200 (Lmf 投与量：171) | Lmf | 1 | 535 | 358 | 32.9 | 23.3 | 8 | 8 |
| | | 28 | 322 | 281 | 15.9 | 16.0 | 24 | 4 |
| 600 (Lmf 投与量：514) | Lmf | 1 | 423 | 402 | 21.1 | 19.6 | 24 | 8 |
| | | 28 | 386 | 449 | 20.6 | 24.8 | 8 | 0 |
| 1000 (Lmf 投与量：857) | Lmf | 1 | 279 | 350 | 15.8 | 33.2 | 24 | 24 |
| | | 28 | 382 | 630 | 18.0 | 37.9 | 8 | 4 |

Source：4.2.3.2-1- BPK (CH) 1995/079，平均値，M :雄，F :雌

雌雄イヌに Lmf 60, 200, 600 mg/kg/日を 13 週間反復経口投与したとき，最終日における 200 及び 600 mg/kg/日群の Lmf の Cmax は投与初日と比べて増加した。投与後 71 日目における曝露量の用量比例性は用量比を下回った[2.6.5.4N-DMPK(CH)1997/006]。

イヌの神経毒性試験では，雄性イヌに Arm/Lmf（Arm/Lmf 143/857）1000 mg/kg を 7 日間反復経口投与したとき，投与後 3 日と 7 日における Lmf の曝露量は，初日に比べて増加する傾向がみられた[2.6.5.4O-DMPK R0510009A]。

雌雄イヌに Arm/Lmf 60，200，600 mg/kg/日を 4 週間反復経口投与試験[2.6.5.4P-BPK(CH)1995/080]，及び Arm/Lmf 20，60，200 mg/mL/日を 13 週間反復経口投与試験[2.6.5.4Q-BPK(CH)1996/024]ともに，個体間のばらつきが大きく，Lmf の累積性，用量比例性，及び性差については一定の傾向が認められなかった。しかしながら，ほとんどの用量で反復投与の AUC0-24h は初日と比べ反復投与最終日で増加した個体が多かった。

ウサギにおける胚・胎児発生に関する試験では，Arm/Lmf 210（Arm/Lmf 30/180），700（Arm/Lmf 100/600），2100（Arm/Lmf 300/1800） mg/kg/日を 13 日間反復経口投与したとき，Arm/Lmf の用量の増加に伴い Lmf の曝露量は増加した[2.6.5.7.G-BPK(CH)1996/085]。

以上の結果，個体間変動を含めたすべてのデータから判断すると，検討したいずれの動物種でも Arm，DHA 及び Lmf の曝露量に明らかな性差はないと考える。また，イヌ及びラットの Lmf の累積性については，ばらつきが大きく，初回投与に比べ反復投与後に曝露量が増加したものの，一定の傾向はみとめられなかった。

# 4　　分布

## 4.1　　*In vitro* 血漿・血清蛋白結合率及び血球への移行性

### Arm

マウス，ラット，ウサギ，イヌ，及びカニクイザルの血清に [$^{14}$C]-Arm を添加（最終濃度：1 又は 10 μg/mL）し，限外ろ過法を用いて血清蛋白結合率を *in vitro* で検討した。また，ヒトの血

液に [$^{14}$C]-Arm を添加（最終濃度：0.323～10 μg/mL）し，超遠心分離法を用いて血清中蛋白結合率，血漿蛋白結合率，及び血球への移行性を検討した。[2.6.5.6A-1998/004]。

Arm の血清蛋白結合率は，マウス，ラット，ウサギ，イヌ，及びカニクイザルで，それぞれ 98.3%～98.4%，97.3%，97.1%～97.2%，97.1%～97.3%，及び 96.0%～96.2%であり，濃度依存性は認められなかった（最終濃度：0.323～10 μg/mL）。

ヒトにおける Arm の血清蛋白結合率は 97.9%であり，血漿蛋白結合率は 95.4%であった。DHA の血清蛋白結合率は 47%～76%であった。ヒト血漿及び赤血球において，Arm は主に α1-酸性糖蛋白質（33%），次に血清アルブミン（17%），高，低及び超低比重リポ蛋白質（それぞれ 12%，9.3%及び 12%）並びに γ グロブリン（0.4%）に結合した。Arm の 11%が血球に移行し，4.6%は非結合型 Arm であった。

### Lmf

マウス，ラット，ウサギ，イヌ，及びカニクイザルの血清に [$^{14}$C]-Lmf を添加（最終濃度：10 μg/mL）し，限外ろ過法により血清蛋白結合率を *in vitro* で検討した。また，ヒトの血液に [$^{14}$C]-Lmf を添加（最終濃度：1 又は 10 μg/mL）し，超遠心分離法を用いて，血清蛋白結合率，血漿蛋白結合率，及び血球への移行性を検討した[2.6.5.6B-1996/044]。

Lmf の血清蛋白結合率は，マウス，ラット，ウサギ，イヌ，及びカニクイザルで，それぞれ 99.8%，99.9%，99.93%，99.92%，及び 99.94%であった（最終濃度：10 μg/mL）。ヒトにおける Lmf の血清蛋白結合率は 99.9%であり，血漿蛋白結合率は 99.7%であった。Lmf は 1 μg/mL（1.89 μmol/L）の濃度で主にリポ蛋白質，特に高比重リポ蛋白質（99.6%）に結合した。約 62%がヒト血清アルブミンに，約 8%が血球に移行した。

## 4.2 組織及び臓器分布

### Arm

雄性アルビノラットに Arm/Lmf 10 mg/kg（ [$^{3}$H]-Arm として 1.43 mg/kg）を単回静脈内投与したときの投与後 5 分，24 時間，及び 168 時間，並びに Arm/Lmf 20 mg/kg（[$^{3}$H]-Arm として 2.86 mg/kg）を単回経口投与したときの投与後 30 分，24 時間，及び 168 時間，の臓器・組織内放射能濃度を検討した[2.6.5.5A-DMPK(CH)1997/241]。静脈内投与後 5 分で全身に広く分布し，副腎，褐色脂肪，甲状腺，及び脳・脊髄等の神経組織に高い放射能が認められた。投与後 24 時間及び 168 時間では，腎臓，褐色脂肪，及び副腎中の放射能が高かった（Table 4-1）。

ラットに Arm/Lmf 20 mg/kg（[$^{3}$H]-Arm として 2.86 mg/kg）を 1 日 1 回 10 日間反復経口投与したとき，最終投与後 24 時間の血液，脳，及び小腸中の放射能濃度は単回投与後 24 時間と比べて約 5 倍高かった（Table 4-2）。経口投与では，消化管及び肝臓以外の組織への分布は静脈内投与したときの放射能分布パターンと類似していた（Table 4-2）。

**Table 4-1 雄性アルビノラットに Arm/Lmf 10 mg/kg（[$^3$H]-Arm として 1.43 mg/kg）を単回静脈内投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | |
|---|---|---|---|
| | 単回投与後 | | |
| | 5 分 | 24 時間 | 168 時間 |
| | N=3 | N=3 | N=4 |
| 血液 | 2.57 | 0.65 | 0.18 |
| 血漿 | 3.74 | 0.57 | 0.06 |
| 赤血球 | 1.20 | 0.67 | 0.31 |
| 唾液腺 | 5.51 | 1.61 | 1.04 |
| 甲状腺 | 7.81 | 1.76 | 1.18 |
| 食道 | 2.75 | 0.94 | 0.29 |
| 腋窩リンパ節 | 7.23 | 3.24 | 1.72 |
| 胸腺 | 3.05 | 1.38 | 0.99 |
| 肺 | 3.41 | 0.94 | 0.71 |
| 心臓 | 5.33 | 2.29 | 1.22 |
| 大動脈 | 5.43 | 1.64 | 0.68 |
| 肝臓 | 6.87 | 2.77 | 1.68 |
| 膵臓 | 5.99 | 1.52 | 0.88 |
| 脾臓 | 1.58 | 2.81 | 2.23 |
| 副腎 | 17.78 | 4.61 | 2.76 |
| 白色脂肪 | 1.34 | 0.50 | 0.25 |
| 腸間膜リンパ節 | 2.39 | 0.98 | 0.60 |
| 精巣 | 1.58 | 0.55 | 0.30 |
| 前立腺 | 3.12 | 0.93 | 0.46 |
| 膀胱 | 1.58 | 0.74 | 0.29 |
| 腎臓 | 5.39 | 15.29 | 8.51 |
| 筋肉 | 2.52 | 0.41 | 0.16 |
| 坐骨神経 | 3.75 | 0.94 | 0.42 |
| 骨髄 | 1.58 | 1.40 | 0.70 |
| 皮膚 | 2.00 | 0.70 | 0.33 |
| 褐色脂肪 | 8.02 | 6.64 | 5.10 |
| ハーダー腺 | 3.28 | 1.82 | 0.34 |
| 眼 | 1.51 | 0.19 | 0.09 |
| 小脳 | 8.28 | 0.41 | 0.30 |
| 大脳 | 7.06 | 0.43 | 0.33 |
| 延髄 | 9.70 | 0.49 | 0.34 |
| 脳下垂体 | 5.94 | 1.34 | 0.82 |
| 脊髄 | 8.65 | 0.34 | 0.23 |
| 無腺胃 | 1.68 | 0.64 | 0.21 |
| 腺胃 | 3.93 | 2.23 | 0.99 |
| 小腸 | 4.18 | 1.25 | 0.70 |

Source: 4.2.2.2-1-DMPK(CH) 1997/241-Table 5, 6, Table 15 1 nmol/g = 298.38 ng Eq/g

**Table 4-2 雄性アルビノラットに Arm/Lmf 20 mg/kg（ [$^3$H]-Arm として 2.86 mg/kg）を単回又は反復経口投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | | |
|---|---|---|---|---|
| | 単回投与後 | | | 10 日間反復投与後 |
| | 30 分 | 24 時間 | 168 時間 | 24 時間 |
| | N=3 | N=3 | N=3 | N=3 |
| 血液 | 3.10 | 0.67 | 0.32 | 3.03 |
| 血漿 | 4.00 | 0.49 | 0.07 | 1.56 |
| 赤血球 | 2.49 | 0.91 | 0.47 | － |
| 唾液腺 | 3.38 | 0.95 | 0.72 | 2.92 |
| 甲状腺 | 3.97 | 1.14 | 0.60 | 4.74 |
| 食道 | 16.95 | 1.14 | 0.25 | 3.68 |
| 腋窩リンパ節 | 4.56 | 1.42 | 0.48 | 5.19 |
| 胸腺 | 2.43 | 0.73 | 0.48 | 2.41 |
| 肺 | 2.87 | 0.64 | 0.35 | 2.34 |
| 心臓 | 4.17 | 1.26 | 1.02 | 4.05 |
| 大動脈 | 3.54 | 0.90 | 0.37 | 2.25 |
| 肝臓 | 18.65 | 3.18 | 1.67 | 12.39 |
| 膵臓 | 3.73 | 0.89 | 0.62 | 2.93 |
| 脾臓 | 3.31 | 1.64 | 1.39 | 6.65 |
| 副腎 | 6.84 | 2.00 | 1.59 | 7.89 |
| 白色脂肪 | 2.79 | 0.24 | 0.09 | 0.69 |
| 腸間膜リンパ節 | 3.00 | 0.59 | 0.35 | 2.52 |
| 精巣 | 1.51 | 0.39 | 0.18 | 1.25 |
| 前立腺 | 2.53 | 0.46 | 0.22 | － |
| 膀胱 | 5.19 | 0.62 | 0.18 | 2.21 |
| 腎臓 | 10.38 | 4.47 | 3.14 | 15.21 |
| 筋肉 | 1.91 | 0.29 | 0.19 | 1.23 |
| 坐骨神経 | 2.01 | 0.40 | 0.19 | 1.57 |
| 骨髄 | 3.28 | 0.79 | 0.45 | 2.66 |
| 皮膚 | 2.13 | 0.57 | 0.29 | 2.42 |
| 褐色脂肪 | 8.56 | 3.92 | 2.58 | 12.46 |
| ハーダー腺 | 3.33 | 0.67 | 0.18 | 1.71 |
| 眼 | 1.19 | 0.18 | 0.08 | 0.77 |
| 小脳 | 1.16 | 0.27 | 0.17 | － |
| 大脳 | 1.11 | 0.28 | 0.20 | 1.32* |
| 延髄 | 1.21 | 0.29 | 0.20 | － |
| 脳下垂体 | 2.93 | 0.66 | 0.44 | － |
| 脊髄 | 1.13 | 0.21 | 0.17 | － |
| 無腺胃 | 108.7 | 4.80 | 0.40 | 7.33 |
| 腺胃 | 39.47 | 2.98 | 0.93 | 6.59 |
| 小腸 | 16.71 | 1.37 | 0.48 | 7.08 |

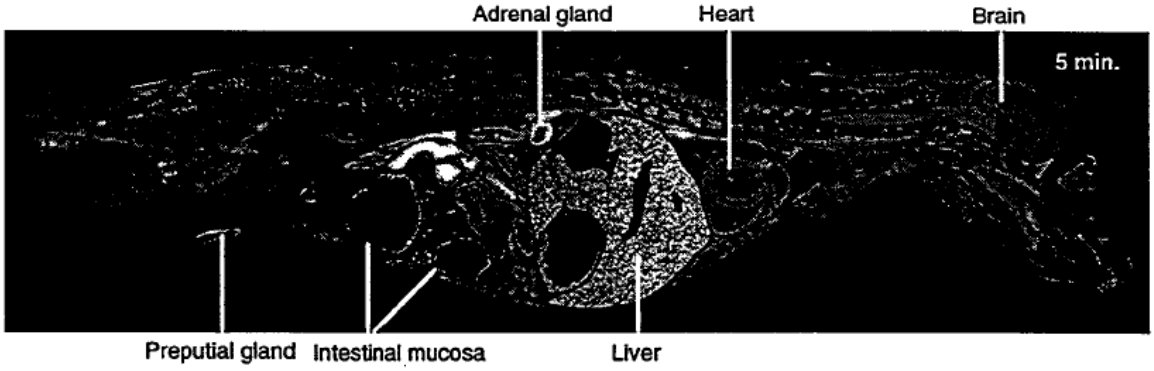
Source: 4.2.2.2-1-DMPK(CH) 1997/241-Table 7, Table 8, Table 9, Table 16 1 nmol/g = 298.38 ng Eq/g
*:脳

また，雄性有色ラットに Arm/Lmf 10 mg/kg（ [$^{14}$C]-Arm として 1.43 mg/kg）を単回静脈内投与したときの投与後 5 分，1 時間，8 時間，24 時間，及び 168 時間の放射能の全身分布を QWBA により検討した。投与後 5 分及び 8 時間の組織中放射能分布をFigure 4-1に，臓器・組織内放射能濃度をTable 4-3に示す。静脈内投与後 5 分で全身に広く分布し，高い放射能が認められた組織は，肝臓（胆管），副腎皮質，小腸，心筋，脳，胃腸管粘膜，及び包皮腺であった。投与後 8 時間では，腎皮質及び腸管内容物の放射能濃度が高く，次いで肝臓（胆管含む），褐色脂肪，脾臓，骨髄，唾液腺，胃粘膜であり，精巣及び肺からも放射能が検出された。

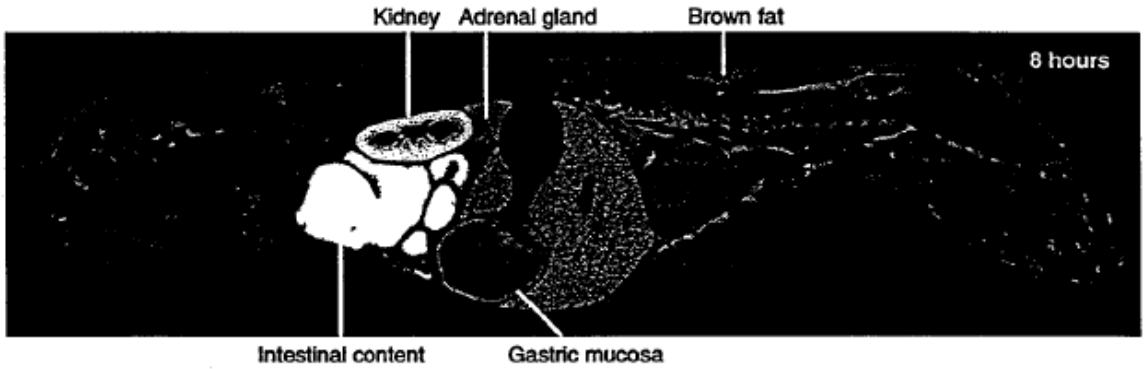
有色ラットの組織内放射能分布はアルビノラットと類似しており，メラニン含有組織である眼から放射能が比較的早く消失することから，メラニンへの親和性は強くないと考える（Table 4-3）。

**Figure 4-1　Arm/Lmf 10 mg/kg （ [$^{14}$C]-Arm として 1.43 mg/kg）を雄性有色ラットに単回静脈内投与したときの投与後 5 分及び 8 時間の組織中放射能分布**

**投与後 5 分**




**投与後 8 時間**




Source：4.2.2.2-1-DMPK(CH) 1997/241-Figure 6
[$^{14}$C] Arm として 1.43 mg/kg
白色部位は放射能の存在を示す

**Table 4-3　雄性有色ラットに Arm/Lmf 10 mg/kg（ [$^{14}$C]-Arm として 1.43 mg/kg）を単回静脈内投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | | | |
|---|---|---|---|---|---|
| | 単回投与後 | | | | |
| | 5 分 | 1 時間 | 8 時間 | 24 時間 | 168 時間 |
| | N=3 | N=3 | N=3 | N=3 | N=3 |
| 血液* | 5.25 | 2.44 | 1.53 | 1.11 | 0.36 |
| 肝臓* | 14.61 | 6.69 | 7.64 | 4.20 | 0.99 |
| 腎臓* | 5.70 | 6.54 | 7.66 | 5.86 | 2.85 |
| 副腎 | 14.74 | 6.58 | 4.79 | 3.64 | 2.26 |
| 脾臓 | 5.73 | 5.52 | 4.73 | 4.01 | 2.02 |
| 肺 | 6.19 | 2.38 | 3.20 | 1.97 | 0.49 |
| 胸腺 | 4.35 | 1.43 | 1.54 | 1.46 | 1.20 |
| 唾液腺 | 6.01 | 2.52 | 2.41 | 2.21 | 1.52 |
| 骨髄 | 4.71 | 3.22 | 3.22 | 1.89 | 0.85 |
| 脊髄 | 12.39 | 0.90 | 0.76 | 0.65 | 0.69 |
| 白色脂肪 | 2.91 | 2.14 | 3.44 | 3.49 | 0.16 |
| 褐色脂肪 | 5.26 | 8.71 | 3.45 | 3.34 | 2.75 |
| 小脳 | 11.21 | 0.69 | 0.58 | 0.64 | 0.69 |
| 大脳 | 11.46 | 0.85 | 0.66 | 0.70 | 0.69 |
| 小腸# | 2.39 | 28.35 | 40.50 | 4.77 | 0.43 |
| 胃# | 2.13 | 0.14 | 0.79 | 4.65 | 0.75 |
| 精巣 | 3.01 | 0.87 | 0.84 | 0.48 | 0.82 |
| 眼 | 1.77 | 0.48 | 0.36 | 0.22 | 0.10 |

Source: 4.2.2.2-1-DMPK(CH) 1997/241-Tabl19　　1 nmol/g = 298.38 ng Eq/g

*: 2 検体の平均値，#: 内容物を含む

### Lmf

雄性アルビノラットに Arm/Lmf 1 mg/kg（ [$^{14}$C]-Lmf として 0.86 mg/kg）を単回静脈内投与したときの投与後 5 分，24 時間，及び 168 時間，並びに Arm/Lmf 20 mg/kg（[$^{14}$C]-Lmf として 17.14 mg/kg）を単回経口投与したときの投与後 3 時間，24 時間，及び 168 時間の臓器・組織内放射能濃度を検討した[2.6.5.5B-DMPK(CH)1997/240]。静脈内投与後 5 分で全身に広く分布し，肝臓，及び血漿に高い放射能が認められた。投与後 24 時間及び 168 時間では副腎の放射能が高かった（Table 4-4）。

経口投与では，消化管及び腸間膜リンパ節以外の組織への分布は静脈内投与したときの放射能分布パターンと類似していた（Table 4-5）。

ラットに Arm/Lmf 20 mg/kg（[$^{14}$C]-Lmf として 17.14 mg/kg）を 1 日 1 回 10 日間反復経口投与したとき，最終投与後 24 時間の腋窩リンパ節，胸腺，白色脂肪，褐色脂肪，及び精巣の放射能濃度は単回投与後 24 時間と比べて 15 倍以上高かった（Table 4-5）。

**Table 4-4 雄性アルビノラットに Arm/Lmf 1 mg/kg（ [$^{14}$C]-Lmf として 0.86 mg/kg）を単回静脈内投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | |
|---|---|---|---|
| | 単回投与後 | | |
| | 5 分 | 24 時間 | 168 時間 |
| | N=3 | N=3 | N=3 |
| 血液 | 10.3 | 0.18 | 0.02 |
| 血漿 | 17.4 | 0.25 | 0.02 |
| 赤血球 | 2.22 | 0.10 | 0.01 |
| 唾液腺 | 0.55 | 1.48 | 0.28 |
| 甲状腺 | 1.42 | 1.77 | 0.40 |
| 食道 | 0.32 | 0.62 | 0.10 |
| 腋窩リンパ節 | 0.60 | 2.17 | 0.97 |
| 胸腺 | 0.25 | 0.84 | 0.37 |
| 肺 | 5.35 | 2.97 | 0.31 |
| 心臓 | 3.85 | 1.80 | 0.15 |
| 大動脈 | 0.44 | 0.48 | 0.12 |
| 肝臓 | 18.4 | 2.28 | 0.28 |
| 膵臓 | 1.73 | 1.88 | 0.34 |
| 脾臓 | 4.93 | 4.46 | 0.38 |
| 副腎 | 3.97 | 16.0 | 1.06 |
| 白色脂肪 | 0.27 | 1.04 | 0.49 |
| 腸間膜リンパ節 | 0.32 | 1.79 | 0.44 |
| 精巣 | 0.14 | 0.13 | 0.08 |
| 前立腺 | 0.26 | 0.70 | 0.22 |
| 膀胱 | 0.29 | 0.52 | 0.07 |
| 腎臓 | 1.69 | 2.22 | 0.33 |
| 筋肉 | 0.17 | 0.72 | 0.13 |
| 坐骨神経 | 0.30 | 0.46 | 0.27 |
| 骨髄 | 3.03 | 2.41 | 0.31 |
| 皮膚 | 0.12 | 0.49 | 0.32 |
| 褐色脂肪 | 0.99 | 3.43 | 1.65 |
| ハーダー腺 | 0.21 | 0.57 | 0.22 |
| 眼 | 0.12 | 0.16 | 0.04 |
| 小脳 | 0.35 | 0.04 | 0.01 |
| 大脳 | 0.25 | 0.02 | 0.01 |
| 延髄 | 0.29 | 0.02 | 0.01 |
| 脳下垂体 | 2.43 | 2.80 | 0.81 |
| 脊髄 | 0.40 | 0.04 | 0.01 |
| 無腺胃 | 0.25 | 0.65 | 0.08 |
| 腺胃 | 0.52 | 0.93 | 0.13 |
| 小腸 | 0.43 | 0.83 | 0.10 |

Source: 4.2.2.2-4- DMPK(CH) 1997/240-Table 10, Table 11, Table 29 1 nmol/g = 528.95 ng Eq/g

**Table 4-5 雄性アルビノラットに Arm/Lmf 20 mg/kg（[$^{14}$C]-Lmf として 17.1 mg/kg）を単回又は反復経口投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | | |
|---|---|---|---|---|
| | 単回投与後 | | | 10 日間反復投与後 |
| | 3 時間 | 24 時間 | 168 時間 | 24 時間 |
| | N=3 | N=3 | N=3 | N=3 |
| 血液 | 2.14 | 0.35 | NS | 2.07 |
| 血漿 | 3.75 | 0.44 | 0.00 | 2.18 |
| 赤血球 | 0.57 | 0.19 | NS | 1.67 |
| 唾液腺 | 0.45 | 3.01 | 0.20 | 33.9 |
| 甲状腺 | 1.15 | 5.80 | 0.28* | 47.2 |
| 食道 | 4.85 | 1.38 | 0.13* | 11.9 |
| 腋窩リンパ節 | 0.66 | 4.01 | 0.42 | 62.1 |
| 胸腺 | 0.26 | 1.82 | 0.32 | 31.6 |
| 肺 | 3.31 | 6.65 | 0.24 | 57.0 |
| 心臓 | 1.82 | 3.86 | 0.12 | 25.8 |
| 大動脈 | 0.19* | 0.96 | 0.19* | 10.4 |
| 肝臓 | 24.6 | 7.02 | 0.18 | 47.0 |
| 膵臓 | 0.73 | 3.97 | 0.27 | 40.6 |
| 脾臓 | 5.77 | 9.29 | 0.31 | 66.7 |
| 副腎 | 14.9 | 44.6 | 0.96 | 251 |
| 白色脂肪 | 0.43 | 2.09 | 0.36 | 33.2 |
| 腸間膜リンパ節 | 24.8 | 6.44 | 0.46 | 45.1 |
| 精巣 | 0.13 | 0.39 | 0.07 | 5.73 |
| 前立腺 | 0.25 | 1.23 | 0.19 | 17.5 |
| 膀胱 | 0.42 | 1.26 | 0.08 | 11.6 |
| 腎臓 | 1.39 | 5.15 | 0.25 | 44.1 |
| 筋肉 | 0.11 | 1.33 | 0.08 | 9.49 |
| 坐骨神経 | 0.00 | 0.78 | 0.27* | 16.11 |
| 骨髄 | 3.03 | 5.57 | 0.33 | 42.2 |
| 皮膚 | 0.16 | 1.35 | 0.18 | 15.8 |
| 褐色脂肪 | 1.61 | 6.89 | 1.23 | 103 |
| ハーダー腺 | 0.14 | 1.27 | 0.19 | 20.2 |
| 眼 | 0.07 | 0.34 | 0.03* | 3.81 |
| 小脳 | 0.04* | 0.05 | 0.00 | 0.85 |
| 大脳 | 0.06* | 0.03* | 0.01* | 0.66 |
| 延髄 | 0.08 | 0.11 | NS | 0.99 |
| 脳下垂体 | 1.22 | 5.98 | 0.80 | 55.7 |
| 脊髄 | 0.05 | 0.00* | NS | 0.79 |
| 無腺胃 | 44.7 | 1.78 | 0.07 | 10.2 |
| 腺胃 | 8.97 | 2.17 | 0.13 | 16.0 |
| 小腸 | 4.60 | 2.39 | 0.08 | 14.1 |

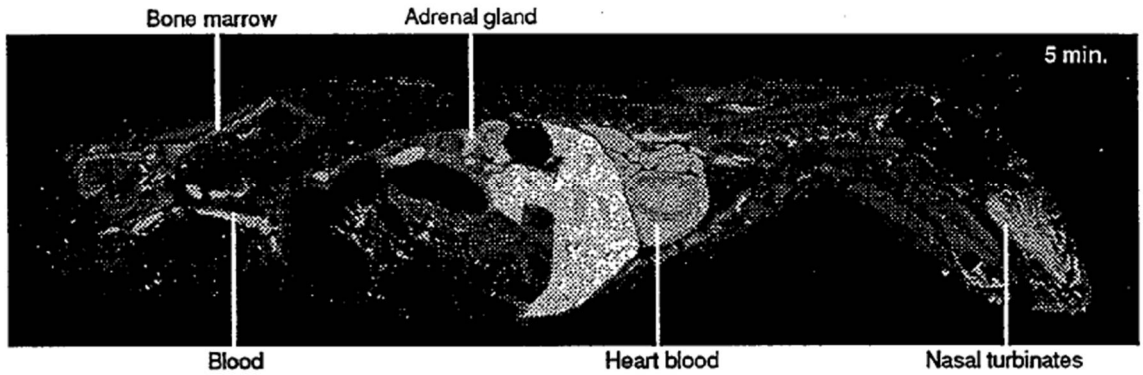
Source: 4.2.2.2-4- DMPK(CH) 1997/240-Table 12, Table 13, Table 29 1 nmol/g = 528.95 ng Eq/g
*:中央値，NS: 定量限界未満かつ検出限界以上の濃度

また，雄性有色ラットに Arm/Lmf 1 mg/kg（ [$^{14}$C]-Lmf として 0.86 mg/kg）を単回静脈内投与したときの投与後 5 分，8 時間，24 時間，及び 168 時間の放射能の全身分布を QWBA より検討した。投与後 5 分及び 8 時間の組織中放射能分布をFigure 4-2に，臓器・組織内放射能濃度をTable 4-6に示す。静脈内投与後 5 分で全身に広く分布し，血液よりも高い放射能が認められた組織は，肝臓及び副腎であった。投与後 8 時間では，副腎の放射能濃度が高く，次いで肝臓，脾臓，骨髄，小腸，肺，腎臓であり，精巣及び眼からも放射能が検出された。

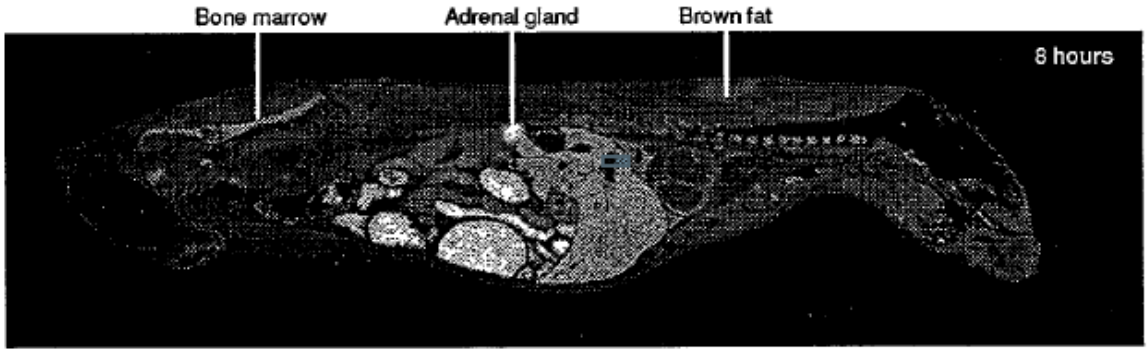
有色ラットの組織内放射能分布はアルビノラットと類似しており，メラニン含有組織である眼から放射能が比較的早く消失することから，メラニンへの親和性は強くないと考える（Table 4-6）。

**Figure 4-2　Arm/Lmf 1 mg/kg（ [$^{14}$C]-Lmf として 0.86 mg/kg）を雄性有色ラットに単回静脈内投与したときの投与後 5 分及び 24 時間の組織中放射能分布**

**投与後約 5 分（0.083 時間）**




**投与後 8 時間**




Source：4.2.2.2-4-DMPK(CH) 1997/240-Figure 8
[$^{14}$C] Lmf として 0.857 mg/kg
白色部位は放射能の存在を示す

**Table 4-6 Arm/Lmf 1 mg/kg （ [$^{14}$C]-Lmf として 0.86 mg/kg）を雄性有色ラットに単回静脈内投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | | | |
|---|---|---|---|---|---|
| | 単回投与後 | | | | |
| | 5 分 | 8 時間 | 24 時間 | 96 時間 | 168 時間 |
| 血液* | 6.64 | 0.54 | 0.22 | 0.06 | 0.03 |
| 肝臓 | 20.4 | 7.19 | 1.82 | 0.23 | 0.12 |
| 腎臓 | 1.43 | 1.46 | 1.16 | 0.24 | 0.13 |
| 副腎 | 8.20 | 25.4 | 22.4 | 6.37 | 2.63 |
| 脾臓 | 4.37 | 5.61 | 2.74 | 0.43 | 0.27 |
| 肺 | 5.52 | 2.02 | 0.91 | 0.19 | 0.10 |
| 胸腺 | 0.21 | 0.37 | 0.53 | 0.47 | 0.35 |
| 唾液腺 | 0.47 | 0.87 | 0.96 | 0.38 | 0.20 |
| 骨髄 | 1.85 | 2.58 | 1.25 | 0.38 | 0.35 |
| 脊髄 | 0.15 | 0.12 | 0.06 | 0.04 | 0.04 |
| 白色脂肪 | 0.21 | 0.53 | 1.09 | 0.50 | 0.40 |
| 褐色脂肪 | 0.98 | 1.67 | 1.76 | 1.16 | 1.29 |
| 小脳 | 0.22 | 0.06 | 0.05 | 0.03 | 0.02 |
| 終脳 | 0.14 | 0.07 | 0.13 | 0.05 | 0.04 |
| 小腸# | 0.07 | 2.32 | 1.13 | 0.29 | 0.40 |
| 胃# | 0.13 | 1.02 | 0.16 | 0.03 | 0.10 |
| 精巣 | 0.07 | 0.12 | 0.16 | 0.19 | 0.22 |
| 眼 | 0.10 | 0.08 | 0.15 | 0.06 | 0.09 |

Source: 4.2.2.2-4- DMPK(CH) 1997/240-Tabl 17 1 nmol/g = 528.95 ng Eq/g
N=1，*: 2 検体の平均値，#: 内容物を含む

## 4.3 胎盤通過性

### Arm

妊娠 13 日目のラットに Arm/Lmf 30 mg/kg（ [$^{3}$H]-Arm として 4.29 mg/kg）をを単回経口投与し，Arm の胎盤通過性及び組織内分布を検討した[2.6.5.7A-DMPK(CH)1997/099]。妊娠 13 日目のラットの臓器・組織内放射能濃度をTable 4-7に示す。

経口投与後，胎盤，羊水，及び胎児中に放射能が認められ，羊水並びに胎児では投与後 1 時間に最高濃度に達した。その他の組織についてもすべて投与後 0.5～8 時間に最高濃度に達した。母体血液中放射能濃度と比べ，胎盤中放射能は高い値を示したが，羊水及び胎児中放射能濃度は低い値であった。胎児組織中放射能の母体血液中放射能に対する比は 0.34～0.74 であり，胎児は Arm 又はその代謝物に曝露されていることが示唆された。

**Table 4-7 Arm/Lmf 30 mg/kg （ [$^3$H]-Arm として 4.29 mg/kg）を妊娠ラットに単回経口投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | | | |
|---|---|---|---|---|---|
| | 単回投与後 | | | | |
| | 0.5 時間 | 1 時間 | 4 時間 | 8 時間 | 24 時間 |
| | N=3 | N=3 | N=3 | N=3 | N=3 |
| 血液 | 3.70 | 3.44 | 3.59 | 3.25 | 1.63 |
| 血漿 | 4.44 | 4.08 | 3.95 | 3.14 | 1.16 |
| 唾液腺 | 3.87 | 3.58 | 3.18 | 2.40 | 1.24 |
| 甲状腺 | 4.95 | 3.86 | 3.52 | 3.36 | 1.81 |
| 食道 | 3.72 | 3.31 | 3.20 | 2.93 | 1.55 |
| 腋窩リンパ節 | 4.35 | 3.75 | 3.01 | 2.26 | 1.03 |
| 胸腺 | 3.02 | 2.59 | 2.56 | 2.04 | 1.40 |
| 肺 | 3.42 | 3.22 | 3.09 | 2.74 | 1.13 |
| 心臓 | 4.18 | 3.92 | 3.93 | 3.74 | 1.86 |
| 大動脈 | 3.83 | 2.46 | 2.64 | 2.06 | 0.82 |
| 腸間膜リンパ節 | 5.53 | 4.09 | 2.90 | 2.45 | 0.88 |
| 肝臓 | 20.02 | 14.11 | 12.60 | 12.09 | 5.55 |
| 膵臓 | 4.07 | 4.04 | 2.95 | 2.43 | 1.22 |
| 脾臓 | 4.74 | 4.25 | 5.22 | 4.99 | 3.67 |
| 副腎 | 11.56 | 8.45 | 7.13 | 6.21 | 3.34 |
| 白色脂肪 | 2.91 | 3.08 | 2.20 | 1.24 | 0.44 |
| 腎臓 | 8.26 | 7.82 | 10.81 | 6.81 | 4.14 |
| 膀胱 | 7.83 | 5.04 | 8.62 | 10.02 | 1.26 |
| 坐骨神経 | 3.18 | 3.00 | 2.47 | 1.73 | 0.64 |
| 筋肉 | 2.14 | 2.11 | 2.87 | 1.49 | 0.58 |
| 骨髄 | 3.88 | 4.91 | 4.54 | 3.85 | 1.80 |
| 皮膚 | 3.04 | 2.58 | 2.39 | 1.87 | 0.96 |
| 褐色脂肪 | 8.26 | 6.99 | 4.43 | 4.77 | 2.92 |
| 眼 | 1.45 | 1.46 | 1.39 | 1.08 | 0.33 |
| ハーダー腺 | 5.69 | 4.53 | 3.21 | 2.59 | 1.26 |
| 脳 | 1.88 | 1.56 | 1.33 | 1.12 | 0.51 |
| 無腺胃 | 197.36 | 86.54 | 32.02 | 12.66 | 6.59 |
| 腺胃 | 34.19 | 24.41 | 11.15 | 6.48 | 2.94 |
| 小腸 | 26.99 | 16.36 | 9.70 | 9.69 | 2.94 |
| 乳腺 | 4.59 | 4.26 | 2.88 | 1.88 | 1.07 |
| 卵巣 | 4.06 | 4.24 | 3.47 | 2.70 | 1.35 |
| 子宮 | 3.24 | 3.59 | 3.65 | 3.28 | 1.36 |
| 胎盤* | 3.91 | 4.87 | 5.14 | 4.14 | 1.99 |
| 羊水* | 1.57 | 1.95 | 1.87 | 1.63 | 0.35 |
| 胎児* | 2.31 | 2.53 | 2.49 | 1.94 | 0.56 |

Source: 4.2.2.3-4- DMPK(CH) 1997/099-Tabl 1~Table 5 1 nmol/g = 298.38 ng Eq/g

*: 6 検体の平均値

**Lmf**

妊娠 13 日目のラットに Arm/Lmf 30 mg/kg（[$^{14}$C]-Lmf として 25.7 mg/kg）をを単回経口投与し，Lmf の胎盤通過性及び組織内分布を検討した[2.6.5.7D-DMPK(CH)1997/139]。妊娠 13 日目のラットの臓器・組織内放射能濃度をTable 4-8に示す。

経口投与後，胎盤，羊水，及び胎児中に放射能が認められ，羊水並びに胎児では投与後 24 時間に最高濃度に達した。胎児組織中放射能の母体血液中放射能に対する比は 0.004～0.262 と小さいが，胎児は Lmf あるいはその代謝物に曝露されていることが示唆された。

**Table 4-8 Arm/Lmf 30 mg/kg （[$^{14}$C]-Lmf として 25.7 mg/kg）を妊娠ラットに単回経口投与したときの臓器・組織内放射能濃度**

| 臓器・組織 | 放射能濃度（nmol/g） | | | |
|---|---|---|---|---|
| | 単回投与後 | | | |
| | 1 時間 | 4 時間 | 8 時間 | 24 時間 |
| | N=3 | N=3 | N=3 | N=3 |
| 血液 | 2.74 | 8.67 | 9.78 | 3.74 |
| 血漿 | 4.51 | 13.80 | 15.72 | 6.44 |
| 赤血球 | 1.24 | 2.97 | 3.43 | 1.08 |
| 羊水# | 0.00 | 0.00 | 0.05 | 0.08 |
| 胎児# | 0.01* | 0.10 | 0.37 | 0.98 |
| 胎盤# | 0.65 | 6.81 | 13.45 | 11.20 |
| 卵巣 | 0.71 | 12.70 | 29.11 | 19.54 |
| 子宮 | 0.18 | 1.68 | 3.57 | 4.86 |
| 唾液腺 | 0.11 | 1.01 | 2.20 | 4.78 |
| 甲状腺 | 0.33 | 3.37 | 7.83 | 11.08 |
| 胸腺 | 0.12 | 0.64 | 1.54 | 4.11 |
| 腋窩リンパ節 | 0.17 | 1.48 | 3.82 | 7.13 |
| 肺 | 0.91 | 9.75 | 16.46 | 12.38 |
| 心臓 | 0.49 | 3.80 | 7.48 | 11.77 |
| 大動脈 | 0.35 | 1.66 | 1.99 | 2.50 |
| 肝臓 | 4.23 | 69.07 | 110 | 43.71 |
| 膵臓 | 0.21 | 1.75 | 3.71 | 8.58 |
| 脾臓 | 1.60 | 26.58 | 41.32 | 30.78 |
| 副腎 | 3.00 | 69.25 | 178 | 244 |
| 白色脂肪 | 0.08 | 0.42 | 0.91 | 2.37 |
| 腸間膜リンパ節 | 11.35 | 79.19 | 49.68 | 22.54 |
| 筋肉 | 0.04 | 0.52 | 0.89 | 2.11 |
| 坐骨神経 | 0.16 | 0.94 | 1.15 | 2.31 |
| 骨髄 | 0.73 | 10.57 | 20.02 | 14.92 |
| 皮膚 | 0.07 | 0.56 | 1.57 | 3.27 |
| 褐色脂肪 | 0.41 | 4.07 | 7.60 | 21.49 |
| 眼 | 0.04 | 0.22 | 0.58 | 0.78 |

| 臓器・組織 | 放射能濃度（nmol/g） | | | |
|---|---|---|---|---|
| | 単回投与後 | | | |
| | 1 時間 | 4 時間 | 8 時間 | 24 時間 |
| | N=3 | N=3 | N=3 | N=3 |
| ハーダー腺 | 0.21 | 1.01 | 0.99 | 1.71 |
| 延髄 | 0.07 | 0.24 | 0.33 | 0.18 |
| 小脳 | 0.06 | 0.26 | 0.26 | 0.16 |
| 大脳 | 0.05 | 0.20 | 0.24 | 0.14 |
| 下垂体 | 0.66 | 3.55 | 6.49 | 13.25 |
| 延髄 | 0.16 | 0.46 | 0.53 | 0.29 |
| 食道 | 1.11 | 1.03 | 1.95 | 2.61 |
| 腎臓 | 0.45 | 3.45 | 7.09 | 10.54 |
| 膀胱 | 0.08 | 0.73 | 1.90 | 2.79 |
| 無腺胃 | 172 | 199 | 224 | 12.19 |
| 腺胃 | 6.04 | 3.98 | 10.33 | 2.82 |
| 小腸 | 23.98 | 34.18 | 17.73 | 11.58 |
| 乳腺 | 0.12 | 1.42 | 3.11 | 8.48 |

Source: 4.2.2.3-5- DMPK(CH) 1997/139-Tabl 1~Table 4 1 nmol/g = 528.95 ng Eq/g

*: 中央値，#: 6 検体の平均値

妊娠 17 日目のウサギに Arm/Lmf 175 mg/kg（ [$^{14}$C]-Arm として 25 mg/kg，又は[$^{14}$C]-Lmf として 150 mg/kg）を単回経口投与し，Arm 及び Lmf の胎盤通過性並びに組織内分布を検討した[2.6.5.7B-DMPK(CH)1997/004] 及び[2.6.5.7E-EMET(EU)29/1996]。投与後 24 時間で，胎盤，羊水，及び胎児中に，Arm 又は Lmf の放射能が認められた。胎児組織中放射能の母体血液中放射能に対する比は，Arm では 0.50～0.99，Lmf では 0.02～0.03 であり，胎児は Arm，Lmf，及びそれらの代謝物に曝露されていることが示唆された。

# 5 代謝（動物種間の比較）

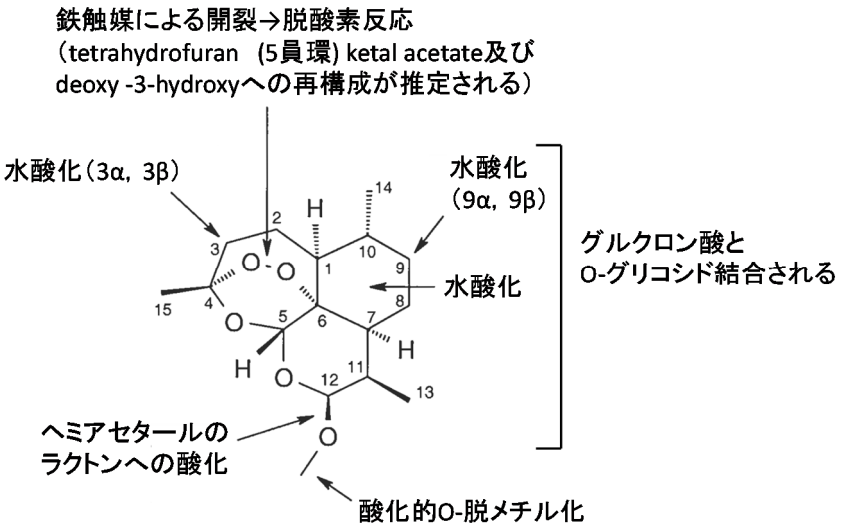
## 5.1 代謝物の推定

Arm 及び Lmf の *in vitro* 代謝について，マウス，ラット，及びヒト肝ミクロソーム，並びにマウス，ラット，イヌの肝 S12 画分を用いて検討した[2.6.510B-DMPK(CH)1997/181]，[2.6.510C-DMPK(CH)1997/156]，[2.6.510D-DMPK(CH)1997/035]，[2.6.510E-DMPK(CH)1997/155]。

[$^{3}$H]-あるいは [$^{14}$C]標識した Arm 又は Lmf を，ラット及びイヌに投与し， *in vivo* 代謝を検討した [2.6.5.9A-DMPK(CH)1997/241]，[2.6.5.9B-DMPK(CH)1997/003]，[2.6.5.9C-DMPK(CH)1997/534]，[2.6.5.9D-DMPK(CH)1997/209]。

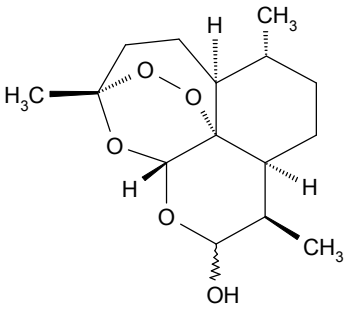
Arm の主な代謝経路は，1）水酸化，2）グルクロン酸抱合，3）O-脱メチル化（薬理的に活性のある代謝物 DHA）と推定された。*In vitro* 代謝試験，各種動物及びヒトの血漿又は血清中並びに排泄物中で推定された代謝部位をFigure 5-1に，DHA の構造式をFigure 5-2に示す。

**Figure 5-1　　Arm の推定代謝部位**




Source: 4.2.2.4-1-DMPK(CH) 1997/534-Figure 9

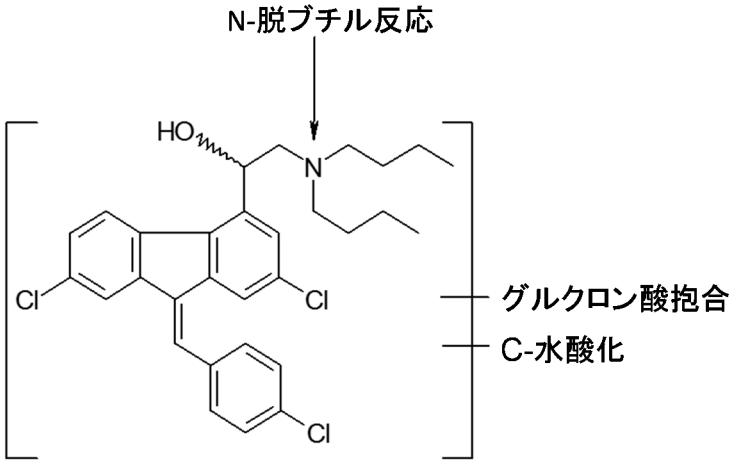
**Figure 5-2　　DHA の構造式**




Source: 4.2.2.4-4-DMPK(CH)1997/181

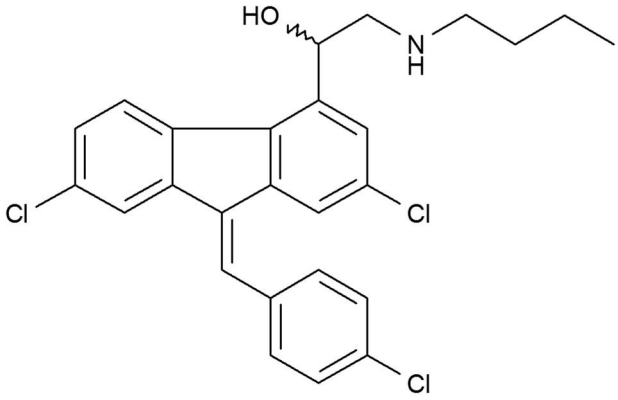
Lmf の主な代謝経路は，1）N-脱ブチル化（薬理学的に活性のある代謝物 desbutyl-Lmf），2）C-水酸化，3）グルクロン酸抱合と推定された。*In vitro* 代謝試験，各種動物及びヒトの血漿又は血清中並びに排泄物中で同定した推定代謝部位をFigure 5-3に，desbutyl-Lmf にFigure 5-4に示す。

**Figure 5-3　　Lmf の推定代謝部位**




Source: 4.2.2.4-2 DMPK(CH) 1997/209-Figure 9

**Figure 5-4　　N- desbutyl-lumefantrine**




Source4.2.2.4-2 DMPK(CH) 1997/209-PT- Table 7

## 5.2　　*In vitro* 代謝

Arm 及び Lmf の代謝に関与する CYP 分子種を，ヒト肝ミクロソーム及びヒト P450 発現系ミクロソームによって，CYP 分子種を同定した [2.6.5.10C-DMPK(CH)1997/156]，[2.6.5.10E-DMPK(CH)1997/155]。Arm 及び Lmf は主として CYP3A4/5 により代謝されることが示唆された (Lefèvre and Thomsen 1999)。

ラット及びイヌ肝 S12 画分を用いた *in vitro* 試験の結果から，Lmf の主要な代謝物は N-desbutyl-Lmf であると考える[Table 2.6.5.10D− 1997/035]

## 5.3 *In vivo* 代謝

### 5.3.1 血漿中代謝物

[$^{3}$H]-Arm 又は[$^{14}$C]-Arm を雄性ラット及び雄性イヌに単回投与したときの血漿中未変化体並びに主代謝物の組成をTable 5-1に示す。いずれも血漿中に未変化体が認められたが，DHA は同定できなかった。

[$^{14}$C]-Lmf を雄性ラット及び雄性イヌに単回投与したときの血漿中未変化体並びに主代謝物の組成は検討していないが，いずれの動物種でも Lmf の未変化体は放射能濃度の 90%以上であった [2.6.5.3D-1997/240]。

**Table 5-1 単回投与後の血漿中 Arm 及び主代謝物の血漿中放射能に対する割合**

| 種 | ラット | ラット | イヌ | イヌ | イヌ |
|---|---|---|---|---|---|
| 性 | 雄 | 雄 | 雄 | 雄 | 雄 |
| Arm/Lmf 投与量 | 10 | 20 | 10 | 20 | 200 |
| 投与経路 | i.v. | p.o. | i.v. | p.o. | p.o. |
| 化合物 | $^{3}$H-Arm | $^{3}$H-Arm | $^{14}$C-Arm | $^{14}$C-Arm | $^{14}$C-Arm |
| 投与後時間(分) | 5 | 30 | 15 | 120 | 120 |
| P52.5 9a-hydroxy-artemether-9-0-glucuronide | 5.0 | 5.9 | 1.5 | 5.5 | 4.8 |
| P33.0 3~,9~-dihydroxy-deoxyartemisinin-9-0-glucuronide | − | − | 1.2 | 4.8 | 2.9 |
| P56.0 | − | − | 8.2 | 16.6 | 16.3 |
| P57.5 3~-hydroxy-deoxyartemisinin− 3~-hydroxy-deoxyartemether-3-0-glucuronide | 3.4 | 6.7 | 10.8 | 15.9 | 15.8 |
| P72.0 3~-hydroxy-deoxyartemether-3-0-glucuronide | − | − | 5.6 | 5.2 | 5.4 |
| P54.0 0-glucoside of an artemisinin derivative | 1.9 | 3.4 | − | − | − |
| 未変化体 | 3.6 | 0.3 | 16.3 | 0.4 | 0.1 |
| 報告書番号 | 1997-241 | | | 1997-003 | |

Source：4.2.2.2-1-DMPK(CH)1997/241-Table 20, 4.2.2.2-2-DMPK(CH)1997/003-Table 7
投与放射能に対する血漿中放射能の割合（%），－：同定できず

ラットに Lmf のラセミ化合物 100 mg/kg，又は *R*-及び *S*-エナンチオマーを各 50 mg/kg をそれぞれ単回経口投与し Lmf の *R*-及び *S*-エナンチオマーの血漿中濃度を測定した [Table 2.6.5.3G-試験 1996/147]，[Table 2.6.5.2 M-試験 1996/147]。また，雌雄イヌにおける 13 週間 TK 試験（200 mg/kg/日；[Table 2.6.5.4Q-試験 1996/024]）及びヒトの A006 試験で採取した検体の血漿中

濃度を測定した。エナンチオマー比に大きな差が認められなかったことから，Lmf の *R*-及び *S*-エナンチオマーのラセミ化はないと考えられる。

### 5.3.2 尿中代謝物

[$^{3}$H]-Arm 又は[$^{14}$C]-Arm を雄性ラット及び雄性イヌに単回投与したときの尿中未変化体並びに主代謝物の組成をTable 5-2に示す。尿中に未変化体はほとんど定量されず，主代謝物は O-グルクロン酸抱合体であった。

なお，[$^{14}$C]-Lmf を雄性ラット及び雄性イヌに単回投与したときの尿中の未変化体並びに主代謝物の組成は検討していない。

**Table 5-2 単回投与後の尿中 Arm 及び主代謝物の尿中放射能に対する割合**

| 種 | ラット | ラット | ラット | ラット | ラット | イヌ | イヌ | イヌ |
|---|---|---|---|---|---|---|---|---|
| 性 | 雄 | 雄 | 雄 | 雄 | 雄 | 雄 | 雄 | 雄 |
| Arm/Lmf 投与量 | 10 | 20 | 1000 | 100 | 20 | 10 | 20 | 200 |
| 投与経路 | i.v. | p.o. | p.o. | p.o. | p.o. | i.v | p.o. | p.o |
| 化合物 | $^{3}$H-Arm | $^{3}$H-Arm | $^{3}$H-Arm | $^{3}$H-Arm | $^{14}$C-Arm | $^{14}$C-Arm | $^{14}$C-Arm | $^{14}$C-Arm |
| P52.5 | 2.2 | 2.5 | 6.5 | 3.8 | 1.6 | 1.2 | 2.7 | 1.4 |
| P33.0 | － | － | － | － | － | 2.7 | 4.8 | 3.6 |
| P57.5 | 1.9 | 1.8 | 3.3 | 2.3 | 1.1 | 7.9 | 9.1 | 8.4 |
| P72.0 | － | － | － | － | － | 0.7 | 0.4 | 0.6 |
| P54.0 | 2.8 | 2 | 3.2 | 1.9 | 1.8 | － | － | － |
| 未変化体 | 0 | 0 | － | － | 0.1 | 0.1 | 0.1 | 0.1 |
| 報告書番号 | 1997-241 | | | | | 1997-003 | | |

Source：4.2.2.2-1-DMPK(CH)1997/241-Table 21~25, 4.2.2.2-2-DMPK(CH)1997/003-Table 8～10
投与放射能に対する尿中放射能の割合（%），－：同定できず

### 5.3.3 糞中及び胆汁中代謝物

[$^{3}$H]-Arm 又は[$^{14}$C]-Arm を雄性ラット及び雄性イヌに単回投与したときの糞中の未変化体並びに主代謝物の組成をTable 5-3に示す。糞中の未変化体は少なく，主代謝物は O-グルクロン酸抱合体であった。

また，雄性ラットを用いて腸肝循環を検討した。胆管にカニュレーション処置したラットに Arm/Lmf 20 mg/kg を経口内投与したとき，投与 72 時間後までに投与量の 58.5%が胆汁から回収され，そのときの主な代謝物は P52.5（9a-hydroxy-artemether-9-0-glucuronided，投与量の約 26%）であった[2.6.5.9A-DMPK(CH) 1997/241]。

**Table 5-3　　単回投与後の糞中 Arm 及び主代謝物の糞中放射能に対する割合**

| 種 | ラット | ラット | ラット | ラット | ラット | イヌ | イヌ | イヌ |
|---|---|---|---|---|---|---|---|---|
| 性 | 雄 | 雄 | 雄 | 雄 | 雄 | 雄 | 雄 | 雄 |
| Arm/Lmf 投与量 | 10 | 20 | 100 | 1000 | 20 | 10 | 20 | 200 |
| 投与経路 | i.v. | p.o. | p.o. | p.o. | p.o. | i.v. | p.o. | p.o. |
| 化合物 | $^{3}$H-Arm | $^{3}$H-Arm | $^{3}$H-Arm | $^{3}$H-Arm | $^{14}$C-Arm | $^{14}$C-Arm | $^{14}$C-Arm | $^{14}$C-Arm |
| P52.5 | 0.9 | 1.2 | 1.1 | 1.2 | 0.2 | 0.6 | 1 | 0.9 |
| P33.0 | － | － | － | － | － | 0.1 | 0.5 | 0.3 |
| P57.5 | 1 | 1.4 | 1.2 | 1.1 | 0 | 0.3 | 0.9 | 0.4 |
| P72.0 | － | － | － | － | － | 1.8 | 2.8 | 2.3 |
| P54.0 | 0.6 | 0.7 | 0.9 | 0.4 | 0.3 | － | － | － |
| 未変化体 | 0.3 | 0.1 | － | － | 0 | 0.2 | 0.2 | 0.2 |
| 報告書番号 | 1997-241 | | | | | 1997-003 | | |

Source：4.2.2.2-1-DMPK(CH)1997/241-Table 21~25, 4.2.2.2-2-DMPK(CH)1997/003-Table 8～10
投与放射能に対する糞中放射能の割合（%），－：同定できず

# 6　　排泄

## 6.1　　尿，糞及び胆汁中への排泄

### Arm

雄性ラット，雌性のウサギ，及び雄性イヌに Arm/Lmf（ [$^{3}$H]-Arm，又は[$^{14}$C]-Arm）を単回投与したときの放射能排泄率をTable 6-1に示す。放射能は速やかに排泄され，投与後 7 日には投与量のほとんどが回収された。ラットの屍体からの放射能の回収率は投与量の 2～9%であった。

胆管にカニュレーション処置をしたラットに Arm/Lmf 20 mg/kg（[$^{14}$C]-Arm として 2.86 mg/kg）を経口投与したとき，投与後 72 時間までの胆汁及び尿中にそれぞれ投与量の約 58%及び約 28%が排泄された。このことから，ラットにおける Arm 及びその代謝物の排泄には，胆汁中排泄の寄与が大きいと考える [2.6.5.14A-DMPK(CH)1997/241]。

**Table 6-1 雄性ラット，雌性ウサギ，雄性イヌに Arm/Lmf を単回投与したときの Arm の放射能排泄率**

| 動物種（試験番号） | 投与量 Arm/Lmf (mg/kg) | 投与経路 | 排泄量(% of dose) 尿 0-24 h | 尿 0-168 h | 糞 0-24 h | 糞 0-168 h | 合計 0-168 h |
|---|---|---|---|---|---|---|---|
| ラット（1997/241） | 10 | i.v. | 38.0 | 43.2 | 33.7 | 44.4 | 87.5 |
| | 20 | p.o. | 41.4 | 46.3 | 33.5 | 41.9 | 88.2 |
| | 100 | p.o. | 53.7 | 60.9 | 34.1 | 42.0 | 102.9[a] |
| | 1000 | p.o. | 45.5 | 65.5 | 14.3 | 32.8 | 98.2[a] |
| ウサギ（1997/004） | 175 | p.o. | 66.5 | NA | 1.9 | NA | NA |
| イヌ（1997/003） | 10 | i.v. | 42.5 | 52.9 | 21.6 | 33.3 | 86.1 |
| | 20 | p.o. | 45.1 | 63.3 | 26.3 | 30.8 | 94.1 |
| | 200 | p.o. | 47.8 | 57.2 | 29.8 | 37.3 | 94.5 |

Source：4.2.2.2-1-DMPK(CH)1997/241-Table 10~13, 4.2.2.2-2-DXMPK(CH)1997/003-Table 1~3, 4.2.2.3-8- DXMPK(CH)1997/004-Table 1

NA: 該当なし，a:採取時間（0-144 h）

### Lmf

雄性ラット，妊娠 17 日目のウサギ，及び雄性イヌに Arm/Lmf（[$^{14}$C]-Lmf）を単回投与したときの放射能排泄率をTable 6-2に示す。

胆管にカニュレーション処置をしたラットに Arm/Lmf 1 mg/kg（[$^{14}$C]-Lmf として 0.86 mg/kg）を静脈内投与したとき，投与後 72 時間までの胆汁及び尿中にそれぞれ投与量の約 39%及び約 2%が排泄された。以上のことから，ラットにおける Lmf 及びその代謝物の排泄には，胆汁中排泄の寄与が大きいと考える[2.6.5.13C-DMPK(CH)1997/240]。

**Table 6-2 雄性ラット，雌性ウサギ，雄性イヌに Arm/Lmf を単回投与したときの Lmf の放射能排泄率**

| 動物種（試験番号） | 投与量 Arm/Lmf (mg/kg) | 投与経路 | 排泄量(% of dose) 尿 0-24 h | 尿 0-168 h | 糞 0-24 h | 糞 0-168 h | 合計 0-168h |
|---|---|---|---|---|---|---|---|
| ラット（1997/240） | 1 | i.v. | 1.33 | 1.95 | 35.4 | 77.5 | 79.5 [a] |
| | 20 | p.o. | 0.16 | 0.24 | 88.7 | 94.1 | 94.3 |
| | 100* | p.o. | 0.27 | 0.38 | 76.6 | 91.3 | 91.7 |
| | 1000* | p.o. | 0.26 | 0.33 | 58.6 | 95.5 | 95.8 |
| ウサギ（29/1996） | 175* | p.o. | 0.3 [b] | NA | 30[c] | NA | NA |
| イヌ（1997/240） | 1 | i.v. | 1.10 | 1.87 | 36.1 | 75.3 | 77.2 |
| | 20 | p.o. | 0.26 | 0.37 | 88.7 | 92.9 | 93.2 |
| | 20* | p.o. | 0.41 | 0.68 | 72.6 | 85.8 | 86.4 |

Source：4.2.2.2-4-DMPK(CH)1997/240-Table 18, 20, 24~28, 4.2.2.3-6-DXMPK(CH)29/1996

NA: データなし，*:自由摂餌

a:投与量の 15.9% が屍体及び消化管から回収された，b: n=2，c: n=1

## 6.2 乳汁中移行

妊娠ラット及び妊娠ウサギに[$^{3}$H]及び[$^{14}$C]-Arm 又は[$^{14}$C]-Lmf を経口投与したとき，投与後 24 時間の乳腺中の放射能が定量可能であったことから，Arm 及び Lmf とそれらの代謝物は乳汁中に移行するすると考える[2.6.5.7A-DMPK(CH)1997/099]，[2.6.5.7B-DMPK(CH)1997/004]，[2.6.5.7D-DMPK(CH)1997/139]，[2.6.5.7E-DMET(EU)29/1996]。

# 7 薬物間相互作用

## 7.1 酵素阻害

ヒト肝ミクロソームを用いて，CYP1A2，CYP2A6，CYP2C9，CYP2C19，CYP2D6，CYP2E1，CYP3A4/5 及び CYP4A9/11 に対する Arm 及び Lmf の阻害能について検討した（Table 7-1）。Arm の CYP1A2 に対する阻害定数（$K_i$ 値）は 216 µmol/L であり，弱い阻害が認められた。しかしながら，熱帯熱マラリア患者での最高血漿中濃度（平均値）は約 0.623 µmol/L（186 ng/mL，A028 試験）であり臨床的には問題ないと考えられた [2.6.5.12A- DMPK(CH)1997/072]。一方，Lmf は CYP2D6 を阻害し，$K_i$ 値は 0.997 µmol/L であった [2.6.5.12B- DMPK(CH)1997/073]。non-immune の患者での最高血漿中濃度（平均値）は約 11 µmol/L（5.72 µg/mL，A2401 試験）であり，$K_i$ 値に比べ高値であることから，治療域の狭い化合物では薬物間相互作用が臨床的に問題となる可能性が示唆された。

**Table 7-1 CYP の各分子種に対する阻害能**

| CYP 分子種 | 酵素活性 | Ki (µmol/L) | |
|---|---|---|---|
| | | Arm | Lmf |
| CYP1A2 | 7-Ethoxyresorufin *O*-dealkylase | ~ 216 | > 2.0 |
| CYP2A6 | Coumarin 7-hydroxylase | > 100 | > 2.0 |
| CYP2C9 | Tolbutamide methyl-hydroxylase** | ~330-470 | > 2.0 |
| CYP2C19 | *S*-Mephenytoin 4'-hydroxylase | 38.4 | > 2.0 |
| CYP2D6 | Dextromethorphan *O*-demethylase* | > 100 | 0.997 |
| CYP2E1 | Chlorzoxazone 6-hydroxylase | > 100 | > 2.0 |
| CYP3A4/5 | Testosterone 6β-hydroxylase | 48.5 | > 2.0 |
| CYP4A9/11 | Lauric Acid 12-hydroxylase | > 100 | > 2.0 |

Source: 4.2.2.4-8-DMPK(CH) 1997/072- Table 3, 4.2.2.4-9-DMPK(CH)1997/073-Table 3

Arm 濃度: 0.1, 1.0, 10 及び 100 µmol/L, *Arm 濃度: 0.133, 1.33, 13.3 及び 100 µmol/L, ** Arm 濃度: 0.1, 0.53, 10 及び 100 µmol/L

Lmf 濃度: 0.05, 0.5, 1.0 及び 2.0 µmol/L, *Lmf 濃度: 0.067, 0.67, 1.33 及び 2.0 µmol/L.

## 7.2 酵素誘導

Arm/Lmf をラット及びイヌに反復経口投与したとき，Arm 及び DHA の血漿中濃度は経時的に減少したことから，自己誘導の可能性が考えられる（3.2 項）。ヒト肝細胞を用いた *in vitro* 試験 [DMPK R0900123]では， Arm，DHA 及び Lmf（それぞれ 2.5，7 及び 200 µM の濃度で検討）は CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19 及び CYP3A を誘導しなかった。一方，Arm

及び DHA 等のアルテミシニン誘導体は，ヒト初代肝細胞及びヒト腸細胞株 LS174T において，CYP2B6，CYP3A4 及び MDR1 を誘導することが報告されている（Burk et al. 2005）。

以上のことから，Arm の CYP 誘導の有無について，*in vitro* 試験では結論が一致しておらず，Arm 及び DHA の CYP 誘導については否定できないと考える。したがって，本剤の CYP3A4 の誘導により，CYP3A4 によって代謝される薬剤と併用した場合，その薬剤の薬物動態に影響が生じる可能性が考えられる。

# 8 その他の薬物動態試験

該当なし。

# 9 考察及び結論

各種動物を用いて Arm 及び Lmf の薬物動態を， *in vivo* 試験及び *in vitro* 試験において検討した。Arm は初回通過を受けやすく，速やかに代謝された。Lmf は絶食時と非絶食時で曝露量が異なり，食事の影響が示唆された。

Arm/Lmf をラット及びイヌに反復投与したとき，Arm 及び DHA の血漿中濃度は経時的に減少した。イヌ及びラットの Lmf の累積性については，初回投与に比べ反復投与後に曝露量が増加したものの，一定の傾向は認められなかった。毒性試験で検討したいずれの動物種でも曝露量に明らかな性差はないと考える。Arm の血清蛋白結合率は，マウス，ラット，ウサギ，イヌ，及びカニクイザルで，それぞれ 98.3%～98.4%，97.3%，97.1%～97.2%，97.1%～97.3%，及び 96.0%～96.2%であり，濃度依存性は認められなかった。Lmf の血清蛋白結合率は，マウス，ラット，ウサギ，イヌ，及びカニクイザルで，それぞれ 99.8%，99.9%，99.93%，99.92%，及び 99.94%であった。ヒト血漿及び赤血球において，Arm は主に α1-酸性糖蛋白質（33%），次に血清アルブミン（17%），高，低及び超低比重リポ蛋白質（それぞれ 12%，9.3%及び 12%）並びに γ グロブリン（0.4%）に結合した。Arm の 11%が血球に移行し，4.6%は非結合型 Arm であった。Lmf は 1 μg/mL（1.89 μmol/L）の濃度で主にリポ蛋白質，特に高比重リポ蛋白質（99.6%）に結合した。約 62%がヒト血清アルブミンに，約 8%が血球に移行した。

[$^{3}$H]-Arm，[$^{14}$C]-Arm，又は[$^{14}$C]-Lmf を有色及びアルビノラットに，単回経口投与並びに単回静脈内投与したとき，Arm，Lmf，及びその代謝物は全身に分布した。有色ラットの組織内放射能分布はアルビノラットと類似しており，メラニン含有組織である眼から放射能が比較的早く消失することから，メラニンへの親和性は強くないと考える。

妊娠ラット及び妊娠ウサギにおいて，Arm 及び Lmf の代謝物は胎盤を通過し胎児へ移行した。乳腺に分布した放射能が定量できたことから，乳汁中移行が示唆された。

Arm 及び Lmf の主な代謝酵素は CYP3A4/5 であった。Arm の主な代謝経路は，1）水酸化，2）グルクロン酸抱合，3）O-脱メチル化（活性代謝物 DHA）であり，Lmf の主な代謝経路は，1）N-脱ブチル化（活性代謝物 desbutyl-Lmf），2）C-水酸化，3）グルクロン酸抱合と推定された。

Arm を経口投与したとき，Arm 及びその代謝物は尿及び糞中にほぼ同じ割合で排泄され，投与後 7 日までにほとんど排泄された。Lmf を経口投与したとき，約 90%が未変化体であり，糞中から回収された。定量された desbutyl-Lmf はわずかであり，投与後 7 日までにほぼ排泄された。

Arm は *in vitro* 試験において，CYP1A2 に対して弱い阻害作用を示したが，臨床的に問題となる程度ではなかった。Lmf は CYP2D6 を阻害し，治療域の狭い化合物では薬物間相互作用は臨床的に問題となる可能性が示唆された。

Arm の CYP 誘導の有無について，*in vitro* 試験では分子種で結論が一致しておらず，Arm 及び DHA の CYP 誘導については否定できないと考える。したがって，CYP3A4 の誘導により，CYP3A4 によって代謝される薬剤の薬物動態に影響が生じる可能性が考えられる。

# 10 参考文献

[Burk O, Arnold KA, Nussler AK, et al. (2005)] Antimalarial Armemisinin drugs induce cytochrome P450 and MDR1 expression by activation of xenosensors pregnane X receptor and constitutive androstane receptor. Mol Pharmacol; 67(6):1954-65.

[Lefèvre G and Thomsen MS (1999)] Clinical pharmacokinetics of Armemether and lumefantrine (Riamet®). Clin Drug Invest; 18(6): 467-80.

[Li QG, Peggins JO, Fleckenstein LL, et al. (1998)] The pharmacokinetics and bioavailability of Dihydroartemisinin, Arteether, Artemether, Artesunic acid and Artelinic acid in rats. J Pharm Pharmacol; 50(2):173-82.

# 2.6.5 薬物動態試験概要表

# 目　次

目　次 ..... 2
略号一覧 ..... 3
2.6.5.1　薬物動態試験：一覧 ..... 5
2.6.5.2　薬物動態試験：分析方法 ..... 17
2.6.5.3　薬物動態試験：単回投与後の吸収 ..... 81
2.6.5.4　薬物動態試験：反復投与後の吸収 ..... 95
2.6.5.5　薬物動態試験：分布 ..... 132
2.6.5.6　薬物動態試験：蛋白結合 ..... 138
2.6.5.7　薬物動態試験：妊娠動物における試験 ..... 145
2.6.5.8　薬物動態試験：その他の分布試験 ..... 155
2.6.5.9　薬物動態試験：*in vivo* における代謝 ..... 156
2.6.5.10　薬物動態試験：*in vitro* における代謝 ..... 184
2.6.5.11　薬物動態試験：推定代謝経路 ..... 204
2.6.5.12　薬物動態試験：薬物代謝酵素の誘導／阻害 ..... 206
2.6.5.13　薬物動態試験：排泄 ..... 211
2.6.5.14　薬物動態試験：胆汁中排泄 ..... 218
2.6.5.15　薬物動態試験：薬物相互作用 ..... 222
2.6.5.16　薬物動態試験：その他 ..... 223

# 略号一覧

| 略号 | 省略していない表現（英） | 省略していない表現（日） |
|---|---|---|
| APCI | atmospheric pressure chemical ionization | 大気圧化学イオン化法 |
| AUC | area under the drug plasma (serum/blood) concentration-time curve | 血漿（血清/血液）中薬物濃度-時間曲線下面積 |
| C1h | concentration at 1 hour | 投与後 1 時間の濃度 |
| C2h | concentration at 2 hours | 投与後 2 時間の濃度 |
| C4h | concentration at 4 hours | 投与後 4 時間の濃度 |
| C8h | concentration at 8 hours | 投与後 8 時間の濃度 |
| CB | concentration in blood | 血液中濃度 |
| CL | clearance | クリアランス |
| Cmax | maximal drug plasma (serum/blood) concentration | 最高血漿（血清/血液）中薬物濃度 |
| CP | concentration in plasma | 血漿中濃度 |
| CV | coefficient of variation | 変動係数（%） |
| CYP | cytochrome P450 | チトクローム P450 |
| DHA | dihydroartemisinin | － |
| GC | gas chromatography | ガスクロマトグラフィー |
| GC-MS | gas chromatography -mass spectrometry | ガスクロマトグラフィー質量分析 |
| GLP | good laboratory practices | 医薬品の安全性に関する非臨床試験の実施の基準 |
| $^{1}$H-NMR | proton nuclear magnetic resonance spectroscopy | プロトン核磁気共鳴スペクトル |
| HPLC | high performance liquid chromatography | 高速液体クロマトグラフィー |
| HPLC-UV | high performance liquid chromatography coupled to ultraviolet absorbance detection | 紫外吸光度検出器を接続した高速液体クロマトグラフィー |
| HSA | human serum albumin | ヒト血清アルブミン |
| Km | michaelis constant | ミカエリス定数 |
| LC-MS | liquid chromatography-mass spectrometry | 液体クロマトグラフィー質量分析 |
| LC-MS/MS | liquid chromatography coupled with tandem mass spectrometry | 液体クロマトグラフィータンデム質量分析 |
| LIMS | laboratory information management system | － |
| LLOQ | lower limit of quantification | 定量下限 |
| LOD | limit of detection | 検出限界 |
| LOQ | limit of quantification | 定量限界 |
| MRM | multiple reaction monitoring | 多重反応モニタリング |
| MS | mass spectrometry | 質量分析 |
| MS/MS | tandem mass spectrometry | タンデム質量分析 |
| NKa | total binding capacity of a protein | 総蛋白結合能 |
| $NK_E$ | total binding capacity of erythrocytes | 総血球結合能 |
| PK | pharmacokinetics | 薬物動態（学） |
| QC | quality control | － |
| $r^2$ | determination coefficient | 決定係数 |
| SDS | sodium dodecyl sulfate | ドデシル硫酸ナトリウム |
| SPF | specific pathogen free | － |

| 略号 | 省略していない表現（英） | 省略していない表現（日） |
|---|---|---|
| T1/2 | elimination half life | 消失半減期 |
| TIS | turbo ion spray | ― |
| TK | toxicokinetics | トキシコキネティクス |
| Tmax | time to reach the maximum drug plasma (serum/blood) concentration following drug administration | 最高血漿（血清/血液）中薬物濃度到達時間 |
| ULOQ | upper limit of quantification | 定量上限 |
| UV | ultraviolet | 紫外線 |
| Vmax | maximum velocity of an enzyme | 酵素の最大反応速度 |
| WBAR | whole body autoradiography | 全身オートラジオグラフィー |

## 2.6.5.1 薬物動態試験：一覧

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| *2.6.5.2 分析法及びバリデーション報告書* | | | | | | | | |
| ***Artemether (Arm)*** | | | | | | | | |
| Analytical methods and validation reports | LC-MS/MS | - | - | Arm dihydroartemisinin (DHA) | yes | [redacted] | [BAPK(EU) R0301212] | 4.2.2.1-1 |
| Analytical methods and validation reports | LC-MS/MS | - | - | Arm, DHA | yes | [redacted] | [BAPK(EU) R0301212-01] | 4.2.2.1-2 |
| Analytical methods and validation reports | LC-MS/MS | - | - | Arm, DHA | yes | [redacted] | [DMPK R0400692] | 4.2.2.1-3 |
| Analytical methods and validation reports | LC-MS/MS | - | - | Arm, DHA | yes | [redacted] | [DMPK R0500059] | 4.2.2.1-4 |
| Analytical methods and validation reports | LC-MS | - | - | Arm, DHA | yes | Novartis | [BAPK(F) R00-1840] | 4.2.2.1-5 |
| Analytical methods and validation reports | Stable isotope Synthesis and release analysis | - | - | [$^{13}CD_3$] Arm | not required | Novartis | [DMPK R0500720-01 (SSE 096-1)] | 4.2.2.1-6 |
| ***Lumefantrine (Lmf)*** | | | | | | | | |
| Analytical methods and validation reports | LC-MS/MS | - | - | Lmf | yes | Novartis | [DMPK R0300924A] | 4.2.2.1-7 |
| Analytical methods and validation reports | LC-MS/MS | - | - | desbutyl lumefantrine (N- desbutyl-Lmf) | yes | Novartis | [DMPK R0300924B] | 4.2.2.1-8 |
| Analytical methods and validation reports | LC-MS/MS | - | - | Lmf | yes | Novartis | [DMPK R0300924C] | 4.2.2.1-9 |
| Analytical methods and validation reports | HPLC-UV | - | - | Lmf | not required | Novartis | [DMPK(F) R00-2105] | 4.2.2.1-10 |
| Analytical methods and validation reports | HPLC-UV | - | - | Lmf N-desbutyl-Lmf | yes | Novartis | [BAPK(F) R00-2105-02] [BAPK(F) R00-2105-02-01 (Amendment-1)] | 4.2.2.1-11 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| Analytical methods and validation reports | HPLC, UV detection | - | - | Lmf | not required | Novartis | [BPK(CH) 1994/038] | 4.2.2.1-12 |
| Analytical methods and validation reports | HPLC, UV detection, enantioselective chromatography | - | - | Lmf enantiomers CGP64455 S(+), CGP64456 R(-) | not required | Novartis | [BPK(CH) 1996/147] | 4.2.2.1-13 |
| Analytical methods and validation reports | Stable isotope synthesis and release analysis | - | - | [$D_9$] Lmf | not required | Novartis | [PCS(EU) R0301253-01 (SSE 072-1)] | 4.2.2.1-14 |
| ***Others*** | | | | | | | | |
| Analytical methods and validation reports | HPLC with fluorenscence detection | - | - | quinine | yes | Novartis | [DMPK(F) R00-1921] | 4.2.2.1-15 |
| ***2.6.5.3 吸収：単回投与*** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Absorption after single dose | Rat:male albino, Tif:RAIf(SPF) | i.v., p.o. | single | Arm/Lmf:<br>i.v.: 10 mg/kg<br>p.o.: 20, 100, 1000 mg/kg containing 1/7of [$^3$H] or [$^{14}$C]-labeled Arm | not required | Novartis | [DMPK(CH) 1997/241] | 4.2.2.2-1 |
| Absorption after single dose | Dog, Beagle, male | i.v., p.o. | single | Arm/Lmf<br>10 mg/kg, i.v.;<br>20 and 200 mg/kg, p.o. containing 1/7 of [$^{14}$C]- Arm | not required | Novartis | [DMPK(CH) 1997/003] | 4.2.2.2-2 |
| Absorption after single dose | Cynomolgus monkey, (Macaca fascicularis), female | p.o. | single | Arm/Lmf<br>350 mg/kg containing 50 mg/kg Arm | not required | Novartis | [BPK(F) 1996/029] | 4.2.2.2-3 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***Lmf*** | | | | | | | | |
| Absorption after single dose | Rat:male albino, Tif:RAIf(SPF)<br>Rat: male pigmented, LE/Mol (SPF) | i.v.; p.o. | single | Arm/Lmf:<br>i.v.: 1 mg/kg<br>p.o.: 20, 100, 1000 mg/kg containing 6/7 of $^{14}$C-labeled Lmf | not required | Novartis | [DMPK(CH) 1997/240] | 4.2.2.2-4 |
| | Dog, Beagle, male | i.v.; p.o. | single | Arm/Lmf:<br>i.v.: 1 mg/kg<br>p.o.: 20 mg/kg containing 6/7 [$^{14}$C]-Lmf | | | | |
| Absorption after single dose | Cynomolgus monkey (Macaca fascicularis), female | p.o., capsule | single | Arm/Lmf<br>350 mg/kg containing 6/7 Lmf | not required | Novartis | [BPK(CH) 1996/095] | 4.2.2.2-5 |
| Absorption after single dose | Rat, albino | i.v. | single | Lmf<br>1 mg/kg | not required | Novartis | [BPK(CH) 1996/026] | 4.2.2.2-6 |
| Absorption after single dose | Rat, male | p.o. | single | Rat: 100 mg/kg Lmf racemate or 50 mg/kg of each enantiomer. | not required | Novartis | BPK(CH) 1996/147 | 4.2.2.1-13 |
| | Dog samples from study BPK(CH) 1996/024. | p.o. | Day 1 & Day 89 | Dog: 200 mg/kg Arm/Lmf. | | | | |
| | Human samples from (Clin study Protocol 56697 01 006). | p.o. | single | Human: 560 mg Arm/Lmf (480 mg Lmf).<br>Enantiomers:<br>CGP64455 S(+)<br>CGP64456 R(-) | | | | |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***2.6.5.4*** **吸収：反復投与** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Absorption after repeated doses | Rat, IGS Wistar Hannover, male, female | oral, gavage | 2 -week | Arm<br>0, 20, 200 mg/kg/day | yes | Novartis | [DMPK R0570030] | 4.2.3.7.7-5 |
| Absorption after repeated doses | Rat, male, female | p.o. | 3-month | Arm/Lmf<br>0, 100, 300, 1000 mg/kg | yes | Novartis | [BPK(F) 1996/007] | 4.2.3.2-2 |
| Absorption after repeated doses | Rat, juvenile, IGS Wistar Hannover, male, female | oral, gavage | day-1 to day-21 p.p. | Arm<br>0,10,30,100 mg/kg | yes | Novartis | [DMPK R0570013] | 4.2.3.5.4-3 |
| Absorption after repeated doses | Dog, Beagle, male | p.o., gavage | 3-8 days | Arm<br>0, 600 300 mg/kg/day<br>Arm/Lmf<br>0, 1000 mg/kg | yes | Novartis | [DMPK R0510009B] | 4.2.3.7.3-2 |
| Absorption after repeated doses | Dog, Beagle, male, female | p.o., capsule | 3-month | Arm/Lmf<br>0, 20, 60, 200 mg/kg | yes | Novartis | [BPK(F) 1996/005] | 4.2.3.2-4 |
| Absorption after repeated doses | Dog, Beagle, male | i.m., solution | 5-day 30 day | Arm<br>0, 20 mg/kg | yes | Novartis | [BPK(F) 1997/004] | 4.2.3.7.3-4 |
| Absorption after repeated doses | Dog, Beagle, male | i.m., solution | 7-day | Arm<br>40 mg/kg/day | yes | Novartis | [DMPK R0410073] | 4.2.3.7.3-5 |
| Absorption after repeated doses | Dog, Beagle, male | i.m., solution | 3 or 8 days | Arm<br>0, 10, 40 mg/kg/day | yes | Novartis | [DMPK R0510001] | 4.2.3.7.3-6 |
| Absorption after repeated doses | Dog, Beagle, male, female | i.m., solution<br>p.o., capsules | 8-day | Arm<br>im: 0,20,40,80 mg/kg<br>po: 0,50,150,600 mg/kg | yes | Novartis | [DMPK(F) 1998/014] | 4.2.3.7.3-3 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***Lmf*** | | | | | | | | |
| Absorption after repeated doses | Rat, male, female | p.o., gavage | 1-month | Arm/Lmf<br>0, 200, 600, 1000 mg/kg | yes | Novartis | [BPK(CH) 1995/079] | 4.2.3.2-1 |
| Absorption after repeated doses | Rat, male, female | p.o., gavage | 3-month | Arm/Lmf<br>0, 100, 300, 1000 mg/kg | yes | Novartis | [BPK(CH) 1996/020] | 4.2.3.2-2 |
| Absorption after repeated doses | Rat, weanling, male, female | p.o., gavage | 13-week | Arm/Lmf<br>0, 100, 300, 1000 mg/kg | yes | Novartis | [DMPK(CH) 1997/177] | 4.2.3.5.4-1 |
| Absorption after repeated doses | Rat, male, female | p.o., gavage | 13-week | Lmf<br>0, 100, 300, 1000 mg/kg | yes | Novartis | [DMPK(CH) 1997/178] | 4.2.3.2-7 |
| Absorption after repeated doses | Dog, Beagle, male, female | p.o., capsule | 13-week | Lmf<br>0, 60, 200, 600 mg/kg | yes | Novartis | [DMPK(CH) 1997/006] | 4.2.2.2-7 |
| Absorption after repeated doses | Dog, Beagle male | p.o., gavage | 3 or 8 days | Arm/Lmf<br>1000 mg/kg/day | yes | Novartis | [DMPK R0510009A] | 4.2.3.7.3-2 |
| Absorption after repeated doses | Dog, Beagle male/female | p.o., capsule | 1-month | Arm/Lmf<br>0, 60, 200, 600 mg/kg | yes | Novartis | [BPK(CH) 1995/080] | 4.2.3.2-3 |
| Absorption after repeated doses | Dog, Beagle, male, female | p.o., capsule | 3-month | Arm/Lmf<br>0, 20, 60, 200 mg/kg | yes | Novartis | [BPK(CH) 1996/024] | 4.2.3.2-4 |
| ***2.6.5.5 組織及び臓器分布*** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Organ distribution | Rats, male albino, Tif:RAIf(SPF)<br>Rats, pigmented, LE/Mol (SPF) | i.v., solution<br>p.o., gavage | single | [$^{3}$H] or [$^{14}$C] Arm<br>i.v.: 10 mg/kg<br>p.o.: 20, 100, 1000 mg/kg | not required | Novartis | DMPK(CH) 1997/241 | 4.2.2.2-1 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***Lmf*** | | | | | | | | |
| Organ distribution | Rat:male albino, Tif:RAIf(SPF)<br>Rat: male pigmented, LE/Mol (SPF) | i.v.; solution<br>p.o., gavage | single | Arm/Lmf:<br>i.v.: 1 mg/kg<br>p.o.: 20, 100, 1000 mg/kg<br>containing 6/7 [$^{14}$C]-Lmf | not required | Novartis | DMPK(CH) 1997/240 | 4.2.2.2-4 |
| ***2.6.5.6 蛋白結合率*** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Plasma protein binding | Serum proteins, human erythrocytes, serum from mouse, rat, dog, rabbit, and cynomolgus monkey | *in vitro* | - | [$^{14}$C]-Arm<br>Plasema: 1, 10 µg/mL<br>Serum: 0.323~10 µg/mL<br>HSA: 0.5, 1, 5, 10 µg/mL | not required | Novartis | [DMPK(F) 1998/004] | 4.2.2.3-1 |
| ***Lmf*** | | | | | | | | |
| Plasma protein binding | human serum proteins, human erythrocytes, and serum proteins from mouse, rat, dog, rabbit, baboon and cynomolgus monkey | *in vitro* | - | [$^{14}$C]-Lmf<br>1, 10 µg/mL | not required | Novartis | [BPK(F) 1996/044] | 4.2.2.3-2 |
| ***Quinine*** | | | | | | | | |
| Plasma protein binding | Ultrafiltration method. *Ex vivo* human plasma of healthy subjects (study 2302) | *ex vivo* | - | quinine<br>1380 − 4250 ng/mL | not required | Novartis | [DMPK(CH) R0101415] | 4.2.2.3-3 |
| ***2.6.5.7 妊娠及び授乳動物*** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Study in pregnant and nursing animals | Rats, pregnant, day 13 of gestation | p.o., gavage | single | Arm/Lmf<br>30 mg/kg containing 1/7 of [$^{3}$H]-Arm | not required | Novartis | [DMPK(CH) 1997/099] | 4.2.2.3-4 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| Study in pregnant and nursing animals | Rabbits, pregnant, day 17 of gestation | p.o., gavage | single | Arm/Lmf<br>175 mg/kg containing 1/7 of [$^{14}$C]-Arm | not required | Novartis | [DMPK(CH) 1997/004] | 4.2.2.3-8 |
| Study in pregnant and nursing animals | Rabbits, pregnant, day 7 to 19 of gestation | p.o., gavage | 13-day | Arm/Lmf<br>0, 210, 700, 2100 mg/kg | yes | Novartis | [BPK(F) 1996/022] | 4.2.3.5.2-4 |
| ***Lmf*** | | | | | | | | |
| Study in pregnant and nursing animals | Rats, pregnant, day 13 of gestation | p.o., gavage | single | Arm/Lmf<br>30 mg/kg containing 6/7 [$^{14}$C]-Lmf | not required | Novartis | [DMPK(CH) 1997/139] | 4.2.2.3-5 |
| Study in pregnant and nursing animals | Rabbit, pregnant, day 17 of gestation | p.o., gavage | single | Arm/Lmf<br>175 mg/kg containing 6/7 [$^{14}$C]-Lmf | not required | Novartis | [DMET(EU) 29/1996] | 4.2.2.3-6 |
| Study in pregnant and nursing animals | Rabbit, pregnant, day 17 of gestation | p.o., gavage | single | Arm/Lmf<br>175 mg/kg containing 6/7 [$^{14}$C] -Lmf | not required | Novartis | [DMPK(CH) 1997/079] | 4.2.2.3-7 |
| Study in pregnant and nursing animals | Rabbit, pregnant, day 7 to 19 of gestation | p.o., gavage | 13-day | Arm/Lmf<br>0, 210, 700, 2100 mg/kg | yes | Novartis | [BPK(CH) 1996/085] | 4.2.3.5.2-4 |
| Study in pregnant and nursing animals | Rabbit, pregnant day 7 to 19 of gestation | p.o., gavage | 13-day | Lmf<br>0, 1000 mg/kg | yes | Novartis | [BPK(CH) 1996/084] | 4.2.3.5.2-10 |
| ***2.6.5.8 その他の分布*** | 該当なし | | | | | | | |
| ***2.6.5.9 代謝：in vivo 試験*** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Metabolism *in vivo* | Rats, male albino, Tif:RAIf(SPF) | i.v., solution<br>p.o., gavage | single | Arm/Lmf:<br>i.v.: 10 mg/kg<br>p.o.: 20, 100, 1000 mg/kg each dose containing 1/7 of [$^{3}$H]- or [$^{14}$C]-Arm | not required | Novartis | DMPK(CH) 1997/241 | 4.2.2.2-1 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| Metabolism *in vivo* | Dog, Beagle, male | i.v., solution<br>p.o., capsule | single | Arm/Lmf:<br>i.v.: 10 mg/kg<br>p.o.: 20, 200 mg/kg each dose containing 1/7 of [$^{14}$C]-Arm | not required | Novartis | DMPK(CH) 1997/003 | 4.2.2.2-2 |
| Metabolism *in vivo* | Rat,<br>samples from study DMPK(CH) 1997/241 | i.v., solution<br>p.o., gavage | single | Arm/Lmf:<br>i.v.: 10 mg/kg<br>p.o.: 20, 100, 1000 mg/kg each dose containing 1/7 of [$^{3}$H] or [$^{14}$C]-Arm | not required | Novartis | [DMPK(CH) 1997/534] | 4.2.2.4-1 |
| Metabolism *in vivo* | Dog, Beagle, male,<br>samples from study DMPK(CH) 1997/003 | i.v., solution<br>p.o., capsule | single | Arm/Lmf:<br>i.v.: 10 mg/kg<br>p.o.: 20, 200 mg/kg each dose containing 1/7 of [$^{3}$H] or [$^{14}$C]-Arm | | | | |
| ***Lmf*** | | | | | | | | |
| Metabolism *in vivo* | Rat,<br>Samples from study DMPK(CH) 1997/240 | i.v., solution<br>p.o., gavage | single | Arm/Lmf:<br>iv: 1 mg/kg<br>po: 20 and 100 mg/kg containing each 6/7 of [$^{14}$C]-Lmf | not required | Novartis | [DMPK(CH) 1997/209] | 4.2.2.4-2 |
| Metabolism *in vivo* | Dog,<br>Samples from study DMPK(CH) 1997/240 | i.v., solution<br>p.o., capsule | single | Arm/Lmf:<br>iv: 1 mg/kg<br>po: 20 mg/kg containing each 6/7 of [$^{14}$C]-Lmf | | | | |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***2.6.5.10* 代謝： *in vitro* 試験** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Metabolism *in vitro* | Rat blood, dog blood and bovine hemoglobin | *in vitro* | - | Arm<br>302 µmol/L<br>(90 µg/mL) | not required | Novartis | [DMPK(CH) 1997/242] | 4.2.2.4-3 |
| Metabolism *in vitro* | Mouse, rat, dog and human postmitochondrial liver fraction S12 | *in vitro* | - | [$^{14}$C]-Arm<br>1.56 − 50 µmol/L<br>(0.465-14.9 µg/mL) | not required | Novartis | [DMPK(CH) 1997/181] | 4.2.2.4-4 |
| Metabolism *in vitro* | Human liver microsomes, recombinant human B-lympho-blastoid cell lines (CYP1B1, 2A6,2B6, 2C9, 2D6, 2E1, 3A4, 4A11) or baculovirus infected insect cell lines (1A1, 1A2, 2C8, 2C19, 3A5) | *in vitro* | - | [$^{3}$H]-Arm<br>10-80 µmol/L<br>(2.98 – 23.9 µg/mL) | not required | Novartis | [DMPK(CH) 1997/156] | 4.2.2.4-5 |
| ***Lmf*** | | | | | | | | |
| Metabolism *in vitro* | Mouse, rat, dog and human postmitochondrial liver fraction S12 | *in vitro* | - | [$^{14}$C]-Lmf<br>5 and 20 µmol/L<br>(2.64 and 10.6 µg/mL) | not required | Novartis | [DMPK(CH) 1997/035] | 4.2.2.4-6 |
| Metabolism *in vitro* | Human liver microsomes, recombinant human B-lympho-blastoid cell line (CYP2D6) or baculovirus infected insect cell lines (CYP2C19, CYP3A4; CYP3A5) | *in vitro* | - | [$^{14}$C]-Lmf | not required | Novartis | [DMPK(CH) 1997/155] | 4.2.2.4-7 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***2.6.5.11 推定代謝経路*** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Possible metabolic pathways | Rat, rabbit, dog, human | - | - | [$^{3}$H] or [$^{14}$C]-Arm | not required | Novartis | DMPK(CH) 1997/534 | 4.2.2.4-1 |
| ***Lmf*** | | | | | | | | |
| Possible metabolic pathways | Rat, dog, human | - | - | [$^{14}$C]-Lmf | not required | Novartis | DMPK(CH) 1997/209 | 4.2.2.4-2 |
| ***2.6.5.12 代謝阻害及び酵素誘導*** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Induction/Inhibition of drug-metabolizing enzymes | Human liver microsomes<br>Marker substrates for: CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, CYP4A9/11 | *in vitro* | - | Arm | not required | [redacted] Cambridge; USA Novartis (sponsor) | [DMPK(CH) 1997/072] | 4.2.2.4-8 |
| ***Lmf*** | | | | | | | | |
| Induction/Inhibition of drug-metabolizing enzymes | Human liver microsomes<br>Marker substrates for: CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, CYP4A9/11 | *in vitro* | - | Lmf | not required | [redacted] Cambridge; USA Novartis (sponsor) | [DMPK(CH) 1997/073] | 4.2.2.4-9 |
| Induction of drug-metabolizing enzymes | Cryopreserved human hepatocytes | *in vitro* | - | Arm, DHA, Lmf | not required | Novartis | [DMPK R0900123] | 4.2.2.4-10 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***2.6.5.13* 排泄** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Excretion | Rats, male albino, Tif:RAIf(SPF) | i.v., solution p.o., , gavage | single | [$^{3}$H] or [$^{14}$C] Arm<br>i.v.: 10 mg/kg<br>p.o.: 20, 100, 1000 mg/kg | not required | Novartis | DMPK(CH) 1997/241 | 4.2.2.2-1 |
| Excretion | Dog, Beagle, male | i.v., solution p.o., gavage | single | [$^{14}$C]Arm<br>i.v.: 10 mg/kg<br>p.o.: 20, 200 mg/kg | not required | Novartis | DMPK(CH) 1997/003 | 4.2.2.2-2 |
| ***Lmf*** | | | | | | | | |
| Excretion | Rat:male albino, Tif:RAIf(SPF) | i.v., solution p.o.,gavage | single | Arm/Lmf:<br>i.v.: 1 mg/kg<br>p.o.: 20, 100, 1000 mg/kg<br>containing 6/7 $^{14}$C-labeled Lmf | not required | Novartis | DMPK(CH) 1997/240 | 4.2.2.2-4 |
| Excretion | Dog:male Beagle | i.v., solution p.o., gavage | single | Arm/Lmf:<br>i.v.: 1 mg/kg<br>p.o.: 20 mg/kg<br>containing 6/7 $^{14}$C-labeled Lmf | not required | Novartis | DMPK(CH) 1997/240 | 4.2.2.2-4 |
| ***2.6.5.14* 胆汁中排泄** | | | | | | | | |
| ***Arm*** | | | | | | | | |
| Excretion into bile | Rats, male albino, Tif:RAIf(SPF) | i.v., solution p.o., gavage | single | [$^{3}$H] or [$^{14}$C] -Arm<br>i.v.: 10 mg/kg<br>p.o.: 20, 100, 1000 mg/kg | not required | Novartis | DMPK(CH) 1997/241 | 4.2.2.2-1 |

| Type of Study | Test System or Species / Strain | Admini-stration | Duration Dosing | Analyte / Doses / Concentrations | GLP Compliance | Testing Facility | Report Number | Location in Module 4 |
|---|---|---|---|---|---|---|---|---|
| ***Lmf*** | | | | | | | | |
| Excretion into bile | Rats, male albino, Tif:RAIf(SPF) | i.v., solution<br>p.o., gavage | single | [$^{14}$C]-Lmf<br>i.v.: 1 mg/kg<br>p.o.: 20 mg/kg | not required | Novartis | DMPK(CH) 1997/240 | 4.2.2.2-4 |
| ***2.6.5.15*** **薬物間相互作用** | 2.6.5.12 代謝阻害及び酵素誘導の項参照 | | | | | | | |
| ***2.6.5.16*** **その他** | 該当なし | | | | | | | |

# 2.6.5.2 薬物動態試験：分析方法

## 2.6.5.2A Pharmacokinetics: Analytical methods and validation reports (BAPK(EU) R0301212)

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in human plasma by LC-MS/MS. Method description and validation. | Study no. BAPK(EU) R0301212 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-1 | |
| Test article: | Arm and DHA | |
| Method: | The objective of this study was to transfer and validate an analytical method for the determination of Arm and its metabolite, DHA, in human plasma and to validate the analytical procedure in terms of recovery (extraction efficiency), intra-batch and inter-batch precision and accuracy at four different concentration levels, specificity, dilution process, carryover, matrix effect, stability of the extracts in the automatic injector at room temperature. The development and the validation of the method was done according to a publication provided by the Novartis Bioanalytical Monitor with the following modifications:<br>Use of a shorter column and a gradient elution<br>Use of MS/MS fragmentation of fragment at m/z= 267.2 for Arm and arteether<br>The method consists of a liquid/liquid extraction with 1-chlorobutane-isooctane (55:45, v:v), followed by reverse phase liquid chromatography with tandem mass spectrometric detection. | |
| Instruments: | | |
| LC-MS/MS system | API 3000, mass spectrometer, Applied Biosystems<br>Series 1100 binary pump, Agilent<br>HTC Pal autosampler, CTC Analytics<br>Series 1100 degasser, Agilent | |
| LC-MS/MS software | Analyst® 1.1 | |
| Software interface | APCI, Applied Biosystems | |
| Data acquisition | Watson® LIMS | |
| **Chromatography:** | Reversed-phase HPLC on an Alltima $C_{18}$ 3 µm (100 x 4.6 mm) column at room temperature using gradient elution (acetic acid solution 0.1% and acetonitrile) at a flow rate of 1.2 mL/min with a total run time of 5 min. | |
| Column | Alltima $C_{18}$, 3 µm (100 x 4.6 mm), Alltech. | |
| Column temperature | Room temperature | |
| Mobile phase | A: Acetic acid solution 0.1%<br>B: Acetonitrile | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in human plasma by LC-MS/MS. Method description and validation. | Study no. BAPK(EU) R0301212 |
|---|---|---|
| Flow rate | 1.2 mL/min | |
| Injection volume | 30 µL | |
| Autosampler temperature | Room temperature | |
| Retention time(s) | Arm: 3.6 min | |
| | DHA: 1.2 min | |
| | Arteether: 4.1 min | |
| | Artemisinin: 2.4 min | |
| Total run time | 5 min | |
| Substance analyzed: | Arm and DHA | |
| Reference compounds: | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$ | |
| | Molecular weight (MW): 298.37 g/mol | |
| DHA | Molecular formula: $C_{15}H_{24}O_5$ | |
| | Molecular weight (MW): 284.35 g/mol | |
| Internal standards: | | |
| Arteether | Molecular formula: $C_{17}H_{28}O_5$ | |
| | Molecular weight (MW): 312.40 g/mol | |
| Artemisinin | Molecular formula: $C_{15}H_{22}O_5$ | |
| | Molecular weight (MW): 282.33 g/mol | |
| Sample preparation: | Liquid-liquid extraction with 1-chlorobutane-isooctane | |
| C standard and QC samples preparation: | | |
| C standards | 7 concentrations in the range of 5 ng/mL to 200 ng/mL | |
| QC samples | 4 concentrations in the range of 5 ng/mL to 160 ng/mL | |
| Matrix: | Human plasma | |
| Detection: | MS/MS detection with positive ion APCI | |
| *Period 1* | Duration: 3 min<br>Polarity: Positive<br>APCI current: 3 µA<br>Source temperature: 400°C<br>Nebuliser gas, pressure: $N_2$, 8 AU | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in human plasma by LC-MS/MS. Method description and validation. | Study no. BAPK(EU) R0301212 |
|---|---|---|
| | Curtain gas: $N_2$, 8 AU | |
| | Collision gas: $N_2$, 4 AU | |
| | Auxillary flow: $N_2$, 5 bars | |
| | Declustering potential: 26 V | |
| | Focusing potential: 110 V for DHA | |
| | 150 V for artemisinin | |
| | Entrance potential: -10 V | |
| | Collision energy: 15 eV for DHA | |
| | 11 eV for artemisinin | |
| | Collision cell exit potential: 16 V for DHA | |
| | 14 V for artemisinin | |
| *Period 2* | Duration: 2 min | |
| | Polarity: Positive | |
| | APCI current: 3 µA | |
| | Source temperature: 400°C | |
| | Nebuliser gas, pressure: $N_2$, 8 AU | |
| | Curtain gas: $N_2$, 8 AU | |
| | Collision gas: $N_2$, 4 AU | |
| | Auxillary flow: $N_2$, 5 bars | |
| | Declustering potential: 21 V | |
| | Focusing potential: 100 V | |
| | Entrance potential: -10 V | |
| | Collision energy: 13 eV | |
| | Collision cell exit potential: 16 V | |
| *DHA transition* | m/z 267.2 →163.0 | |
| | Resolution Q1: unit Q3: Unit | |
| | Dwell time: 250 ms | |
| *Artemisinin transition* | m/z 283.1→209.0 | |
| | Resolution Q1: unit Q3: Unit | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in human plasma by LC-MS/MS. Method description and validation. | Study no. BAPK(EU) R0301212 |
|---|---|---|
| | Dwell time: 100 ms | |
| *Arm and arteether transitions* | m/z 267.2 → 163.0 | |
| | Resolution Q1: unit Q3: Unit | |
| | Dwell time: 400 ms | |
| **Results-1:** | | |
| Specificity | The specificity of the analytical method was investigated by preparing and analyzing blank samples prepared from 6 different individuals. The specificity was assessed by comparing the apparent signal for Arm, DHA, arteether and artemisinin in blank samples to the mean signal obtained for samples spiked with a concentration of Arm and at the LLOQ and with arteether and artemisinin at the working concentration. | |
| *Acceptance criteria* | Arm and DHA: individual interference ≤ 20% of signal at LLOQ | |
| | Arteether and artemisinin: individual interference ≤ 5% of signal at working concentration | |
| *Interference from the human plasma (matrix)* | No peak in the blank extracts at the retention time of the analytes or the internal standard interfered with the analytes by more than 20% of the mean at the LLOQ calibration standard peak signals and by more than 5% of the mean internal standard peak signal. | |
| *Specificity of each analyte* | The specificity of each analyte (Arm and DHA) to the other analyte, in the assay, was verified. The expected retention times for each of the non-spiked analytes were checked to ensure that no peaks were present which represented a peak signal (mean of the 6 determinations) which was greater than 20% of the relevant mean LLOQ calibration standard peak signal and 5% of the mean internal standard peak signal. | |
| Calibration curves | Calibration standards were prepared in duplicate and included the target lower limit of quantification, LLOQ (5 ng/mL) and the target upper limit of quantification, ULOQ (200 ng/mL). One set of calibration standards was analyzed at the start of the analytical run of another one at the end. Concentrations were obtained by least weighted linear regression using 1/x weighting factor. The acceptance criteria for the mean accuracy were met: Mean accuracy: 85% to 115% (80% to 120% at the LLOQ) | |
| *Acceptance criteria* | Bias within ±15% (±20% at the LLOQ) of the nominal values for 70% of the C samples of each series and 6 calibration concentrations included in the final calibration line. | |
| **Results-2:** | | |
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the C samples on each day. | |
| *Intra-day accuracy and precision (within batch)* | The intra-day accuracy and precision were calculated as the mean and precision of all individual concentrations of QC samples analyzed during a single validation day. | |
| *Inter-day accuracy and precision (inter-batch)* | The inter-day accuracy and precision were calculated as the mean accuracy and precision of all individual concentrations of QC samples analyzed during 3 validation days. | |
| *Acceptance criteria* | Mean accuracy 85% to 115% (80% to 120% at LLOQ) | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in human plasma by LC-MS/MS. Method description and validation. | Study no. BAPK(EU) R0301212 |
|---|---|---|
| *Arm* | | |
| Intra-day accuracy and precision | At the LLOQ: Mean accuracy 101.8%, precision 2.99% | |
| | Above the LLOQ: Mean accuracy within the range 94.38% to 102%, precision 3.88% to 5.88% | |
| Inter-day accuracy and precision | At the LLOQ: Mean accuracy 101.6%, precision 12.74% | |
| | Above the LLOQ: Mean accuracy within the range 93.38% to 97.3%, precision 4.38% to 9.23% | |
| *DHA* | | |
| Intra-day accuracy and precision | At the LLOQ: Mean accuracy 105%, precision 6.84% | |
| | Above the LLOQ: Mean accuracy within the range 98.13% to 99.75%, precision 1.1% to 10.23% | |
| Inter-day accuracy and precision | At the LLOQ: Mean accuracy 97.6%, precision 8.65% | |
| | Above the LLOQ: Mean accuracy within the range 96.13% to 100%, precision 4.52% to 7.17% | |
| LLOQ | 5 ng/mL using a sample volume of 0.5 mL for both compounds | |
| Dilution | The dilution procedure (1/10) was verified with samples spiked at 250 ng/mL | |
| **Results-3:** | | |
| Matrix effect | The impact of the matrix effect on the LC-MS/MS method was assessed by analyzing 6 different batches of human plasma at the lowest concentration level (LLOQ) and at the concentration used for the internal standard. These samples were analyzed with calibration standards and QC samples prepared from a different batch of human plasma. No matrix effect was evidenced. | |
| *Acceptance criteria* | Mean accuracy 80% to 120%, precision ≤ 20% | |
| *Arm* | Mean accuracy between 115.6% and 119.4%, precision between 4.11% and 7.37% | |
| *DHA* | Mean accuracy between 90.2% and 106.8%, precision between 3.54% and 18.2% | |
| Absolute recovery | The extraction recovery of Arm and DHAwas determined at 3 concentrations: the low, mid, and high QC levels. The extraction recoveries of the internal standards were determined at the concentrations at which they were used in the assay. Six samples prepared at the low, mid, and high concentration were extracted along with 18 blank human plasma samples (T0/0) containing neither analyte nor internal standard. After extraction, the blank human plasma samples were spiked with analyte and internal standard to ensure that concentrations in the blank extracts were equivalent to those in the low, mid, and high extracted samples. Extraction recovery was calculated by comparing the peak areas obtained from blank extracts spiked after extraction. | |
| *Arm* | Mean recovery 67% (range 61.32% to 72.9%) | |
| *DHA* | Mean recovery 89.59% (range 81.5% to 99.39%) | |
| *Arteether* | Mean recovery 59.4% | |
| *Artemisinin* | Mean recovery 84.6% | |
| Stability | The stability of Arm and DHA was investigated by analyzing quality control of human samples and reconstituted extracts, which were stored under varying conditions together with freshly prepared C standards and QC samples. | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in human plasma by LC-MS/MS. Method description and validation. | Study no. BAPK(EU) R0301212 |
|---|---|---|
| *Acceptance criteria* | Mean accuracy 85% to 115% | |
| *Stability in stock solutions* | No apparent loss after 9 days in a freezer at +5°C ± 5°C. | |
| *Stability in working solutions* | Stable in working solution after 14 days storage in a freezer room at +5°C ± 5°C. | |
| *Stability in extracts* | No apparent loss after 21 hours of storage at room temperature. | |
| Conclusion: | The method is suitable for the routine analysis of Arm and DHA in human plasma with an anticipated limit of quantification of 5 ng/mL using 0.5 mL sample. | |
| | The method is specific for Arm and DHA within the given criteria for acceptance. | |

Additional Information: none

## 2.6.5.2B Pharmacokinetics: Analytical methods and validation reports (BAPK(EU) R0301212-01)

| **Study title:** | Additional development tests for artemether/ dihydroartemisinin assay. Method description and validation: additional data. | Study no. BAPK(EU) R0301212-01 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-2 | |
| Test article: | Arm and DHA | |
| Method: | The objective of this analytical study was to perform additional development work on the LC-MS/MS assay for the determination of Arm and its metabolite DHA in human plasma, developed and validated previously. To date, for the quantification of DHA, only the analytical response (peak area) of alpha-DHA is used. In order to verify that approach was valid, further tests were necessary to determine:<br>If the equilibrium between alpha-DHA and beta-DHA was time and/or temperature dependent.<br>If the use of the peak area of alpha-DHA only for quantification purposes gave comparable results to an approach using the peak areas of both alpha- and beta-DHA. | |
| Instruments: | | |
| LC-MS/MS system | API 3000, mass spectrometer, Applied Biosystems<br>Series 1100 binary pump, Agilent<br>HTC Pal autosampler, CTC analytics<br>Series 1100 degasser, Agilent | |
| LC-MS/MS software | Analyst® | |
| Data acquisition | Watson® LIMS | |
| Chromatography: | Reverse phase HPLC on an Alltima $C_{18}$ 3 µm (100 x 4.6 mm) column at room temperature using gradient elution (acetic acid 0.1% and acetonitrile) at a flow rate of 1.2 mL/min for a total run of 5 min | |
| Autosampler temperature | Room temperature or approximately +5°C | |
| Column | Alltima $C_{18}$, 3 µm (100 x 4.6 mm), Alltech | |
| Column temperature | Room temperature | |
| Mobile phase | A: 0.1% acetic acid solution<br>B: Acetonitrile | |
| Flow rate | 1.2 mL/min | |
| Injection volume | 30 µL | |

| **Study title:** | Additional development tests for artemether/ dihydroartemisinin assay. Method description and validation: additional data. | Study no. BAPK(EU) R0301212-01 |
|---|---|---|
| Retention times | Arm: 3.6 min | |
| | DHA: 1.8 min | |
| | Arteether (internal standard of Arm): 4.1 min | |
| | Artemisinin (internal standard for DHA): 2.4 min | |
| Total run time | 5 min | |
| Substance analyzed: | Arm | |
| Reference compounds: | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$ | |
| | Molecular weight (MW): 298.37 g/mol | |
| Internal standards: | | |
| Arteether | Molecular formula: $C_{17}H_{28}O_5$ | |
| | Molecular weight (MW): 312.4 g/mol | |
| Sample preparation: | Liquid-liquid extraction with 1-chlorobutane-isooctane. | |
| Calibration curves: | No calibration standards or QC samples were prepared. | |
| Stability samples: | Stability samples were spiked with DHA at the concentrations described below. After the stability tests, peak areas were compared. | |
| Stability samples in plasma | Samples for determining the stability of alpha- and beta-DHA in human plasma and human plasma extracts were prepared as pools by spiking human plasma with aliquots of working solutions. The following concentrations were used: | |
| | 2 concentrations: 10 ng/mL and 160 ng/mL | |
| Stability samples in reconstitution phase | Samples for determining the stability of alpha- and beta-DHA in the reconstitution phase were prepared as pools by spiking reconstitution phase with working solutions. The following concentrations were used: | |
| | 2 concentrations: 50 ng/mL and 800 ng/mL | |
| Matrix: | Human plasma with heparin anticoagulant | |
| MS/MS Detection: | MS/MS detection with positive ion APCI | |

| **Study title:** | Additional development tests for artemether/ dihydroartemisinin assay. Method description and validation: additional data. | Study no. BAPK(EU) R0301212-01 |
|---|---|---|
| *Period 1* | Duration: 3 min | |
| | Polarity: Positive | |
| | APCI current: 4 µA | |
| | Source temperature: 400°C | |
| | Nebuliser gas, pressure: $N_2$, 6 AU | |
| | Curtain gas: $N_2$, 8 AU | |
| | Collision gas: $N_2$, 4 AU | |
| | Declustering potential: 26 V | |
| | Focusing potential: 100 V | |
| | Entrance potential: -8 V | |
| | Collision energy: 17 eV | |
| | Collision cell exit potential: 12 V | |
| *Period 2* | Duration: 2 min | |
| | Polarity: Positive | |
| | APCI current: 4 µA | |
| | Source temperature: 400°C | |
| | Nebuliser gas, pressure: $N_2$, 6 AU | |
| | Curtain gas: $N_2$, 8 AU | |
| | Collision gas: $N_2$, 4 AU | |
| | Declustering potential: 21 V | |
| | Focusing potential: 100 V | |
| | Entrance potential: -10 V | |
| | Collision energy: 21 eV | |
| | Collision cell exit potential: 10 V | |
| *DHA transition* | m/z 267.3 → 163.1 | |
| | Resolution Q1: unit Q3: Unit | |
| | Dwell time: 250 ms | |

| **Study title:** | Additional development tests for artemether/ dihydroartemisinin assay. Method description and validation: additional data. | Study no. BAPK(EU) R0301212-01 |
|---|---|---|
| *Artemisinin transition* | m/z 283.3 → 209.1<br>Resolution Q1: unit Q3: Unit<br>Dwell time: 100 ms | |
| *Arm and arteether transitions* | m/z 267.4 → 163.1<br>Resolution Q1: unit Q3: Unit<br>Dwell time: 400 ms | |
| Data processing: | | |
| Software for calculation | Watson® LIMS | |
| Software for additional data processing | Microsoft® Excel | |
| Development tests: | | |
| Influence of temperature/time on alpha-/beta-DHA equilibrium in plasma samples | The first test was designed to determine the influence of temperature and time on the equilibrium between alpha- and beta-DHA in human plasma samples spiked with DHA and Arm at 10 ng/mL and 160 ng/mL. Six sets of six samples were prepared at each concentration. These samples were then treated as follows:<br>1. Three sets of samples at each concentration were kept at room temperature. One set was extracted and injected at t=0, a second set was extracted and injected after 6 hours at room temperature, and a third set was extracted and injected after 24 hours at room temperature.<br>2. Three sets of samples at each concentration were placed in a refrigerator at +5°C ± 5°C. One set was extracted and injected at t=0, a second set was extracted and injected after 6 hours in the refrigerator, and a third set was extracted and injected after 24 hours in the refrigerator. | |
| Influence of temperature/time on alpha-/beta-DHA equilibrium in plasma extracts | The second test was designed to determine the influence of temperature and time on the equilibrium between alpha- and beta-DHA in extract samples obtained from human plasma samples spiked with DHA and Arm at 10 ng/mL and 160 ng/mL. Six sets of six samples were prepared at each concentration. These samples were then treated as follows, prior to the analysis:<br>1. Three sets of samples at each concentration were placed in the autosampler at room temperature. One set was injected at t=0, a second set was injected after 12 hours in the autosampler, and a third set was injected after 24 hours in the autosampler.<br>2. Three sets of samples at each concentration were placed in the autosampler at +5°C ± 5°C. One set was injected at t=0, a second set was injected after 19 hours in the autosampler, a third set was injected after 22 hours in the autosampler, and a fourth set was injected after 24 hours in the autosampler. | |

| **Study title:** | Additional development tests for artemether/ dihydroartemisinin assay. Method description and validation: additional data. | Study no. BAPK(EU) R0301212-01 |
|---|---|---|
| Influence of temperature/time on alpha-/beta-DHA equilibrium in reconstitution phase | The third test was designed to determine the influence of temperature and time on the equilibrium between alpha- and beta-DHA in the reconstitution phase (ethanol/acetic acid [0.1% in water] 50:50, v:v) spiked with DHA and Arm at 50 ng/mL and 800 ng/mL. Six sets of six samples were prepared at each concentration. These samples were then treated as follows, prior to the analysis:<br>1. Three sets of samples at each concentration were placed in the autosampler at room temperature. One set was injected at t=0, a second set was injected after 19 hours in the autosampler, a third set was injected after 19 hours in the autosampler, and a fourth set was injected after 24 hours in the autosampler.<br>2. Three sets of samples at each concentration were placed in the autosampler at approximately +5°C. One set was injected at t=0, a second set was injected after 19 hours in the autosampler, a third set was injected after 22 hours in the autosampler, and a fourth set was injected after 24 hours in the autosampler. | |
| DHA concentration calculations | For the DHA assay, the reference standard used is a mixture of alpha and beta anomers. The exact ratio between the two anomers is not known, but the alpha anomer is the predominant form. Only the analytical response (peak area) for the alpha-DHA is used for quantification purposes and the reference material is therefore considered to be 100% alpha-DHA. In order to verify that the use of the peak area for alpha-DHA only for quantification purposes was valid, calculations comparing this approach with the use of the sum of peak areas for alpha- and beta-DHA were performed on the analytical batches from earlier studies. The results obtained using the two approaches were compared. | |
| **Results-1:** Influence of temperature/time on alpha-/beta-DHA equilibrium in plasma samples | | |
| *At room temperature* | Peak areas for both alpha- and beta-DHA decreased significantly after 24 hours of storage. Under these circumstances, it was not therefore possible to draw a conclusion on the evolution of the peak area ratios between alpha- and beta-DHA. | |
| Peak area ratios for alpha-/beta-DHA | The% difference after 6 hours of storage was 22.7% at 10 ng/mL and 13.6% at 160 ng/mL. The% difference after 24 hours storage was 15.7% at 10 ng/mL and 9.3% at 160 ng/mL. | |
| Peak areas for alpha-DHA | The% difference after 6 hours of storage was -10.6% at 10 ng/mL and -10.0% at 160 ng/mL. The% difference after 24 hours storage decreased significantly: -64.4% at 10 ng/mL and -59.9% at 160 ng/mL. | |
| Peak areas for beta-DHA | The% difference after 6 hours of storage was -17.3% at 10 ng/mL and -20.5% at 160 ng/mL. The% difference after 24 hours storage decreased significantly: -64.6% at 10 ng/mL and -63.2% at 160 ng/mL. | |
| *At +5°C ± 5°C* | No significant evolution of peak area ratios and peak areas was observed. The equilibrium between alpha- and beta-DHA was stable under these conditions. | |
| Peak area ratios for alpha-/beta-DHA | The% difference after 6 hours of storage was 8.8% at 10 ng/mL and 11.0% at 160 ng/mL. The% difference after 24 hours storage was -1.0% at 10 ng/mL and 1.5% at 160 ng/mL. | |
| Peak areas for alpha-DHA | The% difference after 6 hours of storage was -1.2% at 10 ng/mL and 15.4% at 160 ng/mL. The% difference after 24 hours storage was 8.6% at 10 ng/mL and 22.1% at 160 ng/mL. | |
| Peak areas for beta-DHA | The% difference after 6 hours of storage was -7.4% at 10 ng/mL and 3.8% at 160 ng/mL. The% difference after 24 hours storage was 10.8% at 10 ng/mL and 20.3% at 160 ng/mL. | |

| **Study title:** | Additional development tests for artemether/ dihydroartemisinin assay. Method description and validation: additional data. | Study no. BAPK(EU) R0301212-01 |
|---|---|---|

**Results-2:** Influence of temperature/time on alpha-/beta-DHA equilibrium in plasma extracts

| | |
|---|---|
| *At room temperature* | No significant evolution of peak area ratios and peak areas was observed. The equilibrium between alpha- and beta-DHA was stable under these conditions. |
| Peak area ratios for alpha-/beta-DHA | The% difference after 19 hours of storage was 9.5% at 10 ng/mL and 8.1% at 160 ng/mL. The% difference after 22 hours storage was 22.5% at 10 ng/mL and 6.0% at 160 ng/mL. The% difference after 24 hours storage was 27.8% at 10 ng/mL and 22.1% at 160 ng/mL. |
| Peak areas for alpha-DHA | The% difference after 19 hours of storage was -9.2% at 10 ng/mL and -0.8% at 160 ng/mL. The% difference after 22 hours storage was 0.3% at 10 ng/mL and -2.7% at 160 ng/mL. The% difference after 24 hours storage was 24.2% at 10 ng/mL and 11.8% at 160 ng/mL. |
| Peak areas for beta-DHA | The% difference after 19 hours of storage was -16.8% at 10 ng/mL and -8.2% at 160 ng/mL. The% difference after 22 hours storage was -17.1% at 10 ng/mL and -8.1% at 160 ng/mL. The% difference after 24 hours storage was -1.1% at 10 ng/mL and -8.3% at 160 ng/mL. |
| *At approximately +5°C* | Peak area decreased significantly for beta-DHA under these conditions. However, there was no significant evolution of peak areas for alpha-DHA. Consequently, the peak area ratios increased significantly. |
| Peak area ratios for alpha-/beta-DHA | The% difference after 12 hours of storage was 38.5% at 10 ng/mL and 34.6% at 160 ng/mL. The% difference after 24 hours storage was 67.5% at 10 ng/mL and 29.4% at 160 ng/mL. |
| Peak areas for alpha-DHA | The% difference after 12 hours of storage was -1.1% at 10 ng/mL and -2.2% at 160 ng/mL. The% difference after 24 hours storage was 3.6% at 10 ng/mL and 2.7% at 160 ng/mL. |
| Peak areas for beta-DHA | The% difference after 12 hours of storage was -28.1% at 10 ng/mL and -27.3% at 160 ng/mL. The% difference after 24 hours storage was -37.6% at 10 ng/mL and -20.4% at 160 ng/mL. |

**Results-3:** Influence of temperature/time on alpha-/beta-DHA equilibrium in reconstitution phase

| | |
|---|---|
| *At room temperature* | No significant evolution of peak area ratios and peak areas was observed after 19, 22, and 24 hours of storage under these conditions, except for the peak area ratio of the samples at 50 ng/mL, which increased to 33.4% after 24 hours. The equilibrium between alpha- and beta-DHA was stable up to at least 22 hours. |
| Peak area ratios for alpha-/beta-DHA | The% difference after 19 hours of storage was 9.3% at 50 ng/mL and 4.7% at 800 ng/mL. The% difference after 22 hours storage was -7.1% at 50 ng/mL and 2.8% at 800 ng/mL. The% difference after 24 hours storage was 33.4% at 50 ng/mL and 3.8% at 800 ng/mL. |
| Peak areas for alpha-DHA | The% difference after 19 hours of storage was 6.4% at 50 ng/mL and -3.2% at 800 ng/mL. The% difference after 22 hours storage was -0.4% at 50 ng/mL and -1.2% at 800 ng/mL. The% difference after 24 hours storage was 15.6% at 50 ng/mL and 14.2% at 800 ng/mL. |
| Peak areas for beta-DHA | The% difference after 19 hours of storage was -3.3% at 50 ng/mL and -7.5% at 800 ng/mL. The% difference after 22 hours storage was 7.8% at 50 ng/mL and -3.8% at 800 ng/mL. The% difference after 24 hours storage was -13.0% at 50 ng/mL and 10.0% at 800 ng/mL. |
| *At approximately +5°C* | No significant evolution of the peak areas for alpha-DHA was observed. However, the peak areas for beta-DHA decreased significantly. Consequently, the peak area ratios increased slightly. |
| Peak area ratios for alpha-/beta-DHA | The% difference after 12 hours of storage was 19.3% at 50 ng/mL and 19.1% at 800 ng/mL. The% difference after 24 hours storage was 26.1% at 50 ng/mL and 20.9% at 800 ng/mL. |

| **Study title:** | Additional development tests for artemether/ dihydroartemisinin assay. Method description and validation: additional data. | Study no. BAPK(EU) R0301212-01 |
|---|---|---|
| Peak areas for alpha-DHA | The% difference after 12 hours of storage was -6.4% at 50 ng/mL and 5.5% at 800 ng/mL. The% difference after 24 hours storage was 4.2% at 50 ng/mL and 2.7% at 800 ng/mL. | |
| Peak areas for beta-DHA | The% difference after 12 hours of storage was -22.2% at 50 ng/mL and -11.5% at 800 ng/mL. The% difference after 24 hours storage was -16.9% at 50 ng/mL and -15.2% at 800 ng/mL. | |
| DHA concentration calculations | The results obtained from the peak area calculations showed that for each of the eight batches, the% difference between the two concentrations obtained, $C_{cal}$ (concentration calculated using the sum of peak areas) and $C_{ref}$ (concentrations using alpha-DHA peak area only) was between -20% and +20% for at least 2/3 of the samples in each batch. | |
| Special issues: | During the injection of the stability samples corresponding to the tests performed on the plasma extracts and the samples in the reconstitution phase at room temperature, an instrument problem was encountered. Consequently, samples which should have been injected after being retained for 12 hours in the autosampler were not injected. Both tests were therefore repeated. For the repeated tests, an instrument problem was also encountered, which meant that these samples were injected after being retained for 19 hours and 22 hours in the autosampler. | |
| Conclusion: | The storage of human plasma samples at room temperature should not exceed 6 hours. This is in agreement with the stability data obtained and published previously. | |
| | Human plasma samples can be stored at +5°C ± 5°C for up to 24 hours. | |
| | Human plasma extracts can be stored at room temperature up to 24 hours. This is in agreement with the stability data obtained during validation of the method, which showed that plasma extracts could be stored in the autosampler for up to 21 hours. | |
| | When human plasma extracts are stored at +5°C ± 5°C for 12 and 24 hours, beta-DHA is not stable. | |
| | Samples in reconstitution phase can be stored at room temperature for up to 22 hours | |
| | When samples in reconstitution phase are stored at +5°C ± 5°C for 12 and 24 hours, beta-DHA shows some instability. | |
| | The two chromatographic approaches used for the quantification of DHA are: | |
| | The method used to date, where the only the peak areas for alpha-DHA are used. | |
| | The method described in this report, where the sum of the areas of alpha- and beta-DHA is used. Whatever approach is used, the results obtained are similar. The result justifies the use of the peak area for alpha-DHA only. | |

Additional Information: none

## 2.6.5.2C Pharmacokinetics: Analytical methods and validation reports (DMPK R0400692)

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in dog plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0400692 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-3 | |
| Test article: | Arm and DHA | |
| Method: | The method consists of liquid-liquid extraction with 1-chlorobutane-isooctane (55:45, v:v) followed by reverse phase liquid chromatography with tandem mass spectrometric detection. The objective of this analytical study was to develop and to validate an analytical method for the determination of Arm and its metabolite, DHA, in dog plasma. Due to a chromatographic interference that appeared in extract ex-vivo dog samples from toxicokinetic studies on the multiple reaction monitoring (MRM) transition of artemisinin, the chromatographic conditions were modified and a partial revalidation was performed. | |
| Instruments: | | |
| HPLC System | Pump: Series 200 micropump, Perkin Elmer | |
| | Pump: Series 1100 binary pump, Agilent | |
| | Column: Alltima $C_{18}$ 3 µm (100 x 4.6 mm) | |
| | Autosampler: HTC PAL autosampler, CTC analytics | |
| | Autosampler: Series 200 autosampler, Perkin Elmer | |
| MS System | API3000, Applied Biosystems (API3000-III and API3000-IV) | |
| Chromatography: | | |
| Autosampler temperature | Room temperature | |
| Injection volume | 40 µL | |
| Wash 1 (HTC PAL autosampler) | Methanol-$H_2O$ (50:50, v:v) | |
| | Pre-cleans: 0 | |
| | Post-cleans: 2 | |
| | Valve cleans: 1 | |
| Wash 2 (HTC PAL autosampler) | Methanol | |
| | Pre-cleans: 0 | |
| | Post-cleans: 2 | |
| | Valve cleans: 1 | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in dog plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0400692 |
|---|---|---|
| Flush (Series 200 autosampler) | 400 µL | |
| | Pre-injection: 3 | |
| | Post-injection: 3 | |
| Column | Alltima $C_{18}$, 3 µm 100 x 4.6 mm | |
| Column temperature | Room temperature | |
| Mobile phase | A: 0.1% acetic acid solution | |
| | B: Acetonitrile | |
| Flow rate | 1200 µL/min | |
| Retention times (Series 200 micropump) (approximation) | Arm: 5.3 min | |
| | DHA: 3.5 min | |
| | Arteether: 5.8 min | |
| | Artemisinin: 4.3 min | |
| Retention times (Series 1100 pump, Agilent) (approximation) | Arm: 5.4 min | |
| | DHA: 3.7 min | |
| | Arteether: 5.8 min | |
| | Artemisinin: 4.5 min | |
| Total run time | Series 200 micropump: 6.5 min | |
| | Series 1100 pump, Agilent: 9 min | |
| Substance analyzed: | Arm and DHA | |
| Reference compounds: | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$ | |
| | Molecular weight (MW): 298.37 g/mol | |
| DHA | Molecular formula: $C_{15}H_{24}O_5$ | |
| | Molecular weight (MW): 284.35 g/mol | |
| Internal standards: | | |
| Arteether | Molecular formula: $C_{17}H_{28}O_5$ | |
| | Molecular weight (MW): 312.4 g/mol | |
| Artemisinin | Molecular formula: $C_{15}H_{22}O_5$ | |
| | Molecular weight (MW): 282.33 g/mol | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in dog plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0400692 |
|---|---|---|
| Sample preparation: | Liquid-liquid extraction with 1-chlorobutane-isooctane (55:45, v:v) | |
| C standard and QC samples preparation: | | |
| C Standards | 7 concentrations in the range of 5 ng/mL (LLOQ) to 200 ng/mL, expressed as base | |
| QC Samples | 4 concentrations in the range of 5 ng/mL (LLOQ) to 160 ng/mL, expressed as base | |
| Matrix: | Dog plasma | |
| Detection: | MS/MS positive ion mode | |
| MS conditions | Positive ion mode | |
| *Period 1* | Duration 5 min, APCI current: 3 µA (API3000-III) | |
| | Duration 5 min, APCI current: 4 µA (API3000-IV) | |
| *Period 2* | Duration 2 min, APCI current: 3 µA (API3000-III) | |
| | Duration 1.5 min, APCI current: 4 µA (API3000-IV) | |
| *DHA transition* | m/z: 267.1 → 163.1 (API3000-III) | |
| | m/z: 267.3 → 163.1 (API3000-IV) | |
| | Resolution Q1: Unit Q3: Unit | |
| | Dwell time: 250 ms | |
| *Artemisinin transition* | m/z: 283.1 → 209.1 (API3000-III) | |
| | m/z: 283.3 → 209.1 (API3000-IV) | |
| | Resolution Q1: Unit Q3: Unit | |
| | Dwell time: 250 ms | |
| *Arm and arteether transitions* | m/z: 267.1 → 163.1 (API3000-III) | |
| | m/z: 267.4 → 163.1 (API3000-IV) | |
| | Resolution Q1: Unit Q3: Unit | |
| | Dwell time: 400 ms | |
| *Software for data acquisition* and peak integration | Analyst® | |
| **Results-1:** | | |
| Specificity | The specificity of the method was determined by analyzing dog plasma samples prepared and extracted without addition of analytes or internal standards. Dog plasma from 6 different pools (n=2 for each source of matrix) was tested. This method was specific for dog plasma for Arm, DHA, arteether, and artemisinin. No interference at the corresponding retention time in the relevant MRM transition. | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in dog plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0400692 |
|---|---|---|
| *Acceptance criteria* | Arm and DHA: Interference ≤ 20% of signal at LLOQ | |
| | Arteether and artemisinin: Interference ≤ 5% of signal at working concentration | |
| *Interference from the matrix* | No peaks in the blank extracts at the retention time of the analyte or the internal standard and in the relevant mass channel were detected. | |
| **Results-2:** | | |
| Calibration curves | C standards were prepared in duplicate and included the LLOQ (5.00 ng/mL for both compounds) and the ULOQ (200 ng/mL for both compounds). Concentrations of Arm and DHA were obtained by least square weighted linear regression (weighting factor 1/x) of the ratio of response of Arm/internal standard response (arteether) versus the theoretical concentrations and of DHA/internal standard response (artemisinin) versus the theoretical concentrations. | |
| *Acceptance criteria* | Deviation ±15% (±20% at the LLOQ) for 70% of the C standards for nominal values | |
| *Arm* | | |
| At the LLOQ | Mean bias 2.2%, precision 5.91% | |
| Above the LLOQ | Mean bias within the range -2.9% to 2%, precision within the range 2.48% to 6.17% | |
| *DHA* | | |
| At the LLOQ | Mean bias 4.8%, precision 9.81% | |
| Above the LLOQ | Mean bias within the range -4.2% to 2%, precision within the range 4.48% to 7.09% | |
| **Results-3:** | | |
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the C standards on each day. The deviation (bias) from the nominal value is used to evaluate the accuracy in this validation report. | |
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) from the nominal values | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean bias and precision of all individual concentrations of QC samples analyzed during a single validation day. | |
| *Inter-day (inter batch) accuracy and precision* | The inter-day accuracy and precision were calculated as the mean bias and precision of all individual concentrations of QC samples analyzed during two toxicokinetic studies (CP045499 and CP045500). | |
| *Intra-day accuracy and precision for Arm* | At the LLOQ: Bias -5.2%, precision 8.38% | |
| | Above the LLOQ: Bias within the range -7% to 0.88%, precision within the range of 3.53% and 4.47% | |
| | Both were within the acceptance criteria over the entire range | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in dog plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0400692 |
|---|---|---|
| *Inter-day accuracy and precision for Arm* | At the LLOQ: Bias -2%, precision 8.47% | |
| | Above the LLOQ: Bias within the range -8.25% to -6.88%, precision within the range of 4.96% and 12.53% | |
| | Both were within the acceptance criteria over the entire range. | |
| *Intra-day accuracy and precision for DHA* | At the LLOQ: Bias 0.8%, precision 7.56% | |
| | Above the LLOQ: Bias within the range -3.3% to 3.75%, precision within the range of 3.91% and 7.48% | |
| | Both were within the acceptance criteria over the entire range. | |
| *Inter-day accuracy and precision for DHA* | At the LLOQ: bias 5.2%, precision 10.59% | |
| | Above the LLOQ: bias within the range 1.88% to 2.88%, precision within the range of 8.16% and 12.84% | |
| | Both were within the acceptance criteria over the entire range. | |
| LLOQ | 5 ng/mL, expressed as base, using 250 µL of dog plasma | |
| Dilution | Results of dilutions 1/10 and 1/50 with blank dog plasma were within the acceptance criteria. | |
| Matrix effect | The impact on the matrix effect on LC-MS/MS method was assessed by analyzing 6 different batches of dog plasma (n=6 for each animal) spiked before extraction at the lowest concentration level (LLOQ) and at the concentration used for the internal standard. These samples were analyzed with C standards and QC samples prepared from different batch of dog plasma. | |
| *Arm* | Mean bias between -10% and -0.8% | |
| | Precision between 2.86% and 9.12% | |
| *DHA* | Mean bias between 5% and 16% | |
| | Precision between 4.7% and 16.25% | |
| **Results-4:** | | |
| Absolute recovery | The extraction recovery was determined at the three concentrations: the low, mid and high validation QC levels. The recovery of the internal standard was determined at the concentration at which it was used in this assay. | |
| *Arm* | Mean recovery 59.14% (range 48.87% to 69.8%) | |
| *DHA* | Mean recovery 98.35% (range 95.8% to 102.47%) | |
| *Arteether* | Recovery 80.5% | |
| *Artemisinin* | Recovery 85.03% | |
| Stability | The stability of Arm and DHA has been investigated by analyzing quality control dog plasma samples and reconstituted samples stored under varying conditions | |
| | The stability of Arm and DHA in reconstituted extracts and quality control in dog plasma samples which were stored under varying conditions was investigated in 6 replicates together with freshly prepared C standards | |

| **Study title:** | Quantitative determination of artemether and its metabolite dihydroartemisinin in dog plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0400692 |
|---|---|---|
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) from QC plasma samples and reconstituted samples | |
| | Mean difference ±5% for stock or diluted solutions when tested in LC-MS/MS | |
| *Stability in stock solutions* | | |
| Arm | 3 months and 4 days in a fridge +5°C ± 5°C at and for at least 6 hours at room temperature | |
| DHA | 15 days in a fridge +5°C ± 5°C and for at least 6 hours at room temperature | |
| Arteether | 2 months and 7 days in ethanol at +5°C ± 5°C at approximately 500 ng/µL | |
| Artemisinin | 2 months and 7 days in ethanol at +5°C ± 5°C at approximately 500 ng/µL | |
| *Stability in working solutions* | | |
| Arm | For at least 14 days in a fridge +5°C ± 5°C | |
| DHA | For at least 14 days in a fridge +5°C ± 5°C | |
| *Stability in extracts* | At room temperature for 30 and 78.5 hours | |
| **Results-5:** | | |
| Stability | | |
| *Stability in QC samples* | Aliquots of QC samples were stored at the intended storage temperature and analyzed after thawing with freshly prepared C standards. The results were compared to the mean of the concentrations measured at the first determination of stability testing | |
| | No apparent loss of dog plasma after 20 weeks of storage at -75°C ± 10°C | |
| | For DHA, a loss of -18.33% appeared after 20 weeks of storage at -75°C ± 10°C in dog plasma at the 160 ng/mL concentration level. As no apparent loss appeared under the same conditions at the 10 ng/mL concentration level, no conclusion can be drawn on the stability of DHA in dog plasma stored at -75°C ± 10°C after 20 weeks of storage | |
| | Aliquots of QC samples were left 4.5 hours at room temperature. No apparent loss of after 4.5 hours at room temperature | |
| | No apparent after 3 or 5 freeze-thaw cycles, storage at -75°C ± 10°C | |
| Special issues: | Stability in extracts was determined at two concentrations, low and high (n=6), by storing the extracts in the autosampler for 78.5 hours instead of 72 hours as initially planned in the analytical study plan. | |
| | Stability in dog plasma in freezer -75°C ±10°C was determined after 20 weeks (more than 4 months) instead of 3 months as initially planned in the analytical study plan. The 1-month assessment was performed but not presented in this report because the analytical conditions were erroneous. | |
| Conclusion: | It is concluded from these results that the present LC-MS/MS method for the determination of Arm and DHA is validated for the assay of dog plasma and therefore suitable for the analysis of samples from toxicokinetic studies. | |

## 2.6.5.2D Pharmacokinetics: Analytical methods and validation reports (DMPK R0500059)

| **Study title:** | Quantitative determination of artemether and dihydroartemisinin in rat plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0500059 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-4 | |
| Test article: | Arm and DHA | |
| Method: | The method consists of liquid-liquid extraction with 1-chlorobutane-isooctane (55:45, v:v) followed by reverse phase liquid chromatography with tandem mass spectrometric detection. The objective of this analytical study was to develop and to validate a sensitive method for the quantitative determination of Arm and DHA in rat plasma. | |
| Instruments: | | |
| LC System | Pump: Series 1100 binary pump equipped with a series 1100 degasser, Agilent | |
| | Autosampler: Series 200, Perkin Elmer | |
| MS System | API3000 with an APCI (heated nebuliser), Applied Biosystems | |
| Chromatography: | | |
| Autosampler temperature | Room temperature | |
| Column | Alltima $C_{18}$, 3 µm (100 x 4.6 mm), Alltech | |
| Column temperature | Room temperature | |
| Mobile phase | A: 0.1% acetic acid solution | |
| | B: Acetonitrile | |
| Needle wash | Methanol | |
| Injection volume | 25 µL | |
| Injector wash cycles | Pre-flushes: 3 | |
| | Post-flushes: 3 | |
| | Volume of flush: 400 µL | |
| Flow rate | 1.2 mL/min | |
| Retention times | Arm: 5.4 min | |
| | DHA: 3.6 min | |
| | Arteether (internal standard of Arm): 5.8 min | |
| | Artemisinin (internal standard for DHA): 4.4 min | |
| Total run time | 9 min | |

| **Study title:** | Quantitative determination of artemether and dihydroartemisinin in rat plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0500059 |
|---|---|---|
| Substance analyzed: | Arm and DHA | |
| Reference compounds: | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$ | |
| | Molecular weight (MW): 298.37 g/mol | |
| DHA | Molecular formula: $C_{15}H_{24}O_5$ | |
| | Molecular weight (MW): 284.35 g/mol | |
| Internal standards: | | |
| Arteether | Molecular formula: $C_{17}H_{28}O_5$ | |
| | Molecular weight (MW): 312.4 g/mol | |
| Artemisinin | Molecular formula: $C_{15}H_{22}O_5$ | |
| | Molecular weight (MW): 282.33 g/mol | |
| Sample preparation: | Liquid-liquid extraction with 1-chlorobutane-isooctane (55:45, v:v) followed by reverse phase liquid chromatography with tandem mass spectrometric detection | |
| C standard and QC samples preparation: | | |
| C Standards | 7 concentrations in the range of 20 ng/mL (LLOQ) to 200 ng/mL | |
| QC Samples | 4 concentrations in the range of 20 ng/mL (LLOQ) to 160 ng/mL, and 1 concentration (2500 ng/mL) used to test the dilution procedure | |
| Matrix: | Wistar rat plasma (sodium heparin) | |
| Detection: | MS/MS APCI positive ion mode | |
| MS conditions | | |
| *Period 1* | Duration 5 min | |
| | Polarity: Positive | |
| | Corona discharge: 5 µA or 3 µA | |
| | Interface temperature: Set at 450°C | |
| *Period 2* | Duration 4 min | |
| | Polarity: Positive | |
| | Corona discharge: 3 µA | |
| | Interface temperature: Set at 450°C | |

| **Study title:** | Quantitative determination of artemether and dihydroartemisinin in rat plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0500059 |
|---|---|---|
| *DHA transition* | DHA transition : m/z : 267.1 → 163.1 | |
| | Resolution Q1 : Unit Q3 : Unit | |
| | Dwell time: 250 ms | |
| *Artemisinin transition* | Artemisinin transition : m/z : 283.1 → 209.1 | |
| | Resolution Q1 : Unit Q3 : Unit | |
| | Dwell time: 250 ms | |
| *Arm and arteether transitions* | Arm and arteether transition: m/z: 267.1 → 163.1 | |
| | Resolution Q1: Unit Q3: Unit | |
| | Dwell time: 400 ms | |
| *Software for data acquisition and peak integration* | Analyst® | |
| **Results-1:** | | |
| Specificity | The specificity of the method was investigated by preparing and analyzing blank samples prepared from 6 different individuals (n=2 for each source of matrix). The specificity was assessed by comparing the apparent signal of Arm and DHA, arteether and artemisinin in blank samples to the mean signal obtained for C standards spiked with a concentration of Arm and DHA at the LLOQ and with arteether and artemisinin at the working concentration. | |
| | The specificity of the method for each analyte against the other analyte in the assay was evaluated. Each analyte was added to a blank matrix at the validation QC level without addition of other analyte and internal standards (n=6). | |
| *Acceptance criteria* | **Arm and DHA:** Interference ≤ 20% of signal at LLOQ | |
| | **Arteether and artemisinin:** Interference ≤ 5% of signal at working concentration | |
| *Interference from the rat plasma (matrix)* | No peaks in the blank extracts at the retention time of the analyte or the internal standards interfered with the analytes by more that 20% of the mean of the LLOQ C standard peak signals and by more than 5% of the mean internal standard peak signal. | |
| Calibration curves | Calibration standards were prepared in duplicate and included the target lower limit of quantification, LLOQ (20.0 ng/mL) and the target upper limit of quantification, ULOQ (200 ng/mL). One set of calibration standards was analyzed at the start of the analytical run and another at the end. Concentrations were obtained by least square weighted linear regression using 1/x weighting factor. | |
| *Acceptance criteria* | Deviation ±15% (±20% at the LLOQ) from nominal values for 70% of each series and 6 calibration concentrations included in the final calibration line. | |
| *Arm* | | |
| At the LLOQ | Bias 3.5%, precision 7.1% | |
| Above the LLOQ | Bias within the range -3.88% to 1.33%, precision within the range 5.42% to 7.81% | |
| *DHA* | | |

| **Study title:** | Quantitative determination of artemether and dihydroartemisinin in rat plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0500059 |
|---|---|---|
| At the LLOQ | Bias 3.5%, precision 8.36% | |
| Above the LLOQ | Bias within the range -2.63% to 1.33%, precision within the range 4.82% to 7.02% | |
| **Results-2:** | | |
| Accuracy, precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the C standards on each day. The deviation (bias) from the nominal value is used to evaluate the accuracy in this validation report. | |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean bias and precision of all individual concentrations of QC samples analyzed during a single validation day. | |
| *Inter-day (inter- batch) accuracy and precision* | The inter-day accuracy and precision were calculated as the mean bias and precision of all individual concentrations of QC samples analyzed during the 3 validation days. | |
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) from the nominal values | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |
| *Intra-day accuracy and precision for Arm* | At the LLOQ: Bias -7.5%, precision 6.54% | |
| | Above the LLOQ: Bias within the range -13.8% to -9.38%, precision within the range of 5.15% to 6.34% | |
| | Both were within the acceptance criteria over the entire range. | |
| *Inter-day accuracy and precision for Arm* | At the LLOQ: Bias -9%, precision 7.8% | |
| | Above the LLOQ: Bias within the range -12.4% to -8%, precision within the range of 4.95% and 8.31% | |
| | Both were within the acceptance criteria over the entire range | |
| *Intra-day accuracy and precision for DHA* | At the LLOQ: Bias 11.5%, precision 7.8% | |
| | Above the LLOQ: Bias within the range 2.6% to 4.75%, precision within the range of 2.04% and 4.64% | |
| | Both were within the acceptance criteria over the entire range. | |
| *Inter-day accuracy and precision for DHA* | At the LLOQ: Bias -9.5%, precision 11.1% | |
| | Above the LLOQ: Bias within the range -5.8% to -1.5%, precision within the range of 10.38% and 11.17% | |
| | Both were within the acceptance criteria over the entire range. | |
| LLOQ | 20 ng/mL expressed as base using 100 µL of rat plasma | |
| Dilution | Results of dilutions 1/100 with blank rat plasma were within the acceptance criteria. | |
| **Results-3:** | | |
| Matrix effect | The impact on the matrix effect on LC-MS/MS method was assessed by analyzing 6 different batches of matrix (n=6 for each individual) spiked before extraction at the lowest concentration level (LLOQ) and at the concentration used for the internal standards. These samples were analyzed with C standards and QC samples prepared from different batch of matrix. | |

| **Study title:** | Quantitative determination of artemether and dihydroartemisinin in rat plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0500059 |
|---|---|---|
| *Arm* | Mean bias between 1% and 18.5% | |
| | Precision between 3.47% and 8.69% | |
| *DHA* | Mean bias between -6.5% and 5.5% | |
| | Precision between 4.82% and 10.15% | |
| Absolute recovery | The extraction recoveries of Arm and DHA were determined at the 3 concentrations: the low, mid and high validation QC levels. The extraction recoveries of the internal standards were determined at the concentration at which it was used in this assay. Six samples prepared at each concentration of analyte (recovery samples) were extracted along with 18 blank matrix samples containing neither analytes nor internal standards (reference samples). After extraction, the blank matrix samples were spiked with the analytes at the low, mid and high concentrations (n=6 for each concentration level) and spiked with internal standards. The extraction recovery was calculated by comparing the peak signals obtained from the recovery samples with the peak signals obtained from the reference samples. | |
| *Arm* | Mean recovery 80.58% (range 74.35% to 83.85%) | |
| *DHA* | Mean recovery 79.35% (range 75.32% to 84.75%) | |
| *Arteether* | Recovery 73.48% | |
| *Artemisinin* | Recovery 86.44% | |
| **Results-4:** | | |
| Stability | The stability of Arm stock solutions was checked in previous studies after 3 months and 4 days in a fridge at +5°C ± 5°C and after at least 6 hours of storage at room temperature. | |
| | The stability of DHA stock solutions was checked in previous studies after 15 days in a fridge at +5°C ± 5°C and after at least 6 hours of storage at room temperature. | |
| | The stability of Arm and DHA working solutions was checked in previous studies after 14 days in a fridge at +5°C ± 5°C. | |
| | The stability of arteether and artemisinin stock solutions was checked in previous studies after 2 months and 7 days in a fridge at +5°C ± 5°C. | |
| | During this analytical study, the stability of arteether and artemisinin in working solutions was checked by injection in the LC-MS/MS system. | |
| | The stability of Arm and DHA in reconstituted extracts and quality control in matrix samples, which were stored under varying conditions, was investigated together with freshly prepared C standards. | |
| *Acceptance criteria* | The percentage of difference calculated between the fresh and the old working solution should not deviate more that 5%. | |
| *Stability in stock solutions* | | |
| Arm | No apparent loss after 3 months and 4 days of a storage in a fridge at +5°C ± 5°C and no apparent loss after 6 hours at room temperature for Arm | |

| **Study title:** | Quantitative determination of artemether and dihydroartemisinin in rat plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0500059 |
|---|---|---|
| DHA | No apparent loss after 15 days of a storage in a fridge at +5°C ± 5°C and no apparent loss after 6 hours at room temperature for DHA | |
| Arteether and artemisinin | No apparent loss after 2 months and 7 days of a storage in a fridge at +5°C ± 5°C for arteether and artemisinin | |
| **Results-5:** | | |
| Stability | | |
| *Stability in working solutions* | | |
| Arm and DHA | No apparent loss after 14 days of a storage in a fridge at +5°C ± 5°C for Arm and DHA (in mixture) | |
| arteether and artemisinin | No apparent loss after 9 days of a storage in a fridge at +5°C ± 5°C for arteether and artemisinin (in mixture) | |
| *Stability in rat plasma* | | |
| *Stability in extracts* | No apparent loss after 48 hours of a storage at room temperature in the autosampler for both compounds | |
| *Stability in QC samples* | No apparent loss after 3 freeze-thaw cycles at -75°C ± 10°C (thawing at room temperature) for Arm. | |
| | Apparent loss after 2 freeze-thaw cycles at -75°C ± 10°C (thawing at room temperature) for DHA. | |
| | No apparent loss after 3 freeze-thaw cycles at -75°C ± 10°C (thawing on ice) for both compounds. | |
| | No apparent loss after at least 24 hours of storage at room temperature and after at least 4 hours of storage in an ice bath for Arm. | |
| | No apparent loss after at least 4 hours of storage in an ice bath for DHA and apparent loss after at least 4 hours at room temperature for DHA. | |
| | No apparent loss after 22 days of storage at -24°C ± 6°C for Arm and apparent loss after 22 days of storage -24°C ± 6°C for DHA. | |
| | No apparent loss after 5 months at -75°C ± 10°C. | |
| Conclusion: | The method is suitable for the routine analysis of Arm and DHA in rat plasma with an anticipated limit of quantification of 20 ng/mL using a sample volume of 100 µL. | |
| | The method is specific for Arm and DHA within the given criteria for acceptance (apparent peak area for Arm and DHA in zero sample ≤ 20% of mean peak area at LLOQ) in rat plasma. | |
| | It is concluded from these results that the present LC-MS/MS method for the determination of Arm and DHA in rat plasma is validated except for juvenile rat plasma, and therefore suitable for the analysis of samples from pre-clinical studies. | |

| **Study title:** | Quantitative determination of artemether and dihydroartemisinin in rat plasma by LC-MS/MS. Method description and validation. | Study no. DMPK R0500059 |
|---|---|---|
| Special issues: | As DHA was not stable after storage for 4 hours at room temperature and after 3 freeze-thaw cycles at -75°C ± 10°C (thawing at room temperature), it was decided to perform the stability after 4 hours storage in an ice bath, after 2 freeze-thaw cycles at -75°C ± 10°C (thawing at room temperature), and after 3 freeze-thaw cycles at -75°C ± 10°C (thawing in an ice bath).<br>As during a pre-clinical study, rat plasma samples were stored at approximately -20°C for 15 days, the stability of Arm and DHA was checked after 22 days of storage at -24°C ± 6°C.<br>During the analysis of pre-clinical samples, a problem was met with the samples collected from very juvenile rats (7 days). Indeed, for these samples, a tremendous decrease in the chromatographic response was observed for the 2 internal standards. In agreement with the study monitor, blank rat plasma collected from juvenile rats was tested and the problem observed with the pre-clinical samples was not encountered.<br>The analytical batch number 13 was accepted for Arm although the value for $r^2$ (coefficient of determination) was lower than 0.98 ($r^2$=0.978). | |
| Additional assay using juvenile rats plasma: | Accuracy and Precision:<br>Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples prepared either with blank juvenile rat plasma (aged 7 days) together with the calibration standards and validation QC samples. The deviation (bias) from the nominal value is used to evaluate the accuracy.<br>Acceptance criteria:<br>Mean bias ±15% from the nominal values, precision: ≤ 15%<br>Arm Results:<br>In blank juvenile rat plasma: Bias within the range -12% to -6.88%, precision within the range of 3.93% to 5.77%<br>In blank rat plasma: Bias within the range -11.25% to -9.6%, precision within the range of 3.78% to 5.42%<br>DHA Results:<br>In blank juvenile rat plasma: Bias within the range -3.13% to -1.00%, precision within the range of 2.63% to 3.9%<br>In blank rat plasma: Bias within the range -10.63% to -4.8%, precision within the range of 3.03% to 4.23% | |
| Additional Information | Assessment of Arm and DHA stability of stock solutions refers to the method described in Study BAPK (EU) R0301212.<br>Assessment of arteether and artemisinin stability of stock solutions refer to the method described in Study DMPK R0400692. | |

## 2.6.5.2E Pharmacokinetics: Analytical methods and validation reports (BAPK(F) R00-1840)

| **Study title:** | Quantitative determination of artemether and its dihydroartemisinin metabolite in human plasma by liquid chromatography-mass spectrometry method. Method description and validation. | Study no. BAPK(F) R00-1840 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-5 | |
| Test article: | Arm and DHA | |
| Method: | The method consists of liquid-liquid extraction with subsequent evaporation of the supernatant to dryness followed by the analysis of the reconstituted sample by liquid chromatography/ mass spectrometry (HPLC/MS) in a single ion monitoring mode using atmospheric pressure chemical ionization (APCI) as an interface. The method was fully validated on ion 267 and within-day validation was also performed on ion 221. The objective of this study was to develop to validate a sensitive method for the quantitative determination of Arm and its metabolite DHA in human plasma. | |
| Instruments: | | |
| Pump | Hewlett Packard (Agilent), model 1100 | |
| Autosampler | Hewlett Packard (Agilent), model 1050 | |
| Column oven | Hewlett Packard (Agilent), model 1100 | |
| Detector | Thermo-Finnigan, TSQ, API II | |
| Data acquisition | Xcalibur | |
| Chromatography: | Reverse HPLC on an Alltima $C_{18}$, 5 µm (150 x 4.6 mm) at room temperature using isocratic elution (acetonitrile-acetic acid 0.1%, 66:34, v:v) at a flow rate of 1 mL/min for 14 min followed by a flush for 15 min with 100% acetonitrile | |
| Pre-column | Alltima $C_{18}$, 5 µm (7.5 x 4.6 mm), Alltech | |
| Column | Alltima $C_{18}$, 5 µm (150 x 4.6 mm), Alltech | |
| Column temperature | Room temperature | |
| Mobile phase | Acetonitrile/glacial acetic acid 0.1% (66:34, v:v) | |
| Flow rate | 1 mL/min | |
| Flush | 100% acetonitrile for 15 min at a flow rated of 2 mL/min. The duration of this step is given in a rough guide and may change with instrument | |
| Injection volume | 50 µL | |
| Injector wash | Mobile phase | |
| Column pressure | Around 60 bars | |

| **Study title:** | Quantitative determination of artemether and its dihydroartemisinin metabolite in human plasma by liquid chromatography-mass spectrometry method. Method description and validation. | Study no. BAPK(F) R00-1840 |
|---|---|---|
| Retention time(s) | Arm: 10.3 − 11.1 min | |
| | DHA: 3.5 − 3.7 min | |
| | Artemisinin (internal standard): 5 − 5.5 min | |
| Total run time | 14 minutes followed by 15 min flush step | |
| Substance analyzed: | Arm and DHA | |
| Reference compounds: | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$ | |
| | Molecular weight (MW): 298.38 g/mol | |
| DHA | Molecular formula: $C_{15}H_{24}O_5$ | |
| | Molecular weight (MW): 284.35 g/mol | |
| Internal standards: | | |
| Artemisinin | Molecular formula: $C_{15}H_{22}O_5$ | |
| | Molecular weight (MW): 282.34 g/mol | |
| Sample preparation: | Liquid-liquid extraction with 1-chlorobutane-isooctane (55:45, v:v) | |
| C standard and QC samples preparation: | | |
| C Standards | 6 concentrations in the range of 5 ng/mL to 200 ng/mL | |
| QC Samples | 4 concentrations in the range of 5 ng/mL to 160 ng/mL | |
| Matrix: | Human plasma | |
| Detection: | APCI | |
| Source | APCI | |
| Ionization | Sheath gas $N_2$ | |
| | Pressure 85 PSI | |
| | Capillary temperature 300°C | |
| | Vaporizer temperature 400°C | |
| | Corona discharge 5 µA | |
| | Auxiliary gas flow 15 units | |

| **Study title:** | Quantitative determination of artemether and its dihydroartemisinin metabolite in human plasma by liquid chromatography-mass spectrometry method. Method description and validation. | Study no. BAPK(F) R00-1840 |
|---|---|---|
| MS | Manifold temperature 70°C | |
| | Single ion monitoring | |
| | Positive ion mode | |
| | Dynode at 15 kV | |
| | Electron multiplier at 1400 V | |
| | Mass resolution 0.7 amu | |
| Scan time | 1 s for the 3 compounds | |
| Selected masses for Arm | m/z 267.1 or 220.9 | |
| Selected masses for DHA | m/z 267.1 or 220.9 | |
| Selected masses for artemisinin | m/z 282.7 | |
| Data acquisition | Xcalibur | |
| **Results-1:** | | |
| Specificity | The specificity of the method was investigated by preparing and analyzing blank samples prepared from 6 different batches of human plasma. The specificity was assessed by comparing the apparent signal of Arm, DHA and artemisinin in blank samples to the signal obtained for samples spiked with a concentration of Arm and DHA at the LLOQ and with artemisinin at the working concentration. | |
| *Acceptance criteria* | Analytes: Individual interference ≤ 20% of signal at LLOQ | |
| | Internal standards: Individual interference ≤ 5% of signal at working concentration | |
| *Interference from the human plasma (matrix)* | | |
| Ion 267 | Mean apparent peak area for Arm in blank samples from 6 batches of human plasma was within the ranges of 0.7% and 10% of the peak area of Arm in the same batch at the LLOQ | |
| | Mean apparent peak area for DHA in blank samples from 6 batches of human plasma was within the ranges of 2% and 13% of the peak area of DHA in the same batch at the LLOQ | |
| | Mean apparent peak area for artemisinin in blank samples from 6 batches of human plasma was within the ranges of 0.1% and 1% of the peak area of artemisinin in the same batch at the working concentration | |

| **Study title:** | Quantitative determination of artemether and its dihydroartemisinin metabolite in human plasma by liquid chromatography-mass spectrometry method. Method description and validation. | Study no. BAPK(F) R00-1840 |
|---|---|---|

**Results-2:**

| | |
|---|---|
| Ion 221 | Mean apparent peak area for Arm in blank samples from 6 subjects in study CCOA566A2301 was within the ranges of 0.5% and 9% of the peak area of Arm at the LLOQ in a pool of 3 batches of plasma |
| | Mean apparent peak area for DHA in blank samples from 6 subjects in study CCOA566A2301 was within the ranges of 1.5% and 12% of the peak area of DHA at the LLOQ in a pool of 3 batches of plasma |
| | Mean apparent peak area for artemisinin in blank samples from 6 subjects in study CCOA566A2301 was within the ranges of 0% and 0.2% of the peak area of artemisinin at the working concentration in a pool of 3 batches of plasma |
| Tautomeric specificity | The α and β tautomers of DHA were well separated. During the chromatography, the ratio α versus β remained constant at around 3. Only the tautomer α was considered for quantification. |
| Calibration curves | Calibration was performed with a first order polynominal within the range of 5 ng/mL to 200 ng/mL for Arm and DHA using $1/x^2$ weighting. The acceptance criteria for the coefficient of correlation and the mean accuracy were all met. |
| *The acceptance criteria* | Coefficient of correlation, $r \geq 0.95$ |
| | Bias ≤ 15% (20% at the LLOQ) for 2/3 of the C standards |
| *Arm* | coefficients of correlation, $r \geq 0.995$ |
| | 97% ≤ mean of accuracy ≤ 105%, precision ≤ 10% |
| *DHA* | 99% ≤ mean of accuracy ≤ 105%, precision ≤ 10% |

**Results-3:**

| | |
|---|---|
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples that were analyzed together with the C standards on each day. The accuracy and precision of the method was also assessed using ion 221 by analyzing intra-day accuracies and precision. |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean and precision of all individual accuracies of QC samples analyzed during a single validation day. |
| *Inter-day (inter batch) accuracy and precision* | The inter-day accuracy and precision were calculated as the mean accuracy and precision of all individual accuracies of QC samples analyzed during 3 validation days. |
| Acceptance criteria | Mean accuracy 85% to 115% (80% to 120% at LLOQ) |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) |
| *Intra-day accuracy and precision for Arm* | At the LLOQ: Mean accuracy 90% to 105%, precision 3% to 15% |
| | Above the LLOQ: Mean accuracy 88% to 98%, precision 2% to 11% |
| *Intra-day accuracy and precision for DHA* | At the LLOQ: Mean accuracy 97% to 104%, precision 6% to 14% |
| | Above the LLOQ: Mean accuracy 90% to 104%, precision 3% to 9% |

| **Study title:** | Quantitative determination of artemether and its dihydroartemisinin metabolite in human plasma by liquid chromatography-mass spectrometry method. Method description and validation. | Study no. BAPK(F) R00-1840 |
|---|---|---|
| *Inter-day accuracy and precision for Arm* | At the LLOQ: Mean accuracy 97%, precision 13% | |
| | Above the LLOQ: Mean accuracy 91% to 96%, precision 5% to 9% | |
| *Inter-day accuracy and precision for DHA* | At the LLOQ: Mean accuracy 100%, precision 11% | |
| | Above the LLOQ: Mean accuracy 93% to 96%, precision 5% to 9% | |
| **Results-4:** | | |
| LLOQ | 5 ng/mL by using a sample volume of 500 µL for both compounds | |
| Absolute recovery | The absolute recovery was assessed by comparison of the responses obtained from the analysis of reference solutions spiked in the extract of a blank sample and of extracted human plasma samples. It is given as the ratio of the average peak areas obtained from a reference solution relative to the average peak areas obtained from the analysis of extracted plasma samples. | |
| *Arm* | Mean recovery 76% (range 72% to 80%) | |
| | The recovery of Arm does not depend on its concentration. | |
| *DHA* | Mean recovery 84% (range 67% to 109%) | |
| | The recovery of DHA depends on its concentration. For concentrations near the lower limit of quantification, an average recovery of 109% was found. For concentration of 200 ng/mL, an average recovery of 71% was found. | |
| *Artemisinin* | Mean recovery 94% | |
| Stability | The stability of Arm was investigated by analyzing quality control of human samples and reconstituted extracts, which were stored under varying conditions, in triplicate together with freshly prepared C standards and QC samples. | |
| | Arm and DHA did not meet the acceptance criteria of stability after 6 hours at room temperature under daylight at 20 ng/mL concentration. | |
| *Acceptance criteria* | Mean accuracy 85% to 115% (80% to 120% at the LLOQ) | |
| *Stability in stock solutions* | | |
| Arm | No apparent loss after 6 hours at room temperature in darkness and 7 weeks between 2 − 8°C for Arm. | |
| DHA | No apparent loss after 6 hours at room temperature in darkness and 7 weeks between 2 − 8°C for DHA. | |
| Artemisinin | No apparent loss after 6 hours at room temperature in darkness and 6 weeks between 2 − 8°C for artemisinin. | |
| **Results-5:** | | |
| Stability | | |
| *Stability in QC samples* | No apparent change after 6 hours of storage under ambient conditions. | |
| *Stability in frozen QC samples* | No apparent loss after 33 weeks of storage at or below -70°C. | |
| *Stability in actual samples* | No apparent change after 34 weeks of storage at or below -70°C. | |

| | | |
|---|---|---|
| **Study title:** | Quantitative determination of artemether and its dihydroartemisinin metabolite in human plasma by liquid chromatography-mass spectrometry method. Method description and validation. | Study no. BAPK(F) R00-1840 |
| *Effect of freeze-thaw cycles* | No apparent loss after 3 freeze-thaw cycles (freeze temperature at or below at -70°C). The samples were thawed at room temperature. | |
| *Stability in extracts* | No apparent loss after about 40 hours of storage at room temperature for DHA and Arm at 100 ng/mL concentration. At 8 ng/mL concentration, no loss for DHA, but Arm does not meet the acceptance criteria of stability (accuracy 83%). Nevertheless, Arm is stable for at least 14 hours. | |
| Conclusion: | The method is suitable for the routine analysis of Arm and DHA with a limit of quantification of 5 ng/mL with a sample volume of 500 µL using either 267 or 221 ion. | |
| | The method is specific for Arm and DHA within the given criteria for acceptance in human plasma. | |
| | The ion 267 method was applied for the determination of Arm and DHA in an interaction study between Riamet® and quinine. The ion 221 method was applied for an interaction study between Riamet® and ketoconazole because an interference of an unclear origin in all the plasma samples of the tested subjects prevented from using the ion 267 method. The acceptance criteria were met for each analytical run performed. | |

Additional information: none

## 2.6.5.2F Pharmacokinetics: Analytical methods and validation reports (DMPK R0500720-01 (SSE 096-1))

| | | |
|---|---|---|
| **Study title:** | [$^{13}CD_3$] CGP56696 Synthesis and release analysis. | Study No:<br>DMPK R0500720-01<br>(SSE 096-1) |

GLP compliance: not required

Location in CTD: 4.2.2.1-6

Test article: [$^{13}CD_3$] Arm

Type of study: synthesis and analysis

Method: Synthesis: A solution of artemisinin in dichloromethane was treated with di-isobutyl aluminium hydride in tetrahydrofuran at -78°C for 2 hours to give, after work-up, DHA. Subsequent treatment of DHA with $^{3}CD_3OD$ and borontrifluoride etherate at room temperature for 24 hours yielded, after work-up and purification, [$^{13}CD_3$]Arm (CGP 56696).

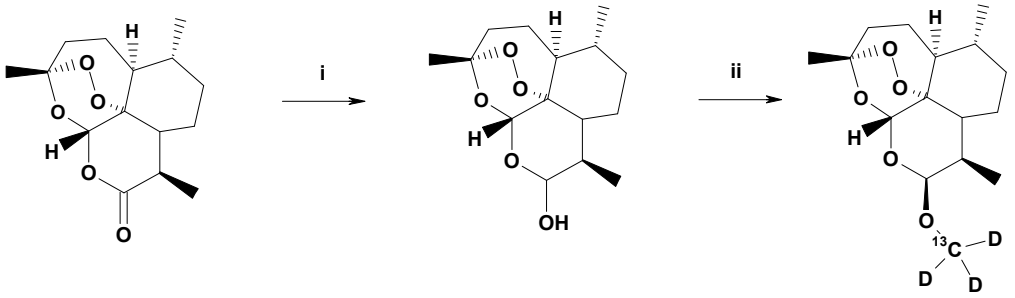
Analysis: The identity of the labeled compound was confirmed by mass spectrometry, nuclear magnetic resonance, and high-performance liquid chromatography.

Results: Analysis

| Test | Method | Requirements | Results | Remarks |
|---|---|---|---|---|
| Identity | HPLC | Elution time corresponds to comparison compound | Elution time corresponds | - |
| Identity | $^{1}$H-NMR spectrum | Corresponds to comparison compound | Corresponds to comparison compound | - |
| Identity | Mass spectrum | Corresponds to comparison compound | Corresponds to comparison compound | Except isotope shift |
| Chemical purity | HPLC-UV | ≥ 95% | 95.9% | - |
| Mass purity | MS | Must contain less than 1% of the molecular ion of the unlabeled comparison compound | < 1% | - |

| **Study title:** | [$^{13}CD_3$] CGP 56696 Synthesis and release analysis. | Study No: [DMPK R0500720-01 (SSE 096-1)] |
|---|---|---|

Results: Synthesis




**Z0** **Z1** **A0**

**Reaction conditions**: i) 1 M DIBALH in THF, $CH_2Cl_2$, -78°C, 2 hours. ii) $BF_3OEt_2$, $^{13}CD_3OD$, RT, 24 hours.

Additional information: none

## 2.6.5.2G Pharmacokinetics: Analytical methods and validation reports (DMPK R0300924A)

| **Study title:** | Quantitative determination of lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924A |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-7 | |
| Test article: | Lmf | |
| Method: | Based on previously described HPLC-UV method, the method was transferred to MS/MS detection and validated for the quantitative determination of Lmf in human plasma. The method consists of liquid-liquid extraction and the analysis of the extracts by high performance liquid chromatography–tandem mass spectrometry (HPLC – MS/MS) in selected reaction monitoring mode using an electrospray source as an interface. | |
| Instruments: | | |
| HPLC System | Pump: Agilent, series 1100 | |
| | Column oven: Agilent, series 1100 set at about 25°C | |
| | Autosampler: CTC Analytics HTS Pal set at about 6°C | |
| MS System | API3000, Applied Biosystems | |
| Chromatography: | | |
| Pre-column | Alltima C18, 5 µm particle size, 2.1 mm ID, from Alltech | |
| Column | Alltima C18, 5 µm particle size, 50 mm length, 2.1 mm ID, from Alltech | |
| Column temperature | 25°C | |
| Mobile phase | Isocratic elution: 96% A − 4% B | |
| | A: Methanol | |
| | B: pH 3.9 buffer (7.71 g ammonium acetate + 28 mL 100% acetic acid, adjusted at 1000 mL with water) | |
| Flow rate | 200 µL/min | |
| Injection volume | 10 µL | |
| Injector wash | Syringe: 4 x methanol-water (80:20, v:v), 4 x methanol-acetic acid (99:1, v:v) | |
| | Valve: 3 x methanol-water (80:20, v:v), 3 x methanol-acetic acid (99:1, v: v) | |
| Chromatography: | | |
| Column pressure | Around 25 bars | |
| Retention times | About 1.8 min (Lmf) and 1.7 min ([$D_9$] Arm/Lmf) | |
| Total run time | 7 min (including the time necessary for the injector wash) | |

| **Study title:** | Quantitative determination of lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924A |
|---|---|---|
| Substance analyzed: | Lmf | |
| Reference compound: | Lmf | |
| | Molecular formula: $C_{30}H_{32}Cl_3NO$ | |
| | Molecular weight (MW): 528.95 g/mol | |
| Internal standard: | Labeled Lmf (name: [$D_9$] Arm/Lmf) | |
| | Molecular formula: $C_{30}H_{23}Cl_3NOD_9$ | |
| | Molecular weight (MW): 538 g/mol | |
| Sample preparation: | Liquid-liquid extraction at pH 4 in hexane | |
| C standard and QC samples preparation: | | |
| C Standards | 7 concentrations in the range of 0.05 µg/mL (LLOQ) to 20 µg/mL | |
| QC Samples | 4 concentrations in the range of 0.05 µg/mL (LLOQ) to 16 µg/mL | |
| Matrix: | Human plasma | |
| Detection: | MS/MS TIS positive ion mode | |
| MS conditions | Source TIS (Turbo ion spray) at 450°C | |
| | MRM positive ion mode | |
| Scan time | 500 ms | |
| Selected masses Lmf | (Precursor) m/z ~ 530, (product) m/z ~ 512 | |
| Selected masses [$D_9$] Arm/Lmf | (Precursor) m/z ~ 539, (product) m/z ~ 521 | |
| Software for data acquisition and peak integration | Analyst | |
| **Results-1:** | | |
| Specificity | The specificity of the analytical method was investigated by preparing and analyzing blank samples prepared from 6 different batches of human plasma (anticoagulant: heparin). | |
| | The specificity was assessed for Lmf and for [$D_9$] Arm/Lmf by comparing the mean apparent signal obtained for the blank samples to the mean apparent signal obtained for samples spiked with a concentration of Lmf at LLOQ and [$D_9$] Arm/Lmf at the working concentration. | |
| *Acceptance criteria* | Lmf: Interference ≤ 20% of signal at LLOQ | |
| | [$D_9$] Arm/Lmf: Interference ≤ 5% of signal at working concentration | |
| *For human plasma* | The method is specific in human plasma for Lmf. | |
| | Mean apparent peak for Lmf in blank samples from 6 batches of human plasma was within the range of 0% to 0.3% of the peak area | |

| **Study title:** | Quantitative determination of lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924A |
|---|---|---|
| | for Lmf at LLOQ. | |
| | Mean apparent peak for [$D_9$] Arm/Lmf in blank samples from 6 batches of human plasma was within the range of 0% to 0.001% of the peak area for [$D_9$] Arm/Lmf at the working concentration. | |
| *With N-desbutylLmf, Arm, and dihydroartemesinin* | No peaks of N-desbutylLmf, Arm, and dihydroartemesinin were observed at the retention time of Lmf and [$D_9$] Arm/Lmf. | |
| Calibration curves | Calibration was performed with linear regression within range of 0.05 µg/mL to 20 µg/mL using $1/x^2$ weighting. | |
| *Acceptance criteria* | Deviation ±15% (± 20% at the LLOQ) for 3/4 of the C standards from nominal values | |
| | The acceptance criteria for the mean bias were met: -3.8% ≤ mean bias ≤ 2% | |
| *At the LLOQ* | Mean bias -0.6%, precision 3.7% | |
| *Above the LLOQ* | Mean bias within the range -3.8% and 2%, precision within the range of 1.1% and 8.7% | |
| **Results-2:** | | |
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the C standards on each day. The deviation (bias) from the nominal value is used to evaluate the accuracy in this validation report. | |
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) from the nominal values | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean bias and precision of all individual concentrations of QC samples analyzed during a single validation day. | |
| *Inter-day (inter-batch) accuracy and precision* | The inter-day accuracy and precision were calculated as the mean bias and precision of all individual concentrations of QC samples analyzed during 3 validation days. | |
| *Intra-day accuracy and precision* | At the LLOQ: Bias within the range -3.4% to 4.2%, precision within the range of 3.3% and 4.6% | |
| | Above the LLOQ: Bias within the range -11.6% to 10.5%, precision within the range of 1.3% and 4.2% | |
| | Both were within the acceptance criteria over the entire range. | |
| *Inter-day accuracy and precision* | At the LLOQ: Bias -0.8%, precision 5.3% | |
| | Above the LLOQ: Bias within the range -4.1% to 9%, precision within the range of 2.2% and 6.8% | |
| | Both were within the acceptance criteria over the entire range. | |
| LLOQ | 0.05 µg/mL using 250 µL of human plasma | |
| Dilution | Dilution with blank human plasma validated (factors 2 and 5) | |
| Matrix effect | The matrix effect is given as the ratio of the average peak areas obtained from analysis of extracted blank samples spiked after extraction with Lmf and [$D_9$] Arm/Lmf relative to the average peak areas obtained from reference solutions. | |

| **Study title:** | Quantitative determination of lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924A |
|---|---|---|
| *Lmf* | Mean recovery 98%, range 97% to 100% | |
| *[$D_9$] Arm/Lmf* | Mean recovery 93% | |
| **Results-3:** | | |
| Absolute recovery | The absolute recovery was given as the ratio of the average peak areas obtained from analysis of extracted spiked matrix samples relative to the average peak areas obtained from analysis of extracted blank samples spiked after extraction with reference solutions. | |
| *Lmf* | Mean recovery 53%, range 47% to 60% | |
| *[$D_9$] Arm/Lmf* | 68% | |
| Stability | The stability of Lmf in reconstituted extracts and in plasma samples used as quality control, which were stored under varying conditions, was investigated in triplicate together with the freshly prepared C standards. | |
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) | |
| *Stability in methanol: acetic acid (99.8:0.2 [v:v])* | Lmf: | |
| | At least 1 month at 0 − 8°C at 2 and 100 µg/mL and 6 hours at room temperature at 2 and 160 µg/mL. | |
| | [$D_9$] Arm/Lmf: | |
| | *In stock solutions (100 µg/mL):* At least 2 months at 0 − 8°C and 22 hours at room temperature. | |
| | *In working solutions (10 µg/mL):* At least 2 weeks at 0 − 8°C and 22 hours at room temperature. | |
| *Stability in plasma extracts* | Stable for at least 3 days at about 6°C on the autosampler | |
| *Stability in QC samples* | Stable for at least 9 months after storage below -70°C in polypropylene tubes. | |
| | Stable for at least 24 hours at room temperature and after 3 freeze-thaw cycles (storage below -70°C). | |
| *Stability in actual samples* | Stable for at least for 10 months after storage below -70°C | |
| Conclusion: | The method is suitable for the routine analysis of Lmf in human plasma with an anticipated limit of quantification of 0.05 µg/mL using a sample volume of 250 µL. | |
| | The method is specific for Lmf within the given criteria for acceptance in human plasma. | |
| | Care has to be taken to check and suppress carryover of Lmf due to the autosampler system. The washing conditions described in this report are suitable to suppress carryover down to a negligible level. | |

Additional information: none

## 2.6.5.2H Pharmacokinetics: Analytical methods and validation reports (DMPK R0300924B)

| **Study title:** | Quantitative determination of desbutyl lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924B |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-8 | |
| Test article: | N-desbutyl-Lmf | |
| Method: | N-desbutyl-Lmf is a metabolite of Lmf in human plasma. Based on previously described HPLC-UV method, the method was transferred to MS/MS detection and validated for the quantitative determination of N-desbutylLmf in human plasma. The method consists of liquid-liquid extraction and the analysis of the extract by high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) in selected reaction monitoring mode using an electrospray source (TIS) as interface. | |
| Instruments: | | |
| HPLC System | Pump: Agilent, series 1100 | |
| | Column oven: Agilent, series 1100 set at about 25°C | |
| | Autosampler: CTC Analytics HTS Pal set at about 6°C | |
| MS System | API3000, Applied Biosystems | |
| Chromatography: | | |
| Pre-column | Alltima C18, 5 µm particle size, 2.1 mm ID, from Alltech | |
| Column | Alltima C18, 5 µm particle size, 50 mm length, 2.1 mm ID, from Alltech | |
| Column temperature | 25°C | |
| Mobile phase | Isocratic elution: 96% A − 4% B | |
| | A: Methanol | |
| | B: pH 3.9 buffer (7.71 g ammonium acetate + 28 mL 100% acetic acid, adjusted at 1000 mL with water) | |
| Flow rate | 200 µL/min | |
| Injection volume | 10 µL | |
| Injector wash | Syringe: 4 x methanol-water (80:20, v:v), 4 x methanol-acetic acid (99:1, v:v) | |
| | Valve: 3 x methanol-water (80:20, v:v), 3 x methanol-acetic acid (99:1, v:v) | |
| Column pressure | Around 25 bars | |
| Retention times | About 1.4 min (N-desbutyl-Lmf) and 1.3 min (halofantrine) | |
| Total run time | 7 min (including the time necessary for the injector wash) | |
| Substance analyzed: | N-desbutyl-Lmf | |

| **Study title:** | Quantitative determination of desbutyl lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924B |
|---|---|---|
| Reference compound: | N-desbutyl-Lmf | |
| | Molecular formula: $C_{26}H_{24}Cl_3NO$ | |
| | Molecular weight (MW): 472.84 g/mol | |
| Internal standard: | Halofantrine | |
| | Molecular formula: $C_{26}H_{30}NOF_3Cl_2$ | |
| | Molecular weight (MW): 500.43 g/mol | |
| Sample preparation: | Liquid-liquid extraction at pH 7 in hexane | |
| C standard and QC samples preparation: | | |
| C Standards | 7 concentrations in the range of 0.005 µg/mL (LLOQ) to 1 µg/mL | |
| QC Samples | 4 concentrations in the range of 0.005 µg/mL (LLOQ) to 0.8 µg/mL | |
| Matrix: | Human plasma | |
| Detection: | MS/MS TIS positive ion mode | |
| MS conditions | Source TIS (Turbo iron spray) at 450°C | |
| | MRM positive ion mode | |
| Scan time | 500 ms | |
| Selected masses N-desbutyl-Lmf | (Precursor) m/z ~ 472, (product) m/z ~ 456 | |
| Selected masses halofantrine | (Precursor) m/z ~ 502, (product) m/z ~ 142 | |
| Software for data acquisition and peak integration | Analyst | |
| **Results-1:** | | |
| Specificity | The specificity of the analytical method was investigated by preparing and analyzing blank samples prepared from 6 different batches of human plasma (anticoagulant: heparin). The specificity was assessed by comparing the mean apparent signal for N-desbutyl-Lmf and for halofantrine in blank samples to the mean signal obtained for samples spiked with concentration of N-desbutyl-Lmf at LLOQ and halofantrine at the working concentration. | |
| *Acceptance criteria* | N-desbutyl-Lmf: Interference ≤ 20% of signal at LLOQ | |
| | Halofantrine: Interference ≤ 5% of signal at working concentration | |
| *For human plasma* | The method is specific in human plasma for N-desbutyl-Lmf and for halofantrine. | |
| | Mean apparent peak area for N-desbutyl-Lmf in blank samples from 6 batches of human plasma was within the range of 0% to 1.3% of the peak area for N-desbutyl-Lmf at LLOQ. | |
| | No apparent peak area for halofantrine in blank samples was observed from 6 batches of human plasma. | |

| **Study title:** | Quantitative determination of desbutyl lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924B |
|---|---|---|
| *With Lmf, Arm, and DHA* | No peak of Lmf, Arm, and DHA were observed at the retention time of N-desbutyl-Lmf and halofantrine. | |
| Calibration curves | Calibration was performed with linear regression within range of 0.005 µg/mL to 1 µg/mL using $1/x^2$ weighting. | |
| *Acceptance criteria* | Deviation ±15% (±20% at the LLOQ) 3/4 of the C standards from nominal values | |
| | The acceptance criteria for the mean bias were met: -2.3% ≤ mean bias ≤ 1.7% | |
| *At the LLOQ* | Mean bias 0%, precision 9.1% | |
| **Results-2:** | | |
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the calibration standards on each day. The deviation (bias) from the nominal value is used to evaluate the accuracy in this validation report. | |
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) from the nominal values | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |
| *Intra-day accuracy and precision* | At the LLOQ: Bias within the range -19.2% to 14.0%, precision within the range of 4.3% and 10.5% | |
| | Above the LLOQ: Bias within the range -14.8% to 8.6%, precision within the range of 1.9% and 11.5% | |
| | Both were within the acceptance criteria over the entire range. | |
| *Inter-day accuracy and precision* | At the LLOQ: Bias 1.7%, precision 16.9% | |
| | Above the LLOQ: Bias within the range -3.5% to 3.2%, precision within the range of 7.6% and 10.6% | |
| | Both were within the acceptance criteria over the entire range. | |
| LLOQ | 0.005 µg/mL using 250 µL of human plasma | |
| Dilution | Dilution with blank human plasma validated (factor 2) | |
| Matrix effect | The matrix effect is given as the ratio of the average peak areas obtained from analysis of extracted blank matrix samples spiked after extraction with N-desbutyl-Lmf and halofantrine relative to the average peak areas obtained from reference solutions. | |
| N-desbutyl-Lmf | Mean recovery 101%, range 100% to 102% | |
| Halofantrine | Mean recovery 101% | |
| Absolute recovery | The absolute recovery was given as the ratio of the average peak areas obtained from analysis of extracted spiked matrix samples relative to the average peak areas obtained from analysis of extracted blank samples spiked after extraction with reference solutions. | |
| *N-desbutyl-Lmf* | Mean recovery 51.9%, range 50% to 53.3% | |
| *Halofantrine* | Mean recovery 90.2% | |
| **Results-3:** | | |
| Stability | The stability of N-desbutyl-Lmf and halofantrine in reconstituted extracts and in plasma samples used as quality control, which were stored under varying conditions, was investigated in triplicate together with the freshly prepared C standards. | |

| **Study title:** | Quantitative determination of desbutyl lumefantrine in human plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924B |
|---|---|---|
| *Acceptance criteria* | Mean bias ±15% | |
| *Stability in stock solutions* | N-desbutyl-Lmf: | |
| | At least 1 month at 0-8°C and 6 hours at room temperature at 0.2 µg/mL and 8 µg/mL | |
| | Halofantrine: | |
| | At least 1 month at 0-8°C and 6 hours at room temperature at 100 µg/mL | |
| *Stability in plasma extracts* | At least 3 days at about 6°C on the autosampler | |
| *Stability in QC samples* | Stable for at least for 4.5 months after storage below -70°C in polypropylene tubes | |
| | Stable for at least for 24 hours at room temperature and after 3 freeze-thaw cycles (storage below -70°C) | |
| Conclusion: | The method is suitable for the routine analysis of N-desbutyl-Lmf in human plasma with an anticipated limit of quantification of 0.005 µg/mL using a sample volume of 250 µL. | |
| | The method is specific for N-desbutyl-Lmf within the given criteria for acceptance in human plasma. | |
| | Care has to be taken to check and suppress carryover of N-desbutyl-Lmf due to the autosampler system. The washing conditions described in this report are suitable to suppress carryover down to a negligible level. | |

Additional information: none

## 2.6.5.2 I Pharmacokinetics: Analytical methods and validation reports (DMPK R0300924C)

| **Study title:** | Quantitative determination of lumefantrine in dog plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924C |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-9 | |
| Test article | Lmf | |
| Method: | The method consists of liquid-liquid extraction and the analysis of the extracts by high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) in selected reaction monitoring mode using an electrospray source (Turbo Ion Spray [TIS]) as an interface. The objective of this study was to develop and validate a sensitive method for the quantitative determination of Lmf in dog plasma. | |
| Instruments: | | |
| HPLC System | Pump: Agilent, series 1100 | |
| | Column oven: Agilent, series 1100 set at about 25°C | |
| | Autosampler: CTC Analytics HTS Pal set at about 5°C | |
| MS System | API3000, Applied Biosystems | |
| Chromatography: | | |
| Pre-column | Alltima C18, 5 µm particle size, 2.1 mm ID, from Alltech | |
| Column | Alltima C18, 5 µm particle size, 50 mm length, 2.1 mm ID, from Alltech | |
| Column temperature | 25°C | |
| Mobile phase | Isocratic elution: 96% A − 4% B | |
| | A: Methanol | |
| | B: pH 3.9 buffer (7.71 g ammonium acetate + 28 mL 100% acetic acid, adjusted at 1000 mL with water) | |
| Flow rate | 200 µL/min | |
| Injection volume | 10 µL | |
| Injector wash | Syringe: 4 x methanol-water (80:20, v:v), 4 x methanol-acetic acid (99:1, v:v) | |
| | Valve: 3 x methanol-water (80:20, v:v), 3 x methanol-acetic acid (99:1, v:v) | |
| Column pressure | Around 25 bars | |
| Retention times | About 1.8 min (Lmf) and 1.7 min ([$D_9$] Arm/Lmf) | |
| Total run time | 7 min (including the time necessary for the injector wash) | |
| Substance analyzed: | Lmf | |

| **Study title:** | Quantitative determination of lumefantrine in dog plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924C |
|---|---|---|
| Reference compound: | Lmf | |
| | Molecular formula: $C_{30}H_{32}Cl_3NO$ | |
| | Molecular weight (MW): 528.95 g/mol | |
| Internal standard: | Labeled Lmf (name: [$D_9$] Arm/Lmf) | |
| | Molecular formula: $C_{30}H_{23}Cl_3NOD_9$ | |
| | Molecular weight (MW): 538 g/mol | |
| Sample preparation: | Liquid-liquid extraction at pH 4 in hexane | |
| C standard and QC samples preparation: | | |
| C Standards | 7 concentrations in the range of 0.1 µg/mL (LLOQ) to 50 µg/mL | |
| QC Samples | 4 concentrations in the range of 0.1 µg/mL (LLOQ) to 40 µg/mL | |
| Matrix: | Dog plasma | |
| Detection: | MS/MS TIS positive ion mode | |
| MS conditions | Source TIS at 450°C | |
| | MRM positive ion mode | |
| Scan time | 500 ms | |
| Selected masses Lmf | (Precursor) m/z ~ 530, (product) m/z ~ 512 | |
| Selected masses [$D_9$] Arm/Lmf | (Precursor) m/z ~ 539, (product) m/z ~ 521 | |
| Software for data acquisition and peak integration | Analyst | |
| **Results-1:** | | |
| Specificity | The specificity of the analytical method was investigated by preparing and analyzing blank samples prepared from 6 different batches of dog plasma (anticoagulant: heparin). The specificity was assessed by comparing the mean apparent signal for Lmf and for [$D_9$] Arm/Lmf in blank samples to mean signal obtained for samples spiked with concentration of Lmf at LLOQ and [$D_9$]Arm/Lmf at the working concentration. | |
| *Acceptance criteria* | Lmf: interference ≤ 20% of signal at LLOQ | |
| | [$D_9$] Arm/Lmf: interference ≤ 5% of signal at working concentration | |
| *Interference from the matrix* | Mean apparent peak area for Lmf in blank samples from 6 batches of dog plasma was 0% of the peak area for Lmf at LLOQ. | |
| | Mean apparent peak area for [$D_9$] Arm/Lmf in blank samples from 6 batches of dog plasma was 0% of the peak area for [$D_9$] Arm/Lmf at the working concentration. | |
| Calibration curves | Calibration was performed with linear regression within range of 0.1 µg/mL to 50 µg/mL using $1/x^2$ weighting | |

| **Study title:** | Quantitative determination of lumefantrine in dog plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924C |
|---|---|---|
| *Acceptance criteria* | Deviation ±15% (±20% at the LLOQ) for 3/4 of the C standards from nominal values | |
| *At the LLOQ* | Mean bias 2% | |
| *Above the LLOQ* | Mean bias within the range -7% to 5.6% | |
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day accuracies and precisions of the QC samples analyzed together with the C standards one day. The deviation (bias) from the nominal value is used to evaluate the accuracy in this validation report. | |
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) from the nominal values | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean bias and precision of all individual concentrations of QC samples analyzed during a single validation day. | |
| *Intra-day accuracy and precision* | At the LLOQ: Bias -4.5%, precision 4.2% | |
| | Above the LLOQ: Bias within the range -9% to -5.8%, precision within the range of 1.9% to 3.6% | |
| | Both were within the acceptance criteria over the entire range. | |
| LLOQ | 0.1 µg/mL using 100 µL of dog plasma | |
| **Results-2:** | | |
| Stability | The stability of Lmf in reconstituted extracts and in dog plasma samples used as quality control, which were stored under varying conditions, was investigated in triplicate together with the freshly prepared C standards. | |
| *Acceptance criteria* | Mean bias ±15% (±20% at LLOQ) | |
| *Stability in methanol: acetic acid (99.8:0.2 [v:v])* | Lmf: | |
| | At least 1 month at 0-8°C at 2 µg/mL and 100 µg/mL and 6 hours at room temperature at 2 µg/mL and 160 µg/mL. | |
| | [$D_9$] Arm/Lmf: | |
| | *In stock solutions (100 µg/mL):* At least 2 months at 0-8°C and 22 hours at room temperature. | |
| | *In working solutions (10 µg/mL):* At least 2 weeks at 0-8°C and 22 hours at room temperature. | |
| *Stability in extracts* | At least 5 days at about 5°C on the autosampler | |
| *Stability in QC samples* | Stable for at least for 17 hours at room temperature and after 3 freeze-thaw cycles, storage below -70°C. No apparent loss after 23 weeks of storage below -70°C. | |

| **Study title:** | Quantitative determination of lumefantrine in dog plasma by liquid chromatography/tandem mass spectrometry. Method description and validation. | Study no. DMPK R0300924C |
|---|---|---|
| Conclusion: | The method is suitable for the routine analysis of Lmf in dog plasma with an anticipated limit of quantification of 0.1 µg/mL using a sample volume of 100 µL.<br>The method is specific for Lmf within the given criteria for acceptance in dog plasma.<br>Care has to be taken to check and suppress carryover of Lmf due to the autosampler system. The washing conditions described in this report are suitable to suppress carryover down to a negligible level. | |

Additional information: none

## 2.6.5.2J Pharmacokinetics: Analytical methods and validation reports (DMPK(F) R00-2105)

| **Study title:** | Quantitative determination of lumefantrine in human plasma by HPLC method with UV detection. Method description and validation. | Study no. DMPK(F) R00-2105 |
|---|---|---|
| GLP compliance: | not required | |
| Location: | 4.2.2.1-10 | |
| Method: | The method consists of the analysis human plasma by liquid chromatography (HPLC) with UV detection after being deproteinized. The objective of this study was to develop to validate a sensitive method for the quantitative determination of Lmf in human plasma. | |
| Instruments: | | |
| Pump | Gilson, model 305 with manometric module, model 805 | |
| Autosampler | Gilson, Aspec XL equipped with a 200 µL injection loop | |
| Column oven | Jones, model 7955 | |
| UV detector | Jasco, model 975 | |
| Data acquisition | X-chrom, version 2.10, LabSystemS, UK | |
| Chromatography: | | |
| Pre-column | Alltima $C_{18}$, 5 µm (7.5 x 4.6 mm), from Alltech | |
| Column | Alltima $C_{18}$, 5 µm (150 x 4.6 mm), from Alltech. | |
| Column temperature | 40°C | |
| Mobile phase | Acetonitrile/ammonium acetate 0.1 mol/L adjusted to pH 3.9 with glacial acetic acid (95:5, v:v) | |
| Flow rate | 2 mL/min | |
| Injection volume | 200 µL | |
| Injector wash | Syringe: Methanol: acetic acid, 98:2 (v:v) | |
| | Valve: Methanol: acetic acid, 98:2 (v:v) | |
| Column pressure | Around 70 bars | |
| Retention time(s) | Lmf: In the range of 9.9 – 11 min | |
| | Halofantrine: In the range of 5.1 – 7.4 min | |
| Total run time | 15 min | |
| Substance analyzed: | Lmf | |
| Reference compounds: | | |
| Lmf | Molecular formula: $C_{30}H_{32}ONCl_3$ | |
| | Molecular weight (MW): 528.95 g/mol | |

| **Study title:** | Quantitative determination of lumefantrine in human plasma by HPLC method with UV detection. Method description and validation. | Study no. DMPK(F) R00-2105 |
|---|---|---|
| Internal standards: | | |
| Halofantrine | Molecular formula: $C_{26}H_{30}NOF_3Cl_2$ | |
| | Molecular weight (MW): 500.43 g/mol | |
| Sample preparation: | Protein precipitation | |
| C standards and QC Sample preparation: | | |
| C Standards | 6 concentrations in the range of 50 ng/mL to 50 µg/mL | |
| QC Samples | 4 concentrations in the range of 50 ng/mL to 40 µg/mL | |
| Matrix: | Human plasma | |
| Detection: | UV fixed at 267 nm | |
| **Results-1:** | | |
| Specificity | The specificity of the analytical method was investigated by preparing and analyzing blank samples prepared from 6 different samples of human plasma. The specificity was assessed by comparing the apparent signal for Lmf and halofantrine in blank samples to the signal obtained for samples spiked with a concentration of Lmf at the LLOQ and with halofantrine at the working concentration. | |
| *Acceptance criteria* | Lmf: Individual interference ≤ 20% of signal at LLOQ | |
| | Halofantrine: Individual interference ≤ 5% of signal at the working concentration | |
| *Interference from the human plasma (matrix)* | Mean apparent peak area of Lmf blank samples from 6 batches of human plasma matrix was within the ranges of 0% to 11% of the peak area of Lmf in the same batch at the LLOQ. | |
| | Mean apparent peak area of halofantrine in blank samples from 6 batches of human plasma matrix was within the range of 0% to 5% of the peak area of halofantrine in the same batch at the working concentration. | |
| Calibration curves | Calibration was performed with a first order polynominal within the range of 50 ng/mL to 50 µg/mL for Lmf using $1/x^2$ weighting. The acceptance criteria for the coefficient of correlation and the mean accuracy were all met. | |
| | Lmf: 96% ≤ mean accuracy ≤ 107%, precision ≤ 7% | |
| *Acceptance criteria* | Coefficient of correlation, r ≥ 0.95 | |
| | Bias ≤15% (20% at the LLOQ) for 2/3 of the C samples | |
| | Coefficients of correlation, r ≥ 0.994 | |
| | Mean accuracy in the range 96% to 107%, all C samples within the range of acceptance | |
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the C samples on each day. | |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean accuracy and precision of all individual accuracies of QC samples analyzed during a single validation day. | |

| **Study title:** | Quantitative determination of lumefantrine in human plasma by HPLC method with UV detection. Method description and validation. | Study no. DMPK(F) R00-2105 |
|---|---|---|
| *Inter-day (inter-batch) accuracy and precision* | The inter-day accuracy and precision were calculated as the mean accuracy and precision of all individual accuracies of QC samples analyzed during 3 validation days. | |
| Acceptance criteria | Mean accuracy 85% to 115% (80% to 120% at LLOQ) | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |
| *Intra-day accuracy and precision of Lmf* | At the LLOQ: Mean accuracy within the range of 96% to 112%, precision within the range of 7% to 13% | |
| | Above the LLOQ: Mean accuracy within the range of 85% to 106%, precision within the range of 2% to 15% | |
| *Inter-day accuracy and precision of Lmf* | At the LLOQ: Mean accuracy 105%, precision 12% | |
| | Above the LLOQ: Mean accuracy within the range of 89% to 100%, precision within the range of 5% to 13% | |
| LLOQ | 50 ng/mL using a sample volume of 250 µL | |
| Stability | The stability of Lmf was investigated by analyzing quality control human samples and reconstituted extracts, which were stored under varying conditions, in triplicate together with freshly prepared C standards and QC samples. | |
| *Acceptance criteria* | Mean accuracy 85% to 115% (80% to 120% at the LLOQ) | |
| *Effect of freeze-thaw cycles* | No apparent loss after 3 freeze-thaw cycles | |
| *Stability in extracts* | No apparent loss after 33 to 34 hours of storage at room temperature | |
| Conclusion: | The method is suitable for the routine analysis of Lmf in human plasma with a limit of quantification of 50 ng/mL with a sample volume of 250 µL. | |
| | The method is specific for Lmf within the given criteria for acceptance in human plasma. | |
| | The method was applied for the determination of Lmf in an interaction study between Riamet® and quinine. The acceptance criteria were met for each analytical run performed. | |

## 2.6.5.2K Pharmacokinetics: Analytical methods and validation reports (BAPK(F) R00-2105-02) & (BAPK(F) R00-2105-02-01 (Amendment 1))

| **Study title:** | Quantitative determination of lumefantrine and its desbutyl metabolite in human plasma by HPLC method with UV detection. Method description and validation. (Amendment no.1) | Study no.<br>BAPK(F) R00-2105-02<br>BAPK(F) R00-2105-02-01<br>(Amendment 1) |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-11 | |
| Test article: | Lmf and N-desbutyl-Lmf | |
| Method: | The method consists of the analysis of human plasma by liquid chromatography (HPLC) with UV detection. The objective of this study was to develop and validate a sensitive method for the quantitative determination of Lmf and its N-desbutylmetabolite in human plasma. | |
| Instruments: | | |
| Pump | Gilson, model 305 with manometric module, model 805 | |
| Autosampler | Gilson, model 234 equipped with 200 µL injection loop and a rack temperature regulator, model 832 set at 5°C | |
| Column oven | CROCO-CIL | |
| UV detector | Waters, model 2487 | |
| Data acquisition | X-Chrom | |
| Chromatography: | | |
| Pre-column | Alltima $C_{18}$, 5 µm (7.5 x 4.6 mm), Alltech | |
| Column | Alltima $C_{18}$, 5 µm (150 x 4.6 mm), Alltech. The column should be equilibrated with mobile phase for 24 hours before use. | |
| Column temperature | 40°C | |
| Mobile phase | Acetonitrile/ammonium acetate 0.1 mol/L adjusted to pH 3.9 with glacial acetic acid (97:3, v:v) | |
| Flow rate | 1.6 mL/min | |
| Injection volume | 90 µL | |
| Injector wash | Syringe: methanol: acetic acid, 99:1 (v:v) | |
| | Valve: methanol: acetic acid, 99:1 (v:v) | |
| Column pressure | Around 50 bars | |
| Retention time(s) | Lmf: In the range of 13.2 – 14.3 min | |
| | N-desbutyl-Lmf: In the range of 5.8 – 6.7 min | |
| | Halofantrine: In the range of 7.5 – 8.1 min | |

| **Study title:** | Quantitative determination of lumefantrine and its desbutyl metabolite in human plasma by HPLC method with UV detection. Method description and validation. (Amendment no.1) | Study no.<br>BAPK(F) R00-2105-02<br>BAPK(F) R00-2105-02-01<br>(Amendment 1) |
|---|---|---|
| Total run time | 16 min | |
| Substance analyzed: | Lmf and N-desbutyl-Lmf | |
| Reference compounds: | | |
| Lmf | Molecular formula: $C_{30}H_{32}ONCl_3$ | |
| | Molecular weight (MW): 528.95 g/mol | |
| N-desbutyl Lmf | Molecular formula: $C_{26}H_{24}ONCl_3$ | |
| | Molecular weight (MW): 472.85 g/mol | |
| Internal standards: | | |
| Halofantrine | Molecular formula: $C_{26}H_{30}NOF_3Cl_2$ | |
| | Molecular weight (MW): 500.43 g/mol | |
| Sample preparation: | Liquid-liquid extraction with hexane | |
| C standard and QC samples preparation: | | |
| C Standards | 6 concentrations in the range of 50 ng/mL to 20 µg/mL and 5 ng/mL to 1 µg/mL of Lmf and N-desbutyl-Lmf, respectively. | |
| QC Samples | 4 concentrations in the range of 50 ng/mL to 16 µg/mL and 5 ng/mL to 0.8 µg/mL of Lmf and N-desbutyl-Lmf, respectively. | |
| Matrix: | Human plasma | |
| Detection: | UV fixed at 335 nm | |
| **Results-1:** | | |
| Specificity | The specificity of the method was investigated by preparing and analyzing blank samples prepared from 6 different samples of human plasma. The specificity was assessed by comparing the apparent signal for Lmf, N-desbutyl-Lmf and halofantrine in blank samples to the signal obtained for samples spiked with a concentration of Lmf and N-desbutyl-Lmf at the LLOQ and with halofantrine at the working concentration. | |
| *Acceptance criteria* | Analytes: Individual interference ≤ 20% of signal at LLOQ | |
| | Internal standards: Individual interference ≤ 5% of signal at working concentration | |
| *Interference from the human plasma (matrix)* | Mean apparent peak area of Lmf in blank samples from 6 batches of human plasma was within the ranges of 5% to 16% of the peak area of Lmf in the same batch at the LLOQ. | |
| | Mean apparent peak area of N-desbutyl-Lmf in blank samples from 6 batches of human plasma was within the ranges of 1% to 10% of the peak area of N-desbutyl-Lmf in the same batch at the LLOQ. | |
| | Mean apparent peak area of halofantrine in blank samples from 6 batches of human plasma was within the ranges of 2% to 4% of the peak area of halofantrine in the same batch at the working concentration. | |

| **Study title:** | Quantitative determination of lumefantrine and its desbutyl metabolite in human plasma by HPLC method with UV detection. Method description and validation. (Amendment no.1) | Study no.<br>BAPK(F) R00-2105-02<br>BAPK(F) R00-2105-02-01<br>(Amendment 1) |
|---|---|---|
| Calibration curves | Calibration was performed with a first order polynominal within the range of 50 ng/mL to 20 µg/mL and 5 ng/mL to 1 µg/mL for Lmf and N-desbutyl-Lmf, respectively, using $1/x^2$ weighting. The acceptance criteria for the coefficient of correlation and the mean accuracy were all met. | |
| | Lmf: 96% ≤ mean accuracy ≤ 105%, precision ≤ 15% | |
| | N-Desbutyl Lmf: 97% ≤ mean accuracy ≤ 102%, precision ≤ 9% | |
| *Acceptance criteria* | Coefficient of correlation, r ≥ 0.95 | |
| | Bias ≤15% (20% at the LLOQ) for 2/3 of the C samples | |
| | Coefficients of correlation, r > 0.997 | |
| | Mean accuracy in the range 96% to 105%, all C samples within the range of acceptance | |
| **Results-2** | | |
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the C samples on each day. | |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean accuracy and the precision of all individual accuracies of QC samples analyzed during a single validation day. | |
| *Inter-day (inter-batch) accuracy and precision* | The inter-day accuracy and precision were calculated as the mean accuracy and precision of all individual accuracies of QC samples analyzed during 3 validation days. | |
| Acceptance criteria | Mean accuracy 85% to 115% (80% to 120% at LLOQ) | |
| | Precision: ≤ 15% (≤ 20% at the LLOQ) | |
| *Intra-day accuracy and precision* | | |
| Lmf | At the LLOQ: Mean accuracy 112%, precision 6% | |
| | Above the LLOQ: Mean accuracy within the range 98% to 108%, precision 1% to 3% | |
| N-desbutyl Lmf | At the LLOQ: Mean accuracy 102%, precision 12% | |
| | Above the LLOQ: Mean accuracy within the range 88% to 115%, precision 1% to 6% | |
| *Inter-day accuracy and precision* | | |
| Lmf | At the LLOQ: Mean accuracy 105%, precision 8% | |
| | Above the LLOQ: Mean accuracy within the range 92% to 101%, precision 4% to 7% | |
| N-Desbutyl Lmf | At the LLOQ: Mean accuracy 99%, precision 13% | |
| | Above the LLOQ: Mean accuracy within the range 89% to 100%, precision 7% to 12% | |

| **Study title:** | Quantitative determination of lumefantrine and its desbutyl metabolite in human plasma by HPLC method with UV detection. Method description and validation. (Amendment no.1) | Study no.<br>BAPK(F) R00-2105-02<br>BAPK(F) R00-2105-02-01<br>(Amendment 1) |
|---|---|---|
| LLOQ | 50 ng/mL and 5 ng/mL using a sample volume of 250 µL and 500 µL for Lmf and N-desbutyl-Lmf, respectively | |
| Absolute recovery | The absolute recovery was assessed by comparison of the responses obtained from the analysis of reference solutions spiked in the extract of a blank sample and of extracted human plasma samples. It is given as the ratio of the average peak areas obtained from a reference solution relative to the average peak areas obtained from the analysis of extracted plasma samples. | |
| *Lmf* | Mean recovery 75% (range 71% to 78%) | |
| | The recovery of Lmf does not depend on its concentration. | |
| *N-desbutyl-Lmf* | Mean recovery 74% (range 67% to 81%) | |
| | The recovery of N-desbutyl-Lmf does not depend on its concentration. | |
| *Halofantrine (IS)* | Mean recovery 83% (Lmf method) and 99% N-desbutyl-Lmf. | |
| Stability | The stability of Lmf was investigated by analyzing quality control of human samples and reconstituted extracts, which were stored under varying conditions, in triplicate together with freshly prepared C standards and QC samples. | |
| *Acceptance criteria* | Mean accuracy 85% to 115% (80% to 120% at the LLOQ) | |
| *Stability in stock solutions* | No apparent loss after 6 hours at room temperature and 1 month at 4°C. | |
| *Stability in QC samples* | No apparent change after 24 hours of storage under ambient conditions. | |
| *Stability in frozen QC samples* | No apparent loss after 4 weeks of storage at or below -70°C. | |
| *Stability in actual samples* | No apparent loss after at least 3.5 months of storage at or below -70°C. | |
| *Effect of freeze-thaw cycles* | No apparent loss after 3 freeze-thaw cycles. | |
| *Stability in extracts* | No apparent loss after 33 to 34 hours of storage at +5°C. | |
| Conclusion: | The method is suitable for the routine analysis of Lmf in human plasma with a limit of quantification of 50 ng/mL with a sample volume of 250 µL and its N-desbutyl-Lmf with a limit of quantification of 5 ng/mL with a sample volume of 500 µL. | |
| | The method is specific for Lmf and N-desbutyl-Lmf within the given criteria for acceptance in human plasma. | |
| | The method was applied for the determination of Lmf in an interaction study between Riamet® and ketoconazole. The acceptance criteria were met for each analytical run performed. | |
| | Cross-validation results for Lmf on actual samples revealed good agreement between this method and the previously described one. | |
| Amendment #1<br>Reason for amendment | This amendment gives the latest stability data obtained in both human plasma and whole blood samples for Lmf compound. | |

| **Study title:** | Quantitative determination of lumefantrine and its desbutyl metabolite in human plasma by HPLC method with UV detection. Method description and validation. (Amendment no.1) | Study no.<br>BAPK(F) R00-2105-02<br>BAPK(F) R00-2105-02-01<br>(Amendment 1) |
|---|---|---|
| Additions to the original document | The stability of Lmf was investigated by analyzing spiked human plasma samples and whole blood samples after 24 hours of storage at 35°C together with freshly prepared C and QC samples. These additional stability tests were initiated in order to support the studies conducted in Africa where samples, either plasma or whole blood, could be exposed to temperature around 35°C.<br>These experiments were performed using the analytical method described in the original validation report without any change (BAPK(F) R00-2105-02).<br>The stability of Lmf was assessed at 200 ng/mL and 10000 ng/mL in plasma and at 400 ng/mL and 6000 ng/mL in whole blood. Analysis was done in the plasma fraction only. | |
| Sample preparation in plasma samples | Two sets of aliquots were spiked (1:10) with appropriate working solutions of Lmf (500 µg/ml) to get the intended concentrations. Three aliquots of each were taken at 0 hours and others were kept at 35°C for 24 hours. Samples were processed with freshly prepared C and QC samples. | |
| Sample preparation in whole blood samples | Two pools of human whole blood were spiked with (1:1250 or 1:83) with the stock solution of Lmf (500 µg/mL) to get the intended concentrations. Each pool of whole blood with Lmf was prepared in triplicate. Immediately after the preparation, 3 aliquots from each pool (5 mL) were centrifuged at 2500 x g for 10 min, and the resulting plasma samples were stored at below -20°C until analysis. The remaining whole blood with Lmf samples were kept at 35°C for 24 hours, and then 3 aliquots from each pool of these samples were centrifuged at 2500 x g for 10 min. These resulting plasma samples were also stored at below -20°C until analysis. For analysis, all plasma samples were thawed and processed together with freshly prepared C and QC samples. | |
| **Results-3:** | | |
| | No apparent loss of Lmf was observed in both human plasma samples and whole blood samples after 24 hours at 35°C. | |

Additional Information: none

## 2.6.5.2 L Pharmacokinetics: Analytical methods and validation reports (BPK(CH) 1994/038)

| Study title: | Quantitative determination of benflumetol in plasma by high-performance liquid chromatography and UV detection. | Study no. BPK(CH) 1994/038 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.1-12 | |
| Test article: | Lmf | |
| Method: | HPLC was performed on a 125 mm x 4 mm i.d. column packed with 100 RP-8 endcapped 5 µm. Detection was performed using ultraviolet detection at 215 nm. The mobile phase contained water, 0.2% SDS, 0.2% acetic acid, and acetonitrile (20/80, v/v), with a flow rate of 1 mL/min. The retention times of Lmf and its internal standard were 11 and 7 min, respectively. | |
| Instrument: | Hewlett-Packard HPLC system (Model 1090)<br>Merck-Hitachi D-2500 integrator<br>Kratos Spectroflow SF 783 variable wavelength ultraviolet detector | |
| Substance analyzed: | Lmf | |
| Reference compounds: | | |
| Lmf | Molecular formula: $C_{30}H_{32}ONCl_3$<br>Molecular weight (MW): 528.95 g/mol | |
| Internal standards: | | |
| Halofantrine | Molecular formula: $C_{26}H_{30}NOF_3Cl_2$<br>Molecular weight (MW): 500.43 g/mol | |
| Sample preparation: | The plasma samples (0.5 mL) containing Lmf and the internal standard (halofantrine) were deproteinised with 1 mL methanol/acetic acid (99/1, v/v). Samples were mixed for 5 min on a Vortex and then were centrifuged for 10 min at 3000 rpm. The solvent extract was then separated and evaporated to dryness under nitrogen stream. The dry residue was dissolved in 200 µL mobile phase. An aliquot (50 µL) of the mixture was transferred to a micro injection vial which was sealed with a PTFE cap. | |

| Study title: | Quantitative determination of benflumetol in plasma by high-performance liquid chromatography and UV detection. | Study no. BPK(CH) 1994/038 |
|---|---|---|
| Results: | | |
| HPLC assay | The chromatograms of extracts of a drug free-human plasma sample (0.5 mL) and of a plasma sample spiked with 27.8 nmol Lmf and 4.95 nmol of internal standard (halofantrine) demonstrated that the two compounds are well separated from plasma constituents.<br>Seven human plasma samples in the concentration range 35 to 1554 nmol/L were analysed on 2 to 5 different days. The inter-assay CV ranged from 0.33 to 14.8% and deviations of the mean found values from the given concentrations ranged from -24.19 to -0.39%.<br>Six spiked human plasma samples (48 to 1549 nmol/L) were analysed 3 times on the same day. The CV ranged from 2.74 to 16.39% and the deviation from theory from -2.85 to 10.33%. | |
| LOQ | 35 nmol/L (18.5 ng/mL) | |
| LOD | 5 nmol/L (2.64 ng/mL) | |
| Relative recovery | Not measured | |
| Stability | Not measured | |

Additional information: none

## 2.6.5.2 M Pharmacokinetics: Analytical methods and validation reports (BPK(CH) 1996/147)

| **Study title:** | Development of an HPLC assay and plasma concentrations of the two enantiomers CGP 64455 S(+) and CGP 64456 R(-) of racemic benflumetol (CGP 56695) in humans, dogs and rats. | Study no. BPK (CH) 1996/147 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.1-13 | |
| Test article: | racemic Lmf | |
| Method: | HPLC assay was performed using a 250 mm x 4 mm i.d. column packed with cellulose 3-chloro-4-methylphenylcarbamate coated on Silica (Nucleosil 4000 – 10 µm). Detection was performed using a variable wavelength ultraviolet detector at 254 nm. | |
| Instrument: | HPLC<br>Kratos Spectroflow 783 variable wavelength ultraviolet detector | |
| Substance analyzed: | CGP 64455 S(+) and CGP 64456 R(-) and the internal standard halofantrine | |
| Reference compound: | CGP 64455 S(+) and CGP 64456 R(-) | |
| *Plasma concentrations* | Human: a single dose of 480 mg Lmf<br>Dogs: repeated doses of 200 mg/kg Lmf<br>Rats: a single dose of 100 mg/kg of Lmf ; and a single dose of either 50 mg/kg S(+) or 50 mg/kg R(-) | |
| Internal standard: | Halofantrine $C_{26}H_{30}NOF_3Cl_2$ | |
| Sample preparation: | | |
| *HPLC assay* | 0.5 mL plasma samples containing racemate / enantiomers were deproteinised with 1 mL methanol / acetic acid. These samples were mixed for 5 min on a Vortex and centrifuged for 10 min at 3000 rpm. The solvent extract was then separated / evaporated to dryness under nitrogen stream at +4°C and dry residue dissolved in 200 µL mobile phase. A 20 µL aliquot was then transferred into microinjection vial sealed with PTFE cap. | |
| Results: | | |
| HPLC assay | The chromatogram of an extract of a drug-free human plasma sample (0.5 mL) spiked with 670 ng of each of the two enantiomers and the internal standard demonstrated that the two enantiomers are well separated from plasma constituents. | |
| *S(+) enantiomer* | Five human plasma samples in the concentration of 450 and 2060 ng/mL, analyzed on 2 to 5 different days, exhibited inter-assay coefficient of variations (CVs) ranging from 3.8 to 26.2% and deviations of the mean found values from the given concentrations ranging from -8.0 to 10.2%.<br>Four spiked human samples analyzed 3 times on the same day demonstrated CVs ranging from 2.6 to 16.5% and a deviation from theory ranging from -8.4 to 12.5%. | |

| | | | |
|---|---|---|---|
| **Study title:** | | Development of an HPLC assay and plasma concentrations of the two enantiomers CGP 64455 S(+) and CGP 64456 R(-) of racemic benflumetol (CGP 56695) in humans, dogs and rats. | Study no. BPK (CH) 1996/147 |
| *R(-) enantiomer* | | Five human plasma samples in the concentration range 480 to 2370 ng/mL analyzed on 4 to 5 different days exhibited inter-assay CVs ranging from 3.1 to 18.1% and deviations of the mean found values from the given concentrations ranging from -5.2 to 10.2%. Four spiked human samples analyzed 3 times on the same day demonstrated CVs ranging from 6.7 to 19.4% and a deviation from theory ranging from -13.3 to 7.4%. | |
| Results: | LOQ: | *S(+) enantiomer* : 1.1 µg/mL | |
| | | *R(-) enantiomer:* 0.5 µg/mL | |
| | LOD | *S(+) enantiomer*: 0.11 µg/mL | |
| | | *R(-) enantiomer*: 0.05 µg/mL | |
| Relative recovery and stability | | Not measured | |
| Plasma concentrations | | Humans: After a single dose of 480 mg Lmf , plasma concentrations of S(+) and R(-) were similar at each time period. | |
| | | Dogs: After repeated doses of 200 mg/kg Lmf given daily over 89 days, plasma concentrations of S(+) and R(-), in both male and female dogs, were similar at each time period. | |
| | | Rats: After a single dose of 100 mg/kg of Lmf , the plasma concentration of the R(-) enantiomer is higher at each time period than the plasma concentration of the S(+) enantiomer. This difference in plasma concentration at each time period was also seen when a single dose of 50 mg/kg S(+) enantiomer or of 50 mg/kg R(-) enantiomer was administered. | |

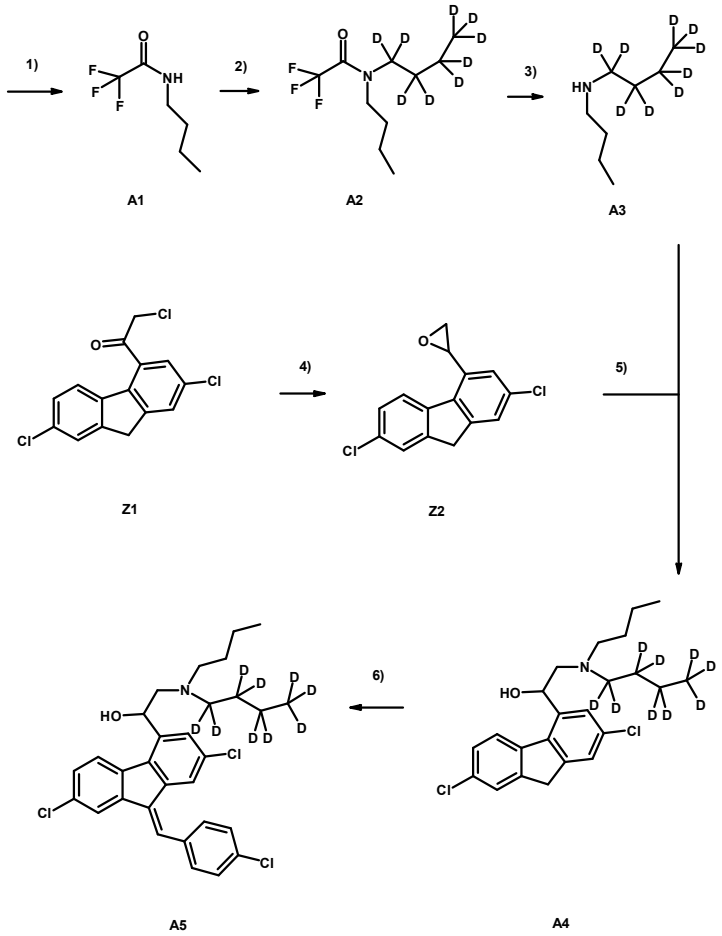
Additional information: none

## 2.6.5.2N Pharmacokinetics: Analytical methods and validation reports (PCS(EU) R0301253-01 (SSE 072-1))

| | | | | | |
|---|---|---|---|---|---|
| **Study title:** | [$D_9$] COA566 Synthesis and Release Analysis | | | | Study No: PCS(EU) R0301253-01 (SSE 072-1) |
| GLP compliance: | not required | | | | |
| Location in CTD: | 4.2.2.1-14 | | | | |
| Test article | [$D_9$] Lmf | | | | |
| Type of study: | synthesis and analysis | | | | |
| Method: | Synthesis: The oxirane was ring-opened with [$D_9$] dibutylamine that was synthesized from commercially available [$D_9$] bromobutane to obtain the alcohol base. Base-catalyzed condensation with chloro-benzaldehyde resulted in [$D_9$] Lmf after final purification.<br>Analysis: The identity of the labeled compound was confirmed by mass spectrometry, nuclear magnetic resonance, and high-performance liquid chromatography | | | | |
| Results: | Analysis | | | | |
| | Test | Method | Requirements | Results | Remarks |
| | Identity | HPLC | Elution time corresponds to reference | Elution time corresponds | - |
| | Identity | $^1$H-NMR spectrum | Corresponds to reference | Corresponds to reference | - |
| | Identity | Mass spectrum | Corresponds to reference | Corresponds to reference | Except isotope shift |
| | Chemical purity | HPLC-UV | ≥ 98% | 99.0% | - |
| | Mass purity | Mass-spectrometry | Must contain less than 1% of molecular ion of the unlabelled reference | < 1% | Mass of unlabelled reference not detectable |

| **Study title:** | [$D_9$] COA566 Synthesis and Release Analysis | Study No:<br>PCS(EU) R0301253-01<br>(SSE 072-1) |
|---|---|---|

Results:




Reaction conditions: 1) N-butylamine, THF, 0°C/15 min, RT/12h, 80%; 2) [$D_9$] bromobutane, NaH, DMPU, RT/12h; 3) NaOH, EtOH, purification, 42%; 4)a) NaBH4, EtOH, 0°C/20 min, *in situ* b) NaOMe, MeOH, 82% ; 5) ethanol, 95°C/20h, flash-chromatography, 80%.

Additional information: none

## 2.6.5.2O Pharmacokinetics: Analytical methods and validation reports (DMPK(F) R00-1921)

| **Study title:** | Quantitative determination of Quinine in human plasma by HPLC method with fluorescence detection. Method description and validation. | Study no. DMPK(F) R00-1921 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.2.1-15 | |
| Test article: | quinine | |
| Method: | The method consists of liquid-liquid extraction with subsequent evaporation of the supernatant to dryness followed by the analysis of the reconstituted sample by liquid chromatography (HPLC) with fluorescence detection. The objective of this study was to develop to validate a sensitive method for the quantitative determination of quinine in human plasma. | |
| Instruments: | | |
| Pump | Gilson, model 305 with manometric module, model 805 | |
| Autosampler | Gilson, model 234 equipped with 200 µL injection loop and a rack temperature regulator, model 832 set at 5°C | |
| Column oven | CROCO-CIL | |
| Fluorescence detector | Merck-Hitachi, model L-7480 | |
| Data acquisition | X-Chrom | |
| Chromatography: | HPLC on an inert silica Inertsil 5 µ (250 x 4.6 mm) column at 24°C using isocratic elution (acetonitrile: methanol containing a 25% ammonia solution ([92.5:7.5, v:v] 78:22, v:v) at a flow rate of 1 mL/min with a total run time of 12 min. Special care was taken to avoid carryover in the autosampler by washing the autosampler system multiple times with methanol | |
| Pre-column | Inertsil silice, 5 µ particle size (10 x 4.6 mm) from Interchim | |
| Column | Inertsil silice, 5 µ particle size 250 x 4.6 mm rom Interchim | |
| Column temperature | 24°C | |
| Mobile phase | Acetonitrile/methanol containing 25% ammonia solution ([92.5:7.5, v:v] 78:22, v:v) | |
| Flow rate | 1 mL/min | |
| Injection volume | 70 µL | |
| Injector wash | Syringe: Methanol | |
| | Valve: Methanol | |
| Column pressure | Around 35 bars | |
| Retention time(s) | Quinine: 7.7 – 7.9 min | |
| | Hydroquinidine: 10.5 – 10.8 min | |
| Total run time | 12 min | |
| Substance analyzed: | Quinine | |

| **Study title:** | Quantitative determination of Quinine in human plasma by HPLC method with fluorescence detection. Method description and validation. | Study no. DMPK(F) R00-1921 |
|---|---|---|
| Reference compounds: | | |
| Quinine | Molecular formula: $C_{20}H_{24}N_2O_2$ | |
| | Molecular weight (MW): 324.4 g/mol | |
| Internal standards: | | |
| Hydroquinidine | Molecular formula: $C_{20}H_{26}N_2O_2$ | |
| | Molecular weight (MW): 326.44 g/mol | |
| Sample preparation: | Liquid-liquid extraction with dichloromethane | |
| C standard and QC samples preparation: | | |
| C Standards | 5 concentrations in the range of 20 ng/mL to 10 µg/mL | |
| QC Samples | 4 concentrations in the range of 40 ng/mL to 8 µg/mL | |
| Matrix: | Human plasma | |
| Detection: | Fluorescence detection | |
| Source | UV | |
| | Excitation wavelength: 325 nm | |
| | Emission wavelength: 375 nm | |
| **Results-1:** | | |
| Specificity | The specificity of the analytical method was investigated by preparing and analyzing blank samples prepared from 6 different batches of human plasma. The specificity was assessed by comparing the apparent signal for quinine and hydroquinidine in blank samples to the mean signal obtained for samples spiked with a concentration of quinine at the LLOQ and with hydroquinidine at the working concentration. | |
| *Acceptance criteria* | Quinine: Individual interference ≤ 20% of signal at LLOQ<br>Hydroquinidine: Individual interference ≤ 5% of signal at working concentration | |
| *Interference from the human plasma (matrix)* | No quantifiable peak at the 6 blank samples at the retention times of the 2 compounds | |
| Calibration curves | Calibration was performed with a first order polynominal within the range of 20 ng/mL to 10000 ng/mL, using $1/x^2$ weighting. The acceptance criteria for the coefficient of correlation and the mean accuracy were all met.<br>98% ≤ mean accuracy ≤ 101%, precision ≤ 11% | |
| *Acceptance criteria* | Coefficient of correlation, $r \geq 0.95$<br>Bias ≤ 15% (20% at the LLOQ) for 2/3 of the C samples | |
| *Results* | Coefficients of correlation, $r > 0.996$<br>Individual accuracy in the range 86% to 111%, all C samples within the range of acceptance | |

| **Study title:** | Quantitative determination of Quinine in human plasma by HPLC method with fluorescence detection. Method description and validation. | Study no. DMPK(F) R00-1921 |
|---|---|---|

**Results-2:**

| | |
|---|---|
| Accuracy, Precision, LLOQ | Accuracy and precision of the method were assessed by analyzing the intra-day and inter-day accuracies and precisions of the QC samples analyzed together with the C samples on each day. |
| *Intra-day (within batch) accuracy and precision* | The intra-day accuracy and precision were calculated as the mean and precision of all individual accuracies of QC samples analyzed during a single validation day. |
| *Inter-day (inter-batch) accuracy and precision* | The inter-day accuracy and precision were calculated as the mean accuracy and precision of all individual accuracies of QC samples analyzed during 3 validation days. |
| Acceptance criteria | Mean accuracy 85% to 115% (80% to 120% at LLOQ)<br>Precision: ≤ 15% (≤ 20% at the LLOQ) |
| *Intra-day accuracy and precision* | At the LLOQ: Mean accuracy within the range of 92% to 109%, precision 8% to 16% |
| | Above the LLOQ: Mean accuracy within the range of 92% to 104%, precision 1% to 12% |
| *Inter-day accuracy and precision* | At the LLOQ: Inter-day accuracy 100%,overall precision 14%<br>Above the LLOQ: Inter-day accuracy within the range 97% to 102%, precision within the range 4% to 8% |
| LLOQ | 20 ng/mL using a sample volume of 500 µL |
| Absolute recovery | The absolute recovery was assessed by comparison of the responses obtained from the analysis of reference solutions spiked in the extract of a blank sample and of extracted human plasma samples. It is given as the ratio of the average peak areas obtained from a reference solution relative to the average peak areas obtained from the analysis of extracted plasma samples. |
| *Quinine* | Mean recovery 74% (range 72% to 76%)<br>The recovery of quinine did not depend on its concentration. For concentrations near the lower limit of quantification, an average recovery of 73% was found. For concentrations of 8000 ng/mL, an average of 72% was found. |
| *Hydroquinidine* | Mean recovery 85% at the working concentration |

**Results-3:** Stability

| | |
|---|---|
| | The stability of quinine was investigated by analyzing quality control human samples and reconstituted extracts, which were stored under varying conditions, in triplicate together with freshly prepared C standards and QC samples. |
| *Acceptance criteria* | Mean accuracy 85% to 115% (80% to 120% at the LLOQ) |
| *Stability in stock solutions* | No apparent loss after 15 days of storage at 4°C |
| *Stability in QC samples* | No apparent change after 6 hours of storage under ambient conditions |
| *Stability in frozen QC samples* | No apparent loss after 6 weeks of storage at or below -70°C |
| *Effect of freeze-thaw cycles* | No apparent loss after 3 freeze-thaw cycles |
| *Stability in extracts* | No apparent loss after about 10 to 11 hours of storage at room temperature |

| **Study title:** | Quantitative determination of Quinine in human plasma by HPLC method with fluorescence detection. Method description and validation. | Study no. DMPK(F) R00-1921 |
|---|---|---|
| Conclusion: | 1. The method is suitable for the routine analysis of quinine in human plasma with a limit of quantification of 20 ng/mL using a sample volume of 500 µL.<br>2. The method is specific for quinine within the given criteria for acceptance in human plasma.<br>3. The method was applied for the determination of quinine in an interaction study with Riamet®.<br>The acceptance criteria were met for each analytical run performed. | |

Additional information: none

# 2.6.5.3 薬物動態試験：単回投与後の吸収

## 2.6.5.3A Pharmacokinetics: Absorption after single dose (DMPK(CH) 1997/241)

| **Study title:** | Disposition studies in rats after administration of Co-artemether (CGP 56 697) containing radiolabelled artemether (CGP 56 696) and unlabelled benflumetol. | Study no. DMPK(CH) 1997/241 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-1 | |
| Test article | $^{3}H$ or $^{14}C$-labeled Arm (1 part) and unlabeled benflumetol (6 parts) | |
| Species, strain, sex, number of animals: | male rat albino / Tif: RAIf (SPF) / 3 per group | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidione (7/3, w/w) for intravenous route<br>0.5% Klucel + 0.1% Tween 80: for oral route | |
| Method/ Route / Duration of administration: | intravenous bolus / tail vain / once<br>gavage / oral / once | |
| Dose (mg/kg): | intravenous dose of 10 once<br>oral dose of 20, 100, or 1000 once | |
| Specific activity: | [$^{3}H$]-Arm (Batch No. Re-54.9A) of 3022.9 MBq/mmol or 10.05 MBq/mg [a]<br>[$^{3}H$]-Arm (Batch No. Re-54.9C) of 3.35 MBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9C1) of 3.35 MBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9D) of 1.39 MBq/mg | [$^{3}H$]-Arm (Batch No. Re-54.9E) of 33 KBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9G )of 273 KBq/mg<br><br>a: taken from the Certificate of Analysis sheet of Re-54.9A (typing error in report). |
| Analyte / Radionuclide: | radioactivity / $^{3}H$ or $^{14}C$ | |
| Assay: | liquid scintillation counting | |

| **Study title:** | Disposition studies in rats after administration of Co-artemeter (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol. | | | | | | Study no. DMPK(CH) 1997/241 | |
|---|---|---|---|---|---|---|---|---|
| **Absorption results**: | | | | | | | | |
| Rat | $^{3}$H mean concentrations µmol/L | | | | | | | |
| Route | intravenous | | single oral (non-fasting) | | | | | |
| Dose (mg/kg) | 10 (fasted) | | 20 (fasted) | | 100 (non-fasted) | | 1000 (non-fasted) | |
| Number of animals | 3 | | 3 | | 3 | | 3 | |
| Time (h) | blood | plasma | blood | plasma | blood | plasma | blood | plasma |
| 0.08 | 4.07 | 4.96 | 0.99 | 1.19 | 1.05 | ns | 1.69 | ns |
| 0.25 | 3.16 | 4.29 | 2.35 | 2.85 | 6.06 | ns | 14.42 | ns |
| 0.5 | 2.90 | 3.97 | 2.55 | 3.06 | 11.00 | ns | 35.14 | ns |
| 1 | 2.73 | 3.69 | 2.25 | 2.55 | 11.87 | ns | 72.47 | ns |
| 2 | 2.28 | 3.14 | 1.64 | 1.76 | 7.33 | ns | 62.86 | ns |
| 4 | 1.74 | 2.29 | 1.49 | 1.58 | 7.61 | ns | 45.87 | ns |
| 8 | 1.37 | 1.58 | 1.51 | 1.42 | 6.55 | ns | 41.95 | ns |
| 24 | 0.73 | 0.66 | 0.86 | 0.64 | 3.42 | ns | 36.73 | ns |
| 48 | 0.46 | 0.38 | 0.53 | 0.33 | 2.09 | ns | 23.47 | ns |
| 72 | 0.34 | 0.23 | 0.37 | 0.22 | 2.00 | ns | 16.70 | ns |
| 96 | 0.27 | 0.16 | 0.34 | 0.16 | 1.29 | ns | 14.04 | ns |
| 120 | 0.22 | 0.11 | 0.27 | 0.13 | 1.58 | ns | 12.09 | ns |
| 144 | 0.19 | 0.08 | 0.26 | 0.09 | 1.46 | ns | 10.89 | ns |
| AUC 0 – 24h | 32.22 | 38.22 | 32.17 | 30.50 | 141 | nc | 1016 | nc |
| AUC 0 – 144h | 74.22 | 68.22 | 81.85 | 59.42 | 348 | nc | 3178 | nc |

ns= no sampling. nc = not calculated.

Additional Information: none

## 2.6.5.3B Pharmacokinetics: Absorption after single dose (DMPK(CH) 1997/003)

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | Study no. DMPK(CH) 1997/003 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-2 | |
| Test article: | $^{14}$C-labeled Arm (1 part) and unlabeled Lmf(6 parts) | |
| Species, strain, sex, number of animals: | male dog / Beagle / 6, n=2 per group | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | PEG 400 / in solution with N-methyl-2-pyrrolidone (7/3, w/w) for intravenous route<br>gelatin capsule for oral route | |
| Method/ Route / Duration of administration: | intravenous bolus / cephalic vein of a foreleg / once<br>gelatin capsule / oral / once | |
| Dose (mg/kg): | intravenous dose of 10, once<br>oral dose of 20 and 200, once | |
| Specific activity: | [$^{14}$C]-Arm (Batch No. Re-84.1C1) of 218 KBq/mg<br>[$^{14}$C]-Arm (Batch No. Re-84.1D2) of 98 KBq/mg<br>[$^{14}$C]-Arm (Batch No. Re-84.1F) of 10 KBq/mg | |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | Arm and DHA determination in plasma by HPLC with online radiodetection or with electromechanical detection in reductive mode;<br>Radioactivity by liquid scintillation counting | |

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | | | | | | Study no. DMPK(CH) 1997/003 | |
|---|---|---|---|---|---|---|---|---|
| **Absorption results**: | | | | | | | | |
| Dog | $^{14}$C mean concentrations µmol/L | | | | | | | |
| Route | intravenous | | | | single oral (non-fasting) | | | |
| Dose (mg/kg) | 10 | | | | 20 | | 200 | |
| Number of animals | 2 | | | | 2 | | 2 | |
| Time (h) | blood | plasma | Arm in plasma | DHA in plasma | blood | plasma | blood | plasma |
| 0.08 | 3.47 | 5.90 | 2.35 | 0.23 | ns | ns | ns | ns |
| 0.25 | 3.97 | 7.35 | 1.09 | 0.27 | 0.28 | 0.52 | ns | ns |
| 0.5 | 4.00 | 6.97 | 0.44 | 0.21 | 2.35 | 3.96 | 0.09 | 0.16 |
| 1 | 3.41 | 6.00 | 0.18 | 0.12 | 7.47 | 12.04 | 3.78 | 6.11 |
| 2 | 2.55 | 4.09 | 0.06 | 0.04 | 6.21 | 10.23 | 25.29 | 39.14 |
| 4 | 1.65 | 2.57 | nd | nd | 2.75 | 3.91 | 31.96 | 48.21 |
| 6 | 1.22 | 1.79 | nd | nd | 1.99 | 3.01 | 20.18 | 29.53 |
| 8 | 1.05 | 1.51 | nd | nd | 1.62 | 2.10 | 14.40 | 20.17 |
| 24 | 0.76 | 1.00 | nd | nd | 0.87 | 1.01 | 10.17 | 11.35 |
| 48 | 0.61 | 0.78 | nd | nd | 0.67 | 0.68 | 5.82 | 5.92 |
| 72 | 0.55 | 0.68 | nd | nd | 0.58 | 0.62 | 4.79 | 4.45 |
| 96 | 0.49 | 0.62 | nd | nd | 0.52 | 0.54 | 4.30 | 4.05 |
| 120 | 0.44 | 0.55 | nd | nd | 0.49 | 0.49 | 4.12 | 3.47 |
| 144 | 0.40 | 0.50 | nd | nd | 0.44 | 0.49 | 3.94 | 3.43 |
| 168 | 0.39 | 0.46 | nd | nd | 0.43 | 0.39 | 3.48 | 3.06 |
| AUC 0 – 168h | 103.7 | 137.8 | 0.81 | 0.30 | 127.10 | 151.26 | 1071.09 | 1175.90 |

nd = not detected (below limit of quantification of 0.034 µmol/L). nc = not calculated. ns= no sampling.

Additional Information: none

## 2.6.5.3C Pharmacokinetics: Absorption after single dose (BPK(F) 1996/029)

| **Study title:** | Artemether and dihydroartemisinin plasma concentrations in female cynomolgus monkeys in a pilot pharmacokinetic study with a single Co-artemether oral dose. | Study no. BPK(F) 1996/029 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-3 | |
| Test article: | Arm/Lmf as 1 part Arm and 6 parts Lmf | |
| Species, strain, sex, number of animals: | cynomolgus monkey, macaca fascicularis, female, n = 3 | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | gelatin capsules | |
| Method of Administration: | oral gavage | |
| Dose (mg/kg): | 350 mg/kg | |
| Sample: | plasma | |
| Analyte: | Arm and DHA | |
| Assay: | HPLC with electrochemical detection operated in the reductive mode | |
| LOQ: | Limits of quantification were 10.1 ng/mL for Arm and 10.4 ng/mL for DHA | |
| Results: | Arm not detected in any of the plasma samples | |
| | DHA measured in only 2 of the plasma samples – 2 h post-dosing in monkey 2000 (14.3 ng/mL) and 4 h post-dosing in monkey 2002 (11.8 ng/mL) | |

Additional Information: the Cynomolgus monkey was not used as toxicity species.

## 2.6.5.3D Pharmacokinetics: Absorption after single dose (DMPK(CH) 1997/240)

| **Study title:** | Disposition studies in rats and dogs after administration of Co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | Study no. DMPK(CH) 1997/240 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-4 | |
| Test article: | [$^{14}$C]-labeled Lmf (6 parts) and unlabeled Arm (1 part) | |
| Species, strain, sex, number of animals: | male rat albino/ Tif: RAIf (SPF) / 3 per group<br>male dog / Beagle / 2 per group | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7/3, w/w) for solution with at intravenous route<br>0.5% Klucel + 0.1% Tween 80 for oral route in rats<br>gelatin capsule for oral route in dogs | |
| Method/ Route / Duration of administration: | rats: intravenous bolus / tail vain / once<br>gavage / oral / once (20 mg/kg)<br>gavage / oral / once (100, 1000 mg/kg)<br>dogs: intravenous bolus / cephalic vein of a foreleg / once<br>gelatin capsule / oral / once | |
| Dose (mg/kg): | rats: intravenous dose of 1 (single)<br>oral dose of 20 (single)<br>oral dose of 100 (single)<br>oral dose of 1000 (single)<br>dogs: intravenous dose of 1 (single)<br>oral dose of 20 (single) | |
| Specific activity: | [$^{14}$C]-Lmf Batch Ko-76.1A2 of 2069 KBq/mg<br>[$^{14}$C]-Lmf Batch Ko-76.1B of 64.1 KBq/mg<br>[$^{14}$C]-Lmf Batch Ko-76.1C of 10.0 KBq/mg<br>[$^{14}$C]-Lmf Batch Ko-76.1D of 960 KBq/mg | [$^{14}$C]-Lmf Batch Ko-76.1F of 159 or 148 KBq/mg<br>[$^{14}$C]-Lmf Batch Ko-761G of 1.147 KBq/mg<br>[$^{14}$C]-Lmf Batch Ot-5.1 of 385.5 KBq/mg |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | HPLC with ultraviolet detection; liquid scintillation counting; liquid chromatography-mass spectrometry | |

| **Study title:** | Disposition studies in rats and dogs after administration of Co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | | | | | | | | Study no. DMPK(CH) 1997/240 | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Absorption results**: | | | | | | | | | | |
| **Rat** | $^{14}$C mean concentrations µmol/L | | | | | | | | | |
| Route | intravenous | | single oral (fasted) | | single oral (non-fasted) | | | | | |
| Dose (mg/kg) | 1 | | 20 | | 20 | | 100 | | 1000 | |
| Number of animals | 3 | | 3 | | 3 | | 3 | | 3 | |
| Time (h) | blood | plasma | blood | plasma | blood | plasma | blood | plasma | blood | plasma |
| 0.08 | 8.28 | 12.75 | ns | ns | ns | ns | ns | ns | ns | ns |
| 0.25 | 6.36 | 10.33 | 0.00 | 0.00 | 0.03 | 0.09 | 0.00 | 0.09 | 0.00 | 0.00 |
| 0.5 | 5.5 | 8.73 | 0.28 | 0.47 | 0.95 | 1.55 | 0.26 | 0.38 | 0.00 | 0.00 |
| 1 | 4.4 | 6.94 | 1.59 | 2.58 | 3.7 | 6.12 | 4.12 | 6.48 | 2.04 | 1.78 |
| 2 | 3.2 | 4.87 | 2.44 | 3.83 | 7.3 | 11.19 | 10.57 | 17.03 | 9.13 | 14.07 |
| 4 | 1.96 | 2.83 | 2.41 | 3.53 | 7.6 | 11.01 | 16.27 | 23.82 | 14.79 | 20.81 |
| 8 | 0.99 | 1.34 | 1.98 | 2.74 | 5.39 | 7.45 | 14.38 | 20.19 | 10.71 | 13.74 |
| 24 | 0.32 | 0.44 | 0.37 | 0.51 | 1.10 | 1.45 | 2.73 | 3.16 | 16.49 | 21.81 |
| 48 | 0.14 | 0.17 | 0.14 | 0.19 | 0.44 | 0.48 | 1.01 | 0.90 | 6.20 | 4.40 |
| 72 | 0.09 | 0.10 | 0.10 | 0.14 | ns | ns | 0.78 | 0.67 | nc | nc |
| 96 | 0.06 | 0.06 | 0.08 | 0.10 | ns | ns | 0.58 | 0.47 | nc | nc |
| 120 | 0.04 | 0.05 | 0.05 | 0.08 | ns | ns | nc | nc | nc | nc |
| 144 | 0.04 | 0.04 | 0.05 | 0.05 | ns | ns | nc | nc | nc | nc |
| AUC 0 – 144h | 42.63 | 59.29 | 48.8 | 68.9 | 123 $_{(0-48)}$ | 170 $_{(0-48)}$ | 327 | 416 | 694 | 764 |

ns= no sampling. nc = not calculated.

| **Study title:** | Disposition studies in rats and dogs after administration of Co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | | | | Study no. DMPK(CH) 1997/240 | |
|---|---|---|---|---|---|---|
| **Absorption results**: | | | | | | |
| **Dog** | $^{14}$C mean concentrations µmol/L | | | | | |
| Route | intravenous | | single oral (fasting) | | single oral (non-fasting) | |
| Dose (mg/kg) | 1 | | 20 | | 20 | |
| Number of animals | 2 | | 2 | | 2 | |
| Time (h) | blood | plasma | blood | plasma | blood | plasma |
| 0.00 | nc | nc | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.08 | 4.37 | 3.74 | ns | ns | ns | ns |
| 0.25 | 1.45 | 2.10 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.5 | 1.60 | 2.67 | 0.00 | 0.00 | 0.00 | 0.09 |
| 1 | 2.22 | 3.71 | 0.47 | 0.76 | 1.36 | 3.34 |
| 2 | 2.68 | 4.30 | 7.63 | 13.62 | 7.73 | 17.29 |
| 4 | 2.12 | 3.46 | 8.63 | 14.98 | 6.21 | 13.15 |
| 6 | 1.47 | 2.72 | 3.98 | 7.44 | 3.57 | 6.94 |
| 8 | 0.96 | 1.70 | 2.11 | 3.45 | 1.80 | 3.75 |
| 24 | 0.14 | 0.24 | 0.25 | 0.30 | 0.54 | 1.15 |
| 48 | 0.06 | 0.10 | 0.14 | 0.15 | 0.28 | 0.81 |
| 72 | 0.04 | 0.07 | 0.06 | 0.09 | 0.06 | 0.20 |
| 96 | 0.03 | 0.05 | 0.00 | 0.04 | 0.00 | 0.14 |
| 120 | 0.02 | 0.04 | 0.00 | 0.00 | 0.00 | 0.12 |
| 144 | 0.02 | 0.03 | 0.00 | 0.00 | 0.00 | 0.11 |
| 168 | 0.02 | 0.03 | 0.00 | 0.00 | 0.00 | 0.08 |
| AUC 0 – 168h | 29.9 | 50.8 | 65.8 | 109 | 67.3 | 155 |

nc = not calculated. LOQ: 0.17 µmol/L

Additional information: none

## 2.6.5.3E Pharmacokinetics: Absorption after single dose (BPK(CH) 1996/095)

| **Study title:** | Benflumetol plasma concentrations in female cynomolgus monkeys from a pilot pharmacokinetic study with a single co-artemether oral dose. | | Study no. BPK(CH) 1996/095 |
|---|---|---|---|
| GLP compliance: | not required | | |
| Location in CTD: | 4.2.2.2-5 | | |
| Test article: | Arm/Lmf: 1 part Arm and 6 parts Lmf | | |
| Species, strain, sex, number of animals: | cynomolgus monkey, macaca fascicularis, female, n = 3 | | |
| Feeding condition: | fed | | |
| Vehicle / Formulation: | gelatin capsules / | | |
| Method of Administration: | oral gavage | | |
| Dose (mg/kg): | 350 mg/kg | | |
| Sample: | plasma | | |
| Analyte: | Lmf | | |
| Assay: | HPLC with ultraviolet detection | | |
| PK parameters: | Animal 2000 | Animal 2002 | Animal 2004 |
| Tmax (h) | 8 | 12 | 12 |
| Cmax (ng/mL) | 6002.2 | 4214.1 | 5698.3 |
| AUC (ng·h/mL) | 114971.8 | 65463.4 | 125539.3 |
| T1/2 (h)<br>(time interval) | 12.3<br>(48-96) | 39.8<br>(72-120) | 27.9<br>(48-120) |

Additional Information: the Cynomolgus monkey was not used as toxicity species.

## 2.6.5.3F Pharmacokinetics: Absorption after single dose (BPK(CH) 1996/026)

| **Study title:** | Plasma concentrations of benflumetol in rats treated with single intravenous doses of 1 mg/kg. | Study no. BPK(CH) 1996/026 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-6 | |
| Test article: | Lmf | |
| Species, strain, sex, number of animals: | male rat albino / 3 per group | |
| Feeding condition: | fasted for 15 h before and 6 h after dose administration; free access to drinking water | |
| Vehicle / Formulation: | PEG 400 N-methyl-2-pyrrolidone (7/3, w/w) solution | |
| Method of Administration: | intravenous tail vein bolus | |
| Dose (mg/kg): | 1 mg/kg | |
| Sample: | plasma | |
| Analyte: | Lmf | |
| Assay: | HPLC with ultraviolet detection | |
| Results: | | |
| Tmax (h) | not measured | |
| Cmax (µg/mL) | not measured | |
| AUC0-24h (µg·h/mL) | 21.36 | |
| AUC(f) a (µg·h/mL) | 20.8 | |
| CL (mL/min·kg$^{-1}$) | 0.8 | |

a: AUC(f): AUC calculated using the fitted (2-compartment model) curve (µg·h/mL)

Additional information: none

## 2.6.5.3G Pharmacokinetics: Absorption after single dose (BPK(CH) 1996/147)

| **Study title** | Development of an HPLC assay and plasma concentrations of the two enantiomers CGP64455 S(+) and CGP 64456 R(-) of racemic benflumetol (CGP 56695) in humans, dogs and rats. | Study no. BPK(CH) 1996/147 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD | 4.2.2.1-13 | |
| Test article | racemic Lmf | |
| Species, strain, sex, number of animals: | rat, male, n=3 per group (VP 1994/034).<br>dog, Beagle, male & female, n=1 per measurement series (BPK1996/024).<br>human healthy volunteers, (HPH 9410 Study 006). | |
| Method: | HPLC assay was performed using a 250 mm x 4 mm i.d. column packed with cellulose 3-chloro-4-methylphenylcarbamate coated on Silica (Nucleosil 4000 – 10 µm). Detection was performed using a variable wavelength ultraviolet detector at 254 nm. | |
| Instrument: | HPLC: Kratos Spectroflow 783 variable wavelength ultraviolet detector | |
| Substance analyzed: | | |
| *HPLC assay* | CGP 64455 S(+) and CGP 64456 R(-) and the internal standard halofantrine | |
| *Plasma concentrations* | CGP 64455 S(+) and CGP 64456 R(-) | |
| Reference compound: | | |
| *HPLC assay* | CGP 64455 S(+) and CGP 64456 R(-) and the internal standard halofantrine | |
| *Plasma concentrations* | Human – a single dose of 480 mg Lmf<br>Dogs – repeated doses of 200 mg/kg Lmf<br>Rats – a single dose of 100 mg/kg of Lmf; and a single dose of either 50 mg/kg S(+) or 50 mg/kg R(-) | |
| Internal standard: | Halofantrine $C_{26}H_{30}NOF_3Cl_2$ | |
| Sample preparation:<br>*HPLC assay* | 0.5 mL plasma samples containing racemate / enantiomers were deproteinised with 1 mL methanol / acetic acid. These samples were mixed for 5 min on a Vortex and centrifuged for 10 min at 3000 rpm. The solvent extract was then separated / evaporated to dryness under nitrogen stream at +4°C and dry residue dissolved in 200 µL mobile phase. An aliquot (20 µL) was then transferred into microinjection vial sealed with PTFE cap. | |

| **Study title** | Development of an HPLC assay and plasma concentrations of the two enantiomers CGP64455 S(+) and CGP 64456 R(-) of racemic benflumetol (CGP 56695) in rats, dogs and humans. | Study no. BPK(CH) 1996/147 |
|---|---|---|
| Results: | | |
| HPLC assay | The chromatogram of an extract of a drug-free human plasma sample (0.5 mL) spiked with 670 ng of each of the two enantiomers and the internal standard demonstrated that the two enantiomers are well separated from plasma constituents. | |
| *S(+) enantiomer* | Five human plasma samples in the concentration of 450 and 2060 ng/mL, analyzed on 2 to 5 different days, exhibited inter-assay coefficient of variations (CVs) ranging from 3.8 to 26.2% and deviations of the mean found values from the given concentrations ranging from -8.0 to 10.2%.<br><br>Four spiked human samples analyzed 3 times on the same day demonstrated CVs ranging from 2.6 to 16.5% and a deviation from theory ranging from -8.4 to 12.5%. | |
| *R(-) enantiomer* | Five human plasma samples in the concentration range 480 to 2370 ng/mL analyzed on 4 to 5 different days exhibited inter-assay CVs ranging from 3.1 to 18.1% and deviations of the mean found values from the given concentrations ranging from -5.2 to 10.2%.<br><br>Four spiked human samples analyzed 3 times on the same day demonstrated CVs ranging from 6.7 to 19.4% and a deviation from theory ranging from -13.3 to 7.4%. | |
| Results: | | |
| LOQ | | |
| *S(+) enantiomer* | 1.1 µg/mL | |
| *R(-) enantiomer* | 0.5 µg/mL | |
| LOD | | |
| *S(+) enantiomer* | 0.11 µg/mL | |
| *R(-) enantiomer* | 0.05 µg/mL | |
| Relative recovery | Not measured | |
| Stability | Not measured | |

| **Study title** | Development of an HPLC assay and plasma concentrations of the two enantiomers CGP64455 S(+) and CGP 64456 R(-) of racemic benflumetol (CGP 56695) in rats, dogs and humans. | | | | Study no. BPK(CH) 1996/147 | | | |
|---|---|---|---|---|---|---|---|---|
| Plasma concentrations: | | | | | | | | |
| Species | **Rat** n = 3 at each time point for each dose | | | | | | | |
| Dose (mg/kg) | 50 | | 100 | | 50 | | 100 | |
| Time (hours) | mean plasma concentrations of S(+) in µg/mL | | | | mean plasma concentrations of R(-) in µg/mL | | | |
| 0 | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| 1 | 0.08 | | 0.79 | | 0.66 | | 0.82 | |
| 2 | 0.32 | | 0.77 | | 0.96 | | 0.90 | |
| 4 | 0.25 | | 1.42 | | 0.56 | | 1.99 | |
| 8 | 0.19 | | 1.14 | | 0.54 | | 2.02 | |
| 24 | 0.00 | | 0.86 | | 0.04 | | 1.22 | |
| Species | **Dog** n = 1 per series (sample source as described in Report BPK(CH) 1996/024) | | | | | | | |
| Dose (mg/kg) | 200 | | | | 200 | | | |
| | plasma concentrations of S(+) in µg/mL | | | | plasma concentrations of R(-) in µg/mL | | | |
| Gender | Male | | Female | | Male | | Female | |
| Time (hours) | Day 1 | Day 89 | Day 1 | Day 89 | Day 1 | Day 89 | Day 1 | Day 89 |
| 0 | not detected | 5.70 | not detected | 5.46 | not detected | 5.62 | not detected | 5.40 |
| 1 | not detected | 4.27 | not detected | 5.63 | not detected | 4.36 | not detected | 5.79 |
| 2 | not measured | not measured | not measured | 4.55 | not measured | not measured | not measured | 4.54 |
| 4 | not measured | not measured | 0.53 | 3.73 | not measured | not measured | 0.36 | 4.08 |
| 6 | not detected | 2.34 | not measured | not measured | not detected | 2.43 | not measured | not measured |
| 8 | not measured | 2.06 | not detected | 2.32 | not measured | 1.96 | not detected | 2.18 |
| 24 | 6.21 | 1.35 | 5.57 | not detected | 5.67 | 1.43 | 5.31 | not detected |

| **Study title** | Development of an HPLC assay and plasma concentrations of the two enantiomers CGP64455 S(+) and CGP 64456 R(-) of racemic benflumetol (CGP 56695) in rats, dogs and humans. | Study no. BPK(CH) 1996/147 |
|---|---|---|
| Species | **Human** n = 1 at all time points for each of the enantiomers (sample source HPH 9410 Study 006) | |
| Dose (mg) | Arm/Lmf 20/120 × 4 tablets | Arm/Lmf 20/120 × 4 tablets |
| Time (hours) | plasma concentrations of S(+) in µg/mL | plasma concentrations of R(-) in µg/mL |
| 1.5 | not detected | not detected |
| 2 | not detected | not detected |
| 3 | 0.21 | 0.17 |
| 4 | 0.46 | 0.39 |
| 6 | 0.39 | 0.43 |
| 8 | 0.28 | 0.35 |
| 10 | 0.19 | 0.29 |
| 12 | 0.24 | 0.20 |
| 15 | not detected | 0.15 |
| 24 | not detected | not detected |
| 34 | not detected | not detected |
| 48 | not detected | not detected |

**Results and conclusions:**

Rats – After a single dose of 100 mg/kg of Lmf, the plasma concentration of the R(-) enantiomer is higher at each time period than the plasma concentration of the S(+) enantiomer. This difference in plasma concentration at each time period was also seen when a single dose of 50 mg/kg S(+) enantiomer or of 50 mg/kg R(-) enantiomer was administered.

Dogs – After repeated doses of 200 mg/kg Lmf given daily over 89 days, plasma concentrations of S(+) and R(-), in both male and female dogs, were similar at each time period.

Humans – After a single dose of 480 mg Lmf, plasma concentrations of S(+) and R(-) were similar at each time period.

Addditional information: none

# 2.6.5.4 薬物動態試験：反復投与後の吸収

## 2.6.5.4A Pharmacokinetics: Absorption after repeated doses (DMPK R0570030)

| Study title: | 2-week oral (gavage) toxicokinetic study in rats. Toxicokinetic report: Determination and toxicokinetics of artemether and its metabolite in rat plasma | Study no.<br>DMPK R0570030 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.7.7-5 | |
| Test article: | Arm | |
| Method and objectives | The objectives of this study are to determine:<br>The concentrations of Arm and its metabolite in rat plasma samples from the toxicity study [0570030].<br>The main pharmacokinetic parameters of Arm and its metabolite DHA in male and female rats after a 2-week repeated oral administration. | |
| Matrix | Rat plasma | |
| Administration form | Suspension in 0.5% Klucel with 0.1% Tween-80 | |
| Route and frequency of administration | Oral administration, once daily for 2 weeks | |
| Species | Male and female IGS Wistar Hannover rats | |
| Dose groups and no. of animals in TK-investigation | Control: Group 1: n=10 per sex and per dose group<br>0 mg/kg/day<br>Arm: Group 2: n=10 per sex and per dose group<br>20 mg/kg/day<br>Arm: Group 3: n=10 per sex and per dose group<br>200 mg/kg/day<br>Note: The control group was dosed with the vehicle. | |
| Sampling schedules | Toxicokinetic blood sampling time:<br>Blood was obtained from all surviving study animals on study days 1-2, 3-4 and 14-15. Two animals/sex/group were bled at 0.5, 1, 2, 8 and 24 hours post-dose. | |
| Bioanalytical method | According to Analytical Method Report [DMPK R0500059]<br>Liquid/liquid extraction with 1-chlorobutane-isooctane (55:45; v/v) followed by reversed phase liquid chromatography with tandem mass spectrometric detection. | |
| *Reference compounds:* | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$<br>Molecular weight (MW): 298.37 g/mol | |

| Study title: | 2-week oral (gavage) toxicokinetic study in rats. Toxicokinetic report: Determination and toxicokinetics of artemether and its metabolite in rat plasma | Study no. DMPK R0570030 |
|---|---|---|

DHA — Molecular formula: $C_{15}H_{24}O_5$; Molecular weight (MW): 284.35 g/mol

*Internal standards:*

Arteether — Molecular formula: $C_{17}H_{28}O_5$; Molecular weight (MW): 312.40 g/mol

Artemisinin — Molecular formula: $C_{15}H_{22}O_5$; Molecular weight (MW): 282.33 g/mol

*LLOQ* — 20.0 ng/mL expressed as base, using 100 µL of rat plasma

*Reporting of concentrations below LLOQ* — Below the lower limit of quantification (20.0 ng/mL)

Results-1:

Stability in matrix — Stable after 4 hours of storage in an ice bath, stable after 3 freeze/thaw cycles (freezing at -75°C±10°C and thawing in an ice bath), stable after 2 months of storage at -75°C±10°C, stable in extracts after 48 hours of storage in the auto sampler at room temperature.

Concentrations in plasma of Arm — Toxicokinetic parameters of Arm in rat plasma

| Gender | Treatment | Day | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) | Cmax/Dose (ng/mL)/(mg/kg/day) | AUC0-24h/Dose (ng·h/mL)/(mg/kg/day) |
|---|---|---|---|---|---|---|---|
| Male | Low (20 mg/kg/day) | 1 | 107 | 0.5 | 104 | 5.35 | 5.21 |
| | | 3 | 132 | 0.5 | 203 | 6.60 | 10.16 |
| | | 14 | 38.5 | 0.5 | 30 | 1.93 | 1.48 |
| | High (200 mg/kg/day) | 1 | 815 | 1 | 3417 | 4.08 | 17.09 |
| | | 3 | 635 | 0.5 | 700 | 3.18 | 3.50 |
| | | 14 | 65.2 | 0.5 | 198 | 0.33 | 0.99 |
| Female | Low (20 mg/kg/day) | 1 | 130 | 0.5 | 309 | 6.50 | 15.43 |
| | | 3 | 174 | 1 | 240 | 8.70 | 12.01 |
| | | 14 | 122 | 0.5 | 177 | 6.10 | 8.83 |
| | High (200 mg/kg/day) | 1 | 3230 | 1 | 16990 | 16.15 | 84.95 |
| | | 3 | 1250 | 1 | 2982 | 6.25 | 14.91 |
| | | 14 | 402 | 1 | 1243 | 2.01 | 6.22 |

| Study title: | 2-week oral (gavage) toxicokinetic study in rats. Toxicokinetic report: Determination and toxicokinetics of artemether and its metabolite in rat plasma | | | | | | Study no. DMPK R0570030 | |
|---|---|---|---|---|---|---|---|---|
| Concentrations in plasma of DHA | Toxicokinetic parameters of DHA in rat plasma | | | | | | | |
| | Gender | Treatment | Day | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) | Cmax/Dose (ng/mL)/ (mg/kg/day) | AUC0-24h/Dose (ng·h/mL)/ (mg/kg/day) |
| | Male | Low (20 mg/kg/day) | 1 | 194 | 0.5 | 234 | 9.70 | 11.68 |
| | | | 3 | 87.3 | 1 | 174 | 4.37 | 8.70 |
| | | | 14 | 57.5 | 0.5 | 45 | 2.88 | 2.27 |
| | | High (200 mg/kg/day) | 1 | 2630 | 2 | 12103 | 13.15 | 60.51 |
| | | | 3 | 435 | 0.5 | 1080 | 2.18 | 5.40 |
| | | | 14 | 141 | 1 | 365 | 0.71 | 1.83 |
| | Female | Low (20 mg/kg/day) | 1 | 83.5 | 1 | 206 | 4.18 | 10.32 |
| | | | 3 | 96.7 | 1 | 115 | 4.84 | 5.73 |
| | | | 14 | 82.3 | 1 | 87 | 4.12 | 4.36 |
| | | High (200 mg/kg/day) | 1 | 1390 | 2 | 7160 | 6.95 | 35.80 |
| | | | 3 | 1400 | 1 | 4384 | 7.00 | 21.92 |
| | | | 14 | 592 | 1 | 2038 | 2.96 | 10.19 |

| Study title: | 2-week oral (gavage) toxicokinetic study in rats. Toxicokinetic report: Determination and toxicokinetics of artemether and its metabolite in rat plasma | Study no. DMPK R0570030 |
|---|---|---|
| Discussion and Conclusion | In the control group, plasma concentrations of Arm and its metabolite were found below the limit of quantification (20.0 ng/mL) in all samples collected except for male rat no. 06 on day 1 at 1 h after dosing. For this animal, the plasma concentration of Arm was 43.4 ng/mL while plasma concentration of DHA was below the LLOQ. In all treated male animals on day 1, plasma concentrations of the metabolite were always higher than plasma concentrations of Arm. This suggests that male rat no. 06 was not exposed to Arm.<br>Maximal plasma concentrations of Arm were observed between 0.5 and 1 h after dosing whatever the dose, the day and the gender while $C_{max}$ of DHA was observed between 0.5 and 2 h for male rats and between 1 and 2 h for female rats, whatever the dose and the day.<br>Based on AUC0-24h, the exposure to Arm and its metabolite was lower on day 3 or day 14 than on day 1 whatever the dose for male and female rats except after dosing with 20 mg/kg/day for male rats for which AUC0-24h of Arm was higher on day 3 compared to day 1.<br>Based on AUC0-24h, it appeared that:<br>In male rats, the exposure to Arm and its metabolite increased more than dose proportionally on day 1 but less than dose proportionally on day 3 and day 14. Arm AUC0-24h increased in a ratio of 33, 3 and 7 on day 1, day 3 and day 14, respectively, for an increase in dose of 10. DHA AUC0-24h increased in a ratio of 52, 6 and 8 on day 1, day 3 and day 14, respectively.<br>In female rats, the exposure to Arm increased more than dose proportionally on day 1 and day 3 but increased less than dose proportionally on day 14. AUC0-24h increased in a ratio of 55, 12 and 7 on day 1, day 3 and day 14, respectively for an increase in dose of 10. By contrast, the exposure to DHA increased more than dose proportionally whatever the day. AUC0-24h increased in a ratio of 35, 38 and 23 on day 1, day 3 and day 14 for an increase in dose of 10. AUC0-24h increased in a ratio of about 2 whatever the day for an increase in dose of 4. | |
| Special issues: | 1. For DHA, the reference standard is a mixture of α and β anomers. The extract ratio between both anomers is not known, the α-anomer being the predominant form. For this reference standard, only the α-anomer was considered and supposed to be 100% of this reference standard. So, only an evaluation of the α-anomer metabolite concentration was done in the plasma samples, thus the metabolite concentrations were reported as indicative levels.<br>2. In the control group, plasma concentrations of Arm and its metabolite were found below the limit of quantification (20.0 ng/mL) in all samples collected except for male rat no. 06 on day 1 at 1 h after dosing. For this animal, the plasma concentration of Arm was 43.4 ng/mL while plasma concentration of DHA was below the LLOQ. In all treated male animals on day 1, plasma concentrations of the metabolite were always higher than plasma concentrations of Arm. This suggests that male rat no. 06 was not exposed to Arm. | |

Additional information: Supplement to the Study no. 0570030. 2-week oral (gavage) toxicokinetic study in rats. No toxicological endpoints were assessed in this study.

## 2.6.5.4B Pharmacokinetics: Absorption after repeated doses (BPK(F) 1996/007)

| | | |
|---|---|---|
| Study title: | Artemether and DHA concentrations in male and female rats on days 1 and 91 of a 3-month pharmacokinetic study following oral administration of co-artemether daily doses of 100, 300 and 1000 mg/kg body weight. | Study no. BPK(F) 1996/007 |

| | |
|---|---|
| GLP compliance: | Yes |
| Location in CTD: | 4.2.3.2-2 |
| Species, strain, sex, number of animals: | male and female rat albino / Tif: RAIf (SPF) / 6 per group |
| Feeding condition: | fed |
| Test article: | Arm/Lmf as 1 part Arm and 6 parts Lmf |
| Vehicle / formulation | suspension / Polysorbate 80 (Tween 80) suspended in 0.5% w/v aqueous cellulose-2-hydroxypropyl ether (Klucel HF) to a final concentration of polysorbate 80 of 0.1% w/v. |
| Method of administration: | oral gavage |
| Duration of dosing: | once daily for 3 months |
| Daily dose (mg/kg) | 0 (control), m+f, n=6<br>100 (group 2), m+f, n=12<br>300 (group 3),m+f, n=12<br>1000 (group 4),m+f, n=12 |
| Sample: | plasma |
| Analyte: | Arm and DHA |
| Assay: | HPLC with electromechanical detection operated in reductive mode |
| Results: | • In the Control Groups, Arm and DHA were not detected in any plasma samples.<br>• In Group 2, Arm was detected on Day 1 at 1 hour post dose in 3 samples, and on Day 91 in 1 sample.<br>• DHA was detected on Day 1 at 1 hour post dose in 5 samples and on Day 91 at 1 hour post dose in 1 sample.<br>• In Group 3, Arm was detected on Day 1 at 1 hour post dose in 6 samples, at 2 hours post dose in 6 samples, and at 4 hours post dose in 3 samples; and on Day 91 at 1 hour post dose in 2 samples and at 4 hours post dose in 1 sample.<br>• DHA was detected on Day 1 at 1 hour post dose in 6 samples, at 2 hours post dose in 5 samples, and at 4 hours post dose in 3 samples; and on Day 91 at 1 hour post dose in 2 samples, at 2 hours post dose in 1 sample, and at 4 hours post dose in 1 sample.<br>• In addition, one sample on Day 1 at 24 hours post dose demonstrated DHA concentrations.<br>• Mean C1h was 285 ng/mL for Arm and 227 ng/mL for DHA; whereas, mean C2h was 47 ng/ml for Arm and 31 ng/ml for DHA. These C2h values represent about 15% of the values for C1h denoting a rapid decrease of both compounds in 1 hour. |

| Study title: | Artemether and DHA concentrations in male and female rats on days 1 and 91 of a 3-month pharmacokinetic study following oral administration of co-artemether daily doses of 100, 300 and 1000 mg/kg body weight. | Study no. BPK(F) 1996/007 |
|---|---|---|

- In Group 4, Arm was detected on Day 1 at 1 hour post dose in 6 samples, at 2 hours post dose in 6 samples, at 4 hours post dose in 5 samples, and at 8 hours post dose in 3 samples; and on Day 91 at 1 hour post dose in 3 samples, at 2 hours post dose in 2 samples, and at 4 hours post dose in 2 samples.
- DHA was detected on Day 1 at 1 hour post dose in 6 samples, at 2 hours post dose in 6 samples, and at 4 hours post dose in 6 samples; and on Day 91 at 1 hours post dose in 4 samples, at 2 hours post dose in 1 sample, at 4 hours post dose in 4 samples, and at 8 hours post dose in 1 sample.
- Mean C1h was 838 ng/mL for Arm and 609 ng/mL for DHA; whereas, mean C2h was 574 ng/mL and 330 ng/mL, and mean C4h was 154 ng/mL and 62 ng/mL for Arm and DHA, respectively. These C2h and C4h values represent about 68% and 18% for Arm and 54% and 10% for DHA of the values for C1h, which denotes a rapid disappearance of both compounds from the plasma.
- This rapid disappearance from the plasma was less notable at 300 mg/kg.
- The rapid disappearance of Arm and DHA from plasma on Day 1 after the first dose indicates a high clearance of both compounds.

Additional information: Supplement to toxicity study no. 946153.

## 2.6.5.4C Pharmacokinetics: Absorption after repeated doses (DMPK R0570013)

| Study title: | An oral (gavage) juvenile development study in rats. Toxicokinetic report: Determination and toxicokinetics of artemether and its metabolite in rat plasma. | Study no. DMPK R0570013 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.5.4-3 | |
| Test article: | Arm | |
| Method and objectives | The objectives of this study are to determine:<br>• The concentrations of Arm and its metabolite in rat plasma samples from toxicity study 0570013.<br>• The main toxicokinetic parameters of Arm and its metabolite (DHA) in juvenile male and female rats. | |
| Matrix | Rat plasma | |
| Administration form | Suspension in 0.5% Klucel with 0.1% Tween-80 | |
| Route and frequency of administration | Oral, single and multiple dosing | |
| *Main study and recovery animals* | Oral, once daily from post partum day 7 to post partum day 21 | |
| *Single dose animals:* | Oral, once on post partum day 7 | |
| Species | IGS Wistar Hannover rats | |
| Dose groups and no. of animals in TK-investigation | Main study animals: n=16 per sex and dose group<br>0, 10, 30, 100 mg/kg<br>Recovery animals: n=12 per sex and dose group<br>0, 10, 30, 100 mg/kg<br>Single dose toxicokinetic group: n=16 per sex and dose group<br>0, 10, 30, 100 mg/kg | |
| Sampling schedules | Toxicokinetic blood sampling times:<br>Main study animals: Plasma samples were obtained on study day 21 at 1, 2, 4 and 24 hours after the final dose (n=3/sex/group).<br>Single dose animals: Plasma samples were collected on day 7 at 1, 2, 4 and 24 hours after the single dose (n=4/sex/group).<br>For Main study animals there was no terminal TK collection for Group 4 due to severe mortality. | |
| Bioanalytical method | According to Analytical Method Report DMPK R0500059.<br>Liquid/liquid extraction with 1-chlorobutane-isooctane (55:45; v/v) followed by reverse phase liquid chromatography with tandem mass spectrometric detection. | |
| *Reference compounds:* | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$<br>Molecular weight (MW): 298.37 g/mol | |

| Study title: | An oral (gavage) juvenile development study in rats. Toxicokinetic report: Determination and toxicokinetics of artemether and its metabolite in rat plasma. | Study no.<br>DMPK R0570013 |
|---|---|---|

DHA — Molecular formula: $C_{15}H_{24}O_5$; Molecular weight (MW): 284.35 g/mol

*Internal standards:*

Arteether — Molecular formula: $C_{17}H_{28}O_5$; Molecular weight (MW): 312.40 g/mol

Artemisinin — Molecular formula: $C_{15}H_{22}O_5$; Molecular weight (MW): 282.33 g/mol

*LLOQ* — 20.0 ng/mL expressed as base using 0.1 mL of rat plasma

*Reporting of concentrations below LLOQ* — Below the lower limit of quantification (20.0 ng/mL)

Additional test performed using the remaining plasma samples from the single-dose TK animals control group — The remaining plasma samples from the Single-Dose TK animals control group were mixed to obtain two different pools. Each pool was spiked at 160 ng/mL with Arm and DHA and was analyzed with C standards and QC samples prepared in blank rat plasma.

Results-1: — For the two different pools, a tremendous decrease of the chromatographic response was observed for the two internal standards and for Arm and DHA. This decrease was erratic and not reproducible from one sample to another. Consequently, the bias and the precision were not acceptable.

Stability in matrix — Stable after 4 hours of storage in an ice bath, stable after 3 freeze/thaw cycles (freezing at -75°C±10°C and thawing in an ice bath), stable after 5 months of storage at -75°C±10°C, stable in extracts after 48 hours of storage in the auto sampler at room temperature.

*Stability for Arm* — Stable after 22 days of storage at -24°C ± 6°C

*Stability for DHA* — Apparent loss after 22 days of storage at -24°C ± 6°C

Concentrations in plasma of Arm and DHA

Toxicokinetic parameters of Arm and DHA in rat plasma
Group 3 (30 mg/kg/day – Day 21)

| | Gender | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) | Cmax/Dose (ng/mL)/ (mg/kg/day) | AUC0-24h/Dose (ng·h/mL)/ (mg/kg/day) |
|---|---|---|---|---|---|---|
| Arm | Male | 129 | 2 | 231.10 | 4.30 | 7.70 |
| | Female | 94.7 | 1 | 263.00 | 3.16 | 8.77 |
| DHA | Male | 129 | 2 | 200.47 | 4.30 | 6.68 |
| | Female | 73.5 | 1 | 73.50 | 2.45 | 2.45 |

Due to analytical problems with juvenile plasma samples collected from the Single-Dose TK groups, no results could be given on day 7. Nevertheless, during the first determination, it was observed that the animals of the Single-Dose TK animals control group were not exposed to the drug as no chromatographic response was detected for Arm and DHA.

Only plasma concentrations obtained in the Main Study animals on day 21 could be used.

In the control group, plasma concentrations of Arm and its metabolite were found below the lower limit of quantification (20.0 ng/mL)

| Study title: | An oral (gavage) juvenile development study in rats. Toxicokinetic report: Determination and toxicokinetics of artemether and its metabolite in rat plasma. | Study no. DMPK R0570013 |
|---|---|---|
| | in all samples collected.<br>In the low dose group (10 mg/kg/day), plasma concentrations of Arm and its metabolite were found below the lower limit of quantification in all samples collected in male and female rats.<br>In the mid dose group (30 mg/kg/day), maximal plasma concentrations of Arm and its metabolite were observed 2 hours after dosing in male rats and 1 hour after dosing in female rats.<br>Based on AUC0-24h:<br>The exposure to Arm was quite comparable in male and female rats;<br>The exposure to DHA was higher in males compared to females;<br>The metabolic ratio between DHA and Arm was higher in males (0.9) compared to females (0.3).<br>Due to severe mortality in the high dose group (100 mg/kg/day), administration was stopped and no terminal TK samples were collected; consequently, no TK parameters were available. | |
| Discussion and Conclusion | 1. Group 4 animals stopped dosing prior to 13-Apr-2005 due to a severe mortality. All remaining Group 4 animals were sacrificed on post partum day 22 for cochlear evaluations.<br>2. For DHA, the reference standard is a mixture of α and β anomers. The exact ratio between both anomers is not known, the α-anomer being the predominant form. For this reference standard, only the α-anomer was considered and supposed to be 100% of this reference standard. Therefore, only an evaluation of the α-anomer metabolite concentration was done in the plasma samples, thus the metabolite concentrations were reported as indicative levels.<br>3. The study samples were stored at approximately -20°C for 15 days at the test facility and then they were transferred at approximately -70°C until the shipment to [redacted] As the study samples were not stored immediately after collection at approximately -70°C, the stability of Arm and DHA in rat plasma at -24°C ± 6°C for 22 days was tested during the analytical method validation (Method Validation Report CP045502 / DMPK R0500059). Arm was stable in rat plasma at -24°C ± 6°C for 22 days; nevertheless, apparent loss was observed for DHA (% Difference/Time=0 between -22.66% to -27.85%).<br>4. During the analysis, a problem was met with the samples collected from the Single-Dose TK animals. It appeared that something disturbed the determination in the plasma of the very juvenile rat (day 7). Indeed, for these samples, the response of the internal standards (arteether and artemisinin) was lower than the response measured for Cs, QCs and samples collected from Main Study animals. Then, during the analytical method validation (Method Validation Report CP045502 / DMPK R0500059), blank rat plasma collected from juvenile rat aged of 7 days was tested, and the problem observed with the plasma of the Single-Dose TK animals group was not encountered.<br>In agreement with the Study Director, the remaining plasma samples from the Single-Dose TK animals control group were used to perform additional tests. For the two internal standards, but also for Arm and DHA, a tremendous decrease of the chromatographic response was observed. This decrease was erratic and not reproducible from one sample to another. Something happened in the plasma of the juvenile rats aged of 7 days from this analytical study, and this phenomenon was not under control. Then, no results were reported on day 7. | |

Additional information: Supplement to toxicity study no. 0570013.

## 2.6.5.4D Pharmacokinetics: Absorption after repeated doses (DMPK R0510009B)

| Study title: | Oral neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and dihydroartemisinin in dog plasma | Study no. DMPK R0510009B |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.7.3-2 | |
| Test article: | Arm | |
| Method and objectives | The objectives of this study are to determine:<br>• The concentrations of Arm and its metabolite (DHA) in dog plasma samples from toxicity study [0510009].<br>• The main toxicokinetic parameters of Arm and its metabolite in male dogs after repeated oral administration. | |
| Matrix | Dog plasma | |
| Administration form | Suspension | |
| Route and frequency of administration | Oral (gavage), once daily administration, at least 3 or 8 days | |
| Species, strain, sex | Beagle dogs, male | |
| Dose groups and no. of animals in TK-investigation | Control: Group 1: n=3, 0 mg/kg/day from toxicity study 0510001.<br>Arm: Group 2, 3, and 4: n=3 per group, and dose group was 600/300 mg/kg/day<br>Note: As of day 2, dose was reduced from 600 to 300 mg/kg/day for groups 2, 3, and 4.<br>Lmf: Group 5, 6, and 7: n=3 per group, and dose group and 857 mg/kg/day Lmf + 143 mg/kg/day Arm | |
| Sampling schedules | Toxicokinetic blood sampling times:<br>Toxicokinetic blood sampling time: On day 1, day 3 and day 7, blood was taken at the following time points: 15 min, 30 min, 1, 2, 3, 6 and 24 hours post-dose<br>Animals of Groups 3 and 4 were grouped together for results and TK analysis, as well as animals of Groups 6 and 7 as they received the same dose<br>Animals of Group 2 (151 − 153) and Group 5 (160 − 162) were excluded from TK sampling<br>210 samples were analyzed | |
| Bioanalytical method | According to Analytical Method Report [DMPK R0500059].<br>Liquid/liquid extraction with 1-chlorobutane-isooctane (55:45; v/v) followed by reverse phase liquid chromatography with tandem mass spectrometric detection (Method validation report CP045360). | |
| *Reference compounds:* | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$<br>Molecular weight (MW): 298.37 g/mol | |
| DHA | Molecular formula: $C_{15}H_{24}O_5$<br>Molecular weight (MW): 284.35 g/mol | |

| Study title: | Oral neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and dihydroartemisinin in dog plasma | Study no. DMPK R0510009B |
|---|---|---|
| *Internal standards:* | | |
| Arteether | Molecular formula: $C_{17}H_{28}O_5$ | |
| | Molecular weight (MW): 312.40 g/mol | |
| Artemisinin | Molecular formula: $C_{15}H_{22}O_5$ | |
| | Molecular weight (MW): 282.33 g/mol | |
| *LLOQ* | 5.00 ng/mL (for both compounds) expressed as base, using 250 µL of plasma | |
| *Reporting of concentrations below LLOQ* | 0 ng/mL | |
| Results: | | |
| Stability in matrix | Stable in extracts at room temperature for 78.5 hours, stable for at least 4.5 hours at room temperature and stable after 3 freeze-thaw cycles (freezing in a freezer at –75°C±10°C) and stable in spiked dog plasma after storage at –75°C±10°C for 4.5 months for Arm (Method validation report CP045360). | |
| Concentrations in plasma of Arm | Mean toxicokinetic parameters of Arm in dog plasma | |

| | | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) | Cmax/Dose (ng/mL)/ (mg/kg/day) | AUC0-24h/ Dose (ng·h/mL)/ (mg/kg/day) |
|---|---|---|---|---|---|---|
| Group 3&4 | | Arm alone | | | | |
| Day 1 (N=6) | Mean | 3358 | 2.33 | 22479 | 5.60 | 37.47 |
| (600 mg/kg/day) | SD | 1666 | 0.82 | 12729 | 2.78 | 21.21 |
| Day 3 (N=6) | Mean | 130 | 1.75 | 602 | 0.44 | 2.01 |
| (300 mg/kg/day) | SD | 131 | 0.61 | 448 | 0.44 | 1.50 |
| Day 7 (N=3) | Mean | 49 | 0.83 | 73 | 0.16 | 0.24 |
| (300 mg/kg/day) | SD | 28 | 0.29 | 28 | 0.09 | 0.10 |
| Group 6&7 | | Arm + Lmf | | | | |
| Day 1## (N=6) | Mean | 486 | 1.17 | 1294 | 3.40 | 9.05 |
| (143 mg/kg/day) | SD | 587 | 0.68 | 1936 | 4.10 | 13.54 |
| Day 3## (N=6) | Mean | 29 | 1.04 | 52 | 0.20 | 0.37 |
| (143 mg/kg/day) | SD | 11 | 0.78 | 28 | 0.08 | 0.20 |
| Day 7## (N=3) | Mean | 18 | 0.33 | 16 | 0.13 | 0.11 |
| (143 mg/kg/day) | SD | 8 | 0.14 | 5 | 0.06 | 0.03 |

| Study title: | Oral neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and dihydroartemisinin in dog plasma | | | | | | Study no. DMPK R0510009B | |
|---|---|---|---|---|---|---|---|---|
| | ##Dose ofArm/Lmf = 143 mg/kg/day of Arm + 857 mg/kg/day of Lmf | | | | | | | |
| Concentrations in plasma of DHA | Mean toxicokinetic parameters of DHA in dog plasma | | | | | | | |
| | | | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) | Cmax/Dose (ng/mL)/ (mg/kg/day) | AUC0-24h/ Dose (ng·h/mL)/ (mg/kg/day) | RAUC0-24h* |
| | Group 3&4 | | Arm alone | | | | | |
| | Day 1 (N=6) | Mean | 2572 | 1.67 | 13375 | 4.29 | 22.29 | 0.70 |
| | (600 mg/kg/day) | SD | 606 | 0.52 | 5570 | 1.01 | 9.28 | 0.25 |
| | Day 3 (N=6) | Mean | 609 | 1.67 | 1389 | 2.03 | 4.63 | 2.17 |
| | (300 mg/kg/day) | SD | 624 | 0.82 | 1374 | 2.08 | 4.58 | 1.37 |
| | Day 7 (N=3) | Mean | 144 | 1.00 | 186 | 0.48 | 0.62 | 2.88 |
| | (300 mg/kg/day) | SD | 44 | 0.00 | 48 | 0.15 | 0.16 | 1.33 |
| | Group 6&7 | | Arm + Lmf | | | | | |
| | Day 1## (N=6) | Mean | 1027 | 1.17 | 2253 | 7.18 | 15.75 | 4.77 |
| | (143 mg/kg/day) | SD | 579 | 0.68 | 1848 | 4.05 | 12.93 | 3.52 |
| | Day 3## (N=6) | Mean | 187 | 1.25 | 363 | 1.31 | 2.54 | 5.61 |
| | (143 mg/kg/day) | SD | 175 | 0.61 | 396 | 1.22 | 2.77 | 3.87 |
| | Day 7## (N=3) | Mean | 9 | 0.50 | 7 | 0.06 | 0.05 | 0.52 |
| | (143 mg/kg/day) | SD | 3 | 0.00 | 4 | 0.02 | 0.03 | 0.39 |
| | ##Dose of Arm/Lmf = 143 mg/kg/day of Arm + 857 mg/kg/day of Lmf | | | | | | | |
| | *Ratio of AUC0-24h between DHA and Arm | | | | | | | |
| Discussion and Conclusion: | Maximal plasma concentrations of Arm and its metabolite (DHA) were observed between 0.25 and 3 h whatever the dose and the day.<br>Based on dose normalized AUC0-24h, it appeared that the exposure to Arm and its metabolite was decreased after repeated administration of Arm alone or in association with Lmf.<br>Based on dose normalized AUC0-24h, it appeared that the exposure to Arm and its metabolite was higher when Arm was administered alone compared to the association with Lmf whatever the dose and the day.<br>Based on AUC0-24h, it appeared that the metabolic ratio between DHA and Arm increased after repeated administration of Arm alone (from 0.70 on day 1 to 2.88 on days 7). After administration of the association Arm/Lmf, the metabolic ratio was higher on day 3 compared to day 1 (5.61 *vs* 4.77) and was lower on day 7 (0.52) compared to day 3 (5.61) and day 1 (4.77).<br>Of note is the higher inter-animal variability observed on AUC0-24h for Arm and its metabolite whatever the dose and the day. | | | | | | | |

| | | |
|---|---|---|
| Study title: | Oral neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and dihydroartemisinin in dog plasma | Study no.<br>DMPK R0510009B |
| Special issues: | For DHA, the reference standard was a mixture of α and β anomers. The exact ratio between both anomers was not known, the α-anomer being the predominant form. For this reference standard, only the α-anomer was assumed to be 100% of this reference standard. So, only an evaluation of the -anomer metabolite concentration was done in the plasma samples, thus the metabolite concentrations were reported as indicative levels.<br>At the request of the Sponsor, the animals of Groups 3 and 4 were grouped together as they received the same dose. Moreover, animals of Groups 6 and 7 were also grouped together. As this process could not be performed by Watson® LIMS, the concentrations and pharmacokinetic tables were transferred into Microsoft® Excel. Then the animals were grouped together and the descriptive statistics were calculated on the two new groups (Groups 10 and 40) using Microsoft® Excel.<br>Due to analytical problems, some samples were extracted and analyzed again.<br>No stability data are available for DHA to storage in dog plasma at –75°C ± 10°C. | |

Additional information: Supplement to toxicity study no. 0510009.

## 2.6.5.4E Pharmacokinetics: Absorption after repeated doses (BPK(F) 1996/005)

| | | |
|---|---|---|
| Study title: | Artemether and dihydroartemisinin concentrations in male and female dogs on days 1 and 89 of a 3-month toxicity study following oral administration of Co-artemether at daily doses of 20, 60 and 200 mg/kg body weight. | Study no. BPK(F) 1996/005 |
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.2-4 | |
| Test article: | Arm/Lmf as 1 part Arm and 6 parts Lmf | |
| Species, strain, sex, number animals | dog, Beagle, M / 12; F / 12 | |
| Feeding condition: | fed | |
| Vehicle / formulation | gelatin capsules | |
| Method of administration: | oral | |
| Duration of dosing: | once daily for 3 months | |
| Daily dose (mg/kg) | 0 (control)<br>20 (group 2)<br>60 (group 3)<br>200 (group 4) | |
| Sample: | plasma | |
| Analyte: | Arm and DHA | |
| Assay: | HPLC with electromechanical detection operated in reductive mode | |
| Results: | In the Control Groups, Arm and DHA were not detected in any plasma samples.<br>In Group 2, Arm and DHA were not detected in any plasma samples or were below the limit of quantification.<br>In Group 3, Arm and DHA were not detected in any plasma samples or were below the limit of quantification.<br>In Group 4, Arm was detected on Day 1 at 1 hour post dose in 1 female. DHA was detected on Day 1 at 2 hours post dose in 1 male.<br>Otherwise, neither Arm nor DHA were detected in any of the other samples. | |

**Additional information**: Supplement to toxicity study no. 946155

## 2.6.5.4F Pharmacokinetics: Absorption after repeated doses (BPK(F) 1997/004)

| | | |
|---|---|---|
| Study title: | Artemether and dihydroartemisinin plasma and cerebrospinal fluid concentrations in male dogs in a 5 days (interim) or 30 days (main) neurotoxicity study following i.m. administration of artemether daily doses of 20 mg/kg body weight. | Study no.<br>BPK(F) 1997/004 |
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.7.3-4 | |
| Species, strain, sex, number animals: | dog, Beagle, M / 8 | |
| Test article: | Arm | |
| Feeding condition: | fed | |
| Vehicle / formulation | injectable solution of Arm dissolved in peanut oil (80 mg/mL). | |
| Method of administration: | intramuscular | |
| Duration of dosing: | once daily<br>4 dogs (1, 3, 4, 5) for 30 days (Dog 5 was sacrificed at Day 28 and was treated for 26 days)<br>4 dogs (2, 6, 7, 8) for 5 days | |
| Daily dose (mg/kg) | 0 (control); 20 (group 2) | |
| Sample: | plasma and cerebrospinal fluid | |
| Analyte: | Arm and DHA | |
| Assay: | HPLC with electrochemical detection operated in reductive mode | |
| Results: | Arm plasma trough levels increased in all animals between Day 2 (mean = 29 ng/mL, n = 6) and Day 5 (mean = 143 ng/mL, n = 6). Then a decrease was observed in almost all animals on Days 8, 15, 22, 29 and 30. Low variability of Arm plasma levels between dogs was noted on Day 4, as assessed by the coefficient of variation of the mean value at each sampling time (range 9 to 21%). In Dogs 3 and 4, Arm concentrations at most sampling times were lower on Day 29 when compared with Day 4.<br>DHA was not detected in plasma before dosing on any day, and plasma concentrations were ≤10% of Arm.<br>The distribution of Arm in cerebrospinal fluid was <10% of that in plasma, while DHA was undetected in any sample.<br>None of the animals in the control group had measurable concentrations of Arm or DHA. Dog 1 had high concentrations of Arm in cerebrospinal fluid despite the absence of any measurable concentrations in the plasma. This was probably the result of interference or artifact. | |

Additional information: Supplement to toxicity study no. 966141.

## 2.6.5.4G Pharmacokinetics: Absorption after repeated doses (DMPK R0410073)

| Study title: | 8-day exploratory neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and dihydroartemisinin in dog plasma | Study no. DMPK R0410073 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.7.3-5 | |
| Test article | Arm | |
| Method and objectives | The objectives of this study are to determine:<br>• The concentrations of Arm and its metabolite (DHA) in dog plasma samples collected from the animal phase of the study referenced 0410073.<br>• The main pharmacokinetic parameters of Arm and its metabolite in male dogs. | |
| Matrix | Dog plasma | |
| Administration form | Intramuscular | |
| Route and frequency of administration | Intramuscular, once daily, seven days a week | |
| Species, strain, gender | Dog, Beagle, male | |
| Dose groups and no. of animals in TK-investigation | Arm: n=2 and dose was 40 mg/kg | |
| Sampling schedules | Toxicokinetic blood sampling times:<br>After the first administration, on day 3 and toward the end of the dosing period (day 7), blood was taken from the 2 study animals at the following time points: 5 and 30 min, and 1, 2, 3, 6 (except for day 3) and 24 hours post-dose. | |
| Bioanalytical method | According to Analytical Method Report DMPK R0500059.<br>Liquid-liquid extraction with 1-chlorobutane-iso-octane (55:45, v/v) followed by reversed phase liquid chromatography with tandem mass spectrometric detection. | |
| *Reference compounds:* | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$<br>Molecular weight (MW): 298.37 g/mol | |
| DHA | Molecular formula: $C_{15}H_{24}O_5$<br>Molecular weight (MW): 284.35 g/mol | |
| *Internal standards:* | | |
| Arteether | Molecular formula: $C_{17}H_{28}O_5$<br>Molecular weight (MW): 312.40 g/mol | |
| Artemisinin | Molecular formula: $C_{15}H_{22}O_5$<br>Molecular weight (MW): 282.33 g/mol | |

| Study title: | 8-day exploratory neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and dihydroartemisinin in dog plasma | Study no. DMPK R0410073 |
|---|---|---|
| *LLOQ* | 5.00 ng/mL expressed as base using 250 µL of dog plasma | |
| *Reporting of concentrations below LLOQ* | 0 ng/mL | |
| Results-1: | | |
| Stability in dog plasma | Stability to long-term storage at −75°C±10°C, stability to 3 freeze/thaw cycles, and stability at room temperature for 4.5 hours were demonstrated (Method Validation Report CP045360). | |

Concentrations in plasma of Arm

Toxicokinetic parameters of Arm in dog plasma

| Animal | Day | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) |
|---|---|---|---|---|
| 349 | 1 | 868 | 0.50 | 6780 |
| | 3 | 1040 | 0.50 | 12051 |
| | 7 | 1060 | 2.00 | 10651 |
| 350 | 1 | 471 | 2.00 | 3639 |
| | 3 | 457 | 0.50 | 5777 |
| | 7 | 416 | 1.00 | 5097 |

Concentrations in plasma of DHA

Toxicokinetic parameters of DHA in dog plasma

| Animal | Day | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) |
|---|---|---|---|---|
| 349 | 1 | 131 | 0.50 | 753 |
| | 3 | 33.0 | 0.50 | 481 |
| | 7 | 33.1 | 1.00 | 480 |
| 350 | 1 | 159 | 1.00 | 980 |
| | 3 | 33.4 | 0.50 | 432 |
| | 7 | 24.0 | 3.00 | 378 |

Discussion and Conclusion

**For Arm**

Maximal Arm plasma concentrations were observed between 30 min and 2 h after dosing whatever the day and the dog.

Based on AUC0-24h, the exposure to Arm was about 2 times higher in dog no. 349 compared to dog no. 350 whatever the day.

Based on AUC0-24h, the exposure to Arm was higher on day 3 and 7 compared to day 1 for both dogs. The ratios AUCD3/D1 and AUCD7/D1 were 1.8 and 1.6 for dog no. 349 and were 1.6 and 1.4 for dog no. 350.

| | | |
|---|---|---|
| Study title: | 8-day exploratory neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and dihydroartemisinin in dog plasma | Study no. DMPK R0410073 |
| | **For DHA**<br>Maximal DHA plasma concentrations were observed between 30 min and 3 h after dosing whatever the day and the dog.<br>Based on AUC0-24h, the ratio Arm/DHA was higher on days 3 and 7 compared to day 1 for both dogs. The ratios Arm/DHA were 9, 25 and 22, respectively on day 1, 3 and 7 for dog no. 349 and 4, 13 and 13, respectively on day 1, 3 and 7 for dog no. 350. | |
| Special issues: | The quantification of Arm and DHA in dog plasma was performed with HPLC gradient used in the validated method for the determination of these analytes in human plasma instead of using gradient conditions mentioned in the dog plasma method. As the calibration curves and the QC samples were within acceptance criteria, the bioanalytical runs performed under these modified conditions were validated.<br>This modification was considered not to have compromised the integrity or validity of this analytical part of the study. | |

Additional information: Supplement to toxicity study no. 0410073.

## 2.6.5.4H Pharmacokinetics: Absorption after repeated doses (DMPK R0510001)

| Study title: | Intramuscular neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and its dihydroartemisinin in dog plasma | Study no. DMPK R0510001 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.7.3-6 | |
| Test article | Arm | |
| Method and objectives | The objectives of this study are to determine:<br>• The concentrations of Arm and its metabolite (DHA) in dog plasma samples collected from the animal phase of the study referenced [0510001]<br>• The main pharmacokinetic parameters of Arm and its metabolite in male dogs after repeated intramuscular administration | |
| Matrix | Dog plasma | |
| Administration form | Intramuscular | |
| Route and frequency of administration | Once daily administration, at least 3 or 8 days | |
| Species, strain, gender | Beagle dogs, male | |
| Dose groups and no. of animals in TK-investigation | Control: Group 1: n=3 , 0 mg/kg/day<br>Arm: Group 2, 3, and 4: n=3 per group and dose group was 10 mg/kg/day<br>Arm: Group 5, 6, and 7: n=3 per group and dose group was 40 mg/kg/day<br>Note: The control group was dosed with the vehicle. | |
| Sampling schedules | Toxicokinetic blood sampling time:<br>On day 1, 3 and 7, blood was taken at the following time points: 5, 30 min, 1, 2, 3, 6 and 24 hours post-dose.<br>Animals of Groups 3 and 4 were grouped together for results and TK analysis, as well as animals of Groups 6 and 7 as they received the same dose.<br>Animals of Group 2 (54-56) and Group 5 (63-65) were excluded from the TK sampling.<br>228 samples were analyzed. | |
| Bioanalytical method | According to Analytical Method Report [DMPK R0500059].<br>Liquid/liquid extraction with 1-chlorobutane-isooctane (55:45; v/v) followed by reverse phase liquid chromatography with tandem mass spectrometric detection (Method validation report CP045360). | |
| *Reference compounds:* | | |
| Arm | Molecular formula: $C_{16}H_{26}O_5$<br>Molecular weight (MW): 298.37 g/mol | |
| DHA | Molecular formula: $C_{15}H_{24}O_5$<br>Molecular weight (MW): 284.35 g/mol | |

| Study title: | Intramuscular neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and its dihydroartemisinin in dog plasma | Study no. DMPK R0510001 |
|---|---|---|
| *Internal standards:* | | |
| Arteether | Molecular formula: $C_{17}H_{28}O_5$ | |
| | Molecular weight (MW): 312.40 g/mol | |
| Artemisinin | Molecular formula: $C_{15}H_{22}O_5$ | |
| | Molecular weight (MW): 282.33 g/mol | |
| *LLOQ* | 5.00 ng/mL (for both compounds) expressed as base, using 250 µL of plasma | |
| *Reporting of concentrations below LLOQ* | 0 ng/mL | |
| Results-1: | | |
| Stability in matrix | Stable in extracts at room temperature for 78.5 hours, stable for at least 4.5 hours at room temperature and stable after 3 freeze-thaw cycles (freezing in a freezer at –75°C ± 10°C) and stable in spiked dog plasma after storage at –75°C±10°C for 4.5 months for Arm (Method validation report CP045360). | |
| Concentrations in plasma of Arm | Mean toxicokinetic parameters of Arm in dog plasma | |

| | | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) | Cmax/Dose (ng/mL)/ (mg/kg/day) | AUC0-24h/Dose (ng·h/mL)/ (mg/kg/day) |
|---|---|---|---|---|---|---|
| Group 3 & 4 | (10 mg/kg/day) | | | | | |
| Day 1 (N=6) | Mean | 82.1 | 1.0 | 949 | 8.21 | 94.85 |
| | SD | 43.0 | 0.5 | 349 | 4.30 | 34.91 |
| Day 3 (N=6) | Mean | 182.0 | 0.8 | 1610 | 18.20 | 160.99 |
| | SD | 49.2 | 0.6 | 396 | 4.92 | 39.62 |
| Day 7 (N=3) | Mean | 209.0 | 0.5 | 1833 | 20.90 | 183.28 |
| | SD | 94.0 | 0.0 | 662 | 9.40 | 66.25 |
| Group 6 & 7 | (40 mg/kg/day) | | | | | |
| Day 1 (N=6) | Mean | 441.8 | 1.8 | 5126 | 11.05 | 128.15 |
| | SD | 101.0 | 0.4 | 876 | 2.52 | 21.91 |
| Day 3 (N=6) | Mean | 770.3 | 1.1 | 10247 | 19.26 | 256.18 |
| | SD | 253.3 | 0.5 | 2163 | 6.33 | 54.07 |
| Day 7 (N=3) | Mean | 689.3 | 1.5 | 7992 | 17.24 | 199.81 |
| | SD | 191.9 | 1.3 | 1150 | 4.80 | 28.74 |

| Study title: | Intramuscular neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and its dihydroartemisinin in dog plasma | | | | | | Study no. DMPK R0510001 |
|---|---|---|---|---|---|---|---|
| Concentrations in plasma of DHA | Mean toxicokinetic parameters of DHA in dog plasma | | | | | | |
| | | | Cmax (ng/mL) | Tmax (h) | AUC0-24h (ng·h/mL) | Cmax/Dose (ng/mL)/ (mg/kg/day) | AUC0-24h/Dose (ng·h/mL)/ (mg/kg/day) |
| | Group 3 & 4 | (10 mg/kg/day) | | | | | |
| | Day 1 (N=6) | Mean | 47.2 | 1.0 | 377 | 4.72 | 37.72 |
| | | SD | 18.8 | 0.0 | 82 | 1.88 | 8.19 |
| | Day 3 (N=6) | Mean | 25.8 | 1.1 | 271 | 2.58 | 27.05 |
| | | SD | 8.4 | 0.7 | 60 | 0.84 | 6.02 |
| | Day 7 (N=3) | Mean | 30.1 | 0.7 | 262 | 3.01 | 26.24 |
| | | SD | 13.5 | 0.3 | 131 | 1.35 | 13.10 |
| | Group 6 & 7 | (40 mg/kg/day) | | | | | |
| | Day 1 (N=6) | Mean | 171.8 | 0.8 | 948 | 4.30 | 23.69 |
| | | SD | 106.1 | 0.3 | 317 | 2.65 | 7.91 |
| | Day 3 (N=6) | Mean | 34.0 | 0.8 | 573 | 0.85 | 14.33 |
| | | SD | 9.8 | 0.6 | 111 | 0.25 | 2.78 |
| | Day 7 (N=3) | Mean | 33.0 | 0.7 | 469 | 0.83 | 11.73 |
| | | SD | 0.7 | 0.3 | 114 | 0.02 | 2.84 |

*Ratio of AUC0-24h between DHA and Arm

| Study title: | Intramuscular neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of artemether and its dihydroartemisinin in dog plasma | Study no. DMPK R0510001 |
|---|---|---|
| Discussion and Conclusion | In the control group, plasma concentrations of Arm and its metabolite were found below the lower limit of quantification (5.00 ng/mL) in all samples collected whatever the day.<br>Maximal plasma concentrations were observed between 0.5 and 3 h after dosing for Arm and between 0.5 and 2 h after dosing for DHA whatever the group and the day.<br>Based on AUC0-24h, the exposure to Arm was higher on Day 3 or Day 7 than on Day 1 after repeated administration of 10 and 40 mg/kg/day of Arm. By contrast, the exposure to DHA was lower on Day 3 or Day 7 than on Day 1.<br>Based on AUC0-24h, the exposure to Arm increased quite dose proportionally when dose increased. AUC0-24h increased in a ratio of about 5 on Day 1, 6 on Day 3 and 4 on Day 7 for an increase in dose of 4.<br>Based on AUC0-24h, the exposure to DHA increased when dose increased, but lower than dose proportionally. AUC0-24 increased in a ratio of about 2 whatever the day for an increase in dose of 4. | |
| Special issues: | For DHA, the reference standard is a mixture of α and β anomers. The exact ratio between both anomers is not known, the α-anomer being the predominant form. For this reference standard, only the α-anomer was assumed to be 100% of this reference standard. So, only an evaluation of the α-anomer metabolite concentration was done in the plasma samples, thus the metabolite concentrations were reported as indicative levels.<br>Due to analytical problems, some samples were extracted and analyzed again.<br>Only sampling times 1 hour and 2 hours were analyzed for control group animals.<br>No stability data are available for DHA to storage in dog plasma at –75°C±10°C.<br>At the request of the Sponsor, the animals of Groups 3 and 4 were grouped together as they received the same dose. Moreover, animals of Groups 6 and 7 were also grouped together. As this process could not be performed by Watson® LIMS, the concentrations and pharmacokinetic tables were transferred into Microsoft® Excel. Then the animals were grouped together and the descriptive statistics were calculated on the two new groups (Groups 10 and 40) using Microsoft® Excel.<br>228 samples were analyzed instead of 315 as specified in the analytical and toxicokinetic working document. This was deemed to have no impact on the integrity of the study for the following reasons:<br>There was an error on the sample accountability, 273 should have been written and not 315 samples.<br>Only the samples time points corresponding to Cmax were analyzed for the control group according to the toxicokinetic protocol. | |

Additional information: Supplement to toxicity study no. 0510001.

## 2.6.5.4 I Pharmacokinetics: Absorption after repeated doses (DMPK(F) 1998/014)

| Study title: | Artemether and dihydroartemisinin plasma concentrations in dogs in a 8 days neurotoxicity study following i.m. and oral administrations of daily doses (intermediate results). | Study no. DMPK(F) 1998/014 |
|---|---|---|
| GLP compliance: | No | |
| Location in CTD: | 4.2.3.7.3-3 | |
| Test article: | Arm | |
| Species, strain, sex, number of animals: | dog, Beagle, M / 32<br>dog, Beagle, F / 32 | |
| Feeding condition: | fed | |
| Vehicle / formulation | i.m.: injectable solution of Arm dissolved in peanut oil (80 mg/mL)<br>oral: gelatin capsules | |
| Method of administration: | intramuscular : into the gluteal or infraspinous muscle alternating both hindlegs and forelegs<br>oral | |
| Duration of dosing: | once daily for 8 days | |
| Daily dose (mg/kg) | intramuscular route<br>0 (control)<br>20 (group 2)<br>40 (group 3)<br>80 (group 4)<br>oral route<br>0 (control)<br>50 (group 2)<br>150 (group 3)<br>600 (group 4) | |
| Sample: | plasma and cerebrospinal fluid (CSF) | |
| Analyte: | Arm and DHA | |
| Assay: | not noted in report | |

| Study title: | Artemether and dihydroartemisinin plasma concentrations in dogs in a 8 days neurotoxicity study following i.m. and oral administrations of daily doses (intermediate results). | | | Study no. DMPK(F) 1998/014 | | |
|---|---|---|---|---|---|---|
| Results: | | | | | | |
| Arm | intramuscular route | | | oral route | | |
| PK parameters: (Day 1 / Day last) | 20 mg/kg | 40 mg/kg | 80 mg/kg | 50 mg/kg | 150 mg/kg | 600 mg/kg |
| Tmax (h) median | 2 / 5 | 2 / 5 | 2 / 5 | incalculable | incalculable | 3 / 1.5 |
| Cmax (ng/mL) | 219 / 294 | 461 / 825 | 985 / 1180 | incalculable | incalculable | 208 / 27.8 |
| AUC0-t (ng·h/mL) | 2050 / 5350 | 5630 / 12800 | 11000 / 20800 | incalculable | incalculable | 1730 / 250 |
| AUC (ng·h/mL) | 2290 / 9340 | 6540 / 18200 | 12700 / 3900 | incalculable | incalculable | not reported |
| T1/2 (h) | 6.8 / 18.7 | 8.9 / 13.6 | 8.1 / 20.7 | incalculable | incalculable | not reported |
| CSF concentration (ng/mL) | 25.2 [a] | 60 [a] | 71.1 [a] | not detected | not detected | not detected |
| DHA | intramuscular route | | | oral route | | |
| PK parameters: (Day 1 / Day last) | 20 mg/kg | 40 mg/kg | 80 mg/kg | 50 mg/kg | 150 mg/kg | 600 mg/kg |
| Tmax (h) median | 2 / 10 | 2 / 5 | 2 / 8 | incalculable | incalculable | 3 / 3.5 |
| Cmax (ng/mL) | 40.3 / 19.0 | 41.4 / 20.4 | 85 / 33.7 | incalculable | incalculable | 795 / 19.2 (n=2) |
| AUC0-t (ng·h/mL) | 314 / 307 | 370 / 335 | 937 / 456 | incalculable | incalculable | 6600 / 125 (n=2) |
| AUC (ng·h/mL)<br>n = 3 | 775 (Day 1)<br>n = 2 | 842 (Day 1) | 790 (Day 1) | incalculable | incalculable | not reported |
| T1/2(h)<br>n = 3 | 14.4 (Day 1)<br>n = 2 | 19.7 (Day 1) | 9.4 (Day 1) | incalculable | incalculable | not reported |
| CSF concentration (ng/mL) | BLQ | BLQ | BLQ | not detected | not detected | not detected |
| BLQ = below the limit of quantification (10 ng/mL). a: mean values. | | | | | | |
| Special issues: | After 50 and 150 mg/kg repeated oral dosing, concentrations at the limit of quantification were found for both Arm and DHA. | | | | | |

Additional information: Supplement to toxicity study no. 970024.

## 2.6.5.4J Pharmacokinetics: Absorption after repeated doses (BPK(CH) 1995/079)

| | | |
|---|---|---|
| Study title: | Plasma concentrations of CGP 56 695 (benflumetol) in rats after repeated daily administration of 200, 600 and 1000 mg/kg of CGP 56 697 (co-artemether) during a 1-month oral toxicity study. | Study no.<br>BPK(CH) 1995/079 |
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.2-1 | |
| Species, strain, sex, number | rat, Tif: RAIf (SPF) / M / 21<br>rat, Tif: RAIf (SPF) / F / 21 | |
| Test article: | Arm/Lmf containing 1 part Arm and 6 parts Lmf | |
| Feeding condition: | fed | |
| Vehicle / formulation | 0.5% Klucel + 0.1% Tween 80 | |
| Method of administration: | oral gavage | |
| Duration of dosing: | once daily for 1 month | |
| Daily dose (mg/kg) | 0 (control),<br>200 (group 2 a/b),<br>600 (group 3 a/b)<br>1000 (group 4 a/b) | |
| Sample: | plasma | |
| Analyte: | Lmf | |
| Assay: | HPLC with ultraviolet detection | |

Results:

| PK parameters (Day 1 / Day last): | 200 mg/kg | 600 mg/kg | 1000 mg/kg |
|---|---|---|---|
| Tmax (h) | M 8 / 24<br>F 8 / 4 | M 24 / 8<br>F 8 / 0 | M 24 / 8<br>F 24 / 4 |
| Cmax (µg/mL) | M 32.87 / 15.85<br>F 23.25 / 15.98 | M 21.09 / 20.61<br>F 19.57 / 24.82 | M 15.77 / 18.00<br>F 33.22 / 37.93 |
| AUC 0-24h (µg·h/mL) | M 535 / 322<br>F 358 / 281 | M 423 / 386<br>F 402 / 449 | M 279 / 382<br>F 350 / 630 |
| Trough concentration (µg/mL) | M 15.27 / 15.85<br>F 7.72 / 5.39 | M 21.09 / 9.35<br>F 18.00 / 13.60 | M 15.77 / 15.42<br>F 33.22 / 25.71 |

None of the animals in the control group had measurable concentrations of Lmf in plasma.

| | | |
|---|---|---|
| Study title: | Plasma concentrations of CGP 56 695 (benflumetol) in rats after repeated daily administration of 200, 600 and 1000 mg/kg of CGP 56 697 (co-artemether) during a 1-month oral toxicity study. | Study no. BPK(CH) 1995/079 |
| Conclusions: | All rats having received the suspension formulation of Arm/Lmf were exposed to Lmf<br>The onset of absorption of Lmf was fast (≤ 1 h); absorption continued over an exended period of time.<br>AUCs and trough values of Lmf showed high variability no clear relationship to the dose. No accumulation was found.<br>There were no obvious differences in sexes. | |

Additional information: Supplement to toxicity study no. 946152.

## 2.6.5.4K Pharmacokinetics: Absorption after repeated doses (BPK(CH) 1996/020)

| Study title: | Plasma concentrations of CGP 56695 (benflumetol) in rats after repeated daily administration of 100, 300 and 1000 mg/kg CGP 56697 (co-artemether) during a 3-month oral toxicity study. | | Study no.<br>BPK(CH) 1996/020 |
|---|---|---|---|
| GLP compliance: | Yes | | |
| Location in CTD: | 4.2.3.2-2 | | |
| Test article: | Arm/Lmf containing 1 part Arm and 6 parts Lmf | | |
| Species, strain, sex, number of animals: | rat, albino, Tif: RAIf (SPF) / Male: 21; Female: 21 | | |
| Feeding condition: | fed | | |
| Vehicle / formulation | 0.5% Klucel + 0.1% Tween 80 | | |
| Method of administration: | oral gavage | | |
| Duration of dosing: | once daily for 3 months | | |
| Daily dose (mg/kg) | 0 (control)<br>100 (group 2 a/b, Lmf: 85.7)<br>300 (group 3 a/b, Lmf: 257.1)<br>1000 (group 4 a/b, Lmf: 857.1) | | |
| Sample: | plasma | | |
| Analyte: | Lmf | | |
| Assay: | HPLC with ultraviolet detection | | |
| Results: | | | |
| PK parameters<br>(Day 1 / Day 91): | 100 mg/kg | 300 mg/kg | 1000 mg/kg |
| Tmax (h) | M 2 / 8<br>F 8 / 4 | M 8 / 8<br>F 8 / 4 | M 24 / 4<br>F 24 / 2 |
| Cmax (µg/mL) | M 15.67 / 12.68<br>F 19.55 / 14.20 | M 18.04 / 24.00<br>F 18.08 / 29.46 | M 13.93 / 18.90<br>F 13.69 / 35.79 |
| AUC0-24h (µg·h/mL) | M 228 / 174<br>F 293 / 262 | M 271 / 434<br>F 298 / 591 | M 227 / 337<br>F 210 / 645 |
| Trough concentration<br>(µg/mL) | M 2.79 / 1.21<br>F 6.68 / 8.58 | M 3.11 / 10.92<br>F 5.87 / 18.24 | M 13.93 / 11.68<br>F 13.69 / 19.71 |
| | None of the animals in the control group had measurable concentrations of Lmf in plasma. | | |

| | | |
|---|---|---|
| Study title: | Plasma concentrations of CGP 56695 (benflumetol) in rats after repeated daily administration of 100, 300 and 1000 mg/kg CGP 56697 (co-artemether) during a 3-month oral toxicity study. | Study no. BPK(CH) 1996/020 |
| Conclusions: | All rats having received the suspension formulation of Arm/Lmfwere exposed to Lmf<br>The onset of absorption of Lmf was fast (≤ 1 h); absorption continued over an exended period of time.<br>AUCs and trough values of Lmf showed high variability no clear relationship to the dose. No accumulation was found.<br>AUC values of Lmf were higher in female than in male rats after repeated administration of 300 and 1000 mg/kg | |

Additional information: Supplement to toxicity study no. 94-7913

## 2.6.5.4 L Pharmacokinetics: Absorption after repeated doses (DMPK(CH) 1997/177)

| **Study title:** | Plasma concentrations of CGP 56695 (benflumetol) in weanling rats after repeated daily administration of 100, 300 and 1000 mg/kg CGP 56697 (co-artemether) during a 13-week oral toxicity study. | | | Study no. DMPK(CH) 1997/177 | | |
|---|---|---|---|---|---|---|
| GLP compliance: | yes | | | | | |
| Location in CTD: | 4.2.3.5.4-1 | | | | | |
| Test article | Arm/Lmf containing 1 part Arm and 6 parts Lmf | | | | | |
| Species, strain, sex, number of animals: | weanling albino rats, Tif: RAIf (SPF) M / 24, F / 24<br>initial age at dosing: 3-5 weeks | | | | | |
| Feeding condition: | fed | | | | | |
| Vehicle / formulation | 0.5% Klucel + 0.1% Tween 80 / Arm/Lmf as 1 part Arm and 6 parts Lmf | | | | | |
| Method of administration: | oral gavage | | | | | |
| Duration of dosing: | 91 days | | | | | |
| Daily dose (mg/kg) | 0 (control)<br>100 (group 2, Lmf: 85.7)<br>300 (group 3, Lmf: 257.1)<br>1000 (group 4, Lmf: 857.1) | | | | | |
| Sample: | plasma | | | | | |
| Analyte: | Lmf | | | | | |
| Assay: | HPLC with ultraviolet detection | | | | | |
| Results: PK parameters: | Day 1 | | | Day 91 | | |
| Dose (mg/kg): | 100 | 300 | 1000 | 100 | 300 | 1000 |
| Number of animals: | M / 3 F / 3 | M / 3 F / 3 | M / 3 F / 3 | M / 3 F / 3 | M / 3 F / 3 | M / 3 F / 3 |
| Mean C8h (µg/mL) | | | | | | |
| Male | 16.75 | 31.82 | 31.74 | 11.01 | 19.29 | 19.00 |
| Female | 13.22 | 38.20 | 26.77 | 14.11 | 31.36 | 30.75 |
| Control group: | None of the animals in the control group had measurable concentrations of Lmf in plasma | | | | | |
| Additional information: | Supplement to toxicity study no: 956088 | | | | | |

## 2.6.5.4 M Pharmacokinetics: Absorption after repeated doses (DMPK(CH) 1997/178)

| | | | |
|---|---|---|---|
| Study title: | Plasma concentrations of CGP 56695 (benflumetol) in rats after repeated daily administration of 100, 300 and 1000 mg/kg CGP 56695 during a 13-week oral toxicity study. | | Study no. DMPK(CH) 1997/178 |
| GLP compliance: | yes | | |
| Location in CTD: | 4.2.3.2-7 | | |
| Test article: | Lmf | | |
| Species, strain, sex, number of animals: | rat, albino, Tif: RAIf (SPF) M / 21<br>rat, albino, Tif: RAIf (SPF) F / 21 | | |
| Feeding condition: | fed | | |
| Vehicle / formulation | 0.5% Klucel + 0.1% Tween 80 | | |
| Method of administration: | oral gavage | | |
| Duration of dosing: | once daily for 13 weeks | | |
| Daily dose (mg/kg) | 0 (control)<br>100 (group 2 a/b, Lmf: 85.7)<br>300 (group 3 a/b, Lmf: 257.1)<br>1000 (group 4 a/b, Lmf: 857.1) | | |
| Sample: | plasma | | |
| Analyte: | Lmf | | |
| Assay: | HPLC with ultraviolet detection | | |
| Results:<br>PK parameters (Day 1 / Day 87): | 100 mg/kg | 300 mg/kg | 1000 mg/kg |
| Tmax (h) | M 8 / 2<br>F 8 / 8 | M 4 / 0<br>F 4 / 4 | M 8 / 24<br>F 8 / 8 |
| Cmax (µg/mL) | M 17.93 / 11.15<br>F 18.45 / 24.72 | M 25.43 / 14.39<br>F 26.69 /25.07 | M 28.55 / 26.02<br>F 42.54 / 38.56 |
| AUC0-24h (µg·h/mL) | M 281.75 / 179.75<br>F 284.78 / 444.70 | M 482.92 / 263.04<br>F 425.00 / 491.14 | M 475.06 / 384.66<br>F 747.21 / 768.44 |
| | None of the animals in the control group had measurable concentrations of Lmf in plasma, while the samples of all treated rats contained Lmf. With one exception, none of the predose samples on Day 1 contained Lmf. The reason for this quite small concentration of Lmf in the plasma of one of the animals could not be determined. | | |
| Conclusions: | • All rats which received the suspension formulation were exposed to the drug. | | |

Additional information: Supplement to toxicity study no. 95-6026.

## 2.6.5.4N Pharmacokinetics: Absorption after repeated doses (DMPK(CH) 1997/006)

| Study title: | Plasma concentrations of CGP 56695 in a 13-week oral (capsule) toxicity study in dogs. | | Study no.<br>DMPK(CH) 1997/006 |
|---|---|---|---|
| GLP compliance: | Yes | | |
| Location in CTD: | 4.2.2.2-7 | | |
| Test article: | Lmf | | |
| Species, strain, sex, number of animals: | Beagle dog, M / 18;<br>Beagle dog, F / 18 | | |
| Feeding condition: | fed | | |
| Vehicle / formulation | gelatin capsules | | |
| Method of administration: | oral | | |
| Duration of dosing: | once daily for 13 weeks | | |
| Daily dose (mg/kg) | 0 (control)<br>60 (group 2, Lmf: 51.4)<br>200 (group 3, Lmf: 171.4)<br>600 (group 4, Lmf: 514) | | |
| Sample: | plasma | | |
| Analyte: | Lmf | | |
| Assay: | HPLC with ultraviolet detection | | |
| PK parameters (Day 1 / Day 71): | 60 mg/kg | 200 mg/kg | 600 mg/kg |
| Tmax (h) | not measured | not measured | not measured |
| Cmax (ng/mL) | M 9020.1 / 6607.8<br>F 6677.4 / 5231.8 | M 160.7 / 17219.8<br>F 0 / 12281.3 | M 12052.3 / 21559.8<br>F 389.2 / 25459.5 |
| AUC0-24h (ng·h/mL) | M 97065.7 (Day 71)<br>F 48649.3 (Day 71) | M 224293.0 (Day 71)<br>F 136285.3 (Day 71) | M 224187.8 (Day 71)<br>F 270011.2 (Day 71) |
| Conclusions: | • After single dosing, Lmf was hardly detected in dog plasma at all dose levels.<br>• Absorption appeared to be delayed.<br>• Accumulation was observed after multiple dosing.<br>• There was no gender difference in exposure to Lmf | | |

Additional information: Supplement to toxicity study no. 956025.

## 2.6.5.4O Pharmacokinetics: Absorption after repeated doses (DMPK R0510009A)

| Study title: | Oral neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of Lmf in plasma. | Study no. DMPK R0510009A |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.7.3-2 | |
| Test article: | Arm/Lmf - fixed dose combination (1:6) of Arm and Lmf | |
| Method and objective | The objective of this study is to describe the measurement of Lmf in dog plasma from toxicity study [0510009]and provide toxicokinetic interpretation of the resulting plasma concentrations | |
| Matrix | Dog plasma | |
| Administration form | Suspension in 0.1% Tween 80 dissolved in aqueous Klucel HF (0.5%) | |
| Route and frequency of administration | Arm or Arm/Lmf by oral gavage to male dogs up to 8 days | |
| Species, strain, sex | Dog, Beagle, male | |
| Dose groups and no. of animals in TK-investigation | Two groups were retained for the Toxicokinetics (TK) evaluation (same administered dose):<br>Group 6: 3 animals, 1000 mg/kg/day for 3 days<br>Group 7: 3 animals, 1000 mg/kg/day for 8 days | |
| Sampling schedules | On days 1, 3 and 7 (day 7: only for group 7), blood was taken at the following time points: 0.25, 0.50, 1, 2, 3, 6 and 24 hours post-dose. | |
| Bioanalytical method | According to Analytical Method Report [R00-1840]<br>Liquid-liquid extraction of plasma samples followed by evaporation of the extract to dryness and analysis of the reconstituted samples by HPLC-MS/MS using Turbo Ion Spray source | |
| *Reference compound:* | | |
| Lmf | Molecular formula: $C_{30}H_{32}Cl_3NO$<br>Molecular weight (MW): 528.95 g/mol | |
| *Internal standard:* | | |
| [$D_9$] Arm/lmf | Molecular formula: $C_{30}H_{23}Cl_3NOD_9$<br>Molecular weight (MW): 538 g/mol | |
| *LLOQ* | 0.100 µg/mL using 100 µL of plasma | |
| *Reporting of concentrations below LLOQ* | 0 µg/mL | |

| | | | | | |
|---|---|---|---|---|---|
| Study title: | Oral neurotoxicity study in dogs. Toxicokinetic report: Determination and toxicokinetics of Lmf in plasma. | | | Study no. DMPK R0510009A | |
| Results: | | | | | |
| Pharmacokinetic parameters: | Mean toxicokinetic parameters (SD) of Lmf in dog plasma | | | | |
| | | | Lmf: 857 mg/kg/day | | |
| | Parameter | Units | Day 1 (n = 6) | Day 3 (n = 6) | Day 7 (n = 3) |
| | Tmax | (h) | 15 (10) | 6 (0) | 18 (10) |
| | Cmax | (µg/mL) | 9.38 (2.86) | 14.4 (3.1) | 18.6 (4.3) |
| | Cmax / dose | (µg/mL) / (mg/kg/day) | 0.0110 (0.0034) | 0.0168 (0.0036) | 0.0217 (0.0050) |
| | AUC0-24h | (µg·h/mL) | 156 (61) | 264 (90) | 337 (38) |
| | AUC0-24 h / dose | (µg·h/mL) / (mg/kg/day) | 0.182 (0.071) | 0.308 (0.105) | 0.393 (0.044) |
| Discussion and Conclusion | All dogs were exposed to Lmf.<br>Cmax was observed between 6 and 24 hours.<br>Based on AUC0-24h/dose and Cmax/dose values, the exposure to Lmf was similar on days 3 and 7, taking into account the inter-animal variability. | | | | |

Additional information: Supplement to toxicity study no. 0510009.

## 2.6.5.4P Pharmacokinetics: Absorption after repeated doses (BPK(CH) 1995/080)

| | | | |
|---|---|---|---|
| Study title: | Plasma concentrations of CGP 56 695 (benflumetol) in dogs after repeated daily administration of 60, 200 and 600 mg/kg of CGP 56 697 (co-artemether) during a 1-month oral toxicity study. | | Study no.<br>BPK(CH) 1995/080 |
| GLP compliance: | Yes | | |
| Location in CTD: | 4.2.3.2-3 | | |
| Test article: | Arm/Lmf containing 1 part Arm and 6 parts Lmf | | |
| Species, strain, sex, number of animals: | Beagle dogs, M / 15,<br>Beagle dogs, F / 15 | | |
| Feeding condition: | fed | | |
| Vehicle / formulation | gelatin capsule | | |
| Method of administration: | oral | | |
| Duration of dosing: | once daily for 1 month | | |
| Daily dose (mg/kg) | 0 (control)<br>60 (group 2, Lmf: 51.4)<br>200 (group 3, Lmf: 171.4)<br>600 (group 4, Lmf: 514) | | |
| Sample: | plasma | | |
| Analyte / Assay: | Lmf/ HPLC with ultraviolet detection | | |
| Results:PK parameters: (Day 1 / Day 25): | 60 mg/kg | 200 mg/kg | 600 mg/kg |
| Tmax (h) | M 8<br>F 24 | M 24<br>F 24 | M 24<br>F 16 |
| Cmax (µg/mL) | M 9.77<br>F 4.48 | M 14.22<br>F 6.2 | M 8.08<br>F 14.86 |
| AUC0-24h (µg·h/mL) | M 134 / 152.67<br>F 69.33 / 115 | M 234.33 / 463<br>F 65.33 / 105.67 | M 108.67 / 168.67<br>F 252 / 235 |

a: median value

None of the animals in the control group had measurable concentrations of Lmf in plasma.

| | | |
|---|---|---|
| Study title: | Plasma concentrations of CGP 56 695 (benflumetol) in dogs after repeated daily administration of 60, 200 and 600 mg/kg of CGP 56 697 (co-artemether) during a 1-month oral toxicity study. | Study no.<br>BPK(CH) 1995/080 |
| Conclusions: | • All dogs were exposed to Lmf<br>• The absorption of Lmf was highly variable with respect to onset and extent.<br>• AUC values and trough concentration values of Lmf showed high variability and no clear relationship to dose or gender.<br>• No accumulation of Lmf was observed despite the slow elimination of the drug from plasma ($T1/2\lambda z$= 31 h) | |

Additional information: Supplement to toxicity study no. 94-6154.

## 2.6.5.4Q Pharmacokinetics: Absorption after repeated doses (BPK(CH) 1996/024)

| | | |
|---|---|---|
| Study title: | Plasma concentrations of CGP 56695 (benflumetol) in dogs after repeated daily administration of 20, 60 and 200 mg/kg of CGP 56697 (co-artemether) during a 3-month oral toxicity study. | Study no. BPK(CH) 1996/024 |
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.2-4 | |
| Test article: | Arm/Lmf as 1 part Arm and 6 parts Lmf | |
| Species, strain, sex, number of animals: | Beagle dogs, M / 12,<br>Beagle dogs, F / 12 | |
| Feeding condition: | fed | |
| Vehicle / formulation | gelatin capsules | |
| Method of administration: | oral | |
| Duration of dosing: | once daily for 3 months | |
| Daily dose (mg/kg) | 0 (control),<br>20 (group 2, Lmf:17.1),<br>60 (group 3, Lmf: 51.4)<br>200 (group 4, Lmf: 171.4) | |
| Sample: | plasma | |
| Analyte: | Lmf | |
| Assay: | HPLC with ultraviolet detection | |

Results:

| PK parameters (Day 1 / Day 89): | 20 mg/kg | 60 mg/kg | 200 mg/kg |
|---|---|---|---|
| Tmax* (h) | M 24 / 1<br>F 8 / 4 | M 24 / 2<br>F 8 / 8 | M 24 / 0<br>F 8 / 8 |
| Cmax (µg/mL) | M 2 / 6.15<br>F 2.86 / 6.03 | M 7.71 / 12.49<br>F 6.65 / 18.35 | M 7.82 / 15.84<br>F 9.51 / 15.65 |
| AUC0-24h (µg·h/mL) | M 19.67 / 86.33<br>F 39.67 / 94.67 | M 100 / 233<br>F 112.33 / 333.67 | M 98.33 / 230.33<br>F 136 / 286 |

*: median value

Three of the animal in the control group had measurable concentrations of Lmf in plasma on Day 89. No reason for this could be found.

| | | |
|---|---|---|
| Study title: | Plasma concentrations of CGP 56695 (benflumetol) in dogs after repeated daily administration of 20, 60 and 200 mg/kg of CGP 56697 (co-artemether) during a 3-month oral toxicity study. | Study no. BPK(CH) 1996/024 |
| Conclusions: | • All dogs having received the capsule formulation of Arm/Lmf were exposed to Lmf<br>• The onset of absorption of Lmf was slow; absorption continued over an extended period of time<br>• Plasma concentration-time profiles, AUC values and trough values of Lmf showed high variability and no clear relationship to the dose<br>• There was no obvious gender difference. | |

Additional information: Supplement to toxicity study no. 94-6155.

# 2.6.5.5 薬物動態試験：分布

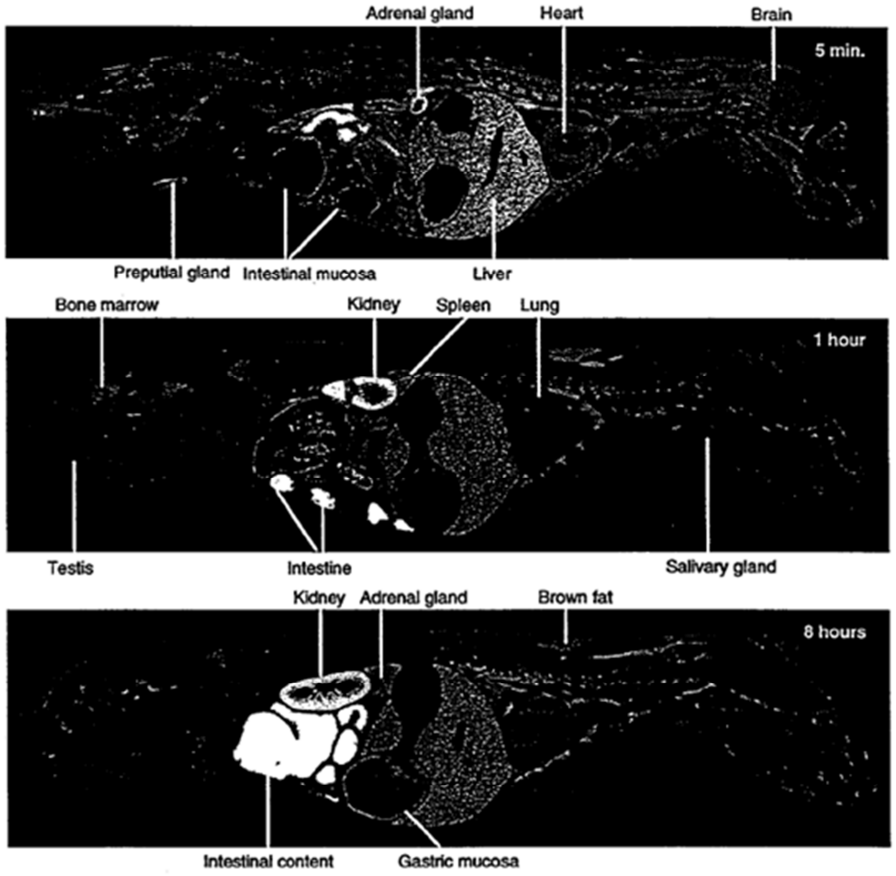
## 2.6.5.5A Pharmacokinetics: Organ distribution (DMPK(CH) 1997/241)

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol. | Study no. DMPK(CH) 1997/241 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-1 | |
| Test article: | $^{3}H$ or $^{14}C$-labeled Arm and unlabeled Lmf | |
| Species, strain, sex, number of animals: | male albino rat / Tif: RAIf (SPF) / n=3 per group<br>male pigmented rat / LE/Mol (SPF) / n=3 per group | |
| Feeding condition: | fasted | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7/3, w/w) for intravenous route<br>0.5% Klucel + 0.1% or 1%Tween 80 for oral route | |
| Method/ Route / Duration of administration: | intravenous bolus / tail vain / once<br>gavage / oral / once or for 10 days | |
| Dose (mg/kg): | intravenous dose of 10 once<br>oral dose of 20, 100, or 1000 once<br>oral dose of 20 once for 10 days | |
| Specific activity: | [$^{3}H$]-Arm (Batch No. Re-54.9A) of 3022.9 MBq/mmol or 10.05 MBq/mg [a]<br>[$^{3}H$]-Arm (Batch No. Re-54.9C) of 3.35 MBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9C1) of 3.35 MBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9D) of 1.39 MBq/mg | [$^{3}H$]-Arm (Batch No. Re-54.9E) of 33 KBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9G )of 273 KBq/mg<br>[$^{14}C$]-Arm (Batch No. Re-84.1A1) of 1.67 MBq/mg (WBAR, i.v.)<br>[$^{14}C$]-Arm (Batch No. Re-84.1C2) of 218 KBq/mg (WBAR, p.o.)<br>a: taken from the Certificate of Analysis sheet of Re-54.9A (typing error in report). |
| Analyte / Radionuclide: | radioactivity / $^{3}H$ or $^{14}C$ | |
| Assay: | whole body autoradiography with a semi-automatic cryostat microtome; liquid scintillation counting | |

| Study title: | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol. | Study no. DMPK(CH) 1997/241 |
|---|---|---|

**whole body autoradiography (5 min, 1 h and 8h; 33 days film exposure): [$^{14}$C]-Arm**

Radioactivity is seen as white areas.



| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol. | Study no. DMPK(CH) 1997/241 |
|---|---|---|

**Distribution results**:

Rat [$^3$H] mean concentrations (nmol/g)

| Route (time post dose) | intravenous (5 min) | intravenous (24 h) | intravenous (168 h) | single oral (30 min) | single oral (24 h) | single oral (168 h) | single oral (144 h) | single oral (144 h) | repeated oral (10 days) (24 h) |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg) | 10 | 10 | 10 | 20 | 20 | 20 | 100 | 1000 | 20 |
| Number of animals | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 |
| Sample | | | | | | | | | |
| Blood | 2.57 | 0.65 | 0.18 | 3.10 | 0.67 | 0.32 | 1.68 | 9.98 | 3.03 |
| Plasma | 3.74 | 0.57 | 0.06 | 4.00 | 0.49 | 0.07 | 0.40 | 2.81 | 1.56 |
| Erythrocytes | 1.20 | 0.67 | 0.31 | 2.49 | 0.91 | 0.47 | ns | ns | ns |
| Liver | 6.87 | 2.77 | 1.68 | 18.65 | 3.18 | 1.67 | 9.10 | 47.54 | 12.39 |
| Spleen | 1.58 | 2.81 | 2.23 | 3.31 | 1.64 | 1.39 | 6.56 | 40.23 | 6.65 |
| Adrenals | 17.78 | 4.61 | 2.76 | 6.84 | 2.00 | 1.59 | 8.42 | 48.75 | 7.89 |
| Bone marrow | 1.58 | 1.40 | 0.70 | 3.28 | 0.79 | 0.45 | 3.62 | 27.62 | 2.66 |
| Pituitary gland | 5.94 | 1.34 | 0.82 | 2.93 | 0.66 | 0.44 | ns | ns | ns |
| Thyroid | 7.81 | 1.76 | 1.18 | 3.97 | 1.14 | 0.60 | 3.05 | 13.60 | 4.74 |

ns = no sampling.

Additional information: none

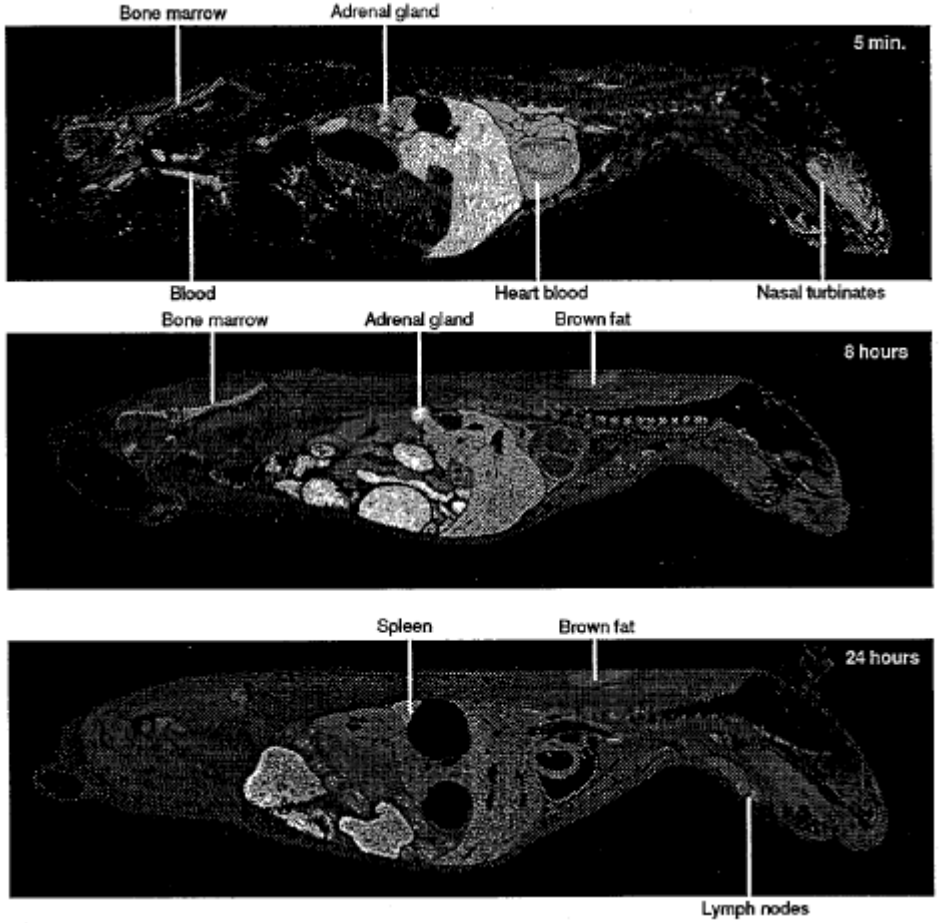
## 2.6.5.5B Pharmacokinetics: Organ distribution (DMPK(CH) 1997/240)

| **Study title:** | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | Study no. DMPK(CH) 1997/240 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-4 | |
| Test article: | $^{14}$C-labeled Lmf (6 parts) and unlabeled Arm (1 part) | |
| Species, strain, sex, number of animals: | male albino rat / Tif: RAIf (SPF), n=3 per group<br>male pigmented rat / LE/Mol (SPF, n=1 per time point, n=5 time points | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7/3, w/w) for intravenous route<br>0.5% Klucel + 0.1% Tween 80 for oral route | |
| Method/ Route / Duration of administration: | rats: intravenous bolus / tail vain / once<br>gavage / oral / once daily for 10 days (20 mg/kg)<br>gavage / oral / once (100, 1000 mg/kg) | |
| Dose (mg/kg): | rats: intravenous dose of 1 once<br>oral dose of 20 for 10 days<br>oral dose of 100 once<br>oral dose of 1000 once | |
| Specific activity: | [$^{14}$C]-Lmf (Batch No. Ko-76.1A-2) of 2069 KBq/mg<br>[$^{14}$C]-Lmf (Batch No. Ko-76.1B) of 64.1 KBq/mg<br>[$^{14}$C]-Lmf (Batch No. Ko-76.1C) of 10.0 KBq/mg<br>[$^{14}$C]-Lmf (Batch No. Ko-76.1D) of 960 KBq/mg | [$^{14}$C]-Lmf (Batch No. Ko-76.1F) of 159 or 148 KBq/mg<br>[$^{14}$C]-Lmf (Batch No. Ko-76.1G) of 1.147 KBq/mg<br>[$^{14}$C]-Lmf (Batch No. Ot-5.1) of 385.5 KBq/mg |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | liquid scintillation counting, whole body autoradiography with a semi-automatic cryostat microtome | |

| Study title: | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | Study no. DMPK(CH) 1997/240 |
|---|---|---|

**whole body autoradiography (5 min, 8 h and 24h; 38 days film exposure): [$^{14}$C]-Lmf**

Radioactivity is seen as white areas.



| **Study title:** | | | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | | | | | Study no. DMPK(CH) 1997/240 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Distribution results**: | | | | | | | | | | | |
| Rat | [$^{14}$C] mean concentrations (nmol/g) | | | | | | | | | | |
| Route (time post dose) | intravenous (5 min) | intravenous (24 h) | intravenous (168 h) | single oral (3 h) at $T_{max}$ | single oral (24 h) | | | single oral (168 h) | | | repeated oral (10 days) |
| Dose (mg/kg) | 1 | 1 | 1 | 20 | 20 | 100 | 1000 | 20 | 100 | 1000 | 20 |
| Number of animals | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Sample | | | | | | | | | | | |
| Blood | 10.3 | 0.18 | 0.02 | 2.14 | 0.35 | 2.82 | 25.5 | ns | 0.12* | ns | 2.07 |
| Plasma | 17.4 | 0.25 | 0.02 | 3.75 | 0.44 | 3.34 | 40.9 | 0.00 | 0.27* | 0.00 | 2.18 |
| Erythrocytes | 2.22 | 0.10 | 0.01 | 0.57 | 0.19 | 1.69 | 10.7 | ns | 0.21* | ns | 1.67 |
| Liver | 18.4 | 2.28 | 0.28 | 24.6 | 7.02 | 58.71 | 409 | 0.18 | 3.84 | 11.71 | 47.0 |
| Spleen | 4.93 | 4.46 | 0.38 | 5.77 | 9.29 | 58 | 207 | 0.31 | 6.44 | 17.74 | 66.7 |
| Adrenals | 3.97 | 16.0 | 1.06 | 14.9 | 44.6 | 302 | 775 | 0.96 | 16.0 | 52.36 | 251 |
| Bone marrow | 3.03 | 2.41 | 0.31 | 3.03 | 5.57 | 33.7 | 129 | 0.33 | 5.30 | 20.15 | 42.2 |
| Pituitary gland | 2.43 | 2.80 | 0.81 | 1.22 | 5.98 | 42.7 | 93.6 | 0.80 | 12.7 | 37.69 | 55.7 |
| Thyroid | 1.42 | 1.77 | 0.40 | 1.15 | 5.80 | 32.1 | 99.7 | 0.28* | 6.32 | 20.97 | 47.2 |

ns = not significant, *:Median

Additional information: none

# 2.6.5.6 薬物動態試験：蛋白結合

## 2.6.5.6A Pharmacokinetics: Plasma protein binding (DMPK(F) 1998/004)

| | | |
|---|---|---|
| Study title: | *In vitro* binding of artemether to human serum proteins, human erythrocytes, and serum proteins from dog, rabbit, rat, mouse, and cynomolgus monkey | Study No: DMPK(F) 1998/004 |
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.3-1 | |
| Test article: | [$^{14}$C]Arm | |
| Study system: | Mouse, rat, rabbit, dog and cynomolgus monkey serum | |
| | human serum proteins and blood from healthy volunteers; protein solutions from 3 batches of human serum albumin (HSA), $\alpha_1$-acid glycoprotein, high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins (VLDL) | |
| Radionuclide: | $^{14}$C | |
| Specific activity: | 1.67 MBq/mg | |
| Method: | ultrafiltration with micropartition system with YMT membrane; erythrocyte partitioning | |
| Analyte / Assay: | radioactivity / liquid scintillation counting | |
| | Ultrafiltration | |

| Protein medium: | Concentration tested (µg/mL) | % Bound |
|---|---|---|
| Human serum | 0.323 | 98.6 |
| | 0.646 | 98.6 |
| | 1 | 98.6 |
| | 3.23 | 98.2 |
| | 6.46 | 97.9 |
| | 10 | 98.1 |

| Study title: | *In vitro* binding of artemether to human serum proteins, human erythrocytes, and serum proteins from dog, rabbit, rat, mouse, and cynomolgus monkey | | | | | Study No: DMPK(F) 1998/004 | | |
|---|---|---|---|---|---|---|---|---|
| | Ultrafiltration | | Erythrocyte partitioning | | | | | |
| Protein medium: | Concentration tested (µg/mL) | % Bound | Protein medium | nKa (L/mol) | NKa | $NK_E$ | r | % Bound |
| Human protein solutions | | | plasma | ND | 20.6 | 3.98 | 0.985 | 95.4 |
| HSA-S1 | 1 | 91.4 | HSA | $6.2 \times 10^3$ | 3.73 | 4.15 | 0.991 | 78.3 |
| HAS-S2 | 0.5 | 87.6 | $\alpha_1$-acid glycoprotein | $3.2 \times 10^5$ | 7.22 | 4.27 | 0.987 | 87.8 |
| | 1 | 87.6 | gamma globulins | $1.3 \times 10^3$ | 0.087 | 3.33 | 0.635 | 8.0 |
| | 5 | 87.8 | high density lipoproteins | $2.1 \times 10^5$ | 2.69 | 3.24 | 0.983 | 72.9 |
| | 10 | 87.6 | low density lipoproteins | $1.7 \times 10^6$ | 2.01 | 3.62 | 0.983 | 66.8 |
| HSA -S3 | 1 | 83.2 | very low density lipoproteins | $2.0 \times 10^7$ | 2.53 | 3.23 | 0.989 | 71.7 |
| $\alpha_1$-acid glycoprotein | 1 | 90.3 | | | | | | |
| | 10 | 51.6 | | | | | | |
| gamma globulins | 1 | 64.1 | | | | | | |
| | 10 | 64.1 | | | | | | |
| Animal serum | | | | | | | | |
| mouse | 1 | 98.3 | | | | | | |
| | 10 | 98.4 | | | | | | |
| rat | 1 | 97.3 | | | | | | |
| | 10 | 97.3 | | | | | | |
| rabbit | 1 | 97.2 | | | | | | |
| | 10 | 97.1 | | | | | | |
| dog | 1 | 97.1 | | | | | | |
| | 10 | 97.3 | | | | | | |
| cynomolgus monkey | 1 | 96.2 | | | | | | |
| | 10 | 96.0 | | | | | | |

ND = not determined
nKa = binding capacity of protein
NKa= total binding capacity
$NK_E$ = total binding capacity of erythrocyte
r = correlation coefficient of the regression lines
% bound = calculated as CB/CP
CB = concentration of bound drug to plasma proteins
CP = total concentrations of Arm in plasma or protein solution

The main protein involved in the binding was found to be $\alpha_1$-acid glycoprotein, considering binding capacity and physiological concentration of this protein. A similar observation was made with arteether, another malarial drug. $\alpha_1$-acid glycoprotein concentration is markedly increased during malarial infection; therefore, alteration in plasma protein binding might be clinically important.
The variability of $NK_E$, determined by the coefficient of variation of the mean (3.7) from 8 experiments, was 11%. Arm concentration in erythrocytes was expected to be 3.7 times higher than free Arm concentrations, and this distribution resulted in a similar ratio of 3 (11/3.7).

Additional information: none

## 2.6.5.6B Pharmacokinetics: Plasma protein binding (BPK(F) 1996/044)

| | | |
|---|---|---|
| Study title: | *In vitro* binding of benflumetol to human serum proteins, human erythrocytes, and serum proteins from mouse, rat, dog, rabbit, baboon, and cynomolgus monkey | Study No: BPK(F) 1996/044 |
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.3-2 | |
| Test article: | [$^{14}$C]-Lmf | |
| Study system: | Mouse, rat, rabbit, dog and cynomolgus monkey, baboon serum | |
| | human serum (or plasma) proteins and blood from healthy volunteers; protein solutions from 1 batch of human serum albumin (HSA), $\alpha_1$-acid glycoprotein, high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins (VLDL) | |
| Radionuclide: | $^{14}$C | |
| Specific activity: | 911 KBq/mg | |
| Method: | ultrafiltration with micropartition system with YMT membrane; erythrocyte partitioning | |
| Analyte / Assay: | radioactivity / liquid scintillation counting | |

**Results**

| | Ultrafiltration | | Erythrocyte partitioning | | | | | |
|---|---|---|---|---|---|---|---|---|
| Protein medium: | Concentration tested (µg/mL) | % Bound | Protein medium | $nK_a$ (L/µmol) | $NK_a$ | $NK_E$ | r | % Bound |
| Human serum (plasma) | | | plasma | ND | 379 | 48.6 | 0.997 | 99.7 |
| C1h (150 µL) | 1 | 100* | HSA | 0.0028 | 1.68 | 9.14 | 0.963 | 62.8 |
| | 10 | 100* | $\alpha_1$-acid glycoprotein | 0.0088 | 0.20 | 23.9 | 0.976 | 16.8 |
| C2h (350 µL) | 10 | 99.93 | high density lipoproteins | 27.5 | 275 | 91.4 | 0.993 | 99.6 |
| | | | low density lipoproteins | 26.0 | 26.0 | 45.2 | 0.972 | 96.3 |
| | | | very low density lipoproteins | 235 | 23.5 | 44.3 | 0.987 | 95.9 |
| Human serum albumin solutions | | | | | | | | |
| HSA | | | C1h = concentration at 1 hour<br>C2h = concentration at 2 hour<br>ND = not determined<br>nKa = binding capacity of protein<br>NKa= total binding capacity | | | | | |
| C1h (400 µL) | 10 | 99.97 | | | | | | |
| C2h (400 µL) | 10 | 99.93 | | | | | | |

| Study title: | | *In vitro* binding of benflumetol to human serum proteins, human erythrocytes, and serum proteins from mouse, rat, dog, rabbit, baboon, and cynomolgus monkey | Study No: BPK(F) 1996/044 |
|---|---|---|---|
| Animal serum | | | $NK_E$ = total binding capacity of erythrocyte<br>r = correlation coefficient of the regression lines<br>% bound = calculated as CB/CP<br>CB = concentration of bound drug to plasma proteins<br>CP = total concentrations of Arm in plasma or protein solution<br>The binding capacity of lipoproteins was high, in particular very low density lipoproteins. However, considering the respective physiological concentration, high density lipoproteins contributed mainly to the binding.<br>The variability of $NK_E$ was evaluated by the standard deviation of the mean value of $NK_E$ determined by 6 experiments was 28. The mean value of $NK_E$ (44) was close to the value obtained in plasma (49), showing that Lmf concentration in erythrocytes was 44 – 49 times higher than free drug concentration. |
| mouse | 10 | 99.82 | |
| rat | 10 | 99.90 | |
| rabbit | 10 | 99.93 | |
| dog | 10 | 99.92 | |
| cynomolgus monkey | 10 | 99.94 | |
| baboon | 10 | 99.95 | |

* poor reliability due to very low values in the ultrafiltrate

Additional information: none

## 2.6.5.6C Pharmacokinetics: Plasma protein binding (DMPK(CH) R0101415)

| **Study title:** | *Ex vivo* plasma protein binding of quinine in healthy subjects given quinine alone or in combination with COA566 (study trial 2302) | Study No: DMPK(CH) R0101415 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.3-3 | |
| Test article: | quinine | |
| Objectives: | To investigate the extent of protein binding of quinine in plasma of healthy subjects given quinine (2-hr *i.v.* infusion of 10 mg/kg) alone or in combination with Arm/Lmf. For details on the study design, please refer to protocol No. CCOA566 2302 (A randomized, double-blind, parallel-group study to evaluate the cardiac effects and pharmacokinetic interaction of the combined [sequential] administration of oral Arm/Lmf and *i.v.* quinine in healthy subjects), dated 08-Sep-2000. The subjects selected for the present binding evaluation were those who received quinine alone (n=14) or the combined administration (n=14). In order to have enough plasma volume for the assay, 2 plasma pools were prepared for each subject after both treatments with the following selected timepoints:<br>62.5, 63, 64, and 65 hours post first dose of Arm/Lmf (or placebo) were pooled for each subject<br>66, 68, 72, and 86 hours post first dose of Arm/Lmf (or placebo) were pooled for each subject | |
| Compound: | Quinine | |
| Matrix | Human volunteers/plasma | |
| Assay Method: | | |
| *Ultrafiltration technique* | The free fraction of quinine was determined by ultrafiltration. After incubation for 30 min at 37°C, the plasma samples were centrifuged at 2000 g for 10 min in the pre-warmed Centrifree devices (Amicon Inc., Beverly, MA, USA, molecular cutoff of 30 kD). The concentration of quinine was determined in the sample introduced into the reservoir before ultrafiltration, and in the ultrafiltrate after ultrafiltration. | |
| Analytical method: | Determination of quinine by a liquid-liquid extraction procedure and analysis of the extract by liquid chromatography/fluorescence detection | |
| Sample preparation: | Liquid-liquid extraction according to the analytical method described in DMPK(F) R00-1921. The same method was used for plasma and ultrafiltration samples. LLOQ was set to 20 ng/mL in both fluids. | |
| Instrumental conditions: | | |
| *Chromatography* | Reverse phase HPLC on an Inertsil silice 5 µ (4.6 x 250 mm), column temperature 24°C, isocratic elution (acetonitrile-methanol containing 25% ammonia solution, [92.5:7.5, v:v], 78:22, v:v) flow rate 1.0 mL/min, injection volume 70 µL | |
| *Detection* | Fluorescence detection set at 325 and 375 nm for excitation and emission waves, respectively | |

| **Study title:** | *Ex vivo* plasma protein binding of quinine in healthy subjects given quinine alone or in combination with COA566 (study trial 2302) | Study No: DMPK(CH) R0101415 |
|---|---|---|
| *Data processing* | Software for data acquisition, X-chrom Version 2.10, LabSystems, UK | |
| | Software for calibration, X-chrom Version 2.10, LabSystems, UK | |
| | Software for additional data processing, Microsoft Excel, Version 97 SR-2 | |
| *Within-study validation* | Different stock solutions of quinine were used for the preparation of calibration (C) and quality control (QC) samples. | |
| Sample preparation: | | |
| *Bound fraction to plasma proteins* | Bound fraction in plasma | |
| | Range 84% to 90% in human volunteers at total quinine plasma concentrations from 1380 to 4250 ng/mL | |
| *Free fraction* | Free(%) = Conc. in ultrafiltration / Conc. in plasma x 100% | |
| *Bound fraction* | Bound = 100% - Free | |
| Reference compound: | | |
| *Quinine* | Molecular formula: $C_{20}H_{24}N_2O_2$ | |
| | Molecular weight (MW): 324.4 g/mol | |
| Internal standards: | | |
| *Hydroquindine* | Molecular formula: $C_{20}H_{26}N_2O_2$ | |
| | Molecular weight (MW): 326.44 g/mol | |
| Results-1: | | |
| *Bound Fraction to plasma proteins* | Bound fraction in plasma:<br>• 86 ± 3% (mean value, n=14) after quinine alone administration for pool A (timepoints 62.5, 63, 64, and 65 hr; mean total plasma concentration = 2776 ng/mL)<br>• 89 ± 2% (mean value, n=14) after quinine alone administration for pool B (timepoints 66, 68, 72, and 86 hr; mean total plasma concentration = 1951 ng/mL)<br>• 90 ± 1% (mean value, n=14) after combined administration of quinine and Arm/Lmf for pool A (timepoints 62.5, 63, 64, and 65 hr; mean total plasma concentration = 3060 ng/mL)<br>• 90 ± 3% (mean value, n=14) after combined administration of quinine and Arm/Lmf for pool B (timepoints 66, 68, 72, and 86 hr; mean total plasma concentration = 2019 ng/mL) | |

| **Study title:** | *Ex vivo* plasma protein binding of quinine in healthy subjects given quinine alone or in combination with COA566 (study trial 2302) | Study No: DMPK(CH) R0101415 |
|---|---|---|
| Discussion and Conclusions: | • The extent of protein binding of quinine was around 90% at total plasma concentrations from 1380 to 4250 ng/mL, as expected from literature data (Silamut K, White NJ, Looareesuwan S, Warrell DA. Binding of quinine to plasma proteins in falciparum malaria. Am. J. Trop. Med. Hyg.. 1985; 34: 681-6 ; and Karbwang J, Davies TME, Looareesuwan S, Molunto P, Bunnag D, White NJ. A comparison of the pharmacokinetic and pharmacodynamic properties of quinine and quinidine in healthy Thai males. J. Clin. Pharmacol. 1993; 35: 265-71.)<br>• The extent of protein binding was similar after quinine alone administration and when combined with Arm/Lmf.<br>• The inter-subject variability of the quinine protein binding was low (CVs in the range 1-3%).<br>• The combined administration of quinine and Arm/Lmf did not modify the extent of quinine protein binding. | |
| Special issues | No major deviation from protocol affecting the conclusions | |

Additional information: none

# 2.6.5.7 薬物動態試験：妊娠動物における試験

## 2.6.5.7A Pharmacokinetics: Study in pregnant and nursing animals (DMPK(CH) 1997/099)

| Study title: | Transfer to the embryo-fetal compartment of pregnant rats after peroral administration of 30 mg/kg of CGP 56697 (co-artemether) containing $^{3}$H-labeled CGP 56696 (artemether) | | | Study No: DMPK(CH) 1997/099 | |
|---|---|---|---|---|---|
| GLP compliance: | not required | | | | |
| Location in CTD: | 4.2.2.3-4 | | | | |
| Test article: | Arm/Lmf: containing 1 part $^{14}$C-labeled Arm and 6 parts unlabeled Lmf | | | | |
| Species / strain: | rat / Tif: RAIf (SPF) | | | | |
| Gestation day / number of animals: | Day 13 / 15; n=3 per time point | | | | |
| Vehicle / formulation: | 0.5% Klucel + 1.0% Tween 80 | | | | |
| Method/ route / duration of administration: | gavage / oral / once | | | | |
| Dose (mg/kg): | 30 (4.29 Arm, 25.7 Lmf) | | | | |
| Radionuclide: | $^{3}$H | | | | |
| Specific activity: | 435 KBq/mg | | | | |
| Analyte/assay (unit): | radioactivity / radiometry by liquid scintillation counting (counts per minutes) | | | | |
| | Mean [$^{3}$H] concentrations (nmol/g) | | | | |
| Time (hr): | 0.5 | 1 | 4 | 8 | 24 |
| Dam | | | | | |
| Blood | 3.70 | 3.44 | 3.59 | 3.25 | 1.63 |
| Plasma | 4.44 | 4.08 | 3.95 | 3.14 | 1.16 |
| Liver | 20.02 | 14.11 | 12.60 | 12.09 | 5.55 |
| Spleen | 4.74 | 4.25 | 5.22 | 4.99 | 3.67 |
| Adrenals | 11.56 | 8.45 | 7.13 | 6.21 | 3.34 |
| Thyroid | 4.95 | 3.86 | 3.52 | 3.36 | 1.81 |
| Mammary glands | 4.59 | 4.26 | 2.88 | 1.88 | 1.07 |
| Fetus | 2.31 | 2.53 | 2.49 | 1.94 | 0.56 |
| Placenta | 3.91 | 4.87 | 5.14 | 4.14 | 1.99 |
| Amniotic fluid | 1.57 | 1.95 | 1.87 | 1.63 | 0.35 |
| $C_{fetus}$ / $C_{blood\ of\ dam}$ | 2.31 / 3.70 = 0.6243 | 2.53 / 3.44 = 0.7355 | 2.49 / 3.59 = 0.6936 | 1.94 / 3.25 = 0.5969 | 0.56 / 1.63 = 0.3436 |

Additional information: none

## 2.6.5.7B Pharmacokinetics: Study in pregnant and nursing animals (DMPK(CH) 1997/004)

| Study title: | Transfer of radioactive substance(s) to the embryo-fetal compartment of rabbits after peroral administration of CGP 56697 (co-artemether) containing $^{14}$C-labeled CGP 56696 (artemether) | Study No: DMPK(CH) 1997/004 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.3-8 | |
| Test article: | Arm/Lmf containing 1 part $^{14}$C -labeled Arm and 6 parts unlabeled Lmf | |
| Species / strain: | pregnant rabbits / New Zealand, albino | |
| Gestation day / number of animals: | Day 17 / 2 | |
| Vehicle / formulation: | 0.5% Klucel + Tween 80 | |
| Method/ route / duration of administration: | gavage / oral / once | |
| Dose (mg/kg): | 175 (25 Arm, 150 Lmf) | |
| Radionuclide: | $^{14}$C | |
| Specific activity: | 43 KBq/mg | |
| Analyte/assay (unit): | radioactivity / radiometry by liquid scintillation counting (counts per min) | |
| | unchanged Arm and DHA in plasma/ HPLC with electromechanical detection in reductive mode | |
| Results: | [$^{14}$C] concentrations (nmol/g) | |
| Time (hr): | 24 | |
| | Rabbit 1 | Rabbit 2 |
| Dam | | |
| Blood | 2.06 | 2.41 |
| Plasma | 2.72 | 2.72 |
| Liver | 34.13 | 46.92 |
| Kidney | 19.15 | 13.69 |
| Mammary glands | 0.86 | 0.84 |
| Fetus | 1.03 | 2.38 |
| Liver | 4.10 | 11.33 |
| Placenta | 3.95 | 7.46 |
| Amniotic fluid | 1.64 | 1.70 |
| Cfetus / Cblood of dam | 1.03 / 2.06 = 0.5 | 2.38 / 2.41 = 0.9875 |

| Study title: | Transfer of radioactive substance(s) to the embryo-fetal compartment of rabbits after peroral administration of CGP 56697 (co-artemether) containing $^{14}$C-labeled CGP 56696 (artemether) | Study No: DMPK(CH) 1997/004 |
|---|---|---|
| Kinetics of $^{14}$C in blood and plasma: | AUC values for radioactivity at 24 hours post dose were 157 and 184 µmol·h/L in blood and 244 and 260 µmol·h/L in plasma for Rabbit 1 and Rabbit 2, respectively.<br>AUC values for Arm were not calculated, but those for DHA, detectable up to 2 hours after dosing, were 0.14 and 0.40 µmol·h/L for Rabbit 1 and Rabbit 2, respectively. | |
| Excretion in urine and feces: | By 24 hours post dose the total recovery of $^{14}$C in excreta was 68.44%, with 66.51% recovered in urine and 1.93% recovered in feces. | |
| Metabolites in urine: | Radioactivity was detected in 37 metabolite peaks in the urine, 21 of which accounted for 56.6% of the dose plus 16 trace peaks accounting for another 9.9%, giving a total of 66.5% analysed. | |

Additional information: none

## 2.6.5.7C Pharmacokinetics: Study in pregnant and nursing animals (BPK(F) 1996/022)

| **Study title:** | Artemether and dihydroartemisinin plasma concentrations in female rabbits in a follow-up dose-range finding study evaluating effects of Co-artemether on embryo and fetal development. | Study No: BPK(F) 1996/022 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.5.2-4 | |
| Test article: | Arm/Lmf containing 1 part Arm and 6 part Lmf | |
| Species, strain, sex, number of animals: | pregnant rabbit, New Zealand, F / 32 | |
| Feeding condition: | fed | |
| Vehicle / formulation | 0.5% Klucel | |
| Method of administration: | oral gavage | |
| Duration of dosing: | once daily for 13 days<br>study performed on pregnant rabbits. Samples were drawn on Day 13 of dosing to Day 19 of gestation. | |
| Daily dose (mg/kg) | 0 (control 1)<br>210 (group 2)<br>700 (group 3)<br>2100 (group 4) | |
| Sample: | plasma | |
| Analyte: | Arm and DHA | |
| Assay: | HPLC with electrochemical detection operated in reductive mode | |
| Results: | • In the Control Groups, Arm and DHA were not detected in any of the samples. | |
| | • In Group 2, no detectable concentrations of Arm or DHA were found. | |
| | • In Group 3, no detectable concentrations of Arm were found, while concentrations of DHA were detected at 1 and 2 hours post dose. The means were 25.7 (n=4) and 17.1 (n=2) ng/mL for $C_{1h}$ and $C_{2h}$, respectively. At 4, 8, and 24 hours post dose, concentrations of DHA were measurable in only 1 sample. | |
| | • In Group 4, Arm was found in 4 of 20 samples at 1, 2, 4, 8, and 24 hours post dose. DHA was detected at 1 and 2 hours post dose. The means were 123 (n=4) and 54.2 (n=4) ng/mL for $C_{1h}$ and $C_{2h}$, respectively. At 4, 8 and 24 hours post dose, concentrations of DHA were measurable in only 2 samples. | |
| | • The rapid disappearance of DHA from plasma after administration of 700 and 2100 mg/kg of Arm/Lmf indicates a high clearance of this metabolite. | |

Additional information: Supplement to Toxicity study no. 95-4049.

## 2.6.5.7D Pharmacokinetics: Study in pregnant or nursing animals (DMPK(CH) 1997/139)

| Study title: | Transfer of radioactive substance(s) to the embryo-foetal compartment of rats after peroral administration of 30 mg/kg CGP 56697 (co-artemether) containing [$^{14}$C]CGP 56695 ([$^{14}$C]benflumetol) and unlabeled CGP 56696 (artemether) | | | Study No: DMPK(CH) 1997/139 |
|---|---|---|---|---|
| GLP compliance: | not required | | | |
| Location in CTD: | 4.2.2.3-5 | | | |
| Test article: | Arm/Lmf containing 1 part unlabeled Arm and 6 parts $^{14}$C-labeled Lmf | | | |
| Species / strain: | rat / Tif: RAIf (SPF) | | | |
| Gestation day / number of animals: | Day 13 / 12 with 8 reserve; n = 3 per time point | | | |
| Vehicle / formulation: | 0.5% Klucel / 1.0% Tween 80 | | | |
| Method/ route / duration of administration: | gavage / oral / once | | | |
| Dose (mg/kg): | 30 | | | |
| Radionuclide: | $^{14}$C | | | |
| Specific activity: | 148 KBq/mg | | | |
| Analyte/assay (unit): | radioactivity / radiometry by liquid scintillation counting (counts per minutes) | | | |
| | Mean $^{14}$C concentrations (nmol/g) | | | |
| Time (hr): | 1 | 4 | 8 | 24 |
| Dam | | | | |
| Blood | 2.74 | 8.67 | 9.78 | 3.74 |
| Plasma | 4.51 | 13.80 | 15.72 | 6.44 |
| Liver | 4.23 | 69.07 | 110 | 43.71 |
| Spleen | 1.60 | 26.58 | 41.32 | 30.78 |
| Adrenals | 3.00 | 69.25 | 178 | 244 |
| Thyroid | 0.33 | 3.37 | 7.83 | 11.08 |
| Mammary glands | 0.12 | 1.42 | 3.11 | 8.48 |
| Fetus | 0.01 (median) | 0.10 | 0.37 | 0.98 |
| Placenta | 0.65 | 6.81 | 13.45 | 11.20 |
| Amniotic fluid | 0.00 | 0.00 | 0.05 | 0.08 |
| Cfetus / Cblood of dam | 0.01 / 2.74 = 0.0036 | 0.10 / 8.67 = 0.0115 | 0.37 / 9.78 = 0.0378 | 0.98 / 3.74 = 0.2620 |

Additional information: none

## 2.6.5.7E Pharmacokinetics: Study in pregnant and nursing animals (DMET(EU) 29/1996)

| | | |
|---|---|---|
| Study title: | Transfer of radioactive substance(s) to the embryo-foetal compartment of rabbits after peroral administration of CGP 56697 (co-artemether) containing $^{14}$C-labeled CGP 56695 (benflumetol) | Study No: DMET(EU) 29/1996 |
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.3-6 | |
| Test article: | Arm/Lmf as 1 part unlabeled Arm and 6 parts $^{14}$C-labeled Lmf | |
| Species / strain: | rabbit / New Zealand albino | |
| Gestation day / number of animals: | Day 17 / 2 | |
| Vehicle / formulation: | 0.5% Klucel + Tween 80 | |
| Method/ route / duration of administration: | gavage / oral / once | |
| Dose (mg/kg): | 175 | |
| Radionuclide: | $^{14}$C | |
| Specific activity: | 9 KBq/mg | |
| Analyte/assay (unit): | radioactivity / radiometry by liquid scintillation counting (counts per min) | |
| Results | Mean $^{14}$C concentrations (nmol/g) | |
| Time (hr): | 24 | |

| | Rabbit 95354 | Rabbit 95442 |
|---|---|---|
| Dam | | |
| Blood | 9.44 | 4.23 |
| Plasma | 12.7 | 6.37 |
| Liver | 212 | 91.6 |
| Kidney | 21.5 | 10.0 |
| Mammary glands | 9.37 | 2.88 |
| Fetus | 0.19 | 0.12 |
| Liver | 0.51 | below the limit of detection |
| Placenta | 55.2 | 26.6 |
| Amniotic fluid | 0.18 | below the limit of detection |
| Cfetus / C blood of dam | 0.19 / 9.44 = 0.0201 | 0.12 / 4.23 = 0.0284 |

| | | |
|---|---|---|
| Study title: | Transfer of radioactive substance(s) to the embryo-foetal compartment of rabbits after peroral administration of CGP 56697 (co-artemether) containing $^{14}$C-labeled CGP 56695 (benflumetol) | Study No: DMET(EU) 29/1996 |
| Kinetics of $^{14}$C in blood and plasma: | AUC of radioactivity values at 24 hours post dose were 157 and 78 µmol·h/L in blood and 266 and 121 µmol·h/L in plasma for Rabbit 95354 and Rabbit 95442, respectively. | |
| Excretion in urine and feces: | Both rabbits excreted very small volumes of urine and feces in the 24 hours post dose. Urine (about 10 mL each rabbit) contained about 0.3% of the radioactive dose. Rabbit 95354 excreted 30% of radioactive dose in about 100 g feces, and Rabbit 95442 did not excrete feces within 24 hours post dose. | |

Additional information: none

## 2.6.5.7F Pharmacokinetics: Study in pregnant and nursing animals (DMPK(CH) 1997/079)

| **Study title:** | Plasma concentrations of benflumetol in pregnant rabbits after peroral administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56695 (benflumetol) and unlabelled CGP 56696 (artemether). | Study No: DMPK(CH) 1997/079 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.3-7 | |
| Test article: | Arm/Lmf: containing 1 part Arm and 6 parts $^{14}$C-labelled Lmf | |
| Species, strain, sex, number of animals: | pregnant rabbits, New Zealand, F / 2 | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | 0.5% Klucel + 0.1% Tween 80 | |
| Method of Administration: | oral gavage; the animals were dosed on Day 17 of gestation | |
| Dose (mg/kg): | 175 mg/kg | |
| Sample: | plasma | |
| Analyte: | Lmf | |
| Assay: | HPLC with ultraviolet detection, method according to Report BPK(CH)1994/038 | |

| PK parameters: | Rabbit 95354 | Rabbit 95442 |
|---|---|---|
| Tmax (h) | 24 | 24 |
| Cmax (µg/mL) | 7.3 | 3.7 |
| AUC0-24h (µg·h/mL) | 145.1 | 56.4 |

Additional information: Study DMPK(CH) 1997/079 was performed on pregnant rabbits on Day 17 of gestation as part of study DMET(EU) 29/1996.

## 2.6.5.7G Pharmacokinetics: Study in pregnant and nursing animals (BPK(CH) 1996/085)

| | | | |
|---|---|---|---|
| Study title: | Plasma concentrations of CGP 56695 (benflumetol) in female rabbits after repeated daily administration of 210, 700 and 2100 mg/kg of CGP 56697 (co-artemether) on day 19 of gestation during a 13-day oral toxicity study. | | Study No: BPK(CH) 1996/085 |
| GLP compliance: | Yes | | |
| Location in CTD: | 4.2.3.5.2-4 | | |
| Test article: | Arm/Lmf: containing 1 part Arm and 6 parts Lmf | | |
| Species, strain, sex, number of animals: | pregnant rabbits, New Zealand, F / 32 | | |
| Feeding condition: | fed | | |
| Vehicle / formulation | 0.5% Klucel | | |
| Method of administration: | oral gavage | | |
| | the study was performed on pregnant rabbits. Samples were drawn on Day 13 of dosing, Day 19 of gestation. | | |
| Duration of dosing: | once daily for 13 days | | |
| Daily dose (mg/kg) | 0 (control 1)<br>210 (group 2)<br>700 (group 3)<br>2100 (group 4) | | |
| Sample: | plasma | | |
| Analyte: | Lmf | | |
| Assay: | HPLC with ultraviolet detection, method according to Report BPK(CH)1994/038 | | |
| Results: | 210 mg/kg | 700 mg/kg | 2100 mg/kg |
| PK parameters: | (180 mg/kg Lmf ) | (600 mg/kg Lmf ) | (1800 mg/kg Lmf ) |
| Tmax (h) | 8 (Day 13) | 8 (Day 13) | 4 (Day 13) |
| Cmax (µg/mL) | 22.8 (Day 13) | 47.5 (Day 13) | 47.9 (Day 13) |
| AUC0-24h (µg·h/mL) | 310 (Day 13) | 841 (Day 13) | 870 (Day 13) |
| Trough concentration (µg/mL) | 7.4 / 2.4 (Day 13/14) | 16.1 / 30.9 (Day 13/14) | 31.3 / 35.0 (Day 13/14) |
| | None of the animals in the control group had measurable concentrations of Lmf in plasma. | | |

Additional information: Supplement to toxicity study no. 95-4092.

## 2.6.5.7H Pharmacokinetics: Study in pregnant or nursing animals (BPK(CH) 1996/084)

| **Study title:** | Plasma concentrations of CGP 56695 (benflumetol) in female rabbits after repeated daily administration of 1000 mg/kg CGP 56695 on day 19 of gestation during a 13-day oral toxicity study. | Study No: BPK(CH) 1996/084 |
|---|---|---|
| GLP compliance: | Yes | |
| Location in CTD: | 4.2.3.5.2-10 | |
| Test article: | Lmf | |
| Species, strain, sex, number of animals: | pregnant rabbits, New Zealand, F / 11 | |
| Feeding condition: | fed | |
| Vehicle / formulation | 0.5% Klucel | |
| Method of administration: | oral gavage | |
| Duration of dosing: | once daily for 13 days<br>Study was performed on pregnant rabbits. Samples were drawn on Day 13 of dosing, Day 19 of gestation. | |
| Daily dose (mg/kg) | 0 (control)<br>1000 (group 2) | |
| Sample: | plasma | |
| Analyte: | Lmf | |
| Assay: | HPLC with ultraviolet detection, method according to Report BPK(CH)1994/038 | |
| PK parameters (Day 1 / Day last): | 1000 mg/kg | |
| Tmax (h) | 10 (Day 13) | |
| Cmax (µg/mL) | 37.9 (Day 13) | |
| AUC0-24h (µg·h/mL) | 592 (Day 13) | |
| Trough concentration (µg/mL) | 15.3 / 14.6<br>(Day 13/14) | |
| Special issues: | None of the animals of the control group had measurable concentrations of Lmf in plasma, while the plasma sample of the treated rabbits all contained Lmf . | |

Additional information: Supplement to toxicity study no. 95071.

## 2.6.5.8 薬物動態試験：その他の分布試験

該当なし

# 2.6.5.9 薬物動態試験：*in vivo* における代謝

## 2.6.5.9A Pharmacokinetics: Metabolism *in vivo* (DMPK(CH) 1997/241)

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol. | Study no. DMPK(CH) 1997/241 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-1 | |
| Test article | Arm/Lmf as 1 part $^{3}H$ or $^{14}C$-labeled Arm and 6 parts unlabeled Lmf | |
| Species, strain, sex, number of animals: | male rat albino / Tif: RAIf (SPF) / n=3 or n=4 per group | |
| Feeding condition: | Fed and Fasted | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7/3, w/w) for intravenous route<br>0.5% Klucel + 0.1% or 1% Tween 80 / for oral route | |
| Method/ Route / Duration of administration: | intravenous bolus / tail vain / once<br>gavage / oral / once or for 10 days | |
| Dose (mg/kg): | intravenous dose of 10 once<br>oral dose of 20, 100, or 1000 once<br>oral dose of 20 once for 10 days | |
| Specific activity: | [$^{3}H$]-Arm (Batch No. Re-54.9A) of 3022.9 MBq/mmol or 10.05 MBq/mg [a]<br>[$^{3}H$]-Arm (Batch No. Re-54.9C) of 3.35 MBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9C1) of 3.35 MBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9D) of 1.39 MBq/mg | [$^{3}H$]-Arm (Batch No. Re-54.9E) of 33 KBq/mg<br>[$^{3}H$]-Arm (Batch No. Re-54.9G )of 273 KBq/mg<br>[$^{14}C$]-Arm (Batch No. Re-84.1A1) of 1.67 MBq/mg (WBAR, i.v.)<br>[$^{14}C$]-Arm (Batch No. Re-84.1C2) of 218 KBq/mg (WBAR, p.o.)<br>a: taken from the Certificate of Analysis sheet of Re-54.9A (typing error in report). |
| Analyte / Radionuclide: | radioactivity / $^{3}H$ or $^{14}C$ | |
| Assay: | HPLC with ultraviolet and radiodetection; liquid scintillation counting | |

a: value from Certificate of analysis, typing error in report.

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | Study no. DMPK(CH) 1997/241 |
|---|---|---|

**Metabolites**

Proportions of metabolites in rat urine and feces after 10 mg/kg Arm/Lmf with [$^3$H] Arm given i.v.(n = 4)

| Peak HPLC Retention Time (minutes) | Peak Number | Feces % of dose | Urine % of dose | Total % of dose |
|---|---|---|---|---|
| 4.0 | 2 | 0.7 | 0.8 | 1.6 |
| 6.5 | 3 | 0.1 | 1.0 | 1.0 |
| 8.0 | 4 | 0.1 | 2.8 | 2.9 |
| 10.0 | 5 | 0.6 | 0.6 | 1.2 |
| 19.5 | 9 | 0.5 | 0.5 | 1.0 |
| 22.5 | 10 | 0.6 | 0.8 | 1.4 |
| 25.5 | 11 | 0.6 | 1.0 | 1.6 |
| 28.0 | 12 | 0.8 | 2.2 | 3.0 |
| 31.0 | 13 | 0.4 | 0.9 | 1.3 |
| 32.5 | 14 | 0.7 | 1.1 | 1.7 |
| 34.5 | 15 | 0.7 | 0.9 | 1.6 |
| 36.0 | 16 | 0.4 | 1.2 | 1.6 |
| 38.0 | 17 | 0.6 | 1.3 | 1.9 |
| 39.5 | 18 | 0.7 | 1.8 | 2.5 |
| 42.5 | 19 | 0.4 | 0.8 | 1.3 |
| 44.0 | 20 | 0.5 | 0.9 | 1.4 |
| 45.5 | 21 | 0.2 | 1.4 | 1.6 |
| 47.5 | 22 | 0.9 | 2.1 | 3.0 |
| 49.5 | 23 | 0.6 | 1.0 | 1.6 |
| 51.0 | 24 | 0.2 | 0.8 | 1.1 |
| 52.5 | 25 | 0.9 | 2.2 | 3.1 |
| 54.0 | 26 | 0.6 | 2.8 | 3.4 |
| 57.5 | 28 | 1.0 | 1.9 | 2.9 |
| 59.5 | 29 | 0.4 | 0.7 | 1.1 |

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | | Study no. DMPK(CH) 1997/241 |
|---|---|---|---|---|---|
| 61.0 | 30 | 0.9 | 1.7 | 2.5 |
| 63.5 | 32 | 0.7 | 0.6 | 1.3 |
| 64.5 | 33 | 0.6 | 0.7 | 1.3 |
| 71.5 | 37 | 0.6 | 0.4 | 1.0 |
| 76.5 | 39 | 1.1 | 0.2 | 1.3 |
| 79.0 | 40 | 1.5 | 0.3 | 1.9 |
| 80.5 | 41 | 0.8 | 0.3 | 1.1 |
| 82.0 | 42 | 0.8 | 2.3 | 3.1 |
| 85.0 | 43 (Arm) | 0.3 | 0.0 | 0.3 |
| total of above 33 peaks | (% dose) | 20.4 | 38.1 | 58.5 |
| total of trace 15 peaks | (% dose) | 3.8 | 3.9 | 7.7 |
| total analyzed | (% dose) | 24.2 | 41.9 | 66.2 |
| mean extraction recovery (%) | | 57.2 | | |
| not extracted from feces | (%) | 18.2 | | |
| total [$^3$H] excreted (% dose) | | 42.4 | 41.9 | 84.3 |

| Proportions of metabolites in rat plasma after single doses of Arm/Lmf with [$^3$H] Arm given i.v. and orally | | | | |
|---|---|---|---|---|
| Dose / Route | 10 mg/kg intravenous n = 3 | | 20 mg/kg oral n = 3 | |
| Time point | 5 minutes | 24 hours | 0.5 hours | 24 hours |
| Total [$^3$H] concentration (µmol/L) | 3.74 | 0.57 | 4.00 | 0.49 |
| Recovery from | | | | |
| processing (%) | 51.9 | 15.8 | 91.8 | 18.2 |
| HPLC (%) | 91.7 | 100 | 96.1 | 100 |
| Total recovery (%) | 47.6 | 15.8 | 88.2 | 18.2 |
| Effectively analyzed (µmol/L) [$^3$H] concentration | 1.78 | 0.09 | 3.53 | 0.09 |

| Study title: | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | | Study no. DMPK(CH) 1997/241 | |
|---|---|---|---|---|---|---|
| | Peak HPLC retention time (minutes) | Peak number | Proportion (%) of total analyzed plasma [$^3$H] in metabolite peak / zone | | | |
| | 22.5 | 10 | 0.4 | 0.8 | 3.1 | 1.5 |
| | 28.0 | 12 | 0.7 | 3.5 | 7.5 | 2.4 |
| | 31.0 | 13 | 0.5 | 0.5 | 1.5 | 1.3 |
| | 39.5 | 18 | 0.5 | 2.1 | 3.8 | 5.6 |
| | 42.5 | 19 | 0.9 | 2.0 | 3.6 | 3.3 |
| | 44.0 | 20 | 0.5 | 3.1 | 1.2 | 2.6 |
| | 47.5 | 22 | 4.8 | 2.3 | 3.3 | 2.2 |
| | 49.5 | 23 | 1.7 | 2.1 | 2.4 | 3.9 |
| | 52.5 | 25 | 5.0 | 1.7 | 5.9 | 8.0 |
| | 54.0 | 26 | 1.9 | 0.9 | 3.4 | 1.5 |
| | 57.5 | 28 | 3.4 | 2.0 | 6.7 | 2.7 |
| | 59.5 | 29 | 4.4 | 0.9 | 1.9 | 1.9 |
| | 61.0 | 30 | 2.0 | 2.4 | 4.0 | 3.5 |
| | 62.0 | 31 | 2.9 | 7.1 | 9.1 | 12.1 |
| | 63.5 | 32 | 3.1 | 0.7 | 2.0 | 1.0 |
| | 66.5 | 34 | 1.2 | 0.5 | 3.6 | 2.3 |
| | 73.5 | 38 | 8.4 | 5.9 | 1.9 | 0.9 |
| | 79.0 | 40 | 15.1 | 4.3 | 1.5 | 2.0 |
| | 80.5 | 41 | 7.7 | 2.0 | 0.7 | 1.4 |
| | 82.0 | 42 | 10.8 | 3.7 | 0.6 | 1.4 |
| | 85.0 | 43 (Arm) | 3.6 | 10.4 | 0.3 | 4.4 |
| | 86.5 | 44 | 2.3 | 10.6 | 0.1 | 2.2 |
| | 89.0 | 45 | 1.3 | 3.2 | 0.0 | 1.9 |
| | 91.0 | 46 | 1.3 | 3.1 | 0.1 | 1.4 |
| | total of 24 major peaks | (%) | 84.4 | 75.9 | 68.3 | 71.4 |

| Study title: | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | | Study no. DMPK(CH) 1997/241 |
|---|---|---|---|---|---|
| sum of 24 trace peaks (<3% each) | (%) | 15.6 | 24.1 | 31.7 | 28.6 |
| total analyzed [$^3$H] | (%) | 100.0 | 100.0 | 100.0 | 100.0 |

Proportions of metabolites in rat urine and feces after 20 mg/kg Arm/Lmf with [$^3$H] Arm given orally (n = 3)

| Peak HPLC Retention Time (minutes) | Peak Number | Feces % of dose | Urine % of dose | Total % of dose |
|---|---|---|---|---|
| 4.0 | 2 | 1.4 | 0.7 | 2.1 |
| 8.0 | 4 | 0.1 | 2.2 | 2.3 |
| 10.0 | 5 | 0.7 | 0.5 | 1.2 |
| 17.0 | 8 | 0.4 | 0.6 | 1.0 |
| 19.5 | 9 | 0.6 | 1.0 | 1.6 |
| 22.5 | 10 | 1.0 | 0.7 | 1.7 |
| 25.5 | 11 | 0.8 | 1.3 | 2.1 |
| 28.0 | 12 | 1.1 | 2.5 | 3.6 |
| 31.0 | 13 | 0.5 | 1.0 | 1.5 |
| 32.5 | 14 | 0.9 | 1.3 | 2.3 |
| 34.5 | 15 | 0.9 | 1.6 | 2.5 |
| 36.0 | 16 | 0.4 | 1.7 | 2.1 |
| 38.0 | 17 | 0.9 | 2.3 | 3.2 |
| 39.5 | 18 | 0.9 | 2.0 | 2.9 |
| 42.5 | 19 | 0.7 | 1.1 | 1.8 |
| 44.0 | 20 | 0.7 | 1.8 | 2.5 |
| 45.5 | 21 | 0.5 | 1.5 | 2.1 |
| 47.5 | 22 | 1.0 | 1.6 | 2.6 |
| 49.5 | 23 | 0.6 | 1.8 | 2.4 |
| 51.0 | 24 | 0.6 | 1.4 | 2.0 |
| 52.5 | 25 | 1.2 | 2.5 | 3.6 |

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | Study no. DMPK(CH) 1997/241 |
|---|---|---|---|---|
| 54.0 | 26 | 0.7 | 2.0 | 2.8 |
| 55.5 | 27 | 0.6 | 0.5 | 1.1 |
| 57.5 | 28 | 1.4 | 1.8 | 3.2 |
| 59.5 | 29 | 0.3 | 0.9 | 1.2 |
| 61.0 | 30 | 1.0 | 1.6 | 2.6 |
| 62.0 | 31 | 1.2 | 0.3 | 1.5 |
| 63.5 | 32 | 0.5 | 0.5 | 1.0 |
| 79.0 | 40 | 1.0 | 0.2 | 1.1 |
| 82.0 | 42 | 0.5 | 2.9 | 3.3 |
| 85.0 | 43 (Arm) | 0.1 | 0.0 | 0.1 |
| total of above 31 peaks | (% dose) | 23.3 | 41.7 | 65.0 |
| total of trace 17 peaks | (% dose) | 4.6 | 3.5 | 8.1 |
| total analyzed | (% dose) | 27.9 | 45.3 | 73.1 |
| mean extraction recovery (%) | | 68.3 | | |
| not extracted from feces | (%) | 12.9 | | |
| total [$^3$H] excreted (% dose) | | 40.8 | 45.3 | 86.1 |

Proportions of metabolites in rat urine and feces after 100 mg/kg Arm/Lmf with [$^3$H] Arm given orally (n = 3)

| Peak HPLC Retention Time (minutes) | Peak Number | Feces % of dose | Urine % of dose | Total % of dose |
|---|---|---|---|---|
| 4.0 | 2 | 0.4 | 0.8 | 1.2 |
| 6.5 | 3 | 0.3 | 0.9 | 1.2 |
| 8.0 | 4 | 0.3 | 2.3 | 2.6 |
| 10.0 | 5 | 0.3 | 0.7 | 1.0 |
| 17.0 | 8 | 0.5 | 1.4 | 1.9 |
| 19.5 | 9 | 1.1 | 1.4 | 2.6 |
| 22.5 | 10 | 0.8 | 1.7 | 2.5 |
| 25.5 | 11 | 1.0 | 2.3 | 3.3 |

| Study title: | | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | Study no. DMPK(CH) 1997/241 |
|---|---|---|---|---|---|
| 28.0 | | 12 | 0.9 | 3.8 | 4.7 |
| 31.0 | | 13 | 0.5 | 1.7 | 2.2 |
| 32.5 | | 14 | 1.3 | 1.7 | 3.0 |
| 34.5 | | 15 | 0.8 | 1.8 | 2.6 |
| 36.0 | | 16 | 0.8 | 2.2 | 3.0 |
| 38.0 | | 17 | 0.7 | 2.5 | 3.2 |
| 39.5 | | 18 | 1.0 | 2.7 | 3.7 |
| 42.5 | | 19 | 0.6 | 0.8 | 1.4 |
| 44.0 | | 20 | 0.7 | 2.2 | 2.9 |
| 45.5 | | 21 | 0.4 | 0.9 | 1.3 |
| 47.5 | | 22 | 0.9 | 3.3 | 4.2 |
| 49.5 | | 23 | 0.7 | 2.5 | 3.1 |
| 51.0 | | 24 | 0.8 | 1.9 | 2.7 |
| 52.5 | | 25 | 1.1 | 3.8 | 4.9 |
| 54.0 | | 26 | 0.9 | 1.9 | 2.9 |
| 55.5 | | 27 | 0.6 | 1.4 | 1.9 |
| 57.5 | | 28 | 1.2 | 2.3 | 3.6 |
| 59.5 | | 29 | 0.4 | 1.0 | 1.4 |
| 61.0 | | 30 | 1.0 | 1.9 | 2.9 |
| 62.0 | | 31 | 1.1 | 0.7 | 1.7 |
| 63.5 | | 32 | 1.1 | 1.2 | 2.3 |
| 76.5 | | 39 | 1.4 | 0.1 | 1.4 |
| 82.0 | | 42 | 0.2 | 2.9 | 3.1 |
| total of above 31 peaks | (% dose) | | 23.8 | 56.6 | 80.4 |
| total of trace 17 peaks | (% dose) | | 4.7 | 3.0 | 7.7 |
| total analyzed | (% dose) | | 28.5 | 59.6 | 88.2 |

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | Study no. DMPK(CH) 1997/241 |
|---|---|---|---|---|
| | mean extraction recovery (%) | 69.7 | | |
| | not extracted from feces (%) | 12.4 | | |
| | total [$^3$H] excreted (% dose) | 40.9 | 59.6 | 100.6 |

Proportions of metabolites in rat urine and feces after 1000 mg/kg Arm/Lmf with [$^3$H] Arm given orally (n = 3)

| Peak HPLC Retention Time (minutes) | Peak Number | Urine % of dose | Feces % of dose | Total % of dose |
|---|---|---|---|---|
| 4.0 | 2 | 0.7 | 0.3 | 1.0 |
| 6.5 | 3 | 0.8 | 0.3 | 1.1 |
| 8.0 | 4 | 4.0 | 1.0 | 5.0 |
| 10.0 | 5 | 0.8 | 0.4 | 1.2 |
| 17.0 | 8 | 1.4 | 0.4 | 1.7 |
| 19.5 | 9 | 1.5 | 0.6 | 2.1 |
| 22.5 | 10 | 0.9 | 0.4 | 1.3 |
| 25.5 | 11 | 1.4 | 0.9 | 2.4 |
| 28.0 | 12 | 4.6 | 0.6 | 5.3 |
| 31.0 | 13 | 1.7 | 0.3 | 2.1 |
| 32.5 | 14 | 2.2 | 0.8 | 3.0 |
| 34.5 | 15 | 2.9 | 0.6 | 3.5 |
| 36.0 | 16 | 1.5 | 0.5 | 2.0 |
| 38.0 | 17 | 1.7 | 0.6 | 2.3 |
| 39.5 | 18 | 2.5 | 0.6 | 3.0 |
| 42.5 | 19 | 1.0 | 0.5 | 1.5 |
| 44.0 | 20 | 3.5 | 0.4 | 3.9 |
| 45.5 | 21 | 0.8 | 0.4 | 1.2 |
| 47.5 | 22 | 2.2 | 0.4 | 2.6 |
| 49.5 | 23 | 3.0 | 0.5 | 3.6 |
| 51.0 | 24 | 1.0 | 0.4 | 1.5 |

| Study title: | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | | Study no. DMPK(CH) 1997/241 |
|---|---|---|---|---|---|
| 52.5 | 25 | 6.5 | 1.2 | 7.7 | |
| 54.0 | 26 | 3.2 | 0.4 | 3.6 | |
| 55.5 | 27 | 1.2 | 0.3 | 1.5 | |
| 57.5 | 28 | 3.3 | 1.1 | 4.4 | |
| 59.5 | 29 | 1.5 | 0.4 | 1.9 | |
| 61.0 | 30 | 2.3 | 0.5 | 2.9 | |
| 62.0 | 31 | 0.7 | 0.8 | 1.6 | |
| 63.5 | 32 | 1.0 | 0.6 | 1.7 | |
| 64.5 | 33 | 0.9 | 0.3 | 1.2 | |
| 68.5 | 35 | 0.6 | 0.5 | 1.1 | |
| total of above 31 peaks | (% dose) | 61.7 | 17.1 | 78.8 | |
| total of trace 17 peaks | (% dose) | 2.8 | 3.5 | 6.4 | |
| total analyzed | (% dose) | 64.6 | 20.6 | 85.1 | |
| mean extraction recovery | (%) | | 65.2 | | |
| not extracted from feces | (%) | | 11.0 | 11.0 | |
| total [$^{3}$H] excreted | (% dose) | 64.6 | 31.6 | 96.1 | |

Proportions of metabolites in rat urine, bile, and feces after 20 mg/kg Arm/Lmf with [$^{14}$C] Arm given orally

| Peak HPLC Retention Time (minutes) | Peak Number | Urine % of dose | Bile % of dose | Feces % of dose | Total % of dose |
|---|---|---|---|---|---|
| 8.0 | 4 | 1.4 | 0.0 | 0.1 | 1.5 |
| 25.5 | 11 | 0.8 | 1.5 | 0.1 | 2.3 |
| 28.0 | 12 | 2.3 | 0.0 | 0.0 | 2.3 |
| 32.5 | 14 | 0.6 | 1.6 | 0.1 | 2.3 |
| 36.0 | 16 | 0.7 | 0.4 | 0.1 | 1.2 |
| 38.0 | 17 | 0.7 | 0.6 | 0.1 | 1.4 |
| 39.5 | 18 | 0.8 | 2.0 | 0.1 | 2.9 |
| 44.0 | 20 | 1.0 | 0.0 | 0.1 | 1.1 |

| Study title: | | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled Lmf . | | | Study no. DMPK(CH) 1997/241 | |
|---|---|---|---|---|---|---|
| 47.5 | | 22 | 0.6 | 1.3 | 0.1 | 2.0 |
| 49.5 | | 23 | 1.2 | 1.7 | 0.2 | 3.0 |
| 51.0 | | 24 | 0.6 | 0.4 | 0.1 | 1.1 |
| 52.5 | | 25 | 1.6 | 26.0 | 0.2 | 27.9 |
| 53.5 | | 25a | 0.7 | 0.0 | 0.0 | 0.7 |
| 54.0 | | 26 | 1.8 | 1.8 | 0.3 | 3.8 |
| 55.5 | | 27 | 0.2 | 2.5 | 0.1 | 2.8 |
| 57.5 | | 28 | 1.1 | 0.9 | 0.0 | 2.0 |
| 59.5 | | 29 | 1.0 | 0.7 | 0.0 | 1.8 |
| 64.5 | | 33 | 0.8 | 3.1 | 0.0 | 3.9 |
| 68.5 | | 35 | 0.9 | 3.8 | 0.0 | 4.7 |
| 73.5 | | 38 | 0.4 | 5.9 | 0.1 | 6.4 |
| 80.5 | | 41 | 0.1 | 1.1 | 0.2 | 1.4 |
| 82.0 | | 42 | 0.9 | 0.2 | 0.1 | 1.2 |
| 85.0 | | 43 (Arm) | 0.1 | 0.8 | 0.0 | 0.9 |
| total of above 23 peaks | (% dose) | | 20.2 | 56.2 | 2.1 | 78.6 |
| total of trace 24 peaks | (% dose) | | 7.7 | 2.2 | 1.0 | 10.9 |
| total analyzed | (% dose) | | 27.9 | 58.5 | 3.1 | 89.5 |
| mean extraction recovery (%) | | | | | 40.4 | |
| not extracted from feces | (%) | | | | 4.5 | 4.5 |
| total [$^{14}$C] excreted (% dose) | | | 27.9 | 58.5 | 7.6 | 94.0 |

Additional information: none

## 2.6.5.9B Pharmacokinetics: Metabolism *in vivo* (DMPK(CH) 1997/003)

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | Study no. DMPK(CH) 1997/003 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-2 | |
| Test article | [$^{14}$C]Arm (1 part) and non-radiolabeled Lmf (6 parts) | |
| Species, strain, sex, number of animals: | male dog / Beagle / 6 | |
| Feeding condition: | fasted | |
| Vehicle / Formulation: | intravenous route: PEG 400<br>oral route: gelatin capsule | |
| Method/ Route / Duration of administration: | intravenous bolus / cephalic vein of a foreleg / once<br>gelatin capsule / oral / once | |
| Dose (mg/kg): | intravenous dose of 10 once<br>oral dose of 20 and 200 once | |
| Specific activity: | [$^{14}$C] -Arm (Batch No.Re-84.1C1) of 218 KBq/mg<br>[$^{14}$C] -Arm (Batch No.-84.1D2) of 98 KBq/mg<br>[$^{14}$C] -Arm (Batch No.Re-84.1F) of 10 KBq/mg | |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | HPLC with online radiodetection or with electromechanical detection in reductive mode; liquid scintillation counting | |

**Metabolites**

Proportions of metabolites in plasma after single intravenous and oral doses

| Dose / Route | | 10 mg/kg intravenous n = 2 | | 20 mg/kg oral n = 2 | | 200 mg/kg oral n = 2 | |
|---|---|---|---|---|---|---|---|
| Time point (hours) | | 0.25 | 4 | 2 | 8 | 2 | 8 |
| Total [$^{14}$C] concentration (mean of 2) | (µmol/L) | 7.35 | 2.57 | 10.23 | 2.10 | 39.14 | 20.17 |
| Recovery | (%) | | | | | | |
| processing | | 54 | 55 | 88 | 49 | 87 | 64 |
| HPLC | | 100 | 97 | 99 | 100 | 99 | 100 |
| total | | 54 | 53 | 87 | 49 | 86 | 64 |

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | | | | | Study no. DMPK(CH) 1997/003 | | |
|---|---|---|---|---|---|---|---|---|
| Effectively analyzed concentration (mean of 2) | (µmol/L) [$^{14}$C] | 3.97 | 1.37 | 8.90 | 1.03 | 33.66 | 12.91 |
| Peak HPLC Retention Time (minutes) | Peak Number | Proportion (%) of total analyzed plasma $^{14}$C in metabolite peak/zone | | | | | |
| 8.5 | 4 | 0.4 | 4.1 | 5.7 | 1.2 | 10.4 | 2.5 |
| 33.0 | 13 | 1.2 | 3.0 | 4.8 | 5.6 | 2.9 | 5.4 |
| 40.0 | 16 | 0.6 | 1.8 | 2.6 | 2.2 | 3.4 | 2.1 |
| 49.0 | 20 | 1.9 | 2.3 | 3.8 | 3.4 | 3.3 | 3.5 |
| 52.5 | 22 | 1.5 | 1.8 | 5.5 | 4.7 | 4.8 | 2.3 |
| 54.5 | 23 | 1.3 | 3.1 | 2.1 | 2.6 | 2.7 | 4.7 |
| 56.0 | 24 | 8.2 | 15.1 | 16.6 | 17.7 | 16.3 | 18.7 |
| 57.5 | 25 | 10.8 | 9.6 | 15.9 | 10.1 | 15.8 | 9.4 |
| 59.0 | 26 | 3.0 | 1.8 | 3.0 | 3.1 | 3.3 | 3.3 |
| 61.5 | 27 | 1.7 | 1.9 | 3.6 | 3.5 | 2.9 | 3.3 |
| 63.5 | 28 | 2.3 | 2.3 | 3.5 | 3.5 | 3.2 | 4.1 |
| 69.5 | 30 | 7.9 | 1.4 | 1.2 | 1.7 | 4.1 | 3.4 |
| 70.0 | 31 | 1.7 | 2.0 | 4.0 | 1.3 | 2.3 | 1.4 |
| 72.0 | 32 | 5.6 | 4.4 | 5.2 | 2.0 | 5.4 | 2.7 |
| 73.5 | 33 | 7.5 | 1.6 | 1.7 | 0.6 | 1.9 | 0.3 |
| 76.5 | 35 | 6.0 | 1.2 | 1.7 | 1.0 | 1.7 | 0.7 |
| 80.5 | 37 | 6.8 | 3.7 | 0.4 | 1.4 | 0.3 | 0.6 |
| 83.0 | 38 | 1.7 | 5.3 | 0.3 | 1.7 | 0.2 | 0.7 |
| 85.0 | 39 (Arm) | 16.3 | 8.1 | 0.4 | 1.5 | 0.1 | 0.6 |
| total of 19 main peaks | (%) | 86.5 | 74.7 | 82.0 | 68.7 | 85.0 | 69.8 |
| sum of 25 trace peaks (<3% each) | (%) | 13.5 | 25.3 | 18.0 | 31.3 | 15.0 | 30.2 |
| total analyzed [$^{14}$C] (%) | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

**Study title:** Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs.

Study no. DMPK(CH) 1997/003

Proportions of metabolites in urine and feces after 10 mg/kg given intravenously (n = 2)

| Peak HPLC Retention Time (minutes) | Peak Number | Feces % of dose | Urine % of dose | Total % of dose |
|---|---|---|---|---|
| 8.5 | 4 | 0.0 | 2.0 | 2.1 |
| 22.0 | 9 | 0.1 | 0.7 | 0.8 |
| 25.5 | 10 | 0.1 | 0.6 | 0.7 |
| 28.0 | 11 | 0.3 | 0.6 | 0.9 |
| 31.0 | 12 | 0.1 | 0.8 | 0.9 |
| 33.0 | 13 | 0.1 | 2.7 | 2.8 |
| 36.0 | 14 | 0.1 | 1.2 | 1.3 |
| 38.0 | 15 | 0.3 | 1.0 | 1.3 |
| 40.0 | 16 | 0.3 | 1.1 | 1.3 |
| 42.5 | 17 | 0.2 | 1.3 | 1.5 |
| 44.0 | 18 | 0.2 | 0.9 | 1.0 |
| 46.5 | 19 | 0.3 | 1.7 | 2.0 |
| 49.0 | 20 | 0.2 | 1.8 | 2.0 |
| 51.5 | 21 | 0.2 | 0.9 | 1.0 |
| 52.5 | 22 | 0.6 | 1.2 | 1.8 |
| 54.5 | 23 | 0.1 | 2.1 | 2.3 |
| 56.0 | 24 | 0.4 | 8.9 | 9.2 |
| 57.5 | 25 | 0.3 | 7.9 | 8.2 |
| 59.0 | 26 | 0.3 | 1.3 | 1.6 |
| 61.5 | 27 | 0.4 | 1.4 | 1.7 |
| 63.5 | 28 | 0.6 | 2.1 | 2.7 |
| 66.5 | 29 | 0.3 | 0.8 | 1.1 |
| 69.5 | 30 | 0.3 | 0.9 | 1.3 |
| 70.0 | 31 | 0.4 | 1.1 | 1.5 |

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | | | Study no. DMPK(CH) 1997/003 |
|---|---|---|---|---|
| 72.0 | 32 | 1.8 | 0.7 | 2.6 |
| 79.0 | 36 | 0.7 | 0.4 | 1.1 |
| 80.5 | 37 | 0.4 | 0.4 | 0.8 |
| 83.0 | 38 | 0.3 | 0.2 | 0.4 |
| 85.0 | 39 (Arm) | 0.2 | 0.1 | 0.2 |
| total of above 29 peaks | (% dose) | 9.5 | 46.7 | 56.1 |
| total of 15 trace peaks | (% dose) | 1.6 | 4.3 | 5.9 |
| total analyzed | (% dose) | 11.0 | 51.0 | 62.0 |
| mean extraction recovery | (%) | 37 | | |
| total [$^{14}$C] excreted | (% dose) | 30.0 | 51.0 | 81.1 |
| Proportions of metabolites in urine and feces after 20 mg/kg given orally (n = 2) | | | | |
| Peak HPLC Retention Time (minutes) | Peak Number | Feces % of dose | Urine % of dose | Total % of dose |
| 8.5 | 4 | 0.1 | 2.0 | 2.1 |
| 22.0 | 9 | 0.4 | 1.3 | 1.7 |
| 25.5 | 10 | 0.4 | 0.9 | 1.3 |
| 28.0 | 11 | 0.6 | 1.5 | 2.1 |
| 31.0 | 12 | 0.2 | 1.5 | 1.7 |
| 33.0 | 13 | 0.5 | 4.8 | 5.2 |
| 36.0 | 14 | 0.3 | 1.4 | 1.7 |
| 38.0 | 15 | 0.5 | 2.1 | 2.5 |
| 40.0 | 16 | 0.4 | 1.9 | 2.3 |
| 42.5 | 17 | 0.3 | 1.5 | 1.8 |
| 44.0 | 18 | 0.3 | 1.2 | 1.5 |
| 46.5 | 19 | 0.5 | 1.3 | 1.8 |
| 49.0 | 20 | 0.4 | 1.9 | 2.3 |
| 51.5 | 21 | 0.3 | 1.5 | 1.8 |

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | | | Study no. DMPK(CH) 1997/003 |
|---|---|---|---|---|
| 52.5 | 22 | 1.0 | 2.7 | 3.6 |
| 54.5 | 23 | 0.4 | 2.2 | 2.6 |
| 56.0 | 24 | 0.9 | 10.9 | 11.8 |
| 57.5 | 25 | 0.9 | 9.1 | 10.0 |
| 59.0 | 26 | 0.5 | 1.6 | 2.1 |
| 61.5 | 27 | 0.5 | 1.5 | 2.1 |
| 63.5 | 28 | 1.0 | 1.5 | 2.5 |
| 66.5 | 29 | 0.7 | 0.8 | 1.5 |
| 69.5 | 30 | 0.5 | 0.9 | 1.5 |
| 70.0 | 31 | 0.4 | 0.6 | 1.0 |
| 72.0 | 32 | 2.8 | 0.4 | 3.2 |
| 79.0 | 36 | 1.0 | 0.1 | 1.1 |
| 80.5 | 37 | 0.9 | 0.2 | 1.1 |
| 83.0 | 38 | 0.2 | 0.2 | 0.4 |
| 85.0 | 39 (Arm) | 0.2 | 0.1 | 0.3 |
| total of above 29 peaks | (% dose) | 17.3 | 57.5 | 74.8 |
| total of 15 trace peaks | (% dose) | 2.7 | 4.8 | 7.5 |
| total analyzed | (% dose) | 20.0 | 62.3 | 82.3 |
| mean extraction recovery | (%) | 68 | | |
| total [$^{14}$C] excreted | (% dose) | 29.4 | 62.2 | 91.6 |

Proportions of metabolites in urine and feces after 200 mg/kg given orally (n = 2)

| Peak HPLC Retention Time (minutes) | Peak Number | Feces % of dose | Urine % of dose | Total % of dose |
|---|---|---|---|---|
| 8.5 | 4 | 0.2 | 1.8 | 2.0 |
| 22.0 | 9 | 0.4 | 0.7 | 1.1 |
| 25.5 | 10 | 0.2 | 0.9 | 1.2 |
| 28.0 | 11 | 0.4 | 1.2 | 1.6 |

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | | | Study no. DMPK(CH) 1997/003 |
|---|---|---|---|---|
| 31.0 | 12 | 0.2 | 0.8 | 1.1 |
| 33.0 | 13 | 0.3 | 3.6 | 3.9 |
| 36.0 | 14 | 0.3 | 1.1 | 1.4 |
| 38.0 | 15 | 0.3 | 1.3 | 1.7 |
| 40.0 | 16 | 0.4 | 1.4 | 1.8 |
| 42.5 | 17 | 0.5 | 1.5 | 2.0 |
| 44.0 | 18 | 0.3 | 1.0 | 1.3 |
| 46.5 | 19 | 0.7 | 1.3 | 2.0 |
| 49.0 | 20 | 0.4 | 2.2 | 2.6 |
| 51.5 | 21 | 0.3 | 0.9 | 1.3 |
| 52.5 | 22 | 0.9 | 1.4 | 2.3 |
| 54.5 | 23 | 0.4 | 3.1 | 3.6 |
| 56.0 | 24 | 0.5 | 10.3 | 10.8 |
| 57.5 | 25 | 0.4 | 8.4 | 8.9 |
| 59.0 | 26 | 0.7 | 1.5 | 2.2 |
| 61.5 | 27 | 0.6 | 1.7 | 2.4 |
| 63.5 | 28 | 0.9 | 1.1 | 2.0 |
| 66.5 | 29 | 0.6 | 0.7 | 1.2 |
| 69.5 | 30 | 0.7 | 1.2 | 1.9 |
| 70.0 | 31 | 0.6 | 0.6 | 1.2 |
| 72.0 | 32 | 2.3 | 0.6 | 3.0 |
| 79.0 | 36 | 2.8 | 0.1 | 2.9 |
| 80.5 | 37 | 1.0 | 0.2 | 1.1 |
| 83.0 | 38 | 4.2 | 0.1 | 4.3 |
| 85.0 | 39 (Arm) | 0.2 | 0.1 | 0.3 |
| total of above 29 peaks | (% dose) | 21.6 | 51.3 | 72.9 |

| Study title: | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | | | Study no. DMPK(CH) 1997/003 |
|---|---|---|---|---|
| total of 15 trace peaks | (% dose) | 2.6 | 4.6 | 7.2 |
| total analyzed | (% dose) | 24.2 | 55.9 | 80.1 |
| mean extraction recovery | (%) | 67 | | |
| total [$^{14}$C] excreted | (% dose) | 35.9 | 56.0 | 92.0 |

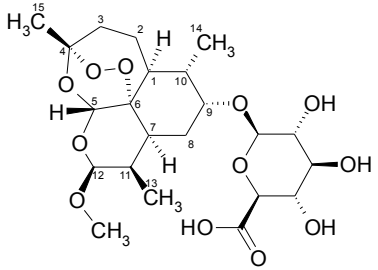
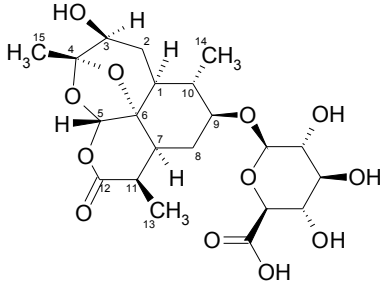
Additional information: none

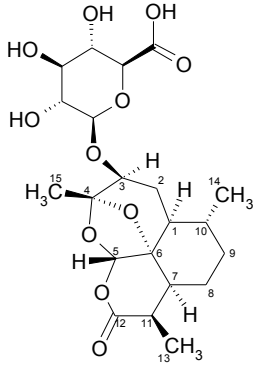
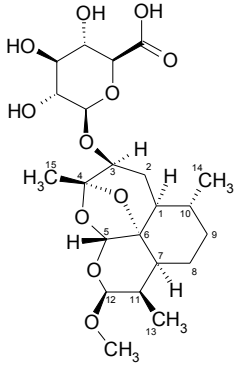
## 2.6.5.9C Pharmacokinetics: Metabolism *in vivo* (DMPK(CH) 1997/534)

| **Study title:** | Characterization of metabolites of artemether (CGP 56696) formed in rats and dogs after single oral doses of co-artemether containing radio-labeled artemether | Study No: DMPK(CH) 1997/534 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-1 | |
| Test article | $^{3}H$ or $^{14}C$-radiolabelled Arm (1 part) and non-radiolabeled Lmf (6 parts) | |
| Gender(M/F) / Number of animals: | rat: M / 2<br>dog: M / 1 | |
| Feeding condition: | fasted | |
| Vehicle / Formulation: | rat: 0.5% Klucel + Tween 80<br>dog: gelatin capsule | |
| Method of Administration: | rat: oral by gavage<br>dog: oral | |
| Dose (mg/kg): | rat: 20 and 1000<br>dog: 200 | |
| Radionuclide: | rat: $^{3}H$ or $^{14}C$<br>dog: $^{14}C$ | |
| Specific activity / radiochemical purity: | rat: 1390-3350 KBq/mg / >98.5% (DMPK(CH) 1997/241)<br>dog: 10 KBq/mg / >98.5% (DMPK(CH) 1997/003) | |
| Analyte/Assay (unit): | radioactivity / HPLC with ultraviolet detection; liquid scintillation counting; and liquid chromatography-mass spectrometry; nuclear magnetic resonance spectroscopy | |

| **Study title:** | Characterization of metabolites of artemether (CGP 56696) formed in rats and dogs after single oral doses of co-artemether containing radio-labeled Arm | Study No: DMPK(CH) 1997/534 |
|---|---|---|

**Results:**

| Species | Sample | Dose (mg/kg) | Time (hours) | Radiolabel | Metabolite name | Metabolite structure |
|---|---|---|---|---|---|---|
| rat | bile | 20 | 0 – 8 | $^{14}C$ | P52.5 | 9α-hydroxy-artemether-9-O-glucuronide  |
| rat | urine | 1000<br>20 | 8 – 24 | $^{3}H$<br>$^{14}C$ | P39.5<br>P54.0 component 1, 2<br>P61.0<br>P63.5 or P64.5 | accounted for <3% of dose; none of the isolated metabolites was sufficiently pure in adequate amounts for clear structure determination; some structural features could be inferred: O-demethylation, hemiacetal oxidation, hydroxylations, and conjugation with glucuronic acid or with glucose. |
| dog | urine | 200 | 0 – 24 | $^{14}C$ | P33.0 | 3β,9β-dihydroxy-deoxyartemisinin-9-O-glucuronide  |

| **Study title:** | | | Characterization of metabolites of artemether (CGP 56696) formed in rats and dogs after single oral doses of co-artemether containing radio-labeled artemether | | | Study No: DMPK(CH) 1997/534 |
|---|---|---|---|---|---|---|
| Species | Sample | Dose (mg/kg) | Time (hours) | Radiolabel | Metabolite name | Metabolite structure |
| dog | urine | 200 | 0 – 24 | $^{14}C$ | P57.5 | 3β-hydroxy-deoxyartemisinin-3-O-glucuronide  |
| | | | | | P72.0 | 3β-hydroxy-deoxyartemether-3-O-glucuronide  |

Additional information: samples were from studies DMPK(CH) 1997/241 and DMPK(CH) 1997/003.

## 2.6.5.9D Pharmacokinetics: Metabolism *in vivo* (DMPK(CH) 1997/209)

| **Study title:** | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | Study no. DMPK(CH) 1997/209 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-2 | |
| Test article: | [$^{14}$C] -Lmf (6 parts) and unlabeled Arm (1 part) | |
| Species, strain, sex, number of animals: | rat, Tif: RAIf (SPF), male, n= 12 / dog, Beagle, male, n=4 | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7/3, w/w) solution for intravenous route<br>0.5% Klucel + 0.1% Tween 80 for oral route in rats<br>gelatin capsule for oral route in dogs | |
| Method/ Route / Duration of administration: | rats: intravenous bolus / tail vain / once<br>gavage / oral / once (20 mg/kg)<br>gavage / oral / once (100 mg/kg)<br>dogs: intravenous bolus / cephalic vein of a foreleg / once<br>gelatin capsule / oral / once | |
| Dose (mg/kg): | rats: intravenous dose of 1 mg/kg<br>oral dose of 20 mg/kg once<br>oral dose of 100 mg/kg once<br>dogs: intravenous dose of 1 mg/kg<br>oral dose of 20 mg/kg once | |
| Specific activity: | [$^{14}$C]-Lmf Batch Ko-76.1B of 64.1 KBq/mg<br>[$^{14}$C]-Lmf Batch Ko-76.1C of 10.0 KBq/mg<br>[$^{14}$C]-Lmf Batch Ko-76.1D of 960 KBq/mg<br>[$^{14}$C]-Lmf Batch Ko-76.1F of 159 KBq/mg | |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | HPLC with ultraviolet detection; liquid scintillation counting; liquid chromatography-mass spectrometry | |

**Study title:** Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs.

Study no. DMPK(CH) 1997/209

**Results:** **HPLC analysis**

Rat feces

| Number | Route | Dose (mg/kg) | | Time (hours) | Radioactivity in chromatographic peak [% of dose] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Arm/Lmf | [$^{14}$C]-Lmf | | P3 6 min | P2 group 9-15 min | P1 18.5 min | Lmf 23 min | P6 24 min | In feces fraction % of dose | In extract for HPLC % of dose |
| 12 | iv | 1 | 0.86 | 0-72 | 2.0 | 11.1 | 15.7 | 15.1 | 1.5 | 61.0 | 47.7 |
| 13 | iv | 1 | 0.86 | 0-72 | 1.9 | 17.1 | 16.6 | 12.0 | 1.2 | 67.7 | 49.1 |
| 14 | iv | 1 | 0.86 | 0-72 | 1.7 | 11.5 | 15.2 | 20.8 | 1.2 | 63.9 | 51.0 |
| | | Arm/Lmf | [$^{14}$C]-Lmf | | P3 5 min | P2 group 10-15 min | P1 18.5 min | Lmf 23 min | P6 24 min | In feces fraction % of dose | In extract for HPLC % of dose |
| 27 | po | 20 | 17.14 | 0-24 | nd | nd | 0.5 | 63.2 | nd | 92.3 | 63.7 |
| 28 | po | 20 | 17.14 | 0-24 | nd | nd | 0.7 | 75.5 | nd | 91.3 | 76.2 |
| 42 | po | 100 | 85.7 | 0-24 | nd | nd | 1.5 | 63.4 | nd | 79.1 | 64.9 |
| 43 | po | 100 | 85.7 | 0-24 | nd | nd | 0.8 | 60.8 | nd | 73.8 | 61.6 |
| 44 | po | 100 | 85.7 | 0-24 | nd | nd | 0.6 | 63.7 | nd | 77.0 | 64.3 |

Rat bile

| Number | Route | Dose (mg/kg) | | Time (hours) | Radioactivity in chromatographic peak [% of dose] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Arm/Lmf | [$^{14}$C]-Lmf | | P4/P5 <5 min | P3 6 min | P2 group 10-13 min | P1 18.5 min | Lmf 23 min | P6 24 min | In bile fraction % of dose |
| 21 | iv | 1 | 0.86 | 0-2 | 68.4 | 7.9 | 12.4 | 1.7 | 9.5 | nd | 1.35 |
| | | | | 2-4 | 68.2 | 13.2 | 9.9 | 0.4 | 8.2 | nd | 2.82 |
| | | | | 4-6 | 60.6 | 17.3 | 11.7 | 1.8 | 8.6 | nd | 2.79 |
| | | | | 6-8 | 58.7 | 19.9 | 10.6 | 1.0 | 8.4 | 1.5 | 2.60 |
| | | | | 8-12 | 56.7 | 22.3 | 9.2 | 1.5 | 8.3 | 2.0 | 4.75 |

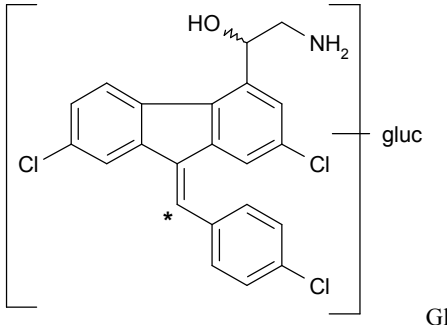
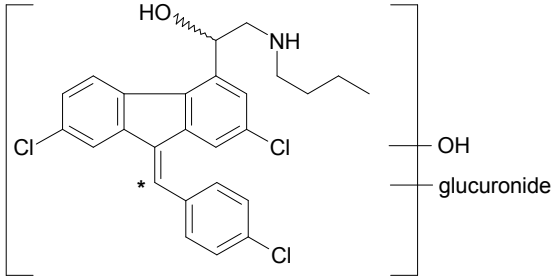
| **Study title:** | | | | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | | | | | | | Study no. DMPK(CH) 1997/209 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 12-24 | 57.5 | 22.6 | 12.8 | 2.5 | 4.6 | nd | 10.73 |
| | 22 | iv | 1 | 0.86 | 0-24 | 52.6 | 29.8 | 12.7 | 0.9 | 3.9 | nd | 14.7 |
| | 23 | iv | 1 | 0.86 | 0-72 | 51.3 | 31.5 | 7.9 | 1.7 | 4.1 | 3.4 | 36.4 |
| | 24 | iv | 1 | 0.86 | 0-24 | 63.1 | 20.3 | 8.7 | 0.7 | 6.1 | nd | 24.2 |

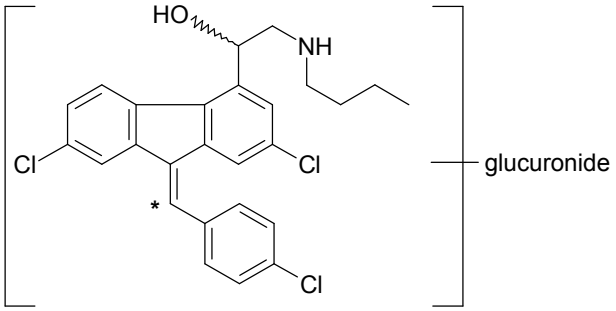
Dog feces

| Number | Route | Dose (mg/kg) | | Time (hours) | Radioactivity in chromatographic peak [% of dose] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Arm/Lmf | [$^{14}$C]-Lmf | | P4/P5 <5 min | P8 7.5 min | P7 9.5 min | P1 18.5 min | Lmf 23 min | In feces fraction % of dose | In extract for HPLC % of dose |
| 1032 | iv | 1 | 0.86 | 0-48 | nd | 8.2 | 14.7 | nd | 11.5 | 54.7 | 40.2 |
| 1040 | iv | 1 | 0.86 | 0-48 | 4.9 | 8.7 | 12.1 | nd | 8.4 | 61.5 | 35.2 |
| 1032 | po | 20 | 17.1 | 0-24 | nd | nd | 2.7 | nd | 64.0 | 87.2 | 66.7 |
| 1040 | po | 20 | 17.1 | 0-24 | nd | nd | nd | nd | 73.6 | 90.3 | 73.6 |

nd = not detected; iv = intravenous; po = oral

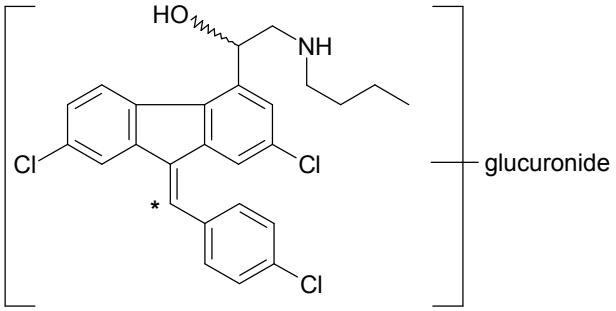
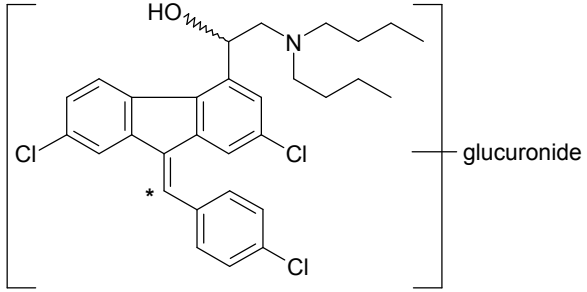
| **Study title:** | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | Study no. DMPK(CH) 1997/209 |
|---|---|---|
| Results: | LC-MS analysis | |
| | Pre-purified rat bile | |

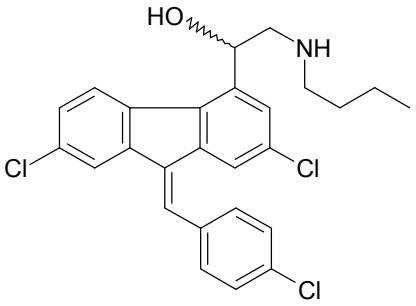
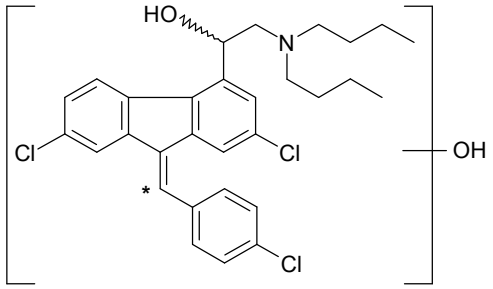
| Retention times | Molecular masses Mr[Da] | Proposed structures |
|---|---|---|
| 22.8 min | 591 |  Gluc: glucuronic acid |
| 24.4 min<br>29.8 min | 663<br>663 |  probably two isomers |

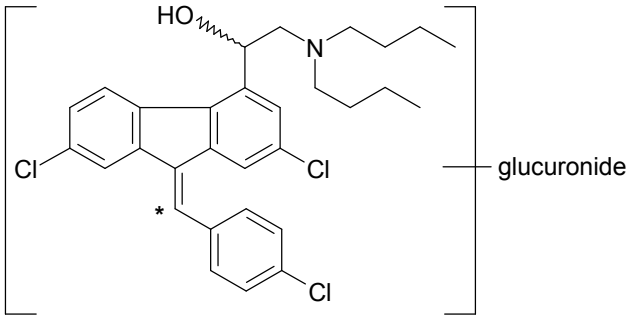
| **Study title:** | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | | Study no. DMPK(CH) 1997/209 |
|---|---|---|---|
| | Pre-purified rat bile | | |
| | Retention times | Molecular masses Mr[Da] | Proposed structures |
| | 29.4 min<br>30.0 min | 647<br>647 |  <br>probably two isomers |
| | 28.6 min<br>40.2 min | 513<br>513 | no structure proposed |
| | 33.5 min | 573 | no structure proposed |
| | 35.1 min<br>35.2 min<br>45.6 min | 497<br>497<br>497 | no structure proposed |
| | 41.0 min | 511 | no structure proposed |

| **Study title:** | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | Study no. DMPK(CH) 1997/209 |
|---|---|---|

Enzymatically hydorolyzed and pre-purified rat bile

| Retention times | Molecular masses Mr[Da] | Proposed structures |
|---|---|---|
| 9.1 min | 647 |  |
| 12.5 min | 703 |  |

| **Study title:** | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | | Study no. DMPK(CH) 1997/209 |
|---|---|---|---|
| | Enzymatically and pre-purified rat bile | | |
| | Retention times | Molecular masses Mr[Da] | Proposed structures |
| | 18.1 min | 471 |  <br>Corresponds to reference compound TA 2133 K (N-desbutyl-Lmf ) |
| | 22.2 min | 543 |  |
| | 24.6 min | 527 | Lmf |

| **Study title:** | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | | Study no. DMPK(CH) 1997/209 |
|---|---|---|---|
| | Pre-purified dog feces | | |
| | Retention times | Molecular masses Mr[Da] | Proposed structures |
| | 12.2 min | 703 |  |



Gluc = glucuronic acid rest ($C_6H_9O_6$ = 177 Da)

Comment: in the report the relative position of the 4-chloro-benzylidene and the N-dibutylamino groups are not correct (typing error).

Additional information:

HPLC analysis of rat and dog feces extracts: After intravenous administration of 1 mg/kg Arm/Lmf (0.86 mg/kg $^{14}$C -labeled Lmf) to rats and dogs, both species demonstrated different patterns of $^{14}$C-labeled metabolites in fecal extracts. In rat feces, 1 main metabolite P1 was found, and 2 minor metabolites, P3 and P6 were detected. In dog feces, 2 different metabolite peaks, P7 and P8, were detected. After oral administration of Arm/Lmf ($^{14}$C -labeled Lmf) to rats and dogs, unchanged $^{14}$C -labeled Lmf was found in almost all of the fecal extracts, with less than 3% of the dose detected as metabolites.

LC-MS analysis of rat bile and dog feces extract: Prior to hydrolysis of rat bile, the structures of 5 putative glucuronide metabolites were partially identified as isomers of desbutylLmf glucuronide, as 2 glucuronides of desbutyl-Lmf probably containing an additional oxygen atom, and as a glucuronide of di- desbutyl-Lmf . From 7 further metabolites, the molecular masses were obtained but without structures being proposed. After the enzymatic hydrolysis of rat bile, minor amounts of glucuronides were still detected. The main metabolite after enzymatic hydrolysis in rat bile was identified as desbutyl-Lmf by comparison (MS and chromatographic retention time) with an authentic reference compound. On the basis of LC-MS analysis of dog feces extracts, the main metabolite was tentatively identified as a glucuronide of [$^{14}$C]-Lmf. However, the precise structure of the glucuronide remains unknown.

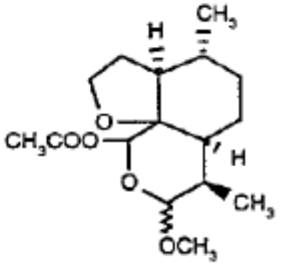
# 2.6.5.10 薬物動態試験：*in vitro* における代謝

## 2.6.5.10A Pharmacokinetics: Metabolism *in vitro* (DMPK(CH) 1997/242)

| **Study title:** | Identification of *in vitro* metabolites of artemether (CGP 56696) in incubations with rat blood, dog blood, and bovine hemoglobin by GC-MS analysis | Study No: DMPK(CH) 1997/242 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-3 | |
| Test article: | Arm | |
| Study system: | bovine hemoglobin | |
| | blood of 2 male albino rats / Tif: RAIf(SPF) | |
| | blood of 1 male Beagle dog | |
| Initial concentration: | stock solution I: CGP 56696 (Arm) 11.79 mg/g; stock solution II: Lmf 11.80 mg/g | |
| Assay: | derivatization and capillary GC-MS | |
| **Results:** | | |
| | The incubation of Arm with bovine hemoglobin or blood obtained from rats or dogs resulted, time-dependently, in the formation of two major degradation products:<br>metabolite III = furano acetate derivative<br>metabolite IV = tentatively characterized as 3-hydroxydeoxy-DHA, which is probably the demethylated derivative of 3α-hydroxydeoxy-β-Arm.<br>The mechanism which yielded IV is unknown. Neither DHA nor hydroxydeoxyArm were detected in the present study.<br>Metabolite V was formed with higher amounts of bovine hemoglobin (150 g/mL).<br>In control incubations with saline after two hours at 37° C, only traces of metabolites III and IV were detected.<br>Similar results were obtained after incubation with bovine hemoglobin at low concentration (5 g/mL).<br>The time-dependent decrease of Arm was more marked in the presence of bovine hemoglobin or rat blood than in the presence of dog blood. | |

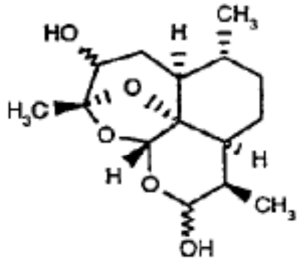
| **Study title:** | Identification of *in vitro* metabolites of artemether (CGP 56696) in incubations with rat blood, dog blood, and bovine hemoglobin by GC-MS analysis | Study No: DMPK(CH) 1997/242 |
|---|---|---|

Metabolites identified in incubations



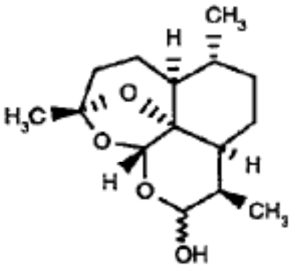
III

Arm furanoacetate metabolite



IV

3-hydroxydeoxy-DHA metabolite



V

deoxy-DHA metabolite

Additional information: none

## 2.6.5.10B Pharmacokinetics: Metabolism *in vitro* (DMPK(CH) 1997/181)

| **Study title:** | *In vitro* biotransformation of CGP 56696; comparison of three animal species and man. | Study No: DMPK(CH) 1997/181 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-4 | |
| Test article: | [$^{14}$C]-Arm | |
| Study system: | liver samples from male and female human and animals for the preparation of each species' respective S12 liver fraction<br>mouse: Tif: MAGf (SPF), pool of 12 males each weighing 30 g<br>rat: Tif: RAIf (SPF), pool of 2 males each weighing 200 to 220 g<br>dog: Beagle pedigree, single male animal weighing 11.6 kg<br>human: livers from 3 male and 3 female human kidney donors. | |
| Initial concentration: | 50 µmol/L | |
| Specific activity: | [$^{14}$C]-Arm (Batch No. Re-84.1A) of 499 MBq/mmol or 1.67 MBq/mg | |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | HPLC coupled to UV and on-line radiodetection / liquid chromatography - mass spectrometry | |

**Results**

**Radioactive compounds in incubations of [$^{14}$C] Arm using mouse liver fraction S12**

| Peak | | [$^{14}$C] concentration (µmol/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number | TR | Incubation time | | | | | Control 1 | Control 2 |
| | (minutes) | 0 min | 15 min | 30 min | 60 min | 120 min | 120 min | 120 min |
| P1 | 3.1 | nd | 0.3 | 0.9 | 1.0 | 2.1 | nd | nd |
| P2 | 5.4 | nd | nd | 0.6 | 1.7 | 4.1 | nd | nd |
| P3 | 7.8 | nd | nd | nd | 0.9 | 2.3 | nd | nd |
| P4 | 9.3 | nd | nd | 0.1 | 0.7 | 2.0 | nd | nd |
| P5 | 11.1 | nd | 1.1 | 1.9 | 2.9 | 4.4 | nd | nd |
| P6 | 12.0 | nd | 1.0 | 1.5 | 2.6 | 3.1 | nd | nd |
| P7 | 14.1 | nd | 0.4 | 0.6 | 1.0 | 0.9 | nd | nd |
| P8 | 15.3 | nd | 3.8 | 6.7 | 9.5 | 10.2 | nd | nd |

| **Study title:** | | *In vitro* biotransformation of CGP 56696; comparison of three animal species and man. | | | | | Study No: DMPK(CH) 1997/181 | |
|---|---|---|---|---|---|---|---|---|
| P9 | 16.1 | nd | 1.8 | 2.4 | 3.3 | 3.6 | nd | nd |
| P10 | 17.2 | nd | 1.0 | 1.8 | 2.5 | 3.4 | nd | nd |
| P11 | 18.0 | nd | 1.1 | 2.1 | 2.7 | 3.2 | nd | nd |
| P12 | 19.3 | nd | 0.6 | 1.0 | 1.2 | 1.0 | nd | nd |
| P13 | 20.7 | nd | 0.3 | 0.5 | 1.0 | 1.2 | nd | nd |
| P14 | 23.3 | nd | nd | nd | nd | 0.6 | nd | nd |
| P15 | 24.2 | nd | 0.5 | 0.5 | 0.5 | 0.2 | nd | 0.5 |
| P16 | 28.4 | nd | 0.3 | 0.3 | 0.5 | 0.2 | nd | nd |
| Arm | 30.3 | 48.4 | 35.5 | 26.0 | 15.7 | 5.2 | 48.9 | 47.2 |
| Total of peaks | | 48.4 | 47.8 | 47.1 | 47.7 | 47.7 | 48.9 | 47.6 |

TR = average chromatographic retention time of the respective peak
Control 1 = 120 min control incubation with liver fraction S12 in the absence of NADPH-regenerating system
Control 2 = 120 min control incubation with heat inactivated (100° C 10 min) liver fraction S12 and an NADPH-regenerating system added
nd = not detected

**Radioactive compounds in incubations of [$^{14}$C] Arm using rat liver fraction S12**

| Peak | | [$^{14}$C] concentration (µmol/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number | TR | Incubation time | | | | | Control 1 | Control 2 |
| | (minutes) | 0 min | 15 min | 30 min | 60 min | 120 min | 120 min | 120 min |
| P1 | 3.1 | nd | 0.1 | 0.4 | 0.9 | 2.7 | nd | nd |
| P2 | 5.4 | nd | nd | 0.7 | 1.6 | 3.8 | nd | nd |
| P3 | 7.8 | nd | nd | nd | 1.3 | 3.0 | nd | nd |
| P4 | 9.3 | nd | nd | nd | 0.2 | 1.4 | nd | nd |
| P5 | 11.1 | nd | nd | 0.9 | 1.8 | 2.4 | nd | nd |
| P6 | 12.0 | nd | 1.8 | 2.7 | 4.6 | 7.2 | nd | nd |
| P7 | 14.1 | nd | 0.6 | 1.0 | 1.7 | 1.7 | nd | nd |
| P8 | 15.3 | nd | 0.5 | 0.9 | 1.7 | 2.3 | nd | nd |
| P9 | 16.1 | nd | 1.8 | 2.8 | 4.2 | 4.6 | 0.4 | 0.2 |

| **Study title:** | | *In vitro* biotransformation of CGP 56696; comparison of three animal species and man. | | | | | | Study No: DMPK(CH) 1997/181 | |
|---|---|---|---|---|---|---|---|---|---|
| | P10 | 17.2 | nd | 1.8 | 3.3 | 4.7 | 4.8 | nd | nd |
| | P11 | 18.0 | nd | 1.1 | 2.0 | 2.4 | 2.9 | nd | nd |
| | P12 | 19.3 | nd | nd | 1.4 | 1.7 | 1.9 | nd | nd |
| | P13 | 20.7 | nd | 1.4 | 1.3 | 1.5 | 1.3 | nd | nd |
| | P14 | 23.3 | nd | nd | nd | 0.7 | 0.9 | nd | nd |
| | P15 | 24.2 | nd | 0.9 | 1.5 | 1.6 | 1.0 | 0.1 | 0.4 |
| | P16 | 28.4 | nd | nd | nd | nd | 0.1 | nd | nd |
| | Arm | 30.3 | 48.3 | 36.0 | 28.6 | 17.4 | 6.0 | 47.0 | 46.2 |
| | Total of peaks | | 48.3 | 46.0 | 47.3 | 47.9 | 48.1 | 47.5 | 46.9 |
| | TR = average chromatographic retention time of the respective peak<br>Control 1 = 120 min control incubation with liver fraction S12 in the absence of NADPH-regenerating system<br>Control 2 = 120 min control incubation with heat inactivated (100° C 10 min) liver fraction S12 and an NADPH-regenerating system added<br>nd = not detected | | | | | | | | |
| | **Radioactive compounds in incubations of [$^{14}$C]** Arm **using dog liver fraction S12** | | | | | | | | |
| | Peak | | [$^{14}$C] concentration (µmol/L) | | | | | | |
| | Number | TR | Incubation time | | | | | Control 1 | Control 2 |
| | | (minutes) | 0 min | 15 min | 30 min | 60 min | 120 min | 120 min | 120 min |
| | P1 | 3.1 | nd | 0.5 | 1.1 | 1.9 | 4.7 | 0.2 | nd |
| | P2 | 5.4 | nd | 0.3 | 1.1 | 2.8 | 5.7 | nd | nd |
| | P3 | 7.8 | nd | 0.1 | 1.2 | 2.3 | 4.3 | nd | nd |
| | P4 | 9.3 | nd | nd | 1.0 | 2.1 | 3.1 | nd | nd |
| | P5 | 11.1 | nd | 1.1 | 1.2 | 1.8 | 2.4 | nd | nd |
| | P6 | 12.0 | nd | 1.4 | 2.2 | 3.4 | 4.3 | nd | nd |
| | P7 | 14.1 | nd | 0.5 | 0.8 | 0.6 | 0.9 | nd | nd |
| | P8 | 15.3 | nd | 2.3 | 3.4 | 4.3 | 3.9 | nd | nd |
| | P9 | 16.1 | nd | 0.9 | 1.4 | 1.6 | 1.4 | nd | 0.3 |
| | P10 | 17.2 | nd | 6.5 | 9.7 | 11.7 | 7.8 | 6.2* | nd |

| **Study title:** | | *In vitro* biotransformation of CGP 56696; comparison of three animal species and man. | | | | | | Study No: DMPK(CH) 1997/181 | |
|---|---|---|---|---|---|---|---|---|---|
| | P11 | 18.0 | nd | 2.0 | 2.5 | 2.8 | 2.3 | nd | nd |
| | P12 | 19.3 | nd | 2.4 | 4.4 | 4.5 | 2.3 | nd | nd |
| | P13 | 20.7 | nd | 2.8 | 3.0 | 3.7 | 2.2 | nd | nd |
| | P14 | 23.3 | nd | 0.3 | 0.6 | 0.6 | 0.7 | nd | nd |
| | P15 | 24.2 | nd | 0.6 | 0.4 | nd | nd | 0.2 | 0.6 |
| | P16 | 28.4 | nd | nd | 0.1 | nd | 0.3 | nd | nd |
| | Arm | 30.3 | 46.3 | 24.2 | 14.3 | 3.9 | 2.2 | 40.4 | 45.6 |
| | Total of peaks | | 46.3 | 45.9 | 48.3 | 47.9 | 48.4 | 47.0 | 46.5 |

TR = average chromatographic retention time of the respective peak
Control 1 = 120 min control incubation with liver fraction S12 in the absence of NADPH-regenerating system
Control 2 = 120 min control incubation with heat inactivated (100° C 10 min) liver fraction S12 and an NADPH-regenerating system added
nd = not detected
* = broad peak from 16 to 18 min (TR = 17.0 min), includes P9 and P10

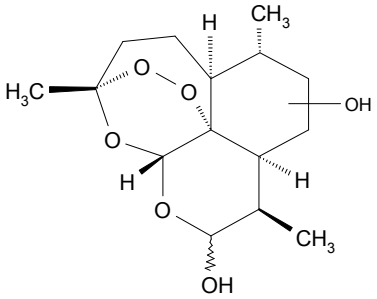
**Radioactive compounds in incubations of [$^{14}$C]** Arm **using human liver fraction S12**

| Peak | | [$^{14}$C] concentration (µmol/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number | TR | Incubation time | | | | | Control 1 | Control 2 |
| | (minutes) | 0 min | 15 min | 30 min | 60 min | 120 min | 120 min | 120 min |
| P1 | 3.1 | nd | 0.5 | 0.9 | 2.2 | 4.7 | 0.2 | nd |
| P2 | 5.4 | nd | 0.9 | 1.6 | 3.7 | 6.8 | nd | nd |
| P3 | 7.8 | nd | nd | 0.8 | 2.2 | 4.7 | nd | nd |
| P4 | 9.3 | nd | nd | 0.8 | 1.5 | 3.0 | nd | nd |
| P5 | 11.1 | nd | 0.4 | nd | 0.5 | 0.3 | nd | nd |
| P6 | 12.0 | nd | 2.1 | 3.1 | 3.9 | 4.2 | nd | nd |
| P7 | 14.1 | nd | 1.4 | 2.0 | 2.3 | 2.0 | nd | nd |
| P8 | 15.3 | nd | 5.4 | 8.3 | 9.6 | 7.6 | nd | nd |
| P9 | 16.1 | 0.1 | 2.2 | 3.0 | 3.7 | 3.0 | 1.5 | 0.5 |
| P10 | 17.2 | nd | 2.0 | 3.5 | 5.0 | 5.0 | nd | nd |

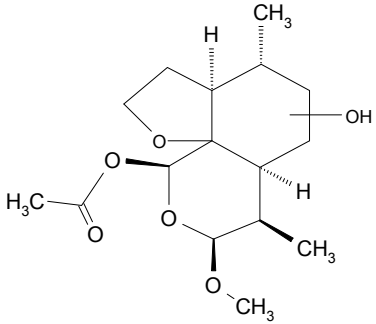
| **Study title:** | | *In vitro* biotransformation of CGP 56696; comparison of three animal species and man. | | | | | | Study No: DMPK(CH) 1997/181 | |
|---|---|---|---|---|---|---|---|---|---|
| | P11 | 18.0 | nd | 1.9 | 2.8 | 4.0 | 3.0 | nd | nd |
| | P12 | 19.3 | nd | 1.1 | 1.4 | 1.6 | 1.7 | nd | nd |
| | P13 | 20.7 | nd | 0.8 | 1.3 | 1.7 | 1.2 | nd | nd |
| | P14 | 23.3 | nd | nd | nd | 0.7 | 0.5 | nd | nd |
| | P15 | 24.2 | nd | 0.4 | 0.6 | 0.2 | nd | 0.3 | 0.5 |
| | P16 | 28.4 | nd | 0.3 | 0.4 | 0.1 | nd | nd | nd |
| | Arm | 30.3 | 47.6 | 28.7 | 18.1 | 6.1 | 1.0 | 45.0 | 46.2 |
| | Total of peaks | | 47.7 | 48.1 | 48.6 | 49.0 | 48.8 | 47.0 | 47.2 |

TR = average chromatographic retention time of the respective peak
Control 1 = 120 min control incubation with liver fraction S12 in the absence of NADPH-regenerating system
Control 2 = 120 min control incubation with heat inactivated (100° C 10 min) liver fraction S12 and an NADPH-regenerating system added
nd = not detected

Proposed chemical structures of metabolites identified in incubates from rat and human S12 fraction

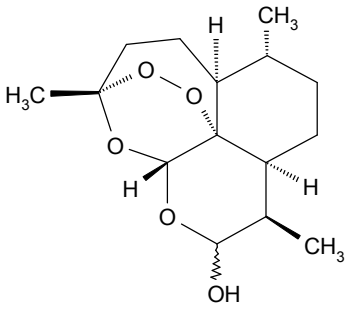



Hydroxylated DHA metabolite
Rt: 9.3 min; human



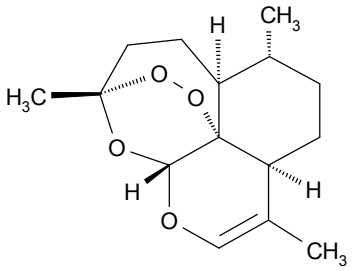

hydroxylated furanoacetate metabolite
Rt: 10.4 and 10.7 min; rat and human

| **Study title:** | *In vitro* biotransformation of CGP 56696; comparison of three animal species and man. | Study No: DMPK(CH) 1997/181 |
|---|---|---|




epimer of DHA

Rt: 17 min; rat and human




Rt: 22 min; human

| | |
|---|---|
| Conclusion | The overall biotransformation of [$^{14}$C]-Arm by the liver fractions S12 of mouse, rat, dog and human, measured as the disappearance of $^{14}$C-labeled Arm, roughly followed Michaelis-Menten kinetics.<br>Similar enzyme kinetic parameters were found for mouse and rat (Km: 7-10 µmol/L; Vmax: approximately 1.2 nmol/min·mg) and for dog and human (Km: 14-17 µmol/L; Vmax: approx. 2.1-2.5 nmol/min·mg).<br>After precipitating the protein from the incubations of the 4 species investigated, a small but distinct amount of radioactivity was tightly associated with the pellet. At 50 µmol/L initial [$^{14}$C]-Arm concentration and after 120 min incubation time, a maximum of 3.6 nmol $^{14}$C/mg protein was precipitated in the dog. The proportion of precipitated radioactivity was dependent on the amount of protein used, the species (dog ≈ human > rat > mouse), the incubation time, and the substrate concentration. The nature of the radioactivity associated with the precipitated proteins was not investigated.<br>Using LC-MS analysis and in comparison with an authentic reference compound, DHA was identified as one of the main *in vitro* metabolites of Arm using rat and human liver fraction S12. On the basis of the LC-MS data, structures for 3 additional metabolites were proposed.<br>Using the HPLC method with radiodetection, a metabolite peak corresponding to DHA was also found in incubations with mouse and dog liver fractions S12. |

Additional information: none

## 2.6.5.10C Pharmacokinetics: Metabolism *in vitro* (DMPK(CH) 1997/156)

| **Study title:** | *In vitro* biotransformation: Identification of the human cytochrome P 450 isoenzyme(s) involved in the biotransformation of artemether. | Study No: DMPK(CH) 1997/156 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-5 | |
| Test article: | [$^{3}$H]Arm | |
| Radionuclide: | radioactivity / $^{3}$H | |
| Specific radioactivity: | Batch No.Re-54.9A of 2.28 MBq/mg; Re-54.9C of 3.35 MBq/mg; Re-54.9C1 of 1.47 MBq/mg | |
| Type of study: | *in vitro* biotransformation of $^{3}$H-labeled Arm by human cytochrome P450 isozymes | |
| Method: | **S12 liver fractions** for metabolic activation: Human liver microsomes, prepared from pieces of the livers of 8 kidney donors, placed in buffer solution and homogenized at 800 rpm and then centrifuged at 12000 g for 15 minutes. The supernatant was centrifuged at 27'000 g for 15 minutes and the resulting supernatant was then fractionated into microsomes and cytosol by ultracentrifugation at 105'000 g for 1 hour at 4° C. The cytosol was removed, and the remaining microsomes were rehomogenized in the buffer solution and centrifuged at 105'000 g for 1 hour. The resulting pellet was rehomogenized in buffer solution and diluted to an equivalent corresponding to 2 g wet liver tissue/mL. The protein content was determined by the method of Bradford, the amount of cytochrome P450 was determined according to the method of Omura and Sato, and the enzyme activity was characterized by the 7-ethoxycoumarin-O-deethylase test of Ullrich and Weber. The preparations were stored frozen in liquid nitrogen.<br>**Pooled liver microsomes** as comparator: A pool or the complete set of human liver microsomes from the Reaction Phenotyping Kit (version 4.0) [redacted], which contained the identified cytochrome P450 isozyme-specific markers CYP2C9, CYP2C19, CYP2D6, CYP2A6, CYP1A2, CYP2B6, CYP2C8, CYP2E1, CYP3A4, CYP3A4/5 and CYP4A9/11, was used as a comparator.<br>**Recombinant human microsomes in culture**: Microsomes prepared from human B-lymphoblastoid cell lines or baculovirus infected insect cell lines heterologously expressing particular human P450s. | |
| DDI CYP3A4 inhibitors | Quinine and ketoconazole to evaluate CYP3A4 inhibition: Cultures of human S12 activated liver microsomes (pooled and individual) with quinine (0 to 50 µmol/L) or ketoconazole (0 to 2 µmol/L) added | |
| Assay | HPLC analysis with online radiodetection | |

| **Study title:** | | | *In vitro* biotransformation: Identification of the human cytochrome P450 isoenzyme(s) involved in the biotransformation of artemether. | | | | Study No: DMPK(CH) 1997/156 | |
|---|---|---|---|---|---|---|---|---|
| **Results-1** | | | | | | | | |
| Sample | Activity[a] | | CYP3A4 | CYP3A4/5 | CYP2B6 | CYP2A6 | CYP2C8 | CYP2C9 |
| | mean | SD | mean activity in pmoles / min x mg protein | | | | | |
| Pool | 1835 | 60 | 160 | 5179 | 227 | 1325 | 180 | 138 |
| 2 | 3137 | 87 | 199 | 12032 | 98 | 129 | 98.8 | 172 |
| 7 | 1093 | 83 | 65 | 2748 | | 690 | 46.6 | 134 |
| 11 | 2781 | 148 | 267 | 7895 | 381 | 1033 | 205.3 | 54 |
| 13 | 1732 | 16 | 89 | 3658 | 80 | 205 | 43.9 | 89 |
| 14 | 753 | 106 | 42 | 1775 | 114 | 500 | 73.6 | 48 |
| 15 | 1265 | 39 | 65 | 2730 | 93 | 1140 | 57.3 | 173 |
| 16 | 3970 | 95 | 434 | 8677 | 692 | 2080 | 341.1 | 230 |
| 17 | 3279 | 166 | 260 | 9132 | 248 | 2785 | 263.0 | 203 |
| 18 | 504 | 20 | 81 | 3407 | 169 | 109 | 68.9 | 122 |
| 19 | 1142 | 59 | 71 | 2899 | 156 | 70 | 80.6 | 129 |
| 20 | 667 | 138 | 44 | 1622 | 54 | 728 | 92.1 | 147 |
| 21 | 1144 | 127 | 105 | 4380 | 295 | 1136 | 128.2 | 43 |
| 22 | 976 | 116 | 59 | 4827 | 56 | 1023 | 237.9 | 136 |
| 23 | 1600 | 24 | 135 | 5746 | 91 | 1612 | 395.1 | 154 |
| 24 | 440 | 87 | 20 | 1077 | 73 | 132 | 42.5 | 83 |
| 25 | 2487 | 97 | 175 | 7442 | 112 | 1094 | 229.1 | 99 |
| t(N-2): | | | 10.3^^ | 8^^ | 3.2^^ | 2.8 | 2.8 | 2.2 |
| correlation coefficient R: | | | 0.94 | 0.90 | 0.65 | 0.59 | 0.58 | 0.49 |

[a]pmoles Arm metabolized per min per mg protein

^^P<0.01

| **Study title:** | *In vitro* biotransformation: Identification of the human cytochrome P 450 isoenzyme(s) involved in the biotransformation of artemether. | | | | | | Study No: DMPK(CH) 1997/156 |
|---|---|---|---|---|---|---|---|
| **Results-1** | | | | | | | |
| Sample | Activity[a] | | CYP2C19 | CYP2D6 | CYP2E1 | CYP4A9/11 | CYP1A2 |
| | mean | SD | | | | | |
| Pool | 1835 | 60 | 139 | 244 | 1865 | 1168 | 48.1 |
| 2 | 3137 | 87 | 8.6 | 106 | 4940 | 535 | 40.5 |
| 7 | 1093 | 83 | 123.8 | 57 | 1670 | 901 | 39.4 |
| 11 | 2781 | 148 | 26.4 | 255 | 1397 | 757 | 114.9 |
| 13 | 1732 | 16 | 30.1 | 182 | 1727 | 861 | 28.1 |
| 14 | 753 | 106 | 47.1 | 524 | 1049 | 761 | 51.4 |
| 15 | 1265 | 39 | 56.2 | 37 | 1656 | 1244 | 21.3 |
| 16 | 3970 | 95 | 253.2 | 365 | 1641 | 1295 | 35.3 |
| 17 | 3279 | 166 | 199.4 | 272 | 2852 | 1190 | 44.1 |
| 18 | 504 | 20 | 141.3 | 188 | 2543 | 1043 | 71.8 |
| 19 | 1142 | 59 | 39.5 | 133 | 1205 | 1066 | 57.9 |
| 20 | 667 | 138 | 34.9 | 40 | 3741 | 820 | 20.1 |
| 21 | 1144 | 127 | 221.8 | 121 | 2024 | 1693 | 107.7 |
| 22 | 976 | 116 | 13.5 | 275 | 2411 | 1441 | 21.8 |
| 23 | 1600 | 24 | 123.3 | 225 | 1886 | 1775 | 32.8 |
| 24 | 440 | 87 | 13.2 | 115 | 2202 | 659 | 29 |
| 25 | 2487 | 97 | 22.5 | 104 | 1770 | 1317 | 15.9 |
| t(N-2): | | | 1.3 | 0.9 | 0.6 | 0.20 | 0.20 |
| correlation coefficient R: | | | 0.32 | 0.23 | 0.16 | 0.04 | 0.04 |

[a]pmoles Arm metabolized per min per mg protein

^^P<0.01

| **Study title:** | *In vitro* biotransformation: Identification of the human cytochrome P 450 isoenzyme(s) involved in the biotransformation of artemether. | Study No: DMPK(CH) 1997/156 |
|---|---|---|
| Results-2: | The *in vitro* biotransformation of Arm was catalyzed by human liver microsomes. The metabolite patterns were similar to those obtained upon incubation with human liver S12 fractions.<br>In the concentration range of Arm tested (10-80 µmol/L), the reaction exhibited apparent Michaelis-Menten type kinetics with a $K_m$ of approximately 160 µmol/L.<br>When examined at an Arm concentration of 20 µmol/L, the rate varied roughly 9-fold in liver microsomes obtained from 16 human individuals.<br>In a set of 16 human individual liver microsomes, the Arm biotransformation activity at 20 µmol/L was highly correlated to testosterone 6β-hydroxylation and dextromethorphan N-demethylation, marker activities of CYP3A4/5. Arm biotransformation was catalyzed by recombinant microsomes containing CYP3A4 or CYP3A5 confirming the involvement of these isoforms.<br>Metabolite patterns produced by CYP3A4 recombinant. microsomes were similar to those obtained with human liver microsomes.<br>Finally, Arm biotransformation by a human liver microsomal pool was greatly reduced at ketoconazole concentrations which inhibit specifically CYP3A4/5.<br>The results suggest that CYP3A4/5 play a predominant role in the *in vitro* biotransformation of Arm. | |
| Conclusions: | Arm biotransformation by a human liver microsomal pool was greatly reduced at ketoconazole concentrations that specifically inhibit CYP3A4/5; whereas, quinine, known to be metabolized by CYP3A4/5, induces a concentration-dependent inhibition of Arm biotransformation *in vitro*. | |

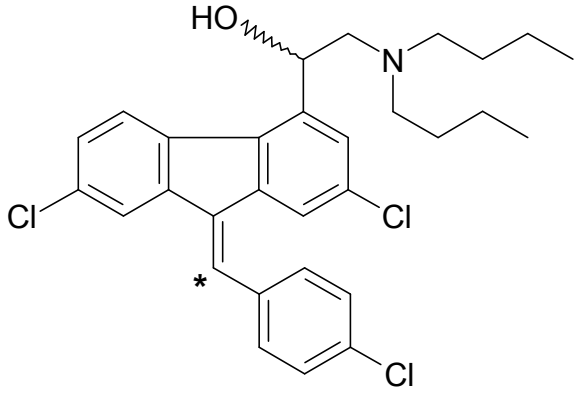
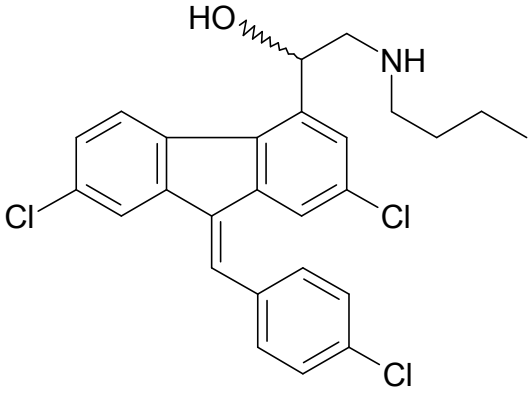
Additional information: none

## 2.6.5.10D Pharmacokinetics: Metabolism *in vitro* (DMPK(CH) 1997/035)

| **Study title:** | *In vitro* biotransformation of CGP 56695; comparison of three animal species and man. | | | | Study No: DMPK(CH) 1997/035 |
|---|---|---|---|---|---|
| GLP compliance: | not required | | | | |
| Location in CTD: | 4.2.2.4-6 | | | | |
| Test article: | [$^{14}$C]-Lmf | | | | |
| Study system: | liver samples from male, human and animals for the preparation of each species' respective S12 liver fraction<br>mouse: Tif: MAGf (SPF), pool of 12 males each weighing 30 g<br>rat: Tif: RAIf (SPF), pool of 12 males each weighing 200 to 220 g<br>dog: Beagle pedigree, single male animal weighing 11.6 kg<br>human: liver from 1 male human kidney donor | | | | |
| Initial concentration: | 5 µmol/L or 20 µmol/L | | | | |
| **Results-1:** | **HPLC with online radiodetection** | | | | |
| **Mouse** | **Radioactivity in chromatographic peak (% of total eluted)** | | | | |
| Sampling time (min): | 0 | 30 | 60 | 180 | 180 (control) |
| Substrate conc. (µmol/L): | 20 | 20 | 20 | 20 | 20 |
| Lmf (retention time 24 minutes) | 99 | 98.2 | 98.3 | 97.7 | 100 |
| P1 (retention time 19 minutes) | 0.4 | 1.2 | 1.3 | 2.3 | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | not detected | not detected |
| Substrate conc: (µmol/L) | 5 | 5 | 5 | 5 | 5 |
| Lmf (retention time 24 minutes) | 100 | 100 | 97.2 | 94.1 | 100 |
| P1 (retention time 19 minutes) | not detected | not detected | 2.8 | 5.9 | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | not detected | not detected |
| **Rat** | **Radioactivity in chromatographic peak (% of total eluted)** | | | | |
| Sampling time (min): | 0 | 30 | 60 | 180 | 180 (control) |
| Substrate conc. (µmol/L): | 20 | 20 | 20 | 20 | 20 |
| Lmf (retention time 24 minutes) | 100 | 99.2 | 98.8 | 96.7 | 100 |
| P1 (retention time 19 minutes) | not detected | 0.8 | 1.2 | 2.6 | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | 0.7 | not detected |
| Substrate conc. (µmol/L): | 5 | 5 | 5 | 5 | 5 |

| **Study title:** | *In vitro* biotransformation of CGP 56695; comparison of three animal species and man. | | | | Study No: DMPK(CH) 1997/035 |
|---|---|---|---|---|---|
| Lmf (retention time 24 minutes) | 100 | 100 | 96.5 | 90.3 | 100 |
| P1 (retention time 19 minutes) | not detected | not detected | 2.9 | 8.4 | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | 1.3 | not detected |
| **Dog** | **Radioactivity in chromatographic peak (% of total eluted)** | | | | |
| Sampling time (min): | 0 | 30 | 60 | 180 | 180 (control) |
| Substrate conc. (µmol/L): | 20 | 20 | 20 | 20 | 20 |
| Lmf (retention time 24 minutes) | 99.6 | 100 | 99.7 | 99.5 | 100 |
| P1 (retention time 19 minutes) | 0.4 | not detected | 0.3 | 0.5 | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | not detected | not detected |
| Substrate conc. (µmol/L): | 5 | 5 | 5 | 5 | 5 |
| Lmf (retention time 24 minutes) | 100 | 100 | 100 | 100 | 100 |
| P1 (retention time 19 minutes) | not detected | not detected | not detected | not detected | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | not detected | not detected |
| **Human** | **Radioactivity in chromatographic peak (% of total eluted)** | | | | |
| Sampling time (min): | 0 | 30 | 60 | 180 | 180 (control) |
| Substrate conc. (µmol/L): | 20 | 20 | 20 | 20 | 20 |
| Lmf (retention time 24 minutes) | 100 | 99.2 | 98.4 | 96.3 | 100 |
| P1 (retention time 19 minutes) | not detected | 0.8 | 1.6 | 3.7 | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | not detected | not detected |
| Substrate conc. (µmol/L): | 5 | 5 | 5 | 5 | 5 |
| Lmf (retention time 24 minutes) | 100 | 100 | 95.1 | 86.5 | 100 |
| P1 (retention time 19 minutes) | not detected | not detected | 4.9 | 13.5 | not detected |
| P2 (retention time 13 minutes) | not detected | not detected | not detected | not detected | not detected |

| **Study title:** | *In vitro* biotransformation of CGP 56695; comparison of three animal species and man. | | | Study No: DMPK(CH) 1997/035 |
|---|---|---|---|---|
| **Results-2:** | **HPLC with offline radiodetection** | | | |
| **Mouse** | **Radioactivity in chromatographic peak (% of total eluted)** | | | |
| Sampling time (min): | 180 | Control | | |
| Substrate conc. (µmol/L): | 5 | 5 | | |
| Lmf (collection 23-25 minutes) | 89.6 | 93.7 | | |
| P1 (collection 19-20 minutes) | 4.4 | 1.9 | | |
| P2 (collection 13-14 minutes) | 0.4 | 0.2 | | |
| **Rat** | **Radioactivity in chromatographic peak (% of total eluted)** | | | |
| Sampling time (min): | 180 | 180 | Control | |
| Substrate conc. (µmol/L): | 5 | 5 | 5 | |
| Lmf (collection 23-25 minutes) | 77.6 | 82.0 | 97.1 | |
| P1 (collection 19-20 minutes) | 8.9 | 7.5 | 0.6 | |
| P2 (collection 13-14 minutes) | 4.3 | 3.6 | 0.1 | |
| **Dog** | **Radioactivity in chromatographic peak (% of total eluted)** | | | |
| Sampling time (min): | 180 | 180 | Control | Control |
| Substrate conc. (µmol/L): | 5 | 5 | 5 | 5 |
| Lmf (collection 23-25 minutes) | 90.5 | 90.6 | 96.9 | 94.3 |
| P1 (collection 19-20 minutes) | 3.9 | 2.9 | 0.9 | 1.2 |
| P2 (collection 13-14 minutes) | 0.5 | 0.5 | 0.1 | 0.2 |
| **Human** | **Radioactivity in chromatographic peak (% of total eluted)** | | | |
| Sampling time (min): | 180 | Control | no NADPH | |
| Substrate conc. (µmol/L): | 5 | 5 | 5 | |
| Lmf (collection 23-25 minutes) | 88.6 | 94.1 | 94.6 | |
| P1 (collection 19-20 minutes) | 7.0 | 1.2 | 1.1 | |
| P2 (collection 13-14 minutes) | 0.4 | 0.1 | 0.2 | |

| | | |
|---|---|---|
| **Study title:** | *In vitro* biotransformation of CGP 56695; comparison of three animal species and man. | Study No: DMPK(CH) 1997/035 |
| **Results-3** | The patterns of metabolite peaks in the incubation supernatants were comparable at the two substrate concentrations investigated, *i.e.* 5 µmol/L and 20 µmol/L [$^{14}$C]-Lmf. Metabolite peak P1 was formed by the liver fractions S12 of the four species. At 20 µmol/L [$^{14}$C]-Lmf after 3 h incubation, P1 accounted for 3.7%, 2.6%, 2.3%, and 0.5% of the radioactivity in the chromatograms obtained from incubation supernatants of liver fraction S12 from human, rat, mouse, and dog, respectively.<br>At 5 µmol/L [$^{14}$C]-Lmf, P1 accounted for approximately 7%, 8%, 4% and 3-4% of the radioactivity in the chromatograms obtained from incubation supernatants of liver fraction S12 from human, rat, mouse, and dog, respectively.<br>The metabolite peak P2 (4% of the radioactivity in the chromatogram) was only detected in the supernatant of incubations with rat liver fraction S12. | |
| Metabolites identified: | On the basis of its chromatographic retention time, which was identical to that of the reference compound TA 2133 K (N-desbutyl-Lmf), the main metabolite peak P1 was tentatively assigned as the N-desbutylderivative of Lmf.<br>No experiments were performed to elucidate the structure of the minor metabolite peak P2. | |
| Conclusions: | The extent of the overall *in vitro* biotransformation of [$^{14}$C]-Lmf by the post-mitochondrial liver fraction S12 was comparable low with that of rat and human, lower with that of mouse, and lowest with that of dog. | |
| Chemical structure of Lmf and N-desbutyl Lmf (TA 2133K) |  <br>Lmf * = $^{14}$C isotope |  <br>N-desbutyl-Lmf |
| | Comment: in the report the relative position of the 4-chloro-benzylidene and the N-dibutylamino groups are not correct (typing error). | |

Additional information: none

## 2.6.5.10E Pharmacokinetics: Metabolism *in vitro* (DMPK(CH) 1997/155)

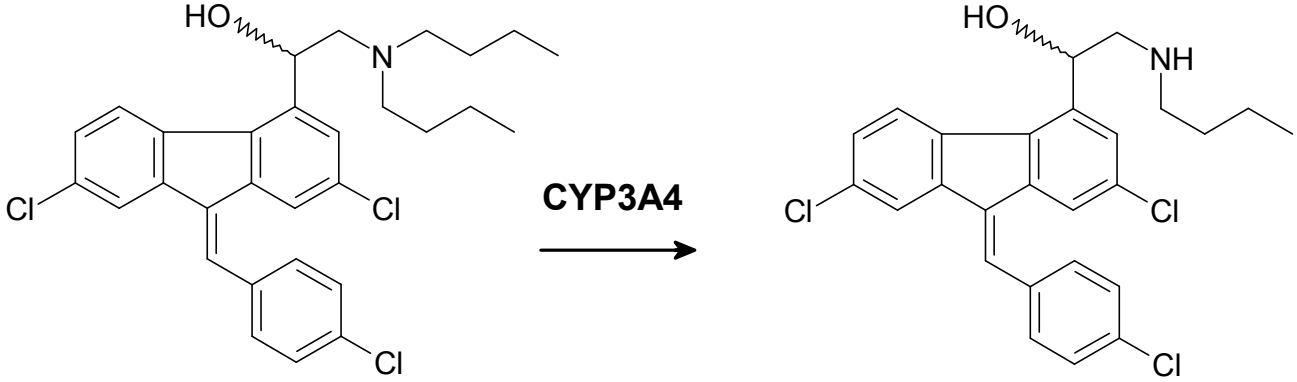
| **Study title:** | *In vitro* biotransformation: Identification of the human cytochrome P 450 isoenzyme(s) involved in the N-dealkylation of benflumetol. | Study No: DMPK(CH) 1997/155 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-7 | |
| Test article: | [$^{14}$C]-Lmf | |
| Type of study: | *in vitro* biotransformation of [$^{14}$C]-Lmf by human cytochrome P450 isozymes | |
| Method: | S12 liver fractions for metabolic activation: Human liver microsomes, prepared from pieces of the livers of 8 kidney donors, were placed in buffer solution and homogenized and then centrifuged at 12000 g for 15 minutes. The supernatant was centrifuged at 27000 g for 15 minutes and the resulting supernatant was then fractionated into microsomes and cytosol by ultracentrifugation at 105000 g for 1 hour. The cytosol was removed, and the remaining microsomes were rehomogenized in the buffer solution and centrifuged at 105000 g for 1 hour. The resulting pellet was rehomogenized in buffer solution and diluted to an equivalent corresponding to 2 g wet liver tissue/mL. The protein content was determined by the method of Bradford, the amount of cytochrome P450 was determined according to the method of Omura and Sato, and the enzyme activity was characterized by the 7-ethoxycoumarin-O-deethylase test of Ullrich and Weber. The preparations were stored frozen in liquid nitrogen.<br>Pooled liver microsomes as comparator: A pool or the complete set of human liver microsomes from the Reaction Phenotyping Kit (version 4.0) [redacted], which contained the identified cytochrome P450 isozyme-specific markers CYP2C9, CYP2C19, CYP2D6, CYP2A6, CYP1A2, CYP2B6, CYP2C8, CYP2E1, CYP3A4, CYP3A4/5 and CYP4A9/11, was used as a comparator.<br>Recombinant human microsomes in culture: Microsomes prepared from human B-lymphoblastoid cell lines or baculovirus infected insect cell lines heterologously expressing particular human P450s.<br>Quinine and halofantrine to evaluate CYP3A4 inhibition: Cultures of human S12 activated liver microsomes (pooled and individual) with quinine (0 to 200 µmol/L), ketoconazole (0 to 1 µmol/L), or halofantrine (0 to 10 µmol/L) added. | |
| Analyte / Assay | radioactivity / HPLC analysis with online radiodetection | |

| **Study title:** | | | *In vitro* biotransformation: Identification of the human cytochrome P 450 isoenzyme(s) involved in the N-dealkylation of lumefantrin. | | | | Study No: DMPK(CH) 1997/155 | |
|---|---|---|---|---|---|---|---|---|
| Results: | | | | | | | | |
| Sample | Activity[a] | | CYP3A4 | CYP3A4/5 | CYP2B6 | CYP2A6 | CYP2C8 | CYP2C9 |
| | mean | SD | mean activity in pmoles / min x mg protein | | | | | |
| Pool | 0.137 | 0.113 | 160 | 5179 | 227 | 1325 | 180.0 | 138 |
| 2 | 0.486 | 0.007 | 199 | 12032 | 98 | 129 | 98.8 | 172 |
| 7 | 0.152 | 0.092 | 65 | 2748 | nd | 690 | 46.6 | 134 |
| 11 | 0.360 | 0.256 | 267 | 7895 | 381 | 1033 | 205.3 | 54 |
| 13 | 0.137 | 0.097 | 89 | 3658 | 80 | 205 | 43.9 | 89 |
| 14 | 0.080 | 0.055 | 42 | 1775 | 114 | 500 | 73.6 | 48 |
| 15 | 0.042 | 0.034 | 65 | 2730 | 93 | 1140 | 57.3 | 173 |
| 16 | 0.201 | 0.080 | 434 | 8677 | 692 | 2080 | 341.1 | 230 |
| 17 | 0.591 | 0.256 | 260 | 9132 | 248 | 2785 | 263.0 | 203 |
| 18 | 0.130 | 0.118 | 81 | 3407 | 169 | 109 | 68.9 | 122 |
| 19 | 0.067 | 0.116 | 71 | 2899 | 156 | 70 | 80.6 | 129 |
| 20 | 0.070 | 0.122 | 44 | 1622 | 54 | 728 | 92.1 | 147 |
| 21 | 0.159 | 0.114 | 105 | 4380 | 295 | 1136 | 128.2 | 43 |
| 22 | 0.075 | 0.066 | 59 | 4827 | 56 | 1023 | 237.9 | 136 |
| 23 | 0.306 | 0.209 | 135 | 5746 | 91 | 1612 | 395.1 | 154 |
| 24 | 0.023 | 0.039 | 20 | 1077 | 73 | 132 | 42.5 | 83 |
| 25 | 0.172 | 0.142 | 175 | 7442 | 112 | 1094 | 229.1 | 99 |
| t(N-2): | | | 3.0 | 6.0 | 1.0 | 2.2 | 2.0 | 1.4 |
| correlation coefficient R: | | | 0.61 ** | 0.84 ** | 0.25 | 0.49 | 0.45 | 0.33 |

[a]pmoles N-desbutyl Lmf formed per min per mg protein
nd = not detected
** P<0.01

| **Study title:** | | | *In vitro* biotransformation: Identification of the human cytochrome P 450 isoenzyme(s) involved in the N-dealkylation of lumefantrin. | | | | Study No: DMPK(CH) 1997/155 |
|---|---|---|---|---|---|---|---|
| Results: | | | | | | | |
| Sample | Activity[a] | | CYP2C19 | CYP2D6 | CYP2E1 | CYP4A9/11 | CYP1A2 |
| | mean | SD | | | | | |
| Pool | 0.137 | 0.113 | 139 | 244 | 1865 | 1168 | 48.1 |
| 2 | 0.486 | 0.007 | 8.6 | 106 | 4940 | 535 | 40.5 |
| 7 | 0.152 | 0.092 | 123.8 | 57 | 1670 | 901 | 39.4 |
| 11 | 0.360 | 0.256 | 26.4 | 255 | 1397 | 757 | 114.9 |
| 13 | 0.137 | 0.097 | 30.1 | 182 | 1727 | 861 | 28.1 |
| 14 | 0.080 | 0.055 | 47.1 | 524 | 1049 | 761 | 51.4 |
| 15 | 0.042 | 0.034 | 56.2 | 37 | 1656 | 1244 | 21.3 |
| 16 | 0.201 | 0.080 | 253.2 | 365 | 1641 | 1295 | 35.3 |
| 17 | 0.591 | 0.256 | 199.4 | 272 | 2852 | 1190 | 44.1 |
| 18 | 0.130 | 0.118 | 141.3 | 188 | 2543 | 1043 | 71.8 |
| 19 | 0.067 | 0.116 | 39.5 | 133 | 1205 | 1066 | 57.9 |
| 20 | 0.070 | 0.122 | 34.9 | 40 | 3741 | 820 | 20.1 |
| 21 | 0.159 | 0.114 | 221.8 | 121 | 2024 | 1693 | 107.7 |
| 22 | 0.075 | 0.066 | 13.5 | 275 | 2411 | 1441 | 21.8 |
| 23 | 0.306 | 0.209 | 123.3 | 225 | 1886 | 1775 | 32.8 |
| 24 | 0.023 | 0.039 | 13.2 | 115 | 2202 | 659 | 29 |
| 25 | 0.172 | 0.142 | 22.5 | 104 | 1770 | 1317 | 15.9 |
| t(N-2): | | | 1.0 | 0.5 | 1.9 | 0.10 | 0.88 |
| correlation coefficient R: | | | 0.25 | 0.13 | 0.43 | 0.02 | 0.22 |

[a]pmoles N-desbutyl Lmf formed per min per mg protein

| **Study title:** | *In vitro* biotransformation: Identification of the human cytochrome P 450 isoenzyme(s) involved in the N-dealkylation of lumefantrin. | Study No: DMPK(CH) 1997/155 |
|---|---|---|
| Conclusions: | Results suggest that CYP3A4 plays a predominant role in the *in vitro* N-debutylation of Lmf. Lmf N-debutylation by the two human liver microsomal pools investigated was greatly reduced at ketoconazole concentrations which specifically inhibit CYP3A4/5. In addition, therapeutic concentrations of quinine and halofantrine, two antimalaria drugs known to be metabolised by CYP3A4/5, are likely as well to inhibit the N-debutylation of Lmf *in vivo*. | |
| Biotransformation pathway of N-debutylation of Lmf |  | |

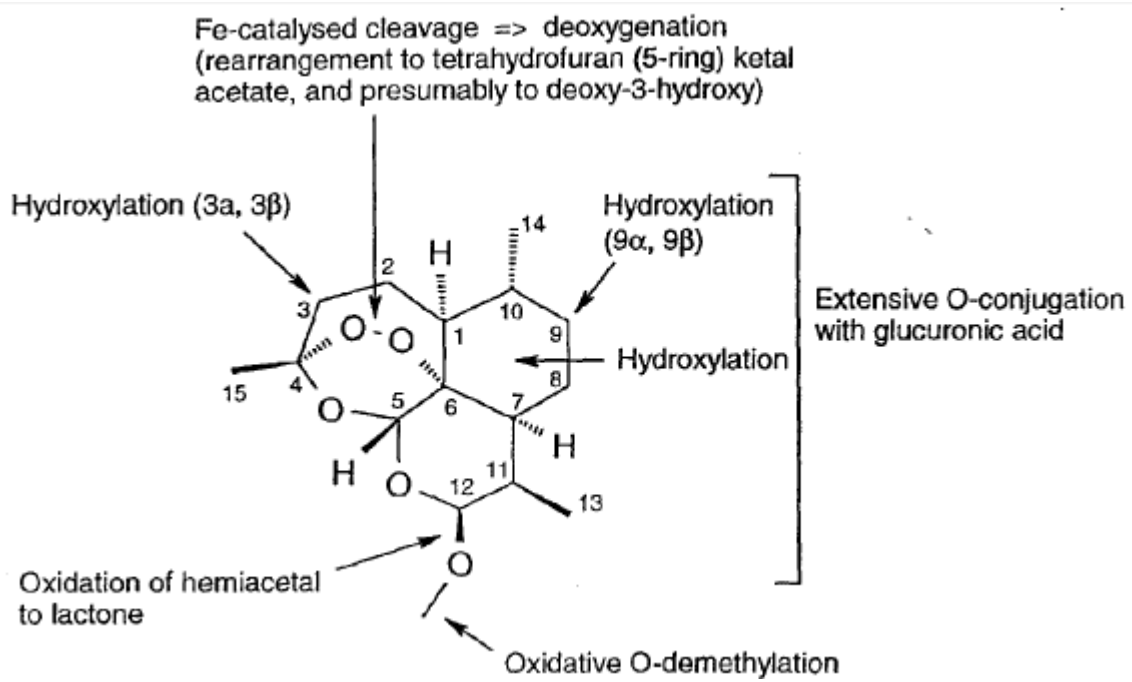
Comment: in the report the relative position of the 4-chloro-benzylidene and the N-dibutylamino groups are not correct (typing error).

Additional information: none

# 2.6.5.11 薬物動態試験：推定代謝経路

## 2.6.5.11A Pharmacokinetics: Possible metabolic pathways (DMPK(CH) 1997/534)

| **Study title:** | Characterization of metabolites of artemether (CGP 56696) formed in rats and dogs after single oral doses of co-artemether containing radio-labeled artemether | Study No: Study DMPK(CH) 1997/534 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-1 | |




Proposed biotransformation pathways in mammals, including rats, rabbits, dogs, and human

additional pathways

hydroxylations at positions. 2, 13, and 14

O-conjugation with glucose

pathways occur in various combinations

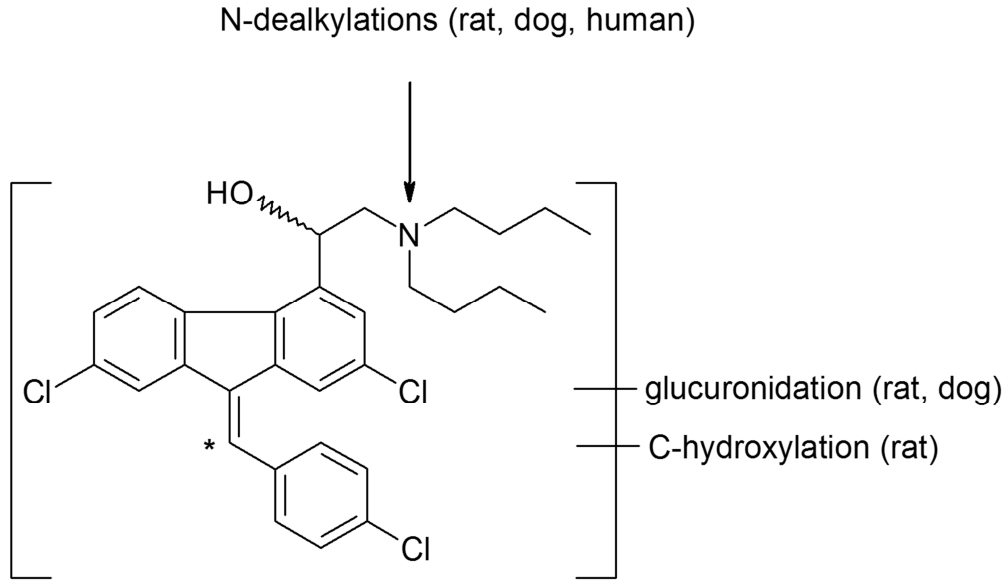
various diastereomers (α/β-epimers)

several pathways occur in all species; species differences in single pathways, stereochemistry and quantities.

## 2.6.5.11B Pharmacokinetics: Possible metabolic pathways (DMPK(CH) 1997/209)

| **Study title:** | Characterization of radioactive substances in disposition studies with $^{14}$C-labelled benflumetol (CGP 56695) after administration of co-artemether (CGP 56697) in rats and dogs. | Study No: DMPK(CH) 1997/209 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-2 | |

**Proposed main biotransformation pathways of Lmf in rats, dogs, and human**



# 2.6.5.12 薬物動態試験：薬物代謝酵素の誘導／阻害

## 2.6.5.12A Pharmacokinetics: Induction/Inhibition of drug-metabolizing enzymes (DMPK(CH) 1997/072)

| **Study title:** | Evaluation of a new chemical entity, CGP 56696, as an inhibitor of human P 450 enzymes. | Study No: DMPK(CH) 1997/072 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-8 | |
| Test article: | Arm | |
| Type of study: | *In vitro* evaluation of the reversible and mechanism-based inhibitions by Arm of the major P450 cytochrome enzymes found in the human liver microsomes CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11 | |
| Method: | Reversible inhibition: Concentrations of Arm at 0, 0.1, 1.0, 10, and 100 µM were added to human liver microsomal samples from a pool of 7 individuals together with the marker substrate at concentrations of Km/2, Km, and 4Km. Reactions were initiated in duplicate with NADPH. The kinetic constants were pre-determined, and the substrate concentrations were selected on the basis of these kinetic constants. Controls, with no Arm, contained the organic solvent used to dissolve Arm and the effect of this additional amount of solvent in the incubation was also determined. The data were analyzed using Dixon plots (Version 1.5 of Enzyme Kinetics from Trinity Software) to determine the type of inhibition (competitive or non-competitive) and the inhibitory constant ($K_i$).<br>Mechanism-based inhibition: Human liver microsomes from a pool of 7 individuals were pre-incubated for 10 minutes in triplicate samples containing Arm and NADPH. An aliquot of microsomes was removed and added to an incubation containing the marker substrate, and to another incubation to measure the residual marker P450 activity. Arm was therefore diluted by a factor of 10-20 for the final incubation with the marker substrate, which minimized any reversible inhibition effects. The concentration of Arm was determined by the results of the reversible inhibition experiments. The highest concentration of Arm that caused less than 20% inhibition was multiplied by the dilution factor to give the concentration of Arm for pre-incubation. After the 10-minute pre-incubation period, an aliquot was diluted 10 fold to measure residual P450 activity. | |

| **Study title:** | Evaluation of a new chemical entity, CGP 56696, as an inhibitor of human P 450 enzymes. | | | Study No: DMPK(CH) 1997/072 |
|---|---|---|---|---|
| Results: | concentrations Arm of 0.1, 1.0, 10, and 100 µM | | | |
| enzyme | P450 activity | Reversible inhibition | | Mechanism-based inhibition |
| | | $K_i$ (µM) | *I* (fractional inhibition) | |
| CYP1A2 | 7-ethoxyresorufin *O*-dealkylase | ~ 216[a] | $4.6 \times 10^{-3}$ | yes |
| CYP2A6 | coumarin 7–hydroxylase | [b] | na | no |
| CYP2C9 | tolbutamide methyl-hydroxylase[c] | ~ 330-470[a] | $3.0 \times 10^{-3}$ | no |
| CYP2C19 | *S*-mephenytoin 4′-hydroxylase | 38.4[a] | 0.025 | no |
| CYP2D6 | dextromethorphan *O*-demethylase[d] | [b] | na | no |
| CYP2E1 | chlorzoxazone 6-hydroxylase | [b] | na | no |
| CYP3A4/5 | testerone 6β-hydroxylase | 48.5 [a] | 0.020 | no |
| CYP4A9/11 | lauric acid 12-hydroxylase | [b] | na | no |

[a] = competitive inhibition
[b] = the $K_i$ value is >100 µM (the highest concentration evaluated), therefore, Arm did not inhibit this enzyme
[c] = concentrations of 0.1, 0.53, 10, and 100 µM
[d] = concentrations of 0.133, 1.33, 13.3, and 100 µM
na = not applicable

Conclusions: Based on the available data at pharmacologically relevant doses, Arm would not be expected to inhibit the metabolism of drugs by the major human P450 enzymes, including CYP2A6, CYP2C9, CYP2C19, CYP3A4/5, CYP2D6, CYP2E1and CYP4A9/11.

The only exception to this is CYP1A2, which was inhibited by Arm in a mechanism-based manner.

Additional information: Study performed at [redacted] USA (Novartis, sponsor)

## 2.6.5.12B Pharmacokinetics: Induction/Inhibition of drug-metabolizing enzymes (DMPK(CH) 1997/073)

| **Study title:** | Evaluation of a new chemical entity, CGP 56695, as an inhibitor of human P 450 enzymes. | Study No: DMPK(CH) 1997/073 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-9 | |
| Test article: | Lmf | |
| Type of study: | *In vitro* evaluation of the reversible and mechanism-based inhibitions by Lmf of the major P450 cytochrome enzymes found in the human liver microsomes CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11 | |
| Method: | Reversible inhibition: Concentrations of Lmf at 0, 0.05, 0.5, 1.0, and 2.0 µM were added to human liver microsomal samples from a pool of 7 individuals together with the marker substrate at concentrations of Km/2, Km, and 4Km. Reactions were initiated in duplicate with NADPH. The kinetic constants were pre-determined, and the substrate concentrations were selected on the basis of these kinetic constants. Controls, with no Lmf, contained the organic solvent used to dissolve Lmf. The effect of this additional amount of solvent in the incubation was also determined. The data were analyzed using Dixon plots (Version 1.5 of Enzyme Kinetics from Trinity Software) to determine the type of inhibition (competitive or non-competitive) and the inhibitory constant ($K_i$).<br>Mechanism-based inhibition: Human liver microsomes from a pool of 7 individuals were pre-incubated for 10 minutes in triplicate samples containing Lmf and NADPH. An aliquot of microsomes was removed and added to an incubation containing the marker substrate, and to another incubation to measure the residual marker P450 activity. Lmf was therefore diluted by a factor of 10-20 for the final incubation with the marker substrate, which minimized any reversible inhibition effects. The concentration of Lmf was determined by the results of the reversible inhibition experiments. The highest concentration of Lmf that caused less than 20% inhibition was multiplied by the dilution factor to give the concentration of Lmf for pre-incubation. After the 10-minute pre-incubation period, an aliquot was diluted 10 fold to measure residual P450 activity. | |

| **Study title:** | Evaluation of a new chemical entity, CGP 56695, as an inhibitor of human P 450 enzymes. | | | | Study No:<br>DMPK(CH) 1997/073 |
|---|---|---|---|---|---|
| Results: | concentrations Lmf of 0.05, 0.5, 1.0, and 2.0 µM | | | | |
| enzyme | P450 activity | Reversible inhibition | | | Mechanism-based inhibition |
| | | $K_i$ (µM) | *i* (fractional inhibition) | $i_{corr}$ (corrected fractional inhibition) | |
| CYP1A2 | 7-ethoxyresorufin *O*-dealkylase | [b] | na | na | no |
| CYP2A6 | coumarin 7–hydroxylase | [b] | na | na | no |
| CYP2C9 | tolbutamide methyl-hydroxylase | [b] | na | na | no |
| CYP2C19 | *S*-mephenytoin 4′ -hydroxylase | [b] | na | na | no |
| CYP2D6 | dextromethorphan *O*-demethylase[c] | 0.997[a] | 0.98 | 0.50 | no |
| CYP2E1 | chlorzoxazone 6-hydroxylase | [b] | na | na | no |
| CYP3A4/5 | testerone 6β-hydroxylase | [b] | na | na | no |
| CYP4A9/11 | lauric acid 12-hydroxylase | [b] | na | na | no |

[a] = competitive inhibition
[b] = the $K_i$ value is >2.0 µM (the highest concentration evaluated), therefore, Lmf did not inhibit this enzyme
[c] = concentrations of 0.067, 0.67, 1.33, and 2.0 µM
na = not applicable

Conclusions:

Lmf has little or no capacity to function as a reversible or mechanism-based inhibitor of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2E1, CYP3A4/5, and CYP4A9/11.

CYP2D6 was competitively inhibited by Lmf with a $K_i$ of 0.997 µM

At the anticipated $C_{max}$ of free Lmf in plasma of humans (≤ 2 µmol/L), the inhibition is sufficiently large to suggest that Lmf is likely to inhibit the metabolism of those drugs that are metabolized by CYP2D6.

Additional information: Study performed at [redacted] USA (Novartis, sponsor)

## 2.6.5.12C Pharmacokinetics: Induction/Inhibition of drug-metabolizing enzymes (DMPK R0900123)

| **Study title:** | Evaluation of artemether, dihydroartemisinin, and Lmf as inducers of drug metabolizing enzymes and metabolism of oral contraceptives in human hepatocytes | Study No: DMPK R0900123 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.4-10 | |
| Test article: | Arm, DHA, and Lmf | |
| Type of study: | *In vitro* CYP450 induction experiment | |
| Method: | The induction potential of Arm was tested at concentrations of 0.025, 0.25, and 2.5 µM, which exceed human plasma concentrations by a factor of more than 10, and which allow assessment of the concentration dependency of induction in a concentration range relevant to the clinical situation. Likewise, DHA was assessed at concentrations of 0.07, 0.7, and 7 µM, and Lmf was tested at concentrations of 2, 20, and 200 µM.<br>The effects of up to 48 h of treatment with Arm, DHA, and Lmf were tested in primary human hepatocytes of at least three individual donors. Evaluation of changes in CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A activity were assessed by measuring metabolism of CYP-selective probe substrates using quantitative LC/MS/MS analysis after the induction period in the livers. Changes of relative mRNA levels of CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 were determined by real-time PCR (RT-PCR).<br>Rifampicin (RIF), evaluated at 0.1, 1, and 20 µM, was used as a positive control for pregnane X receptor (PXR) and/or constitutive androstane receptor (CAR) activation (CYP2B/2C/3A induction). Phenobarbital (PB) at a concentration of 1 mM, was also included as a well-known positive control for CYP2B/2C/3A induction. β-napthoflavone (BNF) was used at a concentration of 10 µM as a positive control for activation of aryl hydrocarbon receptor (AhR) leading to induction of CYP1A enzymes.<br>In this study, incubation concentrations of 1 nM ethinyl estradiol and 20 nM levonorgestrel were used to mimick the therapeutic situation. | |
| Conclusions: | The three test substances were determined not to be inducers of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP3A enzyme activity in hepatocytes. In addition, the test substances did not induce CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or CYP3A5 mRNA. Metabolism of ethinyl estradiol and levonorgestrel was determined not to be induced by Arm, DHA, and Lmf.<br>These conclusions were based upon the levels of mRNA or activity at least less than 2-fold, with respect to the vehicle control, and/or less than 40% of the maximal positive control induction response, indicative of a non-inducer *in vitro*. | |

# 2.6.5.13 薬物動態試験：排泄

## 2.6.5.13A Pharmacokinetics: Excretion (DMPK(CH) 1997/241)

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol | Study no.<br>DMPK(CH) 1997/241 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-1 | |
| Test article | [$^{3}$H]- or [$^{14}$C]-Arm (1part) and unlabeled Lmf (6 parts) | |
| Number of animals: | male rat albino/ Tif: RAIf (SPF) / 3 per group | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7/3, w/w): in solution for intravenous route<br>0.5% Klucel + 0.1% Tween 80: oral route in rats | |
| Method/ Route / Duration of administration: | intravenous bolus / tail vain / once<br>gavage / oral / once | |
| Dose (mg/kg): | intravenous dose of 10 once<br>oral dose of 20, 100, or 1000 once | |
| Specific activity: | [$^{3}$H]-Arm (Batch No. Re-54.9A) of 3022.9 MBq/mmol or 10.05 MBq/mg [a]<br>[$^{3}$H]-Arm (Batch No. Re-54.9C) of 3.35 MBq/mg<br>[$^{3}$H]-Arm (Batch No. Re-54.9C1) of 3.35 MBq/mg<br>[$^{3}$H]-Arm (Batch No. Re-54.9D) of 1.39 MBq/mg | [$^{3}$H]-Arm (Batch No. Re-54.9E) of 33 KBq/mg<br>[$^{3}$H]-Arm (Batch No. Re-54.9G )of 273 KBq/mg<br>[$^{14}$C]-Arm (Batch No. Re-84.1A1) of 1.67 MBq/mg (WBAR, i.v.)<br>[$^{14}$C]-Arm (Batch No. Re-84.1C2) of 218 KBq/mg (WBAR, p.o.)<br>a: taken from the Certificate of Analysis sheet of Re-54.9A (typing error in report). |
| Analyte / Radionuclide: | radioactivity / $^{3}$H or $^{14}$C | |
| Assay: | liquid scintillation counting | |

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled lumefantrin. | | | | | | Study no. DMPK(CH) 1997/241 | |
|---|---|---|---|---|---|---|---|---|
| **Excretion results**: | | | | | | | | |
| Rat | Excretion mean (% of administered radioactivity) | | | | | | | |
| Route | intravenous | | single oral | | | | | |
| Dose (mg/kg) | 10 | | 20 | | 100 | | 1000 | |
| Number of animals | 4 | | 3 | | 3 | | 3 | |
| Sample | urine | feces | urine | feces | urine | feces | urine | feces |
| Time interval (h) | | | | | | | | |
| 0 – 8* | 18.19 | ns | 17.98 | ns | 25.25 | ns | 15.67 | ns |
| 8 – 24 | 19.77 | 33.74 | 23.43 | 33.53 | 28.48 | 34.14 | 29.82 | 14.34 |
| 24 – 48 | 2.76 | 5.83 | 2.91 | 5.69 | 4.91 | 5.57 | 17.24 | 14.61 |
| 48 – 72 | 0.88 | 1.96 | 0.84 | 1.09 | 1.16 | 1.23 | 1.82 | 2.62 |
| 72 – 96 | 0.53 | 1.08 | 0.40 | 0.54 | 0.55 | 0.52 | 0.50 | 0.66 |
| 96 – 120 | 0.40 | 0.73 | 0.33 | 0.55 | 0.31 | 0.33 | 0.26 | 0.26 |
| 120 – 144 | 0.36 | 0.56 | 0.23 | 0.27 | 0.19 | 0.22 | 0.15 | 0.27 |
| 144 – 168 | 0.29 | 0.46 | 0.14 | 0.22 | ns | ns | ns | ns |
| 0 – 168 | 43.17 | 44.35 | 46.26 | 41.89 | $60.86_{(0\text{-}144)}$ | $42.02_{(0\text{-}144)}$ | $65.47_{(0\text{-}144)}$ | $32.76_{(0\text{-}144)}$ |

| Rat carcass | | |
|---|---|---|
| Route | intravenous | single oral |
| Dose (mg/kg) | 10 | 20 |
| Number of animals | 4 | 3 |
| $^{3}H$ in mean% of administered dose | 7.44 | 1.85 |

*: Faces sample collected between 0 and 24 hours post dose. ns= no sampling
Additional information: none

## 2.6.5.13B Pharmacokinetics: Excretion (DMPK(CH) 1997/003)

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | Study no.<br>DMPK(CH) 1997/003 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-2 | |
| Test article: | [$^{14}$C]-Arm (1 part) and unlabeled Lmf (6 parts) | |
| Species, strain, sex, number of animals: | male dog / Beagle / 6 | |
| Feeding condition: | fasted | |
| Vehicle / Formulation: | PEG 400 / in solution with N-methyl-2-pyrrolidone for intravenous route<br>gelatin capsule for oral route in dogs | |
| Method/ Route / Duration of administration: | intravenous bolus / cephalic vein of a foreleg / once<br>gelatin capsule / oral / once | |
| Dose (mg/kg): | intravenous dose of 10 once<br>oral dose of 20 and 200 once | |
| Specific activity: | [$^{14}$C]-Arm Batch Re-84.1C1 of 218 KBq/mg<br>[$^{14}$C]-Arm Batch Re-84.1D2 of 98 KBq/mg<br>[$^{14}$C]-Arm Batch Re-84.1F of 10 KBq/mg | |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | liquid scintillation counting | |

| **Study title:** | Absorption and disposition of CGP 56696 (artemether) after administration of CGP 56697 (co-artemether) containing $^{14}$C-labelled CGP 56696 (artemether) to male dogs. | Study no. DMPK(CH) 1997/003 |
|---|---|---|

**Excretion results**:

| Dog | Excretion mean (% of administered radioactivity) | | | | | |
|---|---|---|---|---|---|---|
| Route | intravenous | | single oral | | | |
| Dose (mg/kg) | 10 | | 20 | | 200 | |
| Number of animals | 2 | | 2 | | 2 | |
| Sample | urine | feces | urine | feces | urine | feces |
| Time interval (h) | | | | | | |
| 0 – 24 | 42.49 | 21.64 | 45.14 | 26.26 | 47.84 | 29.77 |
| 24 – 48 | 6.96 | 5.64 | 17.07 | 3.16 | 8.19 | 6.16 |
| 48 – 72 | 1.57 | 2.75 | 0.72 | 0.75 | 0.70 | 0.84 |
| 72 – 96 | 0.88 | 1.50 | 0.17 | 0.27 | 0.26 | 0.24 |
| 96 – 120 | 0.35 | 1.02 | 0.08 | 0.13 | 0.11 | 0.12 |
| 120 – 144 | 0.37 | 0.39 | 0.10 | 0.13 | 0.07 | 0.08 |
| 144 – 168 | 0.23 | 0.34 | 0.04 | 0.08 | 0.05 | 0.04 |
| 0 – 168 | 52.85 | 33.29 | 63.32 | 30.79 | 57.22 | 37.25 |

Additional information: none

## 2.6.5.13C Pharmacokinetics: Excretion (DMPK(CH) 1997/240)

| **Study title:** | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether. | Study no. DMPK(CH) 1997/240 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-4 | |
| Test article: | [$^{14}$C]Lmf(6 parts) / 1 part unlabeled Arm | |
| Species, strain, sex, number of animals: | male, rat / Tif: RAIf (SPF) / 12<br>male, dog / Beagle / 4 | |
| Feeding condition: | fasted | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7:3, w/w): for intravenous route<br>0.5% Klucel + 0.1% Tween 80: oral route in rats<br>gelatin capsule: oral route in dogs | |
| Method/ Route / Duration of administration: | rats: intravenous bolus / tail vain / once<br>gavage / oral / once (20 mg/kg)<br>gavage / oral / once (100 mg/kg)<br>gavage / oral / once / (1000 mg/kg)<br>dogs: intravenous bolus / cephalic vein of a foreleg / once<br>gelatin capsule / oral / once | |
| Dose (mg/kg): | rats: intravenous dose of 1 mg/kg<br>oral dose of 20 mg/kg once<br>oral dose of 100 mg/kg once<br>oral dose of 1000 mg/kg once<br>dogs: intravenous dose of 1 mg/kg<br>oral dose of 20 mg/kg once | |
| Specific activity: | $^{14}$C Lmf Batch Ko-76.1A-2 of 2069 KBq/mg<br>$^{14}$C Lmf Batch Ko-76.1B of 64.1 KBq/mg<br>$^{14}$C Lmf Batch Ko-76.1C of 10.0 KBq/mg<br>$^{14}$C Lmf Batch Ko-76.1D of 960 KBq/mg<br>$^{14}$C Lmf Batch Ko-76.1F of 159 KBq/mg | |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | liquid scintillation counting | |

| **Study title:** | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether. | | | | | | Study no. DMPK(CH) 1997/240 | |
|---|---|---|---|---|---|---|---|---|
| **Excretion results**: | | | | | | | | |
| **Rat** | Excretion mean (% of administered radioactivity) | | | | | | | |
| Route | intravenous (fasting) | | single oral (fasting) | | single oral (non-fasting) | | | |
| Dose (mg/kg) | 1 | | 20 | | 100 | | 1000 | |
| Number of animals | 3 | | 3 | | 3 | | 3 | |
| Sample | urine | feces | urine | feces | urine | feces | urine | feces |
| Time interval (h) | | | | | | | | |
| 0 – 8* | 0.82 | ns | 0.08 | ns | 0.12 | ns | 0.11 | ns |
| 8 – 24 | 0.51 | 35.36 | 0.08 | 88.69 | 0.15 | 76.64 | 0.15 | 58.56 |
| 24 – 48 | 0.19 | 18.44 | 0.04 | 4.12 | 0.04 | 10.54 | 0.05 | 34.06 |
| 48 – 72 | 0.12 | 10.39 | 0.02 | 0.54 | 0.02 | 1.81 | 0.01 | 2.17 |
| 72 – 96 | 0.09 | 5.37 | 0.01 | 0.30 | 0.02 | 0.91 | 0.01 | 0.31 |
| 96 – 120 | 0.07 | 3.49 | 0.00 | 0.15 | 0.01 | 0.56 | 0.00 | 0.19 |
| 120 – 144 | 0.08 | 2.42 | 0.00 | 0.13 | 0.01 | 0.40 | 0.00 | 0.11 |
| 144 – 168 | 0.06 | 2.07 | 0.00 | 0.15 | 0.01 | 0.44 | 0.00 | 0.09 |
| 0 – 168 | 1.95 | 77.54 | 0.24 | 94.08 | 0.38 | 91.30 | 0.33 | 95.49 |

*: Feces sample collected between 0 and 24 hours post dose. ns = not sampled

| **Study title:** | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether. | | | | | Study no. DMPK(CH) 1997/240 |
|---|---|---|---|---|---|---|
| **Rat carcass** | | | | | | |
| Route | intravenous | | single oral (fasting) | | single oral (non-fasting) | |
| Dose (mg/kg) | 1 | | 20 | | 100 | 1000 |
| Number of animals | 3 | 4[a] | 3 | 4[a] | 3 | 3 |
| $^{14}$C in mean% of administered dose | 15.89 | 42.05 | 0.38 | 0.40 | 1.47 | 0.33 |
| [a] = bile-duct cannulated | | | | | | |
| **Dog** | Excretion (% of administered radioactivity) | | | | | |
| Route | intravenous (fasting) | | single oral (fasting) | | single oral (non-fasting) | |
| Dose (mg/kg) | 1 | | 20 | | 20 | |
| Number of animals | 2 | | 2 | | 2 | |
| Sample | urine | feces | urine | feces[a] | urine | feces[a] |
| Time interval (h) | | | | | | |
| 0 – 8 | 0.00 | ns | 0.00 | ns | 0.00 | ns |
| 8 – 24 | 1.10 | 36.09 | 0.26 | 88.73 | 0.41 | 72.57 |
| 24 – 48 | 0.32 | 21.99 | 0.05 | 2.09 | 0.15 | 6.05 |
| 48 – 72 | 0.17 | 8.15 | 0.02 | 0.91 | 0.06 | 4.18 |
| 72 – 96 | 0.11 | 3.18 | 0.01 | 0.42 | 0.02 | 1.73 |
| 96 – 120 | 0.07 | 2.81 | 0.01 | 0.37 | 0.02 | 0.42 |
| 120 – 144 | 0.06 | 1.78 | 0.00 | 0.20 | 0.01 | 0.51 |
| 144 – 168 | 0.04 | 1.31 | 0.00 | 0.14 | 0.01 | 0.29 |
| 0 – 168 | 1.87 | 75.31 | 0.37 | 92.86 | 0.68 | 85.75 |

[a] = one sample collected between 0 and 24 hours post dose

Additional information: none. ns = not sampled

# 2.6.5.14 薬物動態試験：胆汁中排泄

## 2.6.5.14A Pharmacokinetics: Excretion into bile (DMPK(CH) 1997/241)

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol. | Study no. DMPK(CH) 1997/241 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-1 | |
| Test article | $^{3}$H-labeled Arm (1 part) / unlabeled Lmf (6 parts) | |
| Species, strain, sex, number of animals: | male rat albino/ Tif: RAIf (SPF) / 3 per group | |
| Feeding condition: | fed | |
| Vehicle / Formulation: | 0.5% Klucel + 0.1% Tween 80 | |
| Method/ Route / Duration of administration: | gavage / oral / once | |
| Dose (mg/kg): | oral dose of 20 mg/kg | |
| Specific activity: | [$^{3}$H]-Arm Re-54.9A of 3022.9 MBq/mmol or 10.05 MBq/mg [a]<br>[$^{3}$H]-Arm Re-54.9C of 3.35 MBq/mg<br>[$^{3}$H]-Arm Re-54.9C1 of 3.35 MBq/mg<br>[$^{3}$H]-Arm Re-54.9D of 1.39 MBq/mg.<br>[$^{3}$H]-Arm Re-54.9E of 33 KBq/mg<br>[$^{3}$H]-Arm Re-54.9G of 273 KBq/mg<br>a: value from the Certificate of analysis, typing error in the report. | |
| Analyte / Radionuclide: | radioactivity / $^{3}$H | |
| Assay: | liquid scintillation counting | |

| **Study title:** | Disposition studies in rats after administration of co-artemether (CGP 56697) containing radiolabelled artemether (CGP 56696) and unlabelled benflumetol. | | | Study no. DMPK(CH) 1997/241 |
|---|---|---|---|---|
| Excretion results (including bile): | | | | |
| Rat | Excretion mean (% of administered radioactivity) | | | |
| Route | single oral | | | |
| Dose (mg/kg) | 20 | | | |
| Number of animals | 3 | | | |
| Samples | urine | feces[a] | bile | total |
| Time intervals (h) | | | | |
| 0 – 8 | 15.23 | ns | 47.54 | 62.77 |
| 8 – 24 | 10.91 | 4.14 | 9.53 | 24.58 |
| 24 – 48 | 1.37 | 2.62 | 1.04 | 5.03 |
| 48 – 72 | 0.42 | 0.82 | 0.36 | 1.06 |
| 0 – 72 | 27.93 | 7.59 | 58.46 | 93.98 |

[a] = one sample collected between 0 and 24 hours post dose. ns = not sampled

Additional information: none

## 2.6.5.14B Pharmacokinetics: Excretion into bile (DMPK(CH) 1997/240)

| **Study title:** | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | Study no. DMPK(CH) 1997/240 |
|---|---|---|
| GLP compliance: | not required | |
| Location in CTD: | 4.2.2.2-4 | |
| Test article: | [$^{14}$C]Lmf(6 part) / unlabeled Arm (1 part) | |
| Species, strain, sex, number of animals: | male, rat / Tif: RAIf (SPF), n=3 per group | |
| Feeding condition: | fasted | |
| Vehicle / Formulation: | PEG 400 / N-methyl-2-pyrrolidone (7/3, w/w) for intravenous route<br>0.5% Klucel + 0.1% Tween 80: oral route in rats | |
| Method/ Route / Duration of administration: | rats: intravenous bolus / tail vain / once (1 mg/kg)<br>gavage / oral / once (20 mg/kg) | |
| Dose (mg/kg): | rats: intravenous dose of 1 mg/kg<br>oral dose of 20 mg/kg | |
| Specific activity: | $^{14}$C Lmf Batch Ko-76.1B of 64.1 KBq/mg<br>$^{14}$C Lmf Batch Ko-76.1C of 10.0 KBq/mg<br>$^{14}$C Lmf Batch Ko-76.1D of 960 KBq/mg<br>$^{14}$C Lmf Batch Ko-76.1F of 159 KBq/mg | |
| Analyte / Radionuclide: | radioactivity / $^{14}$C | |
| Assay: | liquid scintillation counting | |

| **Study title:** | Disposition studies in rats and dogs after administration of co-artemether (CGP 56697) containing $^{14}$C-labelled benflumetol (CGP 56695) and unlabelled artemether | | | | | | | | | Study no. DMPK(CH) 1997/240 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Excretion results (including bile): | | | | | | | | | | | | |
| Rat | Excretion mean (% of administered radioactivity) | | | | | | | | | | | |
| Route | intravenous | | | single oral (without bile replacement) | | | single oral (with bile replacement) method A | | | single oral (with bile replacement) method B | | |
| Dose (mg/kg) | 1 | | | 20 | | | 20 | | | 20 | | |
| Number of animals | 4 | | | 4 | | | 3 | | | 4 | | |
| Samples | urine[a] | feces[b] | bile | urine[a] | feces[b] | bile | urine[a] | feces[b] | bile | urine[a] | feces[b] | bile |
| Time intervals (h) | | | | | | | | | | | | |
| 0 – 2 | | | 1.00 | | | 0.01 | | | 0.04 | | | 0.10 |
| 2 – 4 | | | 2.28 | | | 0.02 | | | 0.04 | | | 0.35 |
| 4 – 6 | | | 2.64 | | | 0.02 | | | 0.04 | | | 0.38 |
| 6 – 8 | 0.91 | | 2.41 | 0.09 | | 0.03 | 0.50 | | 0.04 | 0.54 | | 0.34 |
| 8 – 12 | | | 4.12 | | | 0.05 | | | 0.06 | | | 0.44 |
| 12 – 24 | 0.47 | 1.01 | 10.46 | 0.08 | 76.67 | 0.09 | 0.39 | 50.00 | 0.09 | 0.39 | 18.08 | 0.99 |
| 24 – 32 | | | 4.77 | | | 0.04 | | | 0.05 | | | 0.25 |
| 32 – 48 | 0.22 | 1.81 | 6.69 | 0.02 | 15.15 | 0.04 | 0.16 | 30.33 | 0.06 | 0.20 | 48.29 | 0.61 |
| 48 – 72 | 0.41 | 1.35 | 4.72 | 0.01 | 2.69 | nc | | | | | | |
| 0 – 72 | 2.01 | 4.17 | 39.08 | 0.20 | 94.50 | 0.32 | $1.05_{0-48}$ | $80.33_{0-48}$ | $0.43_{0-48}$ | $1.13_{0-48}$ | $66.37_{0-48}$ | $3.15_{0-48}$ |
| Residuals: Carcass+Intestine+Bone | 42.05 + 3.28 + 0.05 = 45.38 | | | 0.4 + 0.77 + 0.0 = 1.17 | | | not measured | | | not measured | | |
| Residuals + Excreta | 83.76 | | | 96.19 | | | not determined | | | not determined | | |

[a] = samples collected at 3 or 4 time intervals (0 − 8, 8 − 24, 24 − 48, or 48 − 72 hours post dose)

[b] = samples collected at 2 or 3 time intervals ( 0 – 24, 24 – 48 or 48 – 72 hours post dose). nc= not calculated.

Additional information: none

## 2.6.5.15 薬物動態試験：薬物相互作用

該当なし

## 2.6.5.16 薬物動態試験：その他

該当なし