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 3
略号一覧 4
補足事項 5
1 まとめ 6
1.1 吸収及び薬物動態 7
1.2 分布 7
1.3 代謝 8
1.4 排泄 9
1.5 薬物間相互作用 9
1.5.1 酵素阻害 9
1.5.2 酵素誘導 10
1.5.3 トランスポーター 10
2 分析法 11
2.1 同位体標識 LCZ 及び関連成分 11
2.2 放射能の分析 12
2.3 AHU，LBQ 及び VAL の分析 12
2.4 代謝物の特性評価及び構造解析 12
3 吸収 12
3.1 吸収 12
3.2 ADME 試験及び TK 試験での薬物動態パラメータ 14
4 分布 20
4.1 血漿蛋白結合及び血球移行 20
4.2 組織及び臓器分布 21
4.3 胎盤通過 21
5 LCZ の代謝（動物種間の比較） 22
5.1 AHU の *in vitro* 代謝 22
5.2 VAL の *in vitro* 代謝 22
5.3 LCZ の *in vivo* 代謝 22
5.4 LCZ の代謝経路 23
6 排泄 24
7 薬物動態学的薬物間相互作用 26
7.1 CYP 阻害 26
7.2 酵素誘導 26

7.3 *In vitro* 膜透過性評価及びトランスポーターを介した相互作用 27
7.4 *In vitro* トランスポーター阻害 28
8 その他の薬物動態試験 30
9 考察及び結論 30
10 参考文献 31

表 一 覧

Table 1-1 LCZ696 及び AHU377 の ADME 試験並びに TK 試験 6
Table 3-1 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における吸収パラメータ 13
Table 3-2 放射性標識 AHU 又は LCZ を単回経口投与したときの血漿中又は血液中総放射能の曝露量（Cmax 及び AUC）（ADME 試験） 14
Table 3-3 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における AHU の曝露量（Cmax 及び AUC） 15
Table 3-4 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における LBQ の曝露量（Cmax 及び AUC） 16
Table 3-5 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における VAL の曝露量（Cmax 及び AUC） 17
Table 3-6 [^{14}C]LCZ 又は [^{14}C]AHU を単回経口投与後の血漿中又は血液中 Cmax 及び AUC の用量補正値 18
Table 3-7 [^{14}C]LCZ 又は [^{14}C]AHU を単回経口投与後の LBQ のバイオアベイラビリティ 19
Table 3-8 [^{14}C] LCZ 又は [^{14}C] AHU を経口投与後の放射能濃度，AHU，LBQ 及び VAL の消失半減期 19
Table 3-9 [^{14}C] AHU 又は[^{14}C] LBQ を静脈内投与後の血漿又は血液クリアランス及び分布容積 20
Table 6-1 [^{14}C]LCZ 又は[^{14}C]AHU を単回投与したときの主要代謝物及び総放射能の排泄率 25

図 一 覧

Figure 2-1 ^{14}C による LCZ，AHU 及び LBQ の標識体 11
Figure 5-1 AHU から LBQ への代謝 22
Figure 5-2 LCZ の推定代謝経路 24

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，代謝及び排泄
AGP	α_1-acid glycoprotein	α_1-酸性糖蛋白質
AUC0-t	area under the drug plasma (serum/blood) concentration-time curve (time 0 to t)	血漿（血清/血液）中薬物濃度-時間曲線下面積（時間 0～t）
AUCinf	area under the serum concentration-time curve (time 0 to infinity)	血清中濃度-時間曲線下面積（0～無限大）
AUClast	area under the drug plasma (serum/blood) concentration-time curve (time 0 to the last measurable concentration sampling time)	血漿（血清/血液）中薬物濃度-時間曲線下面積（0～最終定量可能時点）
BCRP	breast cancer resistance protein	乳癌耐性蛋白質
CL/F	clearance for extravascular administration	血管外投与時のクリアランス
Cmax	maximum plasma/serum/blood concentration after a single dose	単回投与後の最高血漿／血清／血液中濃度
CYP	cytochrome P450	チトクローム P450
HPLC	high performance liquid chromatography	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
IC50	50% inhibitory concentration	50%阻害濃度
Jmax	maximum transport rate	最大輸送速度
Ki	inhibition constant	阻害定数
Km	michaelis constant	ミカエリス定数
LC-MS/MS	liquid chromatography – tandem mass spectrometry	液体クロマトグラフィー／タンデム質量分析
MRP2	multidrug resistance associated protein 2	多剤耐性関連蛋白質 2
NEP	neprilysin; neutral endopeptidase 24.11	ネプリライシン；中性エンドペプチダーゼ 24.11
NMR	nuclear magnetic resonance spectroscopy	核磁気共鳴分析
OAT	organic anion transporter	有機アニオントランスポーター
OATP	organic anion transporter polypeptide	有機アニオントランスポーター
OCT	organic cation transporter	有機カチオントランスポーター
P-gp	P-glycoprotein	P-糖蛋白質
QWBA	quantitative whole-body autoradiographic analysis	定量的全身オートラジオグラフィー
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）中濃度到達時間
UV	ultraviolet	紫外線
Vz/F	apparent distribution volume	見かけの分布容積

補足事項

用語	定義
薬剤名の表記方法	文章及び図表中では以下の表記を用いた。

一般名	本書での表記
LCZ696（サクビトリルバルサルタンナトリウム水和物，エンレスト）	LCZ
AHU377（サクビトリル）	AHU
LBQ657（Sacubitrilat）	LBQ
VAL149（バルサルタン）	VAL

1 まとめ

サクビトリルバルサルタンナトリウム水和物（LCZ696，以下 LCZ）は，アンジオテンシン受容体-ネプリライシン阻害薬に分類される初めての薬剤である。LCZ696 を投与後，中性エンドペプチダーゼ阻害薬（NEPi）であるサクビトリル（AHU377，以下 AHU）及びアンジオテンシン II 受容体拮抗薬であるバルサルタン（以下 VAL）として全身に移行する。AHU はエステラーゼにより代謝され NEPi の活性代謝物である sacubitrilat（LBQ657，以下 LBQ） に変換される。

本項では，実験動物種における LCZ 並びに AHU，LBQ 及び VAL の吸収，分布，代謝及び排泄（ADME）について検討した試験結果について記載する。^{14}C 標識体を用いた *in vivo* ADME 試験は静脈内及び経口投与により実施した。標識あるいは非標識 LCZ 又は AHU 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・用量をTable 1-1に示す。なお，本概要文に記載した LCZ 及び AHU の投与量は遊離塩基として示し，三ナトリウムヘミ五水和物塩(LCZ)及びカルシウム塩（AHU）としての用量は，換算係数 1.13 及び 1.046 をそれぞれ使用した。各試験での用量は，LCZ 又はその関連成分である AHU 及び VAL の毒性試験と同じ用量範囲とし，同様の投与方法を用いた。

各動物種から血液又は血漿試料を経時的に採取し，尿及び糞試料検体も採取した。有色ラット及び妊娠ラットにおける放射能の組織分布は，定量的全身オートラジオグラフィーにより検討した。ラット，イヌ，サル及びヒトの血漿蛋白結合率は超遠心法により測定した。

Table 1-1 LCZ696 及び AHU377 の ADME 試験並びに TK 試験

動物種	放射性核種	投与量[mg/kg]（投与薬剤）	投与形態	投与経路	投与期間	報告書番号
マウス	^{14}C	30 (LBQ)	溶液	静脈内	単回投与	R1200673
	^{14}C	150 (AHU)	懸濁液	経口	単回投与	R1200673
	-[a]	400/日 (AHU)	懸濁液	経口	反復投与	R0770896
	-	800/日(AHU)	懸濁液	経口	反復投与	R0770896
	-	1200/日 (AHU)	懸濁液	経口	反復投与	R0770896
ラット	^{14}C	15 (AHU)	溶液	静脈内	単回投与	R0300247-1
	^{14}C	45 (AHU)	溶液	経口	単回投与	R0300247-1
	^{14}C	45[b] (AHU)	溶液	経口	単回投与	R0300247-2
	^{14}C	25[c] (LCZ)	溶液	経口	単回投与	R1200674
	^{14}C	30[d] (LCZ)	溶液	経口	単回投与	R1200622
	-	30/日(LCZ)	懸濁液	経口	反復投与	R0670283
	-	100/日 (LCZ)	懸濁液	経口	反復投与	R0670283
	-	200/日 (LCZ))	懸濁液	経口	反復投与	R0670283
	-	400/日 (AHU)	懸濁液	経口	反復投与	R0770711
	-	800/日 (AHU)	懸濁液	経口	反復投与	R0770711
	-	1200/日 (AHU)	懸濁液	経口	反復投与	R0770711

動物種	放射性核種	投与量[mg/kg]（投与薬剤）	投与形態	投与経路	投与期間	報告書番号
	-	30/日 (LCZ)	懸濁液	経口	反復投与	R0670390
	-	100/日 (LCZ)	懸濁液	経口	反復投与	R0670390
	-	200/日 (LCZ)	懸濁液	経口	反復投与	R0670390
ウサギ	-	3/日 (LCZ)	懸濁液	経口	反復投与	R0670280
	-	10/日 (LCZ)	懸濁液	経口	反復投与	R0670280
	-	30/日 (LCZ)	懸濁液	経口	反復投与	R0670280
イヌ	^{14}C	3 (LBQ)	溶液	静脈内	単回投与	R0301244
	^{14}C	15 (AHU)	溶液	経口	単回投与	R0301244
サル	^{14}C	10 (LBQ)	溶液	静脈内	単回投与	R1200729
	^{14}C	30 (LCZ)	懸濁液	経口	単回投与	R1200729
	-	30/日 (LCZ)	懸濁液	経口	反復投与	R0670621
	-	100/日 (LCZ)	懸濁液	経口	反復投与	R0670621
	-	300/日 (LCZ)	懸濁液	経口	反復投与	R0670621

Source: [Table 2.6.5.3A-R1200673], [Table 2.6.5.4A-R0770896], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.5- R0300247-2], [Table 2.6.5.8-R1200674], [Table 2.6.5.9I-R1200622], [Table 2.6.5.4B-R0670283], [Table 2.6.5.4B-R0770711], [Table 2.6.5.7A-R0670390], [Table 2.6.5.7B-R0670280], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [Table 2.6.5.4C-R0670621]

[a] 非標識体，[b] 有色ラットにおける組織分布（全身オートラジオグラフィー），[c] 妊娠ラットにおける組織分布（全身オートラジオグラフィー），[d] 乳汁移行試験

1.1 吸収及び薬物動態

[^{14}C] LCZ 又は [^{14}C] AHU を経口投与したときの AHU 由来の総放射能濃度に基づく吸収率は，検討したいずれの動物種においても良好であった（65%～100%）。ヒトにおける吸収率は，[^{14}C] LCZ を経口投与後の尿中への AHU 由来の総放射能回収率に基づき 61%以上であると推定された。吸収は各動物種（マウス，ラット，イヌ，ウサギ，サル及びヒト）において比較的速やかであり，総放射能濃度の Tmax は 0.25～2 時間の範囲であった。[^{14}C]LCZ 又は [^{14}C] AHU を経口投与後の LBQ のバイオアベイラビリティは各動物種で 41～100%の範囲であり，ヒトでは 50%以上と推定された。[^{14}C]AHU（マウス，ラット及びイヌ）又は [^{14}C] LCZ（サル及びヒト）を単回経口投与後，血漿中総放射能濃度は消失半減期 1.3～26 時間で低下した。AHU の消失半減期は 1.3～3.2 時間，LBQ は 1.1～12 時間であり，VAL の消失半減期はサルで 6.5 時間及びヒトで 21 時間であった。TK 試験において，いずれの動物種（マウス，ラット，イヌ及びサル）においても AUC は概ね用量の増加に伴い増加した。曝露量に明らかな性差は認められなかった。ラット及びサルに反復経口投与時に累積性は認められなかった。イヌに LCZ を投与したときの VAL の曝露量は，VAL 単独投与時と比べて高くなる傾向が認められた。

1.2 分布

AHU，LBQ 及び VAL の血球移行はほとんど認められなかった。LBQ の血漿蛋白結合率はラット，イヌ，サル及びヒト血漿でそれぞれ，90%，80%，93%及び 97%であり，AHU の血漿蛋白結

合率はサル及びヒトでそれぞれ 91%及び 97%であった。ヒトにおける AHU 及び LBQ の主要結合蛋白は血清アルブミンであった。同様に，VAL の血清蛋白結合率は動物種間で異なり，94%～98%の範囲であった。VAL のヒト血漿及び血清蛋白結合率はそれぞれ 94%及び 96%であり，主として血清アルブミンに結合した。

[^{14}C] AHU をラットに単回静脈内又は経口投与したとき，放射能は多くの組織に広範かつ速やかに分布した 。静脈内投与後速やかに腎臓，肝臓及び胆汁中で高い放射能（血液中濃度の 2～6 倍）が認められた。脳，眼，精嚢及び脊髄中の放射能濃度は低く，AHU 関連放射能の血液–脳関門及び血液–精巣関門の透過性は低かった。メラニン含有組織（有色皮膚及びブドウ膜）への AHU 関連放射能の移行性も低かった。総放射能の組織分布は，経口投与後と静脈内投与後で類似していた。多くの組織において，放射能濃度は経口投与後 1 時間で最高値に達し，腎臓及び肝臓で高い濃度が認められた。投与後 24 時間における組織中放射能濃度は，小腸及び腎臓を除いて，定量下限未満であった。

[^{14}C]VAL を静脈内投与したとき，比較的高い放射能濃度が肝臓及び腎臓で認められた。[^{14}C]VAL 由来の放射能の脳及び精巣への移行は少なかった。経口投与後の組織分布は静脈内投与後と類似していた。いずれの投与経路においても，投与後 7 日での残存放射能は，肝臓を除くすべての組織で定量下限未満であった。

[^{14}C] AHU を妊娠ラットに投与したとき，母動物の血液から胚・胎児組織への AHU 由来放射能の移行は少なく，胎児の Tmax における胎児/母動物血液中濃度比は平均で 0.51 以下であった。妊娠ウサギに LCZ を投与したときの胎児/母動物血漿中濃度比は平均で 0.21 以下であった。

1.3 代謝

LCZ は経口投与後，AHU 及び VAL に解離して吸収される。[^{14}C]AHU をラット，イヌ及びヒトの肝スライスとともにインキュベーションしたとき，エステラーゼにより加水分解を受け，活性代謝物である LBQ が主に生成した。LBQ は肝スライスとのインキュベーションにより，代謝をほとんど受けなかった。マウス，ラット，ウサギ，イヌ，マーモセット及びヒト肝ミクロソームによる VAL の *in vitro* での酸化的代謝は緩徐かつ軽微であった。ヒト肝ミクロソームでの VAL の 4-ヒドロキシバレリル体生成に関与する酵素は CYP2C9 であった。

すべての動物種において，AHU はエステル加水分解を受けて LBQ が主代謝物として生成した。マウス，ラット及びヒトにおける AHU から LBQ への代謝は速やかであり，血漿中で検出された主成分は LBQ であった。一方，イヌ及びサルにおいては，AHU 及び LBQ が血漿中に認められた。各動物種の血漿試料中でその他数種の微量代謝物も検出された。検討したすべての動物種において，LBQ は排泄物中から回収された主成分であり，経口投与量の約 70%以上を占めていた。AHU を経口投与後の排泄物中における未変化体の回収率は，投与量の 1%未満～14%であった。VAL の代謝については，既に報告されている（Waldmeier et al. 1997）。

1.4 排泄

[^{14}C]AHU をマウス，ラット及びイヌに経口投与後，放射能は投与量の 74%～98%が糞中に排泄された。一方，サル及びヒトでは[^{14}C]LCZ 経口投与後，放射能は投与量の 42%～65%が尿中に排泄された。血漿中濃度推移及び薬物動態パラメータから，明らかな腸肝循環はないと考えられた。マウス，ラット，イヌ，マーモセット及びヒトにおいて，VAL は主に胆汁を介して糞中に排泄された（Waldmeier et al. 1997）。ヒトにおいて，VAL 投与量の 13%及び 86%がそれぞれ尿及び糞中に排泄された。LCZ 又は AHU 投与後，AHU は主に LBQ として排泄され，AHU の未変化体の排泄は微量であった（投与量の約 3%，例外としてイヌでは約 13%）。また，動物及びヒトのいずれにおいても，排泄物中に数種類の微量代謝物が確認された。

1.5 薬物間相互作用

1.5.1 酵素阻害

ヒト肝ミクロソームを用いた *in vitro* 試験において，AHU はヒトチトクローム P450（CYP）の各分子種 CYP1A2，2C9，2D6，2E1，3A4/5 に対する阻害作用をほとんど示さず（IC50：>100 μM），CYP2C8（IC50：15 μM），2C19（IC50：20 μM），及び 2B6（IC50：65 μM）に対して弱い阻害作用を示したのみであった。慢性心不全患者に LCZ 200 mg を 1 日 2 回反復経口投与後の AHU の Cmax,ss（2421 ng/mL: 約 5.5 μM）に基づくと，AHU と併用薬間で CYP を介した薬物間相互作用が生じる可能性は低いと考えられた（A2117 試験及び B2223 試験）。LBQ は *in vitro* において CYP2C9 に対して弱い阻害作用（IC50：40 μM）を示した。臨床試験において CYP2C9 阻害作用の影響を検討した結果，LCZ と CYP2C9 により代謝される薬物であるワルファリンの間での薬物間相互作用は認められなかった。LBQ は主として代謝を受けずに排泄されることから，その消失は CYP 阻害剤により影響を受けないと予想される。したがって，LBQ と併用薬の間で CYP を介した薬物間相互作用が生じる可能性は低いと考えられた。また，AHU 及び LBQ は，100 μM でも CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，並びに CYP3A4/5 に，明らかな時間依存的阻害を示さなかった。VAL は CYP1A2，2A6，2C19，2D6，2E1 及び 3A4/5 に対して有意な阻害作用を示さず，*in vitro* で CYP2C9 を比較的高い Ki 値（135 μM）で阻害した（Taavitsainen et al. 2000）。また，*in vivo* においてもワルファリンと LCZ との薬物相互作用試験において，VAL の薬物動態に相互作用は認められなかった。VAL の 4-ヒドロキシバレリル体生成に関与する酵素は CYP2C9 であったが，VAL は主に未変化体として尿及び糞中に排泄されたことから（Waldmeier et al. 1997），VAL と併用薬の間での CYP を介した薬物間相互作用は無視できると考えられた。

1.5.2 酵素誘導

ヒト初代培養肝細胞を用いた試験において，AHU 及び LBQ は濃度 100 μM まで，CYP1A2, 2B6, 2C9 及び 3A4 の mRNA 発現及び酵素活性を誘導しなかった。また，ヒト初代培養肝細胞を用いた試験において，VAL も CYP1A2, 2B6, 2C9 及び 3A4 の mRNA 発現及び酵素活性を誘導しなかった。

1.5.3 トランスポーター

AHU の能動輸送に P-糖蛋白質（P-gp）の関与が *in vitro* 試験により示唆されたが，AHU と P-gp との親和性は低く（>100 μM），各動物種で高い吸収率（>65%）を示したことから，AHU の経口投与後の吸収時に P-gp による影響は大きくないと予測される。AHU は 50 μM までの濃度で多剤耐性蛋白質 2（MRP2）及び P-gp を阻害せず，乳癌耐性蛋白質（BCRP）に対しては弱い阻害作用を示した 。以上より，AHU が MRP2，P-gp 及び BCRP に対して有意な阻害作用を示す可能性は低いと考えられた。同様に，LBQ の P-gp 及び BCRP に対する阻害作用は認められなかったことから，P-gp 及び BCRP の基質となる薬剤と併用投与したとき薬物動態学的相互作用を示す可能性は低いと考えられた。

In vitro 試験から，LBQ の肝臓への取り込みに有機アニオン輸送ポリペプチド 1B1（OATP1B1）及び OATP1B3 による能動輸送の寄与が示唆された。LBQ は *in vitro* で OATP1B1 阻害作用 （IC50：126 μM）を示したが，OATP1B3 阻害作用は示さなかった。臨床試験で認められた LBQ の Cmax,ss（16531 ng/mL: 約 43 μM）に基づくと，LBQ が OATP1B1 阻害により併用薬の全身曝露量を増加させることはないと考えられた。

AHU，LBQ 及び VAL は，*in vitro* において有機カチオントランスポーター1（OCT1）及び OCT2 を阻害しなかった。AHU は OATP1B1，OATP1B3 及び有機アニオントランスポーター3（OAT3）に対して阻害作用を示し，その IC50 値はそれぞれ 1.9 μM，3.8 μM 及び 0.8 μM であった。したがって，臨床試験で報告された AHU の Cmax,ss（約 5.5 μM）を考慮すると，AHU は OATP1B1/3 及び OAT3 の基質となる薬剤の全身曝露量を増加させる可能性がある。

LBQ は *in vitro* で OAT3 の基質であるとともに阻害作用（IC50：15 μM）を示したが，OAT1 の阻害剤及び基質ではなかった。VAL は *in vitro* で OAT1 及び OAT3 の基質であった。しかしながら，LBQ 及び VAL のヒトでの腎排泄の結果から，これらの *in vitro* での結果は臨床上大きく影響しないことが示されている。LCZ 400 mg を単回経口投与後，投与量の約 55%が LBQ として尿中に排泄された。また，VAL の尿中排泄は投与量の 11%程度であったことから，LBQ による OAT3 を介した VAL の尿中排泄への影響は大きくないと考えられた。

AHU 及び LBQ はいずれも MATE1 と MATE2-K の弱い阻害剤であったが，臨床試験で認められた AHU 及び LBQ の Cmax,ss に基づくと，これらのトランスポーターとの間で薬物間相互作用が生じる可能性は低いと考える。

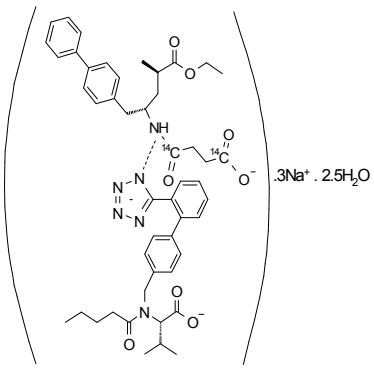
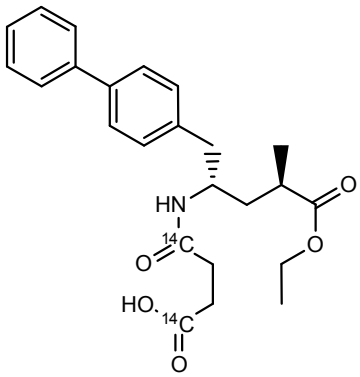
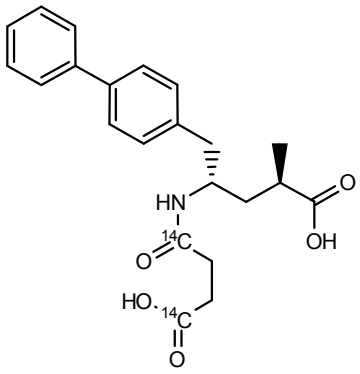
ヒト肝細胞及び OATP1B1/MRP2 を発現させた MDCKII 細胞を用いた試験系において，VAL は OATP1B1，OATP1B3 及び MRP2 の *in vitro* 基質であることが報告されている（Yamashiro et al. 2006）。したがって，これらの取り込み又は排出型トランスポーターの阻害剤と併用投与したとき，VAL の全身曝露量に影響を及ぼす可能性が示唆された。

2 分析法

2.1 同位体標識 LCZ 及び関連成分

放射性標識 LCZ，AHU 及び LBQ は，ノバルティス ファーマのアイソトープ研究所（米国，イーストハノーバー）で合成した。カルボキシプロピオニルアミノ基の 2 つのカルボニル基に ^{14}C 標識した[^{14}C]LCZ，[^{14}C]AHU 及び[^{14}C]LBQ を調製した。^{14}C 標識体は，血漿蛋白結合試験，*in vitro* 代謝試験，*in vitro* 薬物間相互作用試験，ラット QWBA（定量的全身オートラジオグラフィー）試験，マウス，ラット，イヌ，サル及びヒト ADME 試験に使用した。それぞれの ^{14}C 標識体を標識位置と併せて Figure 2-1 に示す [Table 2.6.5.2B-R0900365], [Table 2.6.5.2B-R0600259], [Table 2.6.5.2B-1200840A]。なお，[^{14}C]LCZ の VAL の構成部分は ^{14}C で標識していないため，[^{14}C]LCZ 投与後の VAL 関連放射能のプロファイルは検討していない。AHU，LBQ 及び VAL 測定時の内部標準物質として，$^{13}C_4$-標識 AHU，$^{13}C_4$-標識 LBQ 及び D_9-標識 VAL の安定同位体を調製し，使用した。

Figure 2-1 ^{14}C による LCZ，AHU 及び LBQ の標識体

[^{14}C]LCZ696	[^{14}C]AHU377	[^{14}C]LBQ657
		
[R0900365]	[R0600259]	[R1200840A]

^{14}C 標識体の化学的同一性は，HPLC における保持時間，並びに紫外分光（UV），質量分析（MS）及び NMR スペクトルを非標識標準品と比較することにより確認した。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 放射能の分析

放射能は液体シンチレーションカウンターにより測定した。試料に直接，又は組織ホモジネートを調製後，シンチレーションカクテルを添加し，その一部を用いて放射能を測定した。代謝物プロファイリング試験では，マイクロプレートシンチレーションカウンターを用いて放射能を測定した。放射能の組織分布は QWBA により測定した。

2.3 AHU，LBQ 及び VAL の分析

LCZ 関連成分（AHU，LBQ 及び VAL）の血漿及び組織中濃度はバリデートされたタンデム質量分析装置を接続した液体クロマトグラフィー（LC-MS/MS）により測定した [Table 2.6.5.2A-R0600891]，[Table 2.6.5.2A-R0700990]，[Table 2.6.5.2A-R0600533]，[Table 2.6.5.2A-R0600483]，[Table 2.6.5.2A-R0700176] 。

定量限界は，血漿及び尿試料で 1～10 ng/mL，胎児組織試料で 300 ng/g であった。各分析の精度及び真度は適切な基準を満たしていた。実験動物及びヒトに ^{14}C 標識体を投与したときの排泄物中の薬物濃度は HPLC-放射能検出により測定した[Table 2.6.5.3A-R0300247-1]，[Table 2.6.5.3A-R0301244]，[Table 2.6.5.3A-R1200729]，[Table 2.6.5.3A-R1200673]，[5.3.3.1-3-B2105 試験]。

2.4 代謝物の特性評価及び構造解析

AHU 及び LBQ 由来の代謝物の同定及び定量は HPLC-放射能検出を用いて実施した。また，可能な場合は LC-MS/MS によって代謝物を同定した [Table 2.6.5.3A-R0300247-1]，[Table 2.6.5.3A-R0301244]，[Table 2.6.5.3A-R1200729]，[Table 2.6.5.3A-R1200673]，[5.3.3.1-3-B2105 試験]。

3 吸収

3.1 吸収

ヒトを含むすべての動物種において懸濁液及びカプセル剤を経口投与後，LCZ 又は AHU は比較的速やかに吸収された（Table 3-1）。放射能，AHU，LBQ 及び VAL の血漿中濃度は，投与後 0.25～4.6 時間に最高値に達した。いずれの動物種においても LCZ 及び AHU の経口投与後の吸収率は高かった（65%～100%）。ヒト ADME 試験においても尿中の総放射能の回収率から，吸収率は 61%を超えると考えられた。Caco-2 細胞を用いた *in vitro* 膜透過性評価において，AHU の膜透過性は比較的高いことが示され [Table 2.6.5.16A-R0301243] ，すべての動物種で良好な吸収が認められた結果と一致した。イヌに LCZ を投与したときの VAL の曝露量は，AHU と VAL を併用投与したときの曝露量（AUC）に比べて 3 倍高く，LCZ として投与した際に VAL の吸収が増加した[Table 2.6.5.3C-R0600309]。

Table 3-1 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における吸収パラメータ

動物種	投与量[mg/kg] (投与薬剤)	最高血漿中濃度到達時間 [h] [a]				吸収率 [対投与量%] [b]	報告書番号
		放射能	AHU377	LBQ657	VAL		
マウス	150 (AHU)	0.25 [0.25]	0.25, -	0.25	-	100	R1200673
	400/日(AHU) [c]	- [d]	1.0, 0.5	1.0, 1.0	-	-	R0770896
	800/日(AHU) [c]	-	2.0, 0.5	2.0, 0.5	-	-	R0770896
	1200/日(AHU) [c]	-	0.5, 0.5	0.5, 1.0	-	-	R0770896
ラット	45 (AHU)	[0.30]	0.60, -	0.50, -	-	>70	R0300247-1
	10/日(LCZ) [e]	-	0.5, 0.5	0.5, 0.5	0.5, 0.5	-	R0670283
	30/日(LCZ) [e]	-	0.5, 0.5	0.5, 0.5	0.5, 0.5	-	R0670283
	100/日(LCZ) [e]	-	0.5, 0.5	0.5, 0.5	0.5, 0.5	-	R0670283
	400/日(AHU) [f]	-	0.5, 0.5	0.5, 0.5	-	-	R0770711
	800/日(AHU) [f]	-	0.5, 0.5	0.5, 0.5	-	-	R0770711
	1200/日(AHU) [f]	-	0.5, 1.0	1.0, 1.0	-	-	R0770711
	30/日(LCZ) [g]	-	-, -	-, 0.5	-, 0.5	-	R0670390
	100/日(LCZ) [g]	-	-, 0.5	-, 1.6	-, 1.6	-	R0670390
	200/日(LCZ) [g]	-	-, 2.3	-, 2.3	-, 1.0	-	R0670390
ウサギ	3/日(LCZ) [h]	-	-, 1.0	-, 1.0	-, 3.8	-	R0670280
	10/日(LCZ) [h]	-	-, 1.0	-, 1.0	-, 6.0	-	R0670280
	30/日(LCZ) [h]	-	-, 2.0	-, 2.0	-, 5.0	-	R0670280
イヌ	15 (AHU)	[0.70]	0.60, -	0.70, -	-, -	90～100	R0301244
サル	30 (LCZ)	0.83 [0.83]	0.75, -	0.83, -	1.1, -	40～65	R1200729
	30/日(LCZ) [i]	-	0.75, 0.63	1.0, 0.88	4.6, 2.0	-	R0670621
	100/日(LCZ) [i]	-	0.63, 0.63	0.75, 1.0	2.3, 3.5	-	R0670621
	300/日 (LCZ) [i]	-	1.6, 1.1	2.0, 2.0	2.4, 1.6	-	R0670621
ヒト	2.55 (LCZ) [j]	2.0 [2.0]	0.5,-	2.0, -	1.5, -	> 61[k]	CLCZ696B2105

[a] 値がカンマで区切られている場合，それぞれ雄及び雌の値を示す。血液中濃度の結果を[ ]内に示す。

[b] バイオアベイラビリティ又は排泄率（対投与量%）に基づく推定値。

[c] 非標識体を用いた 13 週間反復投与試験の 11 週目の結果。

[d] 測定せず又は該当せず。

[e] 非標識体を用いた 13 週間反復投与試験の 76 日目の結果。

[f] 非標識体を用いた 13 週間反復投与試験の 11 週目（72 日目）の結果。

[g] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 17 日の結果。

[h] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 20 日の結果。

[i] 非標識体を用いた 39 週間反復投与試験の 272 日目の結果。

[j] 平均体重 78.53 kg，投与量 200 mg に基づく計算値。

[k] ヒト尿中回収率から 61%以上と推定した。

Source：[Table 2.6.5.3A-R1200673], [Table 2.6.5.4A-R0770896], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.4B-R0670283], [Table 2.6.5.4B-R0770711], [Table 2.6.5.7A-R0670390], [Table 2.6.5.7B-R0670280], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [Table 2.6.5.4C-R0670621], [5.3.3.1-3- B2105 試験-Table 12-2~Table 12-6]

3.2 ADME 試験及び TK 試験での薬物動態パラメータ

放射性標識及び非標識 LCZ 又は AHU を投与後の曝露量（Cmax 及び AUC）を各 LCZ 由来成分（AHU，LBQ 及び VAL）ごとに，放射能濃度の結果とともにそれぞれ要約した（総放射能：Table 3-2，AHU：Table 3-3，LBQ：Table 3-4及び VAL：Table 3-5）。なお，動物種間での用量間での比較を容易にするため， 用量補正した Cmax 及び AUC もそれぞれ示す。

放射能の全身曝露量について，各動物種と参考としてヒト ADME 試験結果をTable 3-2に示す。総放射能濃度から算出した Cmax 及び AUC の用量補正値もヒトで高値を示した。

Table 3-2 放射性標識 AHU 又は LCZ を単回経口投与したときの血漿中又は血液中総放射能の曝露量（Cmax 及び AUC）（ADME 試験）

動物種	投与量 [mg/kg] (投与薬剤)	Cmax [nM] [a]	AUCinf [nM•h][a]	用量補正値		報告書番号
				Cmax [nM]/[µmol/kg]	AUC [nM·h]/[µmol/kg]	
マウス	150 (AHU)	57900 [46700]	66900 [50900]	159 [128]	184 [140]	R1200673
ラット	45 (AHU)	[5600]	[20700]	[512]	[189]	R0300247-1
イヌ	15 (AHU)	[13800]	[36500]	[379]	[1000]	R0301244
サル	30 (LCZ)	49700 [24700]	114000 [57600]	1400 [697]	3220 [1630]	R1200729
ヒト	2.55[b] (LCZ)	26400 [12700]	228000 [108000]	8770 [4220]	75700 [35900]	CLCZ696B2105

[a] 値はすべて有効数字 3 桁で示す。血液中濃度の結果を[ ]内に示す。

[b] 平均体重 78.53 kg，投与量 200 mg に基づく計算値。

Source： [Table 2.6.5.3A-R1200673], [Table 2.6.5.3A-R0300247-1], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [5.3.3.1-3-B2105 試験-Table 12-2, Table 12-3]

AHU，LBQ 及び VAL 濃度に関して，すべての動物種（マウス，ラット，ウサギ及びサル，妊娠ラット及びウサギを含む）において，AUC 値は概ね用量に依存して増加した。ラット及びサルに 13 又は 39 週間にわたり反復経口投与しても，明らかな累積性は認められなかった。また，明らかな性差も認められなかった（Table 3-3，Table 3-4，Table 3-5）。

[^{14}C]LCZ 又は [^{14}C] AHU のいずれを投与した場合にも，LCZ 関連成分（放射能，AHU 及び LBQ）の用量補正後の曝露量（Cmax 及び AUC）はヒトで高値を示した（Table 3-6）。

Table 3-7に示すように，単回経口投与後の LBQ のバイオアベイラビリティは 41%～100%（119%）であった。静脈内投与後，LBQ の糞中排泄率は動物種によって異なるものの，投与量の 20%～96%であった。したがって，LCZ を経口投与後，ヒトで LBQ のバイオアベイラビリティは，尿中排泄率から算出された値（投与後 24 時間までの LBQ の尿中排泄量は投与量の約 50%）に比べて高いと推定された。

幼若ラットに AHU を反復経口投与後，AHU 及び LBQ の曝露量に明らかな性差は認められなかった。投与 7 日目の総曝露量（AHU 及び LBQ）は用量増加に比例して増加したが，64 日目には，特に最高用量で，用量比を上回る（約 2 倍）増加を示した。幼若ラット（初回投与後 7 日目）の総曝露量は，成熟ラット（初回投与後 64 日目）に比べて 4～11 倍高い曝露量を示した[Table 2.6.5.7A-R0870734]。

Table 3-3 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における AHU の曝露量（Cmax 及び AUC）

動物種	投与量 [mg/kg] (投与薬剤)	Cmax [nM] [a, b]	AUC0-24h [nM·h] [a, b]	用量補正値		報告書番号
				Cmax [nM]/[µmol/kg]	AUC [nM·h]/[µmol/kg]	
マウス	150 (AHU)	6250, - [d]	3140 (AUCinf), -	17.1, -	8.61, -	R1200673
	400/日 (AHU) [c]	6830, 11500	8210, 13100	7.03, 11.8	8.45, 13.5	R0770896
	800/日 (AHU) [c]	6030, 68300	19400, 76100	3.10, 35.1	10.0, 39.1	R0770896
	1200/日 (AHU) [c]	41800, 43500	134000, 82100	14.3, 14.9	45.8, 28.2	R0770896
ラット	45 (AHU)	[128], -	[238], -	[1.17], -	[2.18], -	R0300247-1
	10/日 (LCZ) [e]	24.3, 38.6	-, -	2.06, 3.27	-, -	R0670283
	30/日 (LCZ) [e]	46.2, 28.9	-, -	1.30, 0.816	-, -	R0670283
	100/日 (LCZ) [e]	532, 306	838, 707	4.51, 2.59	7.10, 5.99	R0670283
	400/日 (AHU) [f]	5760, 4500	23600, 13600	5.93, 4.63	24.3, 14.0	R0770711
	800/日 (AHU) [f]	6100, 8070	28900, 59500	3.14, 4.15	14.9, 30.6	R0770711
	1200/日 (AHU) [f]	10600, 5810	96000, 59800	3.63, 1.99	32.9, 20.5	R0770711
	30/日 (LCZ) [g]	-, -	-, -	-, -	-, -	R0670390
	100/日 (LCZ) [g]	-, 382	-, 1240	-, 3.23	-, 10.5	R0670390
	200/日 (LCZ) [g]	-, 520	-, 2000	-, 2.20	-, 8.45	R0670390
ウサギ	3/日 (LCZ) [h]	-, 36.5	-, 129	-, 10.3	-, 36.3	R0670280
	10/日 (LCZ) [h]	-, 137	-, 736	-, 11.6	-, 62.4	R0670280
	30/日 (LCZ) [h]	-, 348	-, 2130	-, 9.81	-, 60.2	R0670280
イヌ	15 (AHU)	[7740], -	[5980 (AUCinf)], -	[212], -	[164], -	R0301244
サル	30 (LCZ)	6500, -	8750 (AUCinf), -	184, -	247, -	R1200729
	30/日 (LCZ) [i]	3090, 2480	6420, 4860	87.1, 70.0	181, 137	R0670621
	100/日 (LCZ) [i]	31800, 28400	50500, 48800	270, 241	428, 414	R0670621
	300/日 (LCZ) [i]	89700, 93100	227000, 270000	253, 263	641,762	R0670621
ヒト	2.55 (LCZ) [j]	5140, -	6220 (AUCinf), -	1710, -	2070, -	CLCZ696B2105

[a] 値はすべて有効数字 3 桁で示す。

[b] 値がカンマで区切られている場合，それぞれ雄及び雌の値を示す。血液中濃度の結果を[ ]内に示す。

[c] 非標識体を用いた 13 週間反復投与試験の 11 週目の結果。

[d] 測定せず又は該当せず。

[e] 非標識体を用いた 13 週間反復投与試験の 76 日目の結果。

[f] 非標識体を用いた 13 週間反復投与試験の 11 週目（72 日目）の結果。

[g] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 17 日の結果。

[h] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 20 日の結果。

[i] 非標識体を用いた 39 週間反復投与試験の 272 日目の結果。

[j] 平均体重 78.53 kg，投与量 200 mg に基づく計算値。

Source：[Table 2.6.5.3A-R1200673], [Table 2.6.5.4A-R0770896], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.4B-R0670283], [Table 2.6.5.4B-R0770711], [Table 2.6.5.7A-R0670390], [Table 2.6.5.7B-R0670280], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [Table 2.6.5.4C-R0670621], [5.3.3.1-3-B2105 試験-Table 12-5]

Table 3-4 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における LBQ の曝露量（Cmax 及び AUC）

動物種	投与量 [mg/kg] (投与薬剤)	Cmax [nM] [a, b]	AUC0-24h [nM·h] [a, b]	用量補正値		報告書番号
				Cmax [nM]/[µmol/kg]	AUC [nM·h]/[µmol/kg]	
マウス	150 (AHU)	51400, - [d]	55800 (AUCinf), -	141, -	153, -	R1200673
	400/日 (AHU) [c]	261000, 216000	276000, 289000	268, 222	284, 298	R0770896
	800/日 (AHU) [c]	116000, 681000	464000, 879000	59.8, 350	239, 452	R0770896
	1200/日 (AHU) [c]	600000, 699000	1980000, 1330000	206, 240	679, 456	R0770896
ラット	45 (AHU)	[6340], -	[17400], -	[58.0], -	[159], -	R0300247-1
	10/日 (LCZ) [e]	4300, 3860	5500, 3830	364, 327	466, 325	R0670283
	30/日 (LCZ) [e]	4960, 3960	13500, 12500	140, 112	380, 353	R0670283
	100/日 (LCZ) [e]	33400, 15000	94700, 50300	283, 127	802, 426	R0670283
	400/日 (AHU) [f]	141000, 194000	694000, 558000	145, 200	714, 574	R0770711
	800/日 (AHU) [f]	122000, 186000	793000, 1240000	62.8, 95.5	408, 637	R0770711
	1200/日 (AHU) [f]	2050000, 300000	2100000, 1930000	70.4, 103	722, 661	R0770711
	30/日 (LCZ) [g]	-, 5140	-, 15500	-, 145	-, 438	R0670390
	100/日 (LCZ) [g]	-, 8580	-, 50100	-, 72.7	-, 424	R0670390
	200/日 (LCZ) [g]	-, 13600	-, 76700	-, 57.4	-, 325	R0670390
ウサギ	3/日 (LCZ) [h]	-, 1280	-, 5030	-, 362	-, 1420	R0670280
	10/日 (LCZ) [h]	-, 4720	-, 23300	-, 400	-, 1970	R0670280
	30/日 (LCZ) [h]	-, 27400	-, 266000	-, 773	-, 7510	R0670280
イヌ	15 (AHU)	[7230], -	[9600 (AUCinf)], -	[198], -	[263], -	R0301244
サル	30 (LCZ)	32200, -	72000 (AUCinf), -	909, -	2030, -	R1200729
	30/日 (LCZ) [i]	22600, 17400	79000, 62900	639, 492	2230, 1770	R0670621
	100/日 (LCZ) [i]	116000, 126000	386000, 407000	985, 1070	3270, 3450	R0670621
	300/日 (LCZ) [i]	368000, 381000	1720000, 1480000	1040, 1080	4850,4190	R0670621

動物種	投与量 [mg/kg] (投与薬剤)	Cmax [nM] [a, b]	AUC0-24h [nM·h] [a, b]	用量補正値		報告書番号
				Cmax [nM]/[µmol/kg]	AUC [nM·h]/[µmol/kg]	
ヒト	2.55 (LCZ) [j]	22500, -	186000 (AUCinf), -	7470, -	61900, -	CLCZ696B2105

[a] 値はすべて有効数字 3 桁で示す。

[b] 値がカンマで区切られている場合，それぞれ雄及び雌の値を意味する。血液中濃度の結果を[ ]内に示す。

[c] 非標識体を用いた 13 週間反復投与試験の 11 週目の結果。

[d] 測定せず又は該当せず。

[e] 非標識体を用いた 13 週間反復投与試験の 76 日目の結果。

[f] 非標識体を用いた 13 週間反復投与試験の 11 週目（72 日目）の結果。

[g] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 17 日の結果。

[h] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 20 日の結果。

[i] 非標識体を用いた 39 週間反復投与試験の 272 日目の結果。

[j] 平均体重 78.53 kg，投与量 200 mg に基づく計算値。

Source：[Table 2.6.5.3A-R1200673], [Table 2.6.5.4A-R0770896], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.4B-R0670283], [Table 2.6.5.4B-R0770711], [Table 2.6.5.7A-R0670390], [Table 2.6.5.7B-R0670280], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [Table 2.6.5.4C-R0670621], [5.3.3.1-3-B2105 試験-Table 12-6]

Table 3-5 放射性標識及び非標識 LCZ 又は AHU を用いた単回経口投与 ADME 試験並びに反復経口投与 TK 試験における VAL の曝露量（Cmax 及び AUC）

動物種	投与量 [mg/kg] (投与薬剤)	Cmax [nM] [a, b]	AUC0-24h [nM·h] [a, b]	用量補正値		報告書番号
				Cmax [nM]/[µmol/kg]	AUC [nM·h]/[µmol/kg]	
マウス	150 (AHU)	-, - [d]	-, -	-, -	-, -	R1200673
	400/日 (AHU) [c]	-, -	-, -	-, -	-, -	R0770896
	800/日 (AHU) [c]	-, -	-, -	-, -	-, -	R0770896
	1200/日 (AHU) [c]	-, -	-, -	-, -	-, -	R0770896
ラット	45 (AHU)	-, -	-, -	-, -	-, -	R0300247-1
	10/日 (LCZ) [e]	4570, 2960	11100, 7280	387, 251	941, 617	R0670283
	30/日 (LCZ) [e]	7900, 4360	26400, 23700	223, 123	746, 668	R0670283
	100/日 (LCZ) [e]	19900, 13000	123000, 74900	168, 110	1040, 634	R0670283
	400/日 (AHU) [f]	-, -	-, -	-, -	-, -	R0770711
	800/日 (AHU) [f]	-, -	-, -	-, -	-, -	R0770711
	1200/日 (AHU) [f]	-, -	-, -	-, -	-, -	R0770711
	30/日 (LCZ) [g]	-, 6940	-, 30800	-, 196	-, 869	R0670390
	100/日 (LCZ) [g]	-, 17800	-, 138000	-, 151	-, 1170	R0670390
	200/日 (LCZ) [g]	-, 27800	-, 273000	-, 118	-, 1160	R0670390
ウサギ	3/日 (LCZ) [h]	-, 6540	-, 94400	-, 1850	-, 26600	R0670280
	10/日 (LCZ) [h]	-, 23400	-, 388000	-, 1980	-, 32900	R0670280
	30/日 (LCZ) [h]	-, 46200	-, 918000	-, 1300	-, 25900	R0670280
イヌ	15 (AHU)	-, -	-, -	-, -	-, -	R0301244
サル	30 (LCZ)	1150, -	6300 (AUCinf), -	32.5, -	178, -	R1200729

動物種	投与量 [mg/kg] (投与薬剤)	Cmax [nM] [a, b]	AUC0-24h [nM·h] [a, b]	用量補正値		報告書番号
				Cmax [nM]/[µmol/kg]	AUC [nM·h]/[µmol/kg]	
	30/日(LCZ) [i]	1870, 1890	22900, 16100	52.7, 53.5	648, 454	R0670621
	100/日(LCZ) [i]	6660, 9810	66800, 106000	56.4,83.0	566, 899	R0670621
	300/日 (LCZ) [i]	21400, 24300	160000, 150000	60.5, 68.7	453,423	R0670621
ヒト	2.55 (LCZ) [j]	12300	61800 (AUCinf)	4090	20500	CLCZ696B2105

[a] 値はすべて有効数字 3 桁で示す。

[b] 値がカンマで区切られている場合，それぞれ雄及び雌の値を意味する。

[c] 非標識体を用いた 13 週間反復投与試験の 11 週目の結果。

[d] 測定せず又は該当せず。

[e] 非標識体を用いた 13 週間反復投与試験の 76 日目の結果。

[f] 非標識体を用いた 13 週間反復投与試験の 11 週目（72 日目）の結果。

[g] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 17 日の結果。

[h] 非標識体を用いた反復投与による胚・胎児発生に関する試験の妊娠 20 日の結果。

[i] 非標識体を用いた 39 週間反復投与試験の 272 日目の結果。

[j] 平均体重 78.53 kg，投与量 200 mg に基づく計算値。

Source：[Table 2.6.5.3A-R1200673], [Table 2.6.5.4A-R0770896], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.4B-R0670283], [Table 2.6.5.4B-R0770711], [Table 2.6.5.7A-R0670390], [Table 2.6.5.7B-R0670280], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [Table 2.6.5.4C-R0670621], [5.3.3.1-3-B2105 試験-Table 12-4]

Table 3-6 [^{14}C]LCZ 又は [^{14}C]AHU を単回経口投与後の血漿中又は血液中 Cmax 及び AUC の用量補正値

	動物種	投与量 [mg/kg] (投与薬剤)	用量補正 Cmax[a]	用量補正 AUC[b]
放射能	マウス	150 (AHU)	159	184
	ラット*	45 (AHU)	512	189
	イヌ*	15 (AHU)	379	1000
	サル	30 (LCZ)	1400	3220
	ヒト[c]	2.55 (LCZ)	8770	75700
AHU	マウス	150 (AHU)	17.1	8.61
	ラット*	45 (AHU)	1.17	2.18
	イヌ*	15 (AHU)	212	164
	サル	30 (LCZ)	184	247
	ヒト[c]	2.55 (LCZ)	1710	2070
LBQ	マウス	150 (AHU)	141	153
	ラット*	45 (AHU)	58.0	159
	イヌ*	15 (AHU)	198	263
	サル	30 (LCZ)	909	2030
	ヒト[c]	2.55 (LCZ)	7470	61900

放射能，AHU 及び LBQ のデータは，Table 3-2，Table 3-3 及び Table 3-4 から抜粋した。

[a] (nM)/(µmol/kg)

[b] (nM·h)/(μmol/kg)

[c] [^{14}C]LCZ 200 mg（2.55 mg/kg，平均体重 78.53 kg から算出）を単回経口投与後のヒト ADME 試験。

*全血サンプルを用いた。

Table 3-7 [^{14}C]LCZ 又は [^{14}C]AHU を単回経口投与後の LBQ のバイオアベイラビリティ

動物種	投与量 0[mg/kg]（投与薬剤）	バイオアベイラビリティ	報告書番号
マウス	150 (AHU)	100%(119%)	R1200673
ラット	45 (AHU)	72%	R0300247-1
イヌ	15 (AHU)	77% (60%～110%)	R0301244
サル	30 (LCZ)	41%	R1200729
ヒト	2.55 (LCZ) [a]	> 50% [b]	CLCZ696B2105

[a] 平均体重 78.53 kg，投与量 200 mg に基づく計算値。

[b] 0-24 時間ヒト尿試料に LBQ657 として投与量の約 50%が回収率からの推定値。

Source： [Table 2.6.5.3A-R1200673], [Table 2.6.5.3A-R0300247-1], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [5.3.3.1-3-B2105 試験-Table 12-13]

[^{14}C]AHU（マウス，ラット及びイヌ）又は [^{14}C] LCZ（サル及びヒト）を単回経口投与後，血漿中総放射能濃度は消失半減期 1.3～26 時間で低下した（Table 3-8）。AHU の消失半減期は 1.3～3.2 時間，LBQ は 1.1～12 時間であり，VAL の消失半減期はサルで 6.5 時間及びヒトで 21 時間であった。また，クリアランス（CL）はマウスで 7.8 L/h/kg，ラットで 4.3 L/h/kg，イヌで 2.9 L/h/kg，サルで 0.21 L/h/kg 及びヒトで 0.63 L/h/kg（CL/F）であった（Table 3-9）。定常状態における分布容積は，マウスで 1.3 L/kg，ラットで 2.0 L/kg，イヌで 2.5 L/kg，サルで 0.28 L/kg 及びヒトで 1.1 L/kg (Vz/F)であった。

Table 3-8 [^{14}C] LCZ 又は [^{14}C] AHU を経口投与後の放射能濃度，AHU，LBQ 及び VAL の消失半減期

動物種	投与量[mg/kg]（投与薬剤）	見かけの消失半減期 (h) [a]				報告書番号
		AHU377	LBQ657	VAL	放射能	
マウス	150 (AHU)	1.6	1.1	NA	~1.3	R1200673
ラット [b]	45 (AHU)	1.4	2.7	NA	2.5	R0300247-1
イヌ [b]	15 (AHU)	2.8	3.1	NA	26	R0301244
サル	30 (LCZ)	3.2	5.9	6.5	5.2-7.2	R1200729
ヒト	2.55 (LCZ) [c]	1.3	12	21	8.1-10	CLCZ696B2105

NA： AHU を投与したので，該当しない。

[a] 単回投与。データは有効数字 2 桁で示す。

[b] 血液試料のデータ。

[c] 平均体重 78.53 kg，投与量 200 mg に基づく計算値。

Source： [Table 2.6.5.3A-R1200673], [Table 2.6.5.3A-R0300247-1], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [5.3.3.1-3-B2105 試験-Table 12-2~Table 12-6]

Table 3-9 [^{14}C] AHU 又は[^{14}C] LBQ を静脈内投与後の血漿又は血液クリアランス及び分布容積

動物種	投与量[mg/kg]（投与薬剤）	クリアランス [a] (L/h/kg)	Vss [a] (L/kg)	報告書番号
マウス	30 (LBQ)	7.8	1.3	R1200673
ラット	15 (AHU)	4.3 [b]	2.0 [b]	R0300247-1
イヌ	3 (LBQ)	2.9 [b]	2.5 [b]	R0301244
サル	10 (LBQ)	0.21	0.28	R1200729
ヒト	NA	0.63 ± 0.27 [c]	1.1 ± 0.29 [c]	CLCZ696B2105

[a] 有効数字 2 桁で示す。

[b] 血液試料を測定した。

[c] ヒトの ADME 試験（平均体重：78.53 kg）で得られた AHU の経口クリアランス（CL/F）及び分布容積（Vz/F）を平均値 ± SD で示す。

Source：[Table 2.6.5.3A-R1200673], [Table 2.6.5.3A-R0300247-1], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [5.3.3.1-3-B2105 試験-Table 12-5]

4 分布

4.1 血漿蛋白結合及び血球移行

In vitro 試験において，ラット，イヌ，サル及びヒトで AHU 及び LBQ はほとんど血球中に取り込まれることはなく，血漿中濃度は血液中濃度に比べて高かった [Table 2.6.5.6A-R0301324] [Table 2.6.5.6B-R1200657] 。血液中濃度と血漿中濃度の比（Cb/Cp）の平均値は，濃度範囲 0.02～100 µg/mL で 0.46～0.57 であり，濃度に依存しなかった。*In vivo* 試験でマウス，ラット，サル，イヌ及びヒトに単回経口投与したときの血液／血漿中放射能濃度比についても同様の値が認められた [Table 2.6.5.3A-R0300247-1], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729], [Table 2.6.5.3A-R1200673], [5.3.3.1-3-B2105 試験] 。

ラット，イヌ，サル及びヒト血漿を用いて，[^{14}C] AHU 及び[^{14}C] LBQ の *in vitro* 血漿蛋白結合率を 0.02 ～ 100 µg/mL の濃度範囲で超遠心法により測定した [Table 2.6.5.6A-R0301324] [Table 2.6.5.6B-R1200657]。LBQ の血漿蛋白結合率はラット，イヌ，サル及びヒトでそれぞれ，90%，80%，93%及び 97%，AHU の血漿蛋白結合率はサル及びヒトでそれぞれ 91%及び 97%であった。いずれの動物種においても，血漿蛋白結合率は濃度に依存しないと考えられた。生理学的濃度のヒト血清アルブミン（HSA）及び α_1-酸性糖蛋白質（AGP）を用いた *in vitro* 試験 [Table 2.6.5.6C-R1200658]において，AHU 及び LBQ は HSA に対して高い結合率（99%）を示したが，AGP に対する結合率は低かった（13%～30%）。HSA 濃度（60 及び 600 µM）に応じて，AHU 及び LBQ の HSA との結合率は変化した。したがって，HSA 濃度が大きく低下した場合には，AHU 及び LBQ の血漿蛋白結合率が低下する可能性が示唆される。

平衡透析法により測定した VAL の血清蛋白結合率はラット，イヌ及びマーモセットで 94%～98%であった。VAL のヒト血漿及び血清蛋白結合率はそれぞれ 94%及び 96%であり，主な結合

蛋白は血清アルブミンであった [ディオバン錠，20 mg，40 mg，80 mg；製造承認申請資料添付資料ヘ-14]。

4.2 組織及び臓器分布

有色（Long Evans Hooded）及び非有色（HanWister）ラット [Table 2.6.5.5-R0300247-2]並びに妊娠ラット（4.3 項参照）[Table 2.6.5.8-R1200674]を用いて，定量的全身オートラジオグラフィー法（QWBA）により，組織及び臓器分布試験を実施した。

[^{14}C] AHU をラットに単回静脈内（15 mg/kg）及び経口投与（45 mg/kg）したとき，放射能は多くの組織に広範かつ速やかに分布した [Table 2.6.5.5-R0300247-2] 。静脈内投与後 5 分で，放射能濃度はすべての組織で最高値に達した。特に腎臓，肝臓及び胆汁中に高い放射能濃度（血液中濃度の 2～6 倍）が認められた。静脈内投与後 5 分で脳，眼，精嚢及び脊髄中の放射能濃度は低く，血中濃度の約 1%～2%であった。脳及び精巣中の放射能濃度は静脈内投与後 5 分で最高値に達し，組織／血液中濃度比がそれぞれ 0.02 及び 0.05 であったことから，血液-脳関門及び血液-精巣関門の透過性は低いと考えられた。小腸及び腎臓を除いて，投与後 24 時間から 168 時間まで，組織中放射能濃度は定量下限未満であった。また，有色皮膚及びブドウ膜中に放射能の蓄積性は認められず，[^{14}C] AHU 関連放射能のメラニンへの親和性は低かった。放射能の組織分布は，経口投与後と静脈内投与後で類似していた。大部分の組織において，経口投与後 1 時間で放射能濃度は最高値に達し，腎臓及び肝臓で高い濃度が認められた。経口投与後 24 時間から 168 時間まで，組織中放射能濃度は，腎臓を除いて，定量下限未満であった。

[^{14}C]VAL を 1 mg/kg の用量で静脈内投与したとき，肝臓及び腎臓中に比較的高い放射能濃度が認められたが，他の組織中放射能濃度は血中よりも低かった [ディオバン錠，20 mg，40 mg，80 mg；製造承認申請資料添付資料ヘ-6, -11] 。脳及び脳脊髄液中の放射能濃度は検出限界未満であり，VAL の血液-脳関門の透過性は低いことが示された。経口投与後 15 分の組織分布は，静脈内投与直後にみられた組織分布と類似していたが，組織中の絶対量は約 1/2 であった。いずれの投与経路についても，肝臓を除く検討したすべての組織において，残存放射能濃度は 7 日後には検出限界未満にまで低下した。経口投与後 7 日において，肝臓中に放射能が検出されたが，組織中濃度は低かった（0.12～0.20 nmol/g）。

4.3 胎盤通過

妊娠 12 日及び 17 日のラットに[^{14}C] LCZ を 25 mg/kg の用量で単回経口投与したとき，胎児を含めて，血液及び組織中放射能濃度は投与後 1～3 時間に最高値に達したが，例外として，妊娠 17 日のラットの腎皮質では投与後 6 時間で最高値に達した [Table 2.6.5.8-R1200674]。最高組織中濃度到達時点において，母動物肝臓（約 3～8 倍）及び腎臓（約 2～34 倍）で高い組織／母動物血液中濃度比が認められた。中枢神経系（母動物脳）への薬物関連放射能の分布はわずかであり，脳／血液中濃度比 は 0.014～0.037 であった。投与後 24 時間において，母動物の腎皮質，腎髄質

及び腎盂を除いて，血液及び大部分の組織中放射能濃度は低く，定量下限と同程度であった。薬物関連放射能の胎児臓器への分布はわずかであり，胎児組織の Tmax における胎児／母動物血液中濃度比は 0.246～0.509 であった。

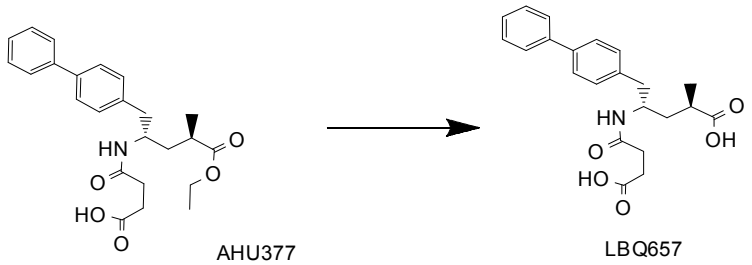
妊娠ウサギに LCZ を 3，10 又は 30 mg/kg の用量で反復経口投与したとき，AHU，LBQ 及び VAL の AUC は用量に依存して増加した [Table 2.6.5.7B-R0670280]。また，胎児組織中の LBQ 及び VAL 曝露量も用量に依存して増加した。投与後 24 時間における胎児中の LBQ 及び VAL の組織中濃度／血漿中濃度比は，3 mg/kg 群でそれぞれ 0.21 及び 0.01，10 mg/kg 群で 0.09 及び 0.01，30 mg/kg 群で 0.06 及び 0.01 であった。以上より，LCZ に由来する活性成分の胎児移行性は低いと考えられた。

5 LCZ の代謝（動物種間の比較）

5.1 AHU の *in vitro* 代謝

[^{14}C] AHU をラット，イヌ及びヒトの肝スライスとインキュベーション後，主に LBQ が検出された（Figure 5-1）。ラット及びイヌ肝スライスによる代謝プロファイルはヒトと類似し，主要代謝物 LBQ が生成し，その後は代謝をほとんど受けないと考えられた[Table 2.6.510A-R0300249]。

Figure 5-1 AHU から LBQ への代謝



5.2 VAL の *in vitro* 代謝

マウス，ラット，ウサギ，イヌ，マーモセット及びヒトの肝ミクロソームを用いて，VAL の *in vitro* 代謝について検討した。酸化的代謝は緩徐かつ軽微であった。VAL の *in vivo* での酸化的代謝も限られていた。ヒトミクロソーム中での 4-ヒドロキシバレリル体の生成に関与する酵素は CYP2C9 であった（Nakashima et al. 2005）。

5.3 LCZ の *in vivo* 代謝

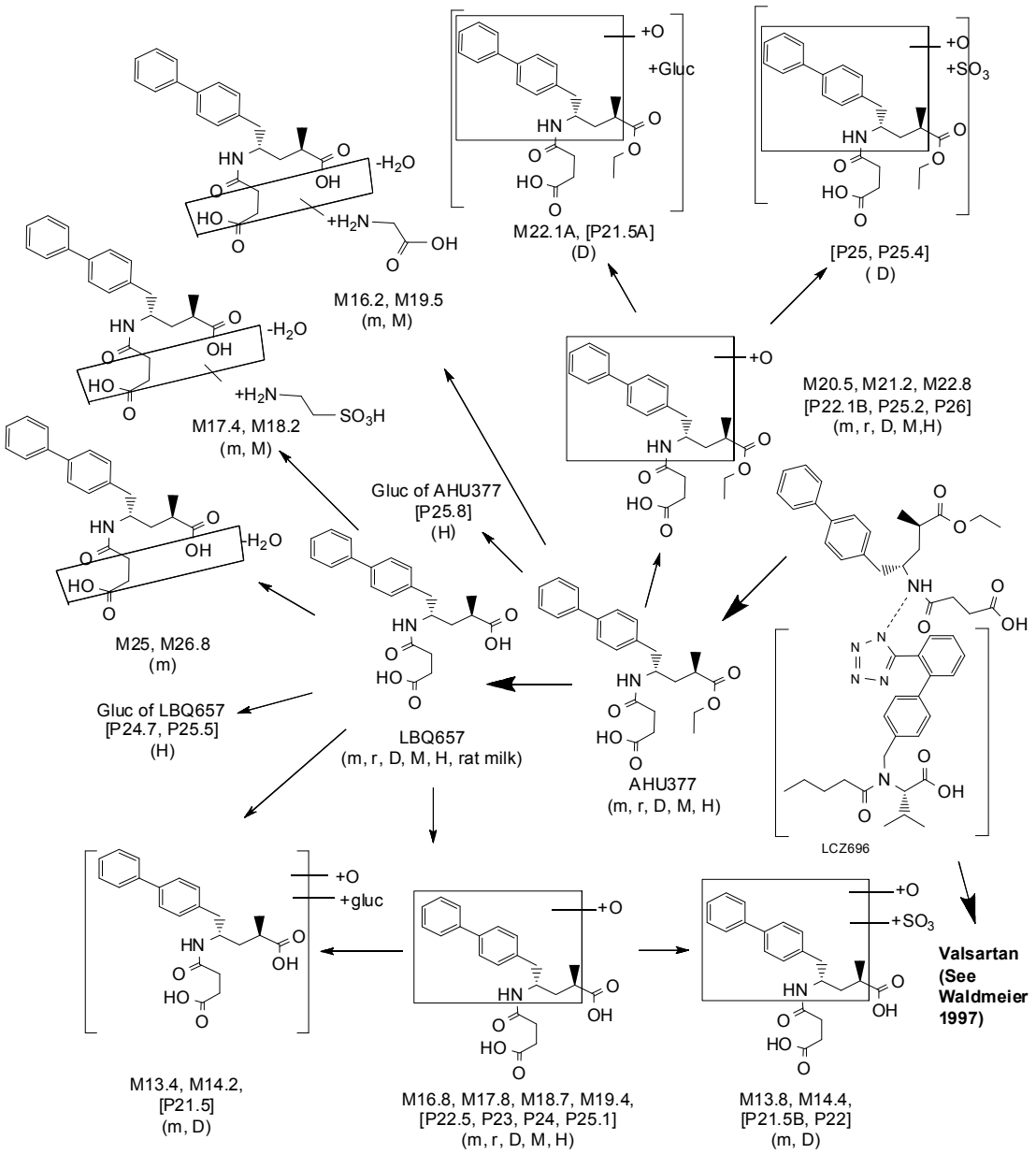
マウス，ラット，イヌ，サル及びヒトに LCZ を経口投与したとき，血漿中に AHU，LBQ 及び VAL が検出された。肝スライスを用いた *in vitro* 試験結果から予想されたように，すべての動物種において，AHU は主にエチルエステル加水分解を受けて LBQ に速やかに代謝された。

[^{14}C]LCZ をサル及びヒトに経口投与したとき，AHU，LBQ 及び VAL が血漿中に検出された [Table 2.6.5.3A-R1200729]，[Table 2.6.5.9J-CLCZ696B2105]。[^{14}C]AHU をマウス，ラット及びイヌに経口投与したとき，AHU 及び LBQ が血漿中に検出された [Table 2.6.5.3A-R1200673]，[Table 2.6.5.3A-R0300247-1]，[Table 2.6.5.3A-R0301244]。すべての動物種において，AHU はエステル加水分解を受けて LBQ が主代謝物として生成した。マウス，ラット及びヒトにおける AHU から LBQ への代謝は速やかであり，血漿中で検出された主成分は LBQ であった（総放射能 AUCinf に対して，マウス（[^{14}C]AHU 投与）：83%，ラット（[^{14}C]AHU 投与）：84%，ヒト（[^{14}C]LCZ 投与）：77%）。一方，イヌ及びサルにおいては，AHU 及び LBQ が血漿中に認められた（総放射能 AUCinf に対して，イヌ（[^{14}C]AHU 投与）では AHU 及び LBQ がそれぞれ 23%及び 36%，サル（[^{14}C]LCZ 投与）では AHU 及び LBQ が 7.7%及び 63%）。微量代謝物として AHU 及び LBQ の水酸化体，グルクロン酸抱合体，硫酸抱合体及びグリシン/タウリン抱合体が各動物種の血漿中に検出された。検討したすべての動物種において，LBQ は排泄物中から回収された主成分であり，経口投与量の約 70%以上を占めていた。AHU を経口投与後の排泄物中における未変化体の回収率は低く，投与量の 1%未満～14%であった。

5.4 LCZ の代謝経路

AHU は主にエステル加水分解を受けて LBQ に代謝される。検討したすべての動物種において，LBQ は循環血中の主要成分であるとともに，糞又は尿中に排泄される主要成分であった。AHU 及び LBQ はさらに加水分解，グルクロン酸抱合，硫酸抱合，グリシン抱合及びタウリン抱合を受け，数種の微量代謝物が生成した。LCZ の推定代謝経路をFigure 5-2に示す。VAL の代謝については，既に報告（Waldmeier et al. 1997）されていることから本項では特に記載しない。

Figure 5-2 LCZ の推定代謝経路



略号 m，r，R，D，M 及び H はそれぞれ，マウス，ラット，ウサギ，イヌ，サル及びヒトでの結果を示す。

代謝物の識別番号として，P に続いて過去の報告書（R0300247-1, R0301244, CLCZ696B2105）に記載されている HPLC 保持時間を付与するか， M に続いて最近の報告書（R1200673, R1200428, R1200729, R1200622）に記載されている HPLC 保持時間を付与した。

6 排泄

放射性標識 LCZ 又は AHU を単回静脈内又は経口投与後の尿中及び糞中排泄量を Table 6-1 に示す。マウス，ラット及びイヌに [^{14}C]AHU 投与後，関連放射能は主に糞中に排泄され（投与量

の約 74%～98%），尿中排泄は微量であった。しかし，サル及びヒトでは [^{14}C]LCZ 投与後，尿中にも比較的多くの放射能（投与量の約 42%～65%）が排泄された。マウス，ラット及びイヌに [^{14}C]AHU 投与後，尿及び糞中には主に LBQ として排泄され，AHU 未変化体としての排泄量は少なかった（投与量の約 3%以下，例外として，イヌでは約 13%）。血漿中濃度推移及び各薬物動態パラメータから判断して，腸肝循環はないと考えられた。概して，投与放射能の排泄は 7 日以内に完了した。

マウス，ラット，イヌ，マーモセット及びヒトにおいて，VAL は主に胆汁排泄により排泄された。ヒトにおいて，VAL 経口投与後に投与量の 13%及び 86%がそれぞれ尿及び糞中に排泄された（Waldmeier et al. 1997）。検討したすべての動物種において，VAL は主に未変化体として排泄された。

Table 6-1　[^{14}C]LCZ 又は[^{14}C]AHU を単回投与したときの主要代謝物及び総放射能の排泄率

動物種	投与量 [mg/kg] (投与薬剤)	投与経路	排泄量（投与量%）					
			尿			糞		
			放射能		LBQ657	放射能		LBQ657
			0-24 h	0-168 h	0-24 h	0-48 h	0-168 h	0-48 h
マウス [a]	30 (LBQ)	静脈内	4.57	4.90	4.48	97.4	98.2	95.8
	150 (AHU)	経口	8.30	8.77	7.89[#]	103 (92.2)**	103 (92.2)**	95.9
ラット [b]	15 (AHU)	静脈内	23.0	23.7	23.0	71.6	74.4	69.6
	45 (AHU)	経口	7.37	8.75	7.40	90.3	91.4	87.0
イヌ [c]	3 (LBQ)	静脈内	10.5	11.0	10.5	79.9	88.8	86.2
	15 (AHU)	経口	8.61	9.94	6.73	83.3	89.2	71.1
サル [d]	10 (LBQ)	静脈内	50.3	56.1 (65.2)*	55.1[#]	21.1	23.0	21.1
	30 (LCZ)	経口	26.1	31.1 (42.1)*	29.0[#]	46.4	47.9	40.6
ヒト [e]	2.55 (LCZ)	経口	54.8	60.7	44.0～53.8	22.4	41.8	31.6～41.5 [f]

[*] ケージ洗液を含む。[**] 総回収率 112%（対投与量%）で補正した値。[#] 0-48 h 試料。

[a] [Table 2.6.5.13A-R1200673]

[b] [Table 2.6.5.13B-R0300247-1]

[c] [Table 2.6.5.13D-R0301244]

[d] [Table 2.6.5.13E-R1200729]

[e] [5.3.3.1-3-B2105 試験-Table 12-12~Table 12-15，ヒト ADME 試験における LCZ696 投与量 200 mg 及び平均体重 78.53 kg に基づいて投与量を算出した。

[f] 排便頻度が異なっていたため，試料のプール時点（少なくとも総放射能の 90%が回収される時間）は被験者ごとに異なり，最長は 0-120 時間であった。

授乳中のラットに[^{14}C] LCZ 30 mg/kg を単回経口投与したとき，LBQ の乳汁移行が認められた [Table 2.6.5.13C-R1200622]。AUC0-24h 及び AUCinf に基づいて算出した総放射能の乳汁／血漿中

濃度比はそれぞれ，0.76 及び 0.91 であった。ラットでのデータをヒトに外挿すると，授乳婦が LCZ を 1 日 400 mg（200 mg 1 日 2 回）を服用し，乳児が毎日 1 L の母乳を摂取したとき，乳児は最大で投与量の 0.889%相当の LBQ を含む AHU 関連成分に曝露されると推定された。LBQ はラット乳汁中の主要成分であった。ラット血漿中には LBQ とともに微量水酸化代謝物（M22.8）も検出された。

授乳中のラットに[^{14}C]VAL を 3 mg/kg の用量で単回経口投与したとき，VAL の乳汁移行が認められた[ディオバン錠，20 mg，40 mg，80 mg；製造承認申請資料添付資料ヘ-23]。

7 薬物動態学的薬物間相互作用

7.1 CYP 阻害

ヒト肝ミクロソームを用いた *in vitro* 試験において，AHU 及び LBQ の CYP 活性阻害を検討した。各 CYP 分子種の選択的基質（フェナセチン，パクリタキセル，ジクロフェナク，S-メフェニトイン，ブフラロール，クロルゾキサゾン，ミダゾラム，テストステロン，及びブプロピオン）を用いて評価した。AHU は CYP1A2，2C9，2D6，2E1，3A4/5 に対して阻害作用をほとんど示さず（IC50：>100 µM），CYP2C8（IC50：15 µM），CYP2C19（IC50：20 µM）及び CYP2B6（IC50：65 µM）に対して弱い阻害作用を示した。また，LBQ は同様に各 CYP 分子種に対して阻害作用をほとんど示さず（IC50：>100 µM），CYP2C9（IC50：40 µM）に対して弱い阻害作用を示した[Table 2.6.5.12A-R0300252] [Table 2.6.5.16N-R1500389]。これらの *in vitro* 阻害結果に基づくと，臨床上高濃度に達しない限り，各 CYP 分子種によって代謝される併用薬の代謝クリアランスを AHU 及び LBQ が阻害する可能性は低いと考えられた。また，AHU 及び LBQ は，100 µM でも CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，並びに CYP3A4/5 に，明らかな時間依存的阻害を示さなかった。VAL が CYP1A2，2A6，2C19，2D6，2E1 及び 3A4 に対して有意な阻害作用を示すことはなく，CYP2C9 に対する Ki 値は 135 µM であった（Taavitsainen et al. 2000）。しかし，CYP2C9 ワルファリン（CYP2C9 基質）との併用投与後，薬物間相互作用は認められなかった。

7.2 酵素誘導

ヒト初代培養肝細胞を用いた *in vitro* 試験において，AHU を 48 時間処理したときの CYP の mRNA 発現量及び酵素活性の誘導作用を評価した [Table 2.6.5.12B-R1200084]。ヒト初代培養肝細胞に AHU 10～100 µM を処理しても，CYP1A2，CYP2B6，CYP2C9 及び CYP3A4 の mRNA 発現及び酵素活性の誘導は認められなかった。溶媒対照と比較した mRNA 発現及び酵素活性は，2 倍未満又は陽性対照の 40%未満であった。インキュベーション 4 時間後の時点で AHU の 50%以上が活性代謝物である LBQ に代謝され，インキュベーション 24 時間後の LBQ への変換率は 100%

であった。以上より，AHU 及び LBQ が *in vivo* で CYP1A2，CYP2B6，CYP2C9 及び CYP3A の発現及び酵素活性を誘導する可能性は低いと考えられた。

ヒト初代肝細胞を用いた *in vitro* 試験において，VAL を 48 時間処理したときの CYP の mRNA 発現量及び酵素活性の誘導作用を評価した [Table 2.6.5.12C-R1200239]。VAL（1～100 μM）で処理しても，CYP1A2，CYP2B6，CYP2C9 及び CYP3A4 の mRNA 発現及び CYP2B6，CYP2C9 及び CYP3A4 酵素活性が誘導されることはなかった。溶媒対照と比較した mRNA 発現及び酵素活性は，2 倍未満又は陽性対照の 40%未満であった。3 検体中 1 検体の肝細胞で VAL 10 μM 処理により CYP1A2 の酵素活性に高値が認められたが，mRNA の発現には変化はなく，他の濃度で VAL を処理後も酵素活性は溶媒対照と同程度であったことから，当該 1 検体の酵素活性の高値は異常値であると判断した。以上より，VAL が *in vivo* で CYP1A2，CYP2B6，CYP2C9 及び CYP3A の mRNA 発現及び酵素活性を誘導する可能性は低いと考えられた。

7.3 *In vitro* 膜透過性評価及びトランスポーターを介した相互作用

[^{14}C] AHU 及び[^{14}C] LBQ を用いて，AHU 及び LBQ の Caco-2 細胞での膜透過性を評価した [Table 2.6.5.16A-R0301243]。2 種類の濃度（約 5.0 及び約 23 μM）の[^{14}C] AHU 及び[^{14}C] LBQ を用いて，定常状態条件下で双方向輸送（頂端膜側→底膜側及び基底膜側→頂端膜側）を検討した。さらに，P 糖蛋白質（P-gp）及び多剤耐性蛋白質 2（MRP2）阻害剤である PSC833（1.0 μM）及びインドメタシン（50 μM）の輸送速度に対する影響について検討した。[^{14}C]AHU の頂端膜側→基底膜側方向及び基底膜側→頂端膜側方向の膜透過係数はそれぞれ，23～28 × 10^{-5} cm/min 及び 56～60 × 10^{-5} cm/min であった。また，同様に[^{14}C] LBQ の膜透過係数はそれぞれ，0.67～0.75 × 10^{-5} cm/min 及び 2.7～4.2 × 10^{-5} cm/min であった。Caco-2 細胞での AHU の輸送速度は PSC833 による影響を受けたが，インドメタシンの影響は受けなかった。PSC833 により[^{14}C] AHU の頂端膜側→基底膜側方向の透過性が約 2 倍上昇するとともに，基底膜側→頂端膜側方向の透過性は約 3 分の 1 に低下した。これらの結果から，AHU は P-gp 基質であることが示唆された。しかし，AHU の P-gp に対する K_m 値は 100 μM を超えることから，臨床での相互作用の影響は大きくないと考えられた。以上より，*in vitro* で推定された膜透過係数から，AHU は中程度の膜透過性を示したが，LBQ の膜透過性は低かった。AHU は P-gp を介して排出されるが，同時に中程度の膜透過性を示すことから，AHU の吸収に大きな影響は生じないと予想された。

有機アニオン輸送ポリペプチド 1B1（OATP1B1）又は有機アニオン輸送ポリペプチド 1B3（OATP1B3）を安定発現するヒト胎児由来腎臓細胞（HEK293）を用いて，LBQ の動態に対する OATP1B1 及び OATP1B3 の寄与について検討した [Table 2.6.5.16F-R1200043]。OATP1B1 及び OATP1B3 発現細胞では，対照細胞に比べて，陽性対照基質である[^{3}H] エストラジオール-17β-グルクロナイド（E_2-17βG：1.6 μM）がそれぞれ，6.6 倍及び 2.6 倍蓄積したことから，OATP トランスポーターを十分発現していることが示された。さらに，OATP のモデル阻害剤である rifamycin SV（25 μM）存在下では，陽性対照基質の OATP 発現細胞への蓄積が対照細胞と同程度

のレベルにまで低下した。検討した濃度範囲（12～490 µM）で OATP1B1 及び OATP1B3 を介した[^{14}C] LBQ の蓄積が認められた。LBQ の OATP1B3 輸送活性の K_m は 174 ± 49.8 µM であったが，OATP1B1 輸送に関する K_m 値は十分な活性の飽和がみられなかったことから算出しなかった。以上より，OATP1B1 及び OATP1B3 による能動輸送が LBQ の肝臓への取り込み及び全身クリアランスに関与し，これらのトランスポーターを阻害する薬剤を併用したときに，LBQ のクリアランスが変化する可能性が示唆された。

ヒト有機アニオントランスポーター1（OAT1）及び有機アニオントランスポーター3（OAT3）を安定発現する HEK 細胞を用いて，LBQ の能動輸送への関与を検討した [Table 2.6.5.16D-R0900341]。対照細胞と比較して， OAT3 発現細胞では，[^{14}C] LBQ（10.8 µM）の細胞内蓄積が 14.5 倍上昇したが， OAT1 発現細胞では，2 倍程度の上昇であった。OAT3 による LBQ の最大輸送速度（J_{max}）及び Michaelis 定数（K_m）はそれぞれ，77.1 ± 3.69 pmol·mg protein^{-1}·min^{-1} 及び 10.6 ± 2.02 µM であった。結論として，LBQ が OAT3 の *in vitro* 基質であることが示され，i*n vivo* で OAT3 阻害剤と併用したとき，LBQ の分布及び消失に影響が生じる可能性があると考えられた。

同様に OAT1 及び OAT3 を安定発現させた細胞系を用いて，VAL の能動輸送への関与を検討した [Table 2.6.5.16E-R0900652]。対照細胞と比較して，OAT1 及び OAT3 発現細胞では，[^{14}C]VAL の細胞内蓄積がそれぞれ，1.5 倍及び 3.2 倍上昇した。OAT3 を介した VAL 輸送に関する J_{max} 及び K_m はそれぞれ，19.9 ± 1.86 pmol·mg protein^{-1}·min^{-1} 及び 1.93 ± 0.77 µM と算出された。以上より，VAL は OAT1 及び OAT3 の *in vitro* 基質であり，i*n vivo* で OAT1 及び OAT3 阻害剤と併用したとき，VAL の分布及び消失に影響が生じる可能性が示唆された。

ヒト肝細胞及び OATP1B1/MRP2 を安定発現させた MDCKII 細胞を用いた試験系において，VAL は OATP1B1，OATP1B3（肝取り込みトランスポーター）及び MRP2（排出トランスポーター）の *in vitro* 基質であることが示された（Yamashiro et al. 2006）。この結果に基づくと，臨床的意義は明らかではないものの，これらの取り込みトランスポーター又は排出トランスポーターを阻害する薬剤と併用したとき，VAL の全身曝露量が増加する可能性が示唆された。

7.4 *In vitro* トランスポーター阻害

AHU の P-gp，BCRP 又は MRP2 を介した排出型トランスポーター活性の阻害作用について，BCRP（T8 細胞），P-gp（MDA435 T0.3 細胞）及び MRP2（MDCKII 細胞）をそれぞれ過剰発現した細胞系を用いて評価した [Table 2.6.5.16C-R0900054] 。BCRP 及び P-gp による蛍光基質 Bodipy FL prazosin（BDP）及び Rhodamine 123（Rho123）の排出に対する AHU の影響をフローサイトメトリーにより評価した。MRP2 のプローブ基質として [^{14}C] VAL を使用した。AHU は濃度 50 µM で BDP 濃度を 1.9 倍（I_{max}）上昇させたが，この阻害の程度は，BCRP の陽性対照である fumitremorgin C（10 µM）による最大抑制の 6.7%（$\%_{max}$）であった。一方，AHU は濃度 50 µM まで，P-gp を発現する MDA435 T0.3 細胞からの Rho123 排出及び MRP2 を発現する MDCKII 細胞からの[^{14}C]VAL 排出を阻害しなかった。以上より，AHU は検討した最高濃度，

BCRP，P-gp 及び MRP2 を阻害しないと考えられた。また，LBQ の P-gp 及び BCRP に対する阻害効果を検討した結果[Table 2.6.5.16B-R1300267]，膜小胞で発現させた P-gp（[^{3}H]*N*-methylquinidine 輸送）及び BCRP（[^{3}H]estrone-3-sulfate 輸送）をほとんど阻害しなかった（IC50：> 50 µM）。

OATP1B1 又は OATP1B3 を安定発現した HEK293 細胞系を用いて，OATP1B1 及び OATP1B3 トランスポーター活性に対する LBQ 及び AHU の阻害作用について検討した[Table 2.6.5.16G-R1200044] [Table 2.6.5.16H-R1200090]。LBQ（OATP1B1 発現細胞系では 500 µM まで，OATP1B3 発現細胞では 250 µM まで）及び AHU 濃度（50 µM まで）を上昇させながら，細胞内への[^{3}H]E_2-17βG の蓄積に対する影響を検討した。検討した最高濃度（50 µM）で AHU は，OATP1B1 及び OATP1B3 発現細胞への[^{3}H]E_2-17βG 取り込みをそれぞれ 98.3%及び 89.9%阻害し，その IC_{50} はそれぞれ，1.91 ± 0.56 µM 及び 3.81 ± 2.2 µM であった。検討した最高濃度（500 µM）で LBQ は OATP1B1 発現細胞への[^{3}H]E_2-17βG 取り込みを 82.8%阻害し，IC50 は 126 ± 26 µM であった。一方，LBQ は 250 µM まで OATP1B3 トランスポーター活性を阻害しなかったため，IC50 は算出しなかった。

慢性心不全患者に LCZ 200 mg を 1 日 2 回反復経口投与後の LBQ の Cmax,ss（16531 ng/mL: 約 43.1 µM）（A2117 試験及び B2223 試験）であったことから，LBQ と OATP1B1 又は OATP1B3 基質の間で薬物間相互作用が生じる可能性は小さいと考えられた。一方，AHU では，慢性心不全患者での LCZ 200 mg1 日 2 回反復投与時の Cmax,ss は 2421 ng/mL（約 5.5 µM，A2117 試験及び B2223 試験）であったことから，これらのトランスポーターを介した薬物間相互作用が生じる可能性がある。

MATE1 及び MATE2-K を安定発現した HEK 細胞系を用いて，AHU 及び LBQ のトランスポーター活性阻害について検討した結果，AHU は検討した最高濃度（50 µM）で MATE1 及び MATE2-K を介した[^{14}C]メトホルミン輸送をそれぞれ 17.3%及び 13.8%阻害し[Table 2.6.5.16L-R1300681]，LBQ は検討した最高濃度（250 µM）でそれぞれ 10.4%及び 27.3%阻害した[[Table 2.6.5.16M- R1300680]。AHU 及び LBQ はいずれも MATE1 と MATE2-K の弱い阻害剤であることが示唆された。慢性心不全患者（A2117 試験及び B2223 試験）の Cmax, ss から AHU 及び LBQ とこれらのトランスポーターとの間で薬物間相互作用が生じる可能性は小さいと考えられた。

OCT1 及び OCT2 を安定発現した HEK 細胞系を用いて，AHU，LBQ 及び VAL のトランスポーター活性阻害について検討した[Table 2.6.5.16J-R1200302]，[Table 2.6.5.16K-R1200303]。AHU，LBQ 及び VAL のいずれについても，検討した濃度範囲内（AHU：1.0～50 µM，LBQ：5.0～500 µM，VAL：1～100 µM）で，OCT1 を介する[^{3}H]Methyl-4-phenylpyridinium acetate *N*-[methyl-^{3}H]（[^{3}H]MPP^+）輸送及び OCT2 を介する[^{14}C]メトホルミン輸送を阻害しなかった。以上より，LCZ の構成成分（AHU，LBQ 及び VAL）は，ヒトで肝臓の OCT1 及び腎臓の OCT2 を阻害しないと考えられた。

OAT1 及び OAT3 を安定発現した HEK 細胞系を用いて，AHU 及び LBQ のトランスポーター活性阻害を検討した [Table 2.6.5.16I-R1300221]， [Table 2.6.5.16D-R0900341]。AHU は検討した最高濃度（50 µM）で OAT1 及び OAT3 を介した 6-carboxyfluorescein（6CF）の輸送をそれぞれ 36.6%及び 91.1%阻害した。OAT3 を介した 6CF 輸送に対する AHU の IC50 は 0.795 ± 0.058 µM であった。LBQ は検討した最高濃度（200 µM）で OAT3 を介した 6CF の輸送を 85.3%阻害したが，OAT1 にはほとんど影響しなかった。OAT3 を介した 6CF 輸送に対する LBQ の IC50 は 15.2 ± 5.60 µM であった。以上より，AHU 及び LBQ は OAT3 の *in vitro* 阻害剤であり，臨床で OAT3 阻害を示す可能性が示唆された。

また同様の細胞系を用いて，VAL による OAT1 及び OAT3 のトランスポーター活性阻害について検討した [Table 2.6.5.16E-R0900652]。OAT1 及び OAT3 による 6CF の取り込みは，検討した最高濃度の VAL で 96.4%及び 100%低下した。OAT1 及び OAT3 を介した 6CF 輸送に対する VAL の IC50 はそれぞれ，14.8 ± 5.42 µM 及び 1.11 ± 0.33 µM であった。これらのデータから，VAL は OAT1 及び OAT3 の *in vitro* 阻害剤であり，OAT1 又は OAT3 の基質である併用薬の腎排泄に影響する可能性が示唆された。

8 その他の薬物動態試験

該当なし。

9 考察及び結論

LCZ を経口投与後の吸収は，検討したいずれの動物種においても良好であり，曝露量は概ね投与量に依存して増加した。いずれの動物種においても，曝露量に明らかな性差は認められなかった。ラット及びサルに反復経口投与後も，明らかな累積性は認められなかった。

AHU 及び LBQ の血漿蛋白結合率は各動物種で 80%～97%であり，ヒト血漿で AHU 及び LBQ ともに 97%であった。ヒト血漿中での AHU，LBQ 及び VAL の主な結合蛋白は血清アルブミンであった。[^{14}C]LCZ 又は[^{14}C]AHU を投与後，放射能は主に腎臓及び肝臓に分布し，脳内移行び精巣への移行は低かった。メラニン含有組織（有色皮膚及びブドウ膜）への蓄積性は認められなかった。妊娠ラット及びウサギでの LCZ 関連放射能の胎児移行性は低かった。授乳中ラットにおいて，LCZ 関連放射能の乳汁移行が認められた。ラットのデータをヒトに外挿することにより，1 日あたりの母乳摂取量を 1 L としたとき，乳児は AHU として成人投与量の約 0.9%に曝露されると推定された。

AHU は *in vitro* で CYP2C8 及び 2C19 活性を阻害したが，慢性心不全患者（LCZ 200 mg を 1 日 2 回反復経口投与）での定常状態における Cmax,ss は約約 5.5 µM であり，血漿蛋白結合率が高いことを考慮すると，AHU と併用薬の間に CYP を介した薬物間相互作用は生じないと予想された。投与した LCZ 関連成分の大部分が糞及び尿中に LBQ 及び VAL として排泄されたことから，CYP 阻害剤が肝臓での LCZ 関連成分の各クリアランスに影響する可能性は低いと予想された。しか

し，腎機能又は肝機能障害はこれらの有効成分の消失に影響する可能性がある。また，エステラーゼ阻害により AHU から LBQ の生成に影響する可能性があると考えられる。

AHU は P-gp の基質であることが *in vitro* 試験結果で示されたが，吸収率が比較的高かったことから臨床上 AHU の吸収に P-gp は大きく影響しないと考えられた。また，AHU，LBQ 及び VAL は，CYP1A2，CYP2B6，CYP2C9 及び CYP3A を誘導せず，OCT1 及び OCT2 を阻害しなかった。AHU は *in vitro* で MRP2 及び P-gp を阻害せず，BCRP の阻害はわずかであった。以上の *in vitro* の結果から，LCZ 投与により，これらの代謝酵素及びトランスポーターを介した併用薬の薬物動態に影響が生じる可能性は低いと考えられた。

In vitro 試験結果から，OATP1B1 及び OATP1B3 による能動輸送が LBQ の肝取り込みに寄与し，これらのトランスポーターを阻害する薬剤を併用したときに，LBQ のクリアランスに影響する可能性が示唆された。LBQ は OATP1B1 に対して *in vitro* 阻害作用を示した（IC50 ：126 µM）。しかし，慢性心不全患者に LCZ 200 mg を 1 日 2 回反復経口投与後の LBQ の Cmax,ss（約 43 µM）に基づくと，LBQ によって OATP1B1 基質となる薬剤の全身曝露量が増加する可能性は低いと考えられた。

AHU は OATP1B1，OATP1B3 及び OAT3 に対して *in vitro* 阻害作用を示し，その IC50 値はそれぞれ，1.91 µM，3.81 µM 及び 0.80 µM であった。慢性心不全患者に LCZ696 を 200 mg の用量で 1 日 2 回反復経口投与したときの AHU の Cmax,ss は約 5.5 µM であったことから，AHU は OATP1B1/1B3 及び OAT3 の基質となる薬剤の全身曝露量を増加させる可能性がある。

VAL は *in vitro* で OAT1 及び OAT3 の基質であった。LBQ は *in vitro* で OAT3 の基質であるとともに阻害作用（IC50：15.2 µM）を示したが，OAT1 の阻害剤及び基質ではなかった。しかしながら，LBQ 及び VAL のヒトでの腎排泄の結果から，これらの *in vitro* での結果は臨床上大きく影響しないことが示されている[5.3.3.1-1-A1101 試験]。LCZ 400 mg を投与後の投与量の約 55%が LBQ として尿中に排泄された。また，VAL の尿中排泄は投与量の 11%程度であったことから LBQ による OAT3 を介した VAL の尿中排泄への影響は大きくないと考えられた。

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2.6.5 薬物動態試験概要表

目　次

目　次 2
略号一覧 4
2.6.5.1 薬物動態試験：一覧 6
2.6.5.2A 薬物動態試験：分析方法 12
2.6.5.2B 薬物動態試験：分析方法 18
2.6.5.3A 薬物動態試験：単回投与後の吸収 22
2.6.5.3B 薬物動態試験：単回投与後の吸収 27
2.6.5.3C 薬物動態試験：単回投与後の吸収 30
2.6.5.4A 薬物動態試験：反復投与後の吸収 32
2.6.5.4B 薬物動態試験：反復投与後の吸収 44
2.6.5.4C 薬物動態試験：反復投与後の吸収 73
2.6.5.5 薬物動態試験：分布 112
2.6.5.6A 薬物動態試験：血球移行性及び血漿たん白結合 117
2.6.5.6B 薬物動態試験：血球移行性及び血漿たん白結合 119
2.6.5.6C 薬物動態試験：血球移行性及び血漿たん白結合 120
2.6.5.7A 薬物動態試験：妊娠動物における試験 121
2.6.5.7B 薬物動態試験：妊娠動物における試験 125
2.6.5.8 薬物動態試験：その他の分布試験 126
2.6.5.9A 薬物動態試験：*in vivo* における代謝 129
2.6.5.9B 薬物動態試験：*in vivo* における代謝 136
2.6.5.9C 薬物動態試験：*in vivo* における代謝 146
2.6.5.9D 薬物動態試験：*in vivo* における代謝 151
2.6.5.9E 薬物動態試験：*in vivo* における代謝 155
2.6.5.9F 薬物動態試験：*in vivo* における代謝 160
2.6.5.9G 薬物動態試験：*in vivo* における代謝 167
2.6.5.9H 薬物動態試験：*in vivo* における代謝 173
2.6.5.9I 薬物動態試験：*in vivo* における代謝 177
2.6.5.9J 薬物動態試験：*in vivo* における代謝 179
2.6.5.9K 薬物動態試験：*in vivo* における代謝 182
2.6.5.10A 薬物動態試験：*in vitro* における代謝 185
2.6.5.10B 薬物動態試験：*in vitro* における代謝 191
2.6.5.11 薬物動態試験：推定代謝経路 199
2.6.5.12A 薬物動態試験：薬物代謝酵素の誘導／阻害 200
2.6.5.12B 薬物動態試験：薬物代謝酵素の誘導／阻害 201
2.6.5.12C 薬物動態試験：薬物代謝酵素の誘導／阻害 211

2.6.5.13A 薬物動態試験：排泄 222
2.6.5.13B 薬物動態試験：排泄 223
2.6.5.13C 薬物動態試験：排泄 224
2.6.5.13D 薬物動態試験：排泄 225
2.6.5.13E 薬物動態試験：排泄 226
2.6.5.13F 薬物動態試験：排泄 227
2.6.5.14 薬物動態試験：胆汁中排泄 228
2.6.5.15 薬物動態試験：薬物相互作用 228
2.6.5.16A 薬物動態試験：その他 229
2.6.5.16B 薬物動態試験：その他 232
2.6.5.16C 薬物動態試験：その他 236
2.6.5.16D 薬物動態試験：その他 239
2.6.5.16E 薬物動態試験：その他 242
2.6.5.16F 薬物動態試験：その他 247
2.6.5.16G 薬物動態試験：その他 251
2.6.5.16H 薬物動態試験：その他 255
2.6.5.16I 薬物動態試験：その他 259
2.6.5.16J 薬物動態試験：その他 263
2.6.5.16K 薬物動態試験：その他 270
2.6.5.16L 薬物動態試験：その他 274
2.6.5.16M 薬物動態試験：その他 276
2.6.5.16N 薬物動態試験：その他 278

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，代謝及び排泄
AGP	α_1-acid glycoprotein	α_1酸性糖蛋白質
AUC0-t	area under the drug plasma (serum/blood) concentration-time curve (time 0 to t)	血漿（血清/血液）中薬物濃度-時間曲線下面積（時間 0～t）
AUCinf	area under the serum concentration-time curve (time 0 to infinity)	血清中濃度-時間曲線下面積（0～無限大）
AUClast	area under the drug plasma (serum/blood) concentration-time curve (time 0 to the last measurable concentration sampling time)	血漿（血清/血液）中薬物濃度-時間曲線下面積（0～最終定量可能時点）
BCRP	breast cancer resistance protein	乳癌耐性蛋白質
CL	clearance	クリアランス
Cmax	maximum plasma/serum/blood concentration after a single dose	単回投与後の最高血漿（血清/血液）中濃度
CMC	carboxymethylcellulose	カルボキシメチルセルロース
CYP	cytochrome P450	チトクローム P450
ESI	Electrospray ionization	エレクトロスプレーイオン化法
EDTA	Ethylenediaminetetraacetic acid	エチレンジアミン四酢酸
GLP	Good laboratory practice	医薬品の安全性に関する非臨床試験の実施の基準
HPC	hydroxypropylcellulose	ヒドロキシプロピルセルロース
HPLC	high performance liquid chromatography	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
IC50	50% inhibitory concentration	50%阻害濃度
i.v.	intravenous	静脈内
Jmax	maximum transport rate	最大輸送速度
Km	Michaelis constant	ミカエリス定数
LC-MS/MS	liquid chromatography – tandem mass spectrometry	液体クロマトグラフィー／タンデム質量分析
LLOQ	lower limit of quantification	定量下限
LOD	limit of detection	検出限界
MRP2	multidrug resistance associated protein 2	多剤耐性関連蛋白質 2
OAT	organic anion transporter	有機アニオントランスポーター
OATP	organic anion transporter polypeptide	有機アニオントランスポーター
OCT	organic cation transporter	有機カチオントランスポーター
P-gp	P-glycoprotein	P-糖蛋白質
PK	pharmacokinetics	薬物動態
p.o.	oral	経口
SRM	selected reaction monitoring	-
TK	toxicokinetics	トキシコキネティクス
T12	elimination half life	消失半減期
Tmax	time to the maximum observed	最高血漿（血清/血液）中濃度到達時間

略号	省略していない表現（英）	省略していない表現（日）
	plasma/serum/blood concentration	
USP	United States Pharmacopeia	米国薬局方
UV	ultraviolet	紫外線
Vz/F	apparent distribution volume	見かけの分布容積

2.6.5.1 薬物動態試験：一覧

Type of Study	Test System or Species / Strain	Admini-stration	Duration of Dosing	Dose	GLP Compliance	Testing Facility	Report Number	Location in CTD
Absorption, Metabolism and Excretion	Rat, Han:Wist	i.v. p.o	single	15 mg/kg [^{14}C]AHU377 45 mg/kg [^{14}C]AHU377	not required	Novartis US	[R0300247-1]	4.2.2.2-1
Absorption, Metabolism and Excretion	Dog, Beagle	i.v. p.o	single	3 mg/kg [^{14}C]LBQ657 15 mg/kg [^{14}C]AHU377	not required	Novartis US	[R0301244]	4.2.2.2-2
Absorption, Metabolism and Excretion	Monkey, Cyno	i.v. p.o	single	10 mg/kg [^{14}C]LBQ657 30 mg/kg [^{14}C]LCZ696	not required	Novartis US	[R1200729]	4.2.2.2-3
Absorption, Metabolism and Excretion	Mouse, CD-1	i.v. p.o.	single	30 mg/kg [^{14}C]LBQ657 150 mg/kg [^{14}C]AHU377	not required	Novartis US	[R1200673]	4.2.2.2-4
Absorption	Dog, Beagle	p.o.	single	11.3 mg/kg LCZ696 5.14 mg/kg VAL489 5.08 mg/kg AHU377	not required	Novartis US	[R0600309]	4.2.2.2-5
Absorption, Metabolism and Excretion	Human	p.o	single	200 mg [^{14}C]LCZ696	not required	Novartis US	[CLCZ696B2105]	5.3.3.1-3
Distribution	Rat, Han:Wist; Dog, Beagle; Monkey, Cyno; Human	In vitro	-	0.02, 0.1, 1, 10 and 100 µg/mL [^{14}C]LBQ657	not required	Novartis US	[R0301324]	4.2.2.3-1
Distribution	Monkey, Cyno; Human	In vitro	-	0.02, 0.1, 1, 10 and 100 µg/mL [^{14}C]AHU377	not required	Novartis US	[R1200657]	4.2.2.3-2
Distribution	Human α_1-acid glycoprotein and albumin	In vitro	-	0.02, 0.1, 1, 10 and 100 µg/mL[^{14}C]LBQ657, [^{14}C]AHU377	not required	Novartis US	[R1200658]	4.2.2.3-3
Distribution	Rat, LEH pigmented and Han:Wist	i.v. p.o	single	15 mg/kg [^{14}C]AHU377 45 mg/kg [^{14}C]AHU377	not required	Novartis US	[R0300247-2]	4.2.2.3-4

Type of Study	Test System or Species / Strain	Admini-stration	Duration of Dosing	Dose	GLP Compliance	Testing Facility	Report Number	Location in CTD
Distribution	Rat, Han:Wist, Pregnant	p.o	single	25 mg/kg [^{14}C]LCZ696	not required	Novartis US	[R1200674]	4.2.2.3-5
Metabolism	Rat, Han:Wist; Dog, Beagle; Human liver slices	In vitro	-	5 and/or 20 µM [^{14}C]AHU377	not required	Novartis US	[R0300249]	4.2.2.4-1
Metabolism	Human liver microsomes and S9	In vitro	-	42 µM [^{14}C]AHU377 or 41 µM [^{14}C]LBQ657	not required	Novartis US	[R0300251]	4.2.2.4-2
Excretion into milk	Rat, Han:Wist, Lactating	p.o.	single	30 mg/kg [^{14}C]LCZ696	not required	Novartis US	[R1200622]	4.2.2.5-1
DDI	Human microsomes	In vitro	-	0, 1, 2, 5, 10, 25, 50, and 100 µM AHU377	not required	Novartis US	[R0300252]	4.2.2.6-1
DDI	Human hepatocytes	in vitro	-	10, 50, and 100 µM AHU377	not required	Novartis US	[R1200084]	4.2.2.6-2
DDI	Human hepatocytes	in vitro	-	1, 10, 25, 50, and 100 µM VAL489	not required	Novartis US	[R1200239]	4.2.2.6-3
Permeability	Caco-2 monolayer	in vitro	-	4.9-24 µM [^{14}C]AHU377 or [^{14}C]LBQ657	not required	Novartis US	[R0301243]	4.2.2.6-4
Transporter	T8/MDA435 T0.3 / MDCKII cells	In vitro	-	0-50 µM [^{14}C]AHU377	not required	Novartis US	[R0900054]	4.2.2.6-5
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	2.4, 4.9 or 7.5 µM [^{14}C]LBQ657	not required	Novartis US	[R0900341]	4.2.2.6-6
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	10 µM [^{14}C]VAL489	not required	Novartis US	[R0900652]	4.2.2.6-7
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	12-490 µM [^{14}C]LBQ657	not required	Novartis US	[R1200043]	4.2.2.6-8
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	2.5-500 µM LBQ657	not required	Novartis US	[R1200044]	4.2.2.6-9
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	1-50 µM AHU377	not required	Novartis US	[R1200090]	4.2.2.6-10
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	1-50 µM AHU377 5-500 µM LBQ657	not required	Novartis US	[R1200302]	4.2.2.6-11

Type of Study	Test System or Species / Strain	Admini-stration	Duration of Dosing	Dose	GLP Compliance	Testing Facility	Report Number	Location in CTD
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	1-100 µM VAL489	not required	Novartis US	[R1200303]	4.2.2.6-12
Transporter	Inside out membrane vesicles	In vitro	-	2.5-50 µM LBQ657	not required	Novartis US	[R1300267]	4.2.2.6-13
Transporter	Human embryonic kidney (HEK) cells	In vitro	-	0.1-50 µM AHU377	not required	Novartis US	[R1300221]	4.2.2.6-14
Toxicokinetic	Mouse, CD-1	p.o.	2 weeks	0 mg/kg/day LCZ696 50 mg/kg/day LCZ696 200 mg/kg/day LCZ696 400 mg/kg/day LCZ696 800 mg/kg/day LCZ696	No	Novartis US	[R0670221]	4.2.3.2-1
Toxicokinetic	Mouse, CD-1	p.o.	13 weeks	0 mg/kg/day LCZ696 50 mg/kg/day LCZ696 100 mg/kg/day LCZ696 200 mg/kg/day LCZ696 400 mg/kg/day LCZ696	Yes	Novartis US	[R0670524]	4.2.3.2-2
Toxicokinetic	Mouse, CD-1	p.o.	13 weeks	0 mg/kg/day AHU377 400 mg/kg/day AHU377 800 mg/kg/day AHU377 1200 mg/kg/day AHU377	Yes	Novartis US	[R0770896]	4.2.3.4.1-4
Toxicokinetic	Mouse, CD-1	p.o.	104 weeks	0 mg/kg/day AHU377 150 mg/kg/day AHU377 400 mg/kg/day AHU377 1200 mg/kg/day AHU377	Yes	Novartis US	[R0870374]	4.2.3.4.1-5
Toxicokinetic	Rat, Han:Wist	p.o.	2 weeks	0 mg/kg/day LCZ696 50 mg/kg/day LCZ696 200 mg/kg/day LCZ696 600 mg/kg/day LCZ696 1200 mg/kg/day LCZ696	Yes	Novartis US	[R0670220]	4.2.3.2-3

Type of Study	Test System or Species / Strain	Admini-stration	Duration of Dosing	Dose	GLP Compliance	Testing Facility	Report Number	Location in CTD
Toxicokinetic	Rat, Han:Wist	p.o.	2 weeks	0 mg/kg/day LCZ696 10 mg/kg/day LCZ696 30 mg/kg/day LCZ696	Yes	Novartis US	[R0670468]	4.2.3.2-4
Toxicokinetic	Rat, Han:Wist	p.o., s.c.	5 days	0 mg/kg/day LCZ696 200 mg/kg/day LCZ696	No	Novartis US	[R0670624]	4.2.3.6-2
Toxicokinetic	Rat, Han:Wist	p.o.	13 weeks	0 mg/kg/day LCZ696 30 mg/kg/day LCZ696 100 mg/kg/day LCZ696 200 mg/kg/day LCZ696	Yes	Novartis US	[R0670283]	4.2.3.2-5
Toxicokinetic	Rat, Han:Wist	p.o.	4 and 13 weeks	0 mg/kg/day AHU377 400 mg/kg/day AHU377 800 mg/kg/day AHU377 1200 mg/kg/day AHU377	Yes	Novartis US	[R0770711]	4.2.3.2-18
Toxicokinetic	Pregnant Rat, Han:Wist	p.o.	gestation days 6- 17	0 mg/kg/day LCZ696 30 mg/kg/day LCZ696 100 mg/kg/day LCZ696 200 mg/kg/day LCZ696	Yes	Novartis US	[R0670390]	4.2.3.5.2-1
Toxicokinetic	Rat, Han:Wist	p.o.	26 weeks	0 mg/kg/day LCZ696 10 mg/kg/day LCZ696 30 mg/kg/day LCZ696 100 mg/kg/day LCZ696	Yes	Novartis US	[R0670620]	4.2.3.2-6
Toxicokinetic	Pregnant Rat, Han:Wist	p.o.	gestation days 6-17	0 mg/kg/day AHU377 75 mg/kg/day AHU377 250 mg/kg/day AHU377 750 mg/kg/day AHU377	Yes	Novartis US	[R0570301]	4.2.3.5.2-4

Type of Study	Test System or Species / Strain	Admini-stration	Duration of Dosing	Dose	GLP Compliance	Testing Facility	Report Number	Location in CTD
Toxicokinetic	Rat, Han:Wist	p.o.	days 7-64 (66) post partum	0 mg/kg/day AHU377 100 mg/kg/day AHU377 400 mg/kg/day AHU377 800 mg/kg/day AHU377	Yes	Novartis US	[R0870734]	4.2.3.5.4-2
Toxicokinetic	Rat, Han:Wist	p.o.	13 weeks	0 mg/kg/day AHU377 100 mg/kg/day AHU377 400 mg/kg/day AHU377	Yes	Novartis US	[R1270139]	4.2.3.7.3-3
Toxicokinetic	Rat, Han:Wist	p.o.	104 weeks	0 mg/kg/day AHU377 50 mg/kg/day AHU377 150 mg/kg/day AHU377 400 mg/kg/day AHU377	Yes	Novartis US	[R0870373]	4.2.3.4.1-6
Toxicokinetic	Pregnant Rabbit, NZW	p.o.	gestation days 7-20	0 mg/kg/day LCZ696 3 mg/kg/day LCZ696 10 mg/kg/day LCZ696 30 mg/kg/day LCZ696	Yes	Novartis US	[R0670280]	4.2.3.5.2-3
Toxicokinetic	Monkey, Cyno	p.o.	1 week	0 mg/kg/day LCZ696 25 mg/kg/day LCZ696 100 mg/kg/day LCZ696	Yes	Novartis US	[R0670259]	4.2.3.2-7
Toxicokinetic	Monkey, Cyno	p.o.	2 weeks	0 mg/kg/day LCZ696 50 mg/kg/day LCZ696 100 mg/kg/day LCZ696 300 mg/kg/day LCZ696 600 mg/kg/day LCZ696	No	Novartis US	[R0670276]	4.2.3.2-8
Toxicokinetic	Monkey, Cyno	p.o.	13 weeks	0 mg/kg/day LCZ696 30 mg/kg/day LCZ696 100 mg/kg/day LCZ696 300 mg/kg/day LCZ696	Yes	Novartis US	[R0670282]	4.2.3.2-9

Type of Study	Test System or Species / Strain	Admini-stration	Duration of Dosing	Dose	GLP Compliance	Testing Facility	Report Number	Location in CTD
Toxicokinetic	Monkey, Cyno	Nasal gavage	39 weeks	0 mg/kg/day LCZ696 30 mg/kg/day LCZ696 100 mg/kg/day LCZ696 300 mg/kg/day LCZ696	Yes	Novartis US	[R0670621]	4.2.3.2-10
Toxicokinetic	Monkey, Marmoset	p.o.	52 weeks	0 mg/kg/day AHU377 25 mg/kg/day AHU377 100 mg/kg/day AHU377 200 mg/kg/day AHU377	Yes	Novartis US	[R0570307]	4.2.3.2-20

2.6.5.2A 薬物動態試験：分析方法

Test article: LCZ696

	Human	Human	Monkey
Study number:	DMPK R0600891	DMPK R0700990	DMPK R0600533
Study title:	Quantitative determination of AHU377, Valsartan and LBQ657 in human plasma by LC-MS/MS	Transfer and validation of the method for quantitative determination of AHU377, LBQ657 and valsartan in human plasma and urine by LC-MS/MS. Method description and validation	Quantitative determination of AHU377, Valsartan and LBQ657 in monkey plasma by LC-MS/MS
GLP	No	No	No
Test facility:	Novartis Pharmaceuticals Corporation Drug Metabolism and Pharmacokinetics, BA-US East Hanover, New Jersey, USA	Novartis Pharma K.K. Drug Metabolism and Pharmacokinetics, BA-J Tsukuba, Japan	Novartis Pharmaceuticals Corporation Drug Metabolism and Pharmacokinetics, BA-US East Hanover, New Jersey, USA
Analyte	AHU377 calcium salt (batch: 0824011),VAL489 (batch: C0115), LBQ657 (batch: 07/2)	AHU377 calcium salt (batch: 0522005),VAL489 (batch: C0162), LBQ657 (batch: 06/1)	AHU377 calcium salt (batch: 0824011),VAL489 (batch: C0115), LBQ657 (batch: 11/3)
Reference compound	AHU377 calcium salt (batch: 0824011: purity 99.2%), VAL489 (batch: C0115, purity 99.1%), LBQ657 (batch: 07/2, purity 98.7%)	AHU377 calcium salt (batch: 0522005, purity 99.2%), VAL489 (batch: C0162, purity 99.1%), LBQ657 (batch: 06/1, purity 98.7%)	AHU377 calcium salt (batch: 0824011: purity 99.2%), VAL489 (batch: C0115, purity 99.1%), LBQ657 (batch: 11/3, purity 97.6%)
Assay	Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)	Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)	Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)
Method description	The objective of this study was to develop and validate a sensitive and selective method for the quantitative determination of AHU377, Valsartan and LBQ657, the metabolite of AHU377, in human plasma. The results of these investigations are presented in this report. The method consists of a liquid-liquid extraction, evaporation of the supernatant to dryness, and analysis of the reconstituted sample residues by liquid chromatography – tandem mass spectrometry (LC-MS/MS) in SRM mode using ESI as the ionization technique. The method is suitable for the determination of AHU377, Valsartan and LBQ657 in human plasma (EDTA) over the range of 1.00 ng/mL	The objective was to validate a sensitive method for the quantitative determination of AHU377, LBQ657 and Valsartan in human plasma and urine, in order to transfer the plasma method to BA-J from BA-US where the original plasma method had been already validated, and then in order to apply the transferred plasma method to urine quantitative analysis. The results of these determinations are presented in this report. The method consists of a liquid-liquid extraction with methyl tert-butyl ether (MTBE) in acidic conditions, an evaporation of the supernatant to dryness and of the analysis of the reconstituted sample by liquid chromatography / tandem mass spectrometry	The objective of this study was to cross-validate a sensitive and selective method for the quantitative determination of AHU377, Valsartan and LBQ657, the metabolite of AHU377, in monkey plasma. The results of these investigations are presented in this report. The method consists of liquid-liquid extraction, evaporation of the supernatant to dryness, and analysis of the reconstituted sample residue by liquid chromatography - tandem mass spectrometry (LCMS/MS) in MRM mode using ESI as the ionization technique. The method was suitable for the determination of AHU377, Valsartan and LBQ657 in monkey plasma (EDTA) over the range of 10.0 ng/mL

	Human	Human	Monkey
	(LLOQ) to 1000 ng/mL using 0.1 mL of human plasma. In addition, 10-fold and 400-fold dilutions of human plasma samples have been validated.	(LC- MS/MS) in selected reaction monitoring (SRM) mode using electrospray ionization (ESI) as an interface.	(LLOQ) to 10,000 ng/mL using 25 µL of monkey plasma. In addition, 100-fold dilutions of monkey plasma samples have been validated.
Sample preparation	Sample preparation was performed using liquid-liquid extraction with incorporation of a Quadra 96 TomTec system for solvent transfers. A 100 µL aliquot of each plasma sample was added to a 96 well 2 mL round-bottom plate. A 50 µL aliquot of internal standard solution of approximately 100 ng/mL (119/124/125 ng/mL $^{13}C_4$-AHU377/$^{13}C_4$-LBQ657/D_9-Valsartan in methanol/water, 50/50,v/v) was added to each well, except for human plasma blanks, and the plate was vortexed briefly. To each well, 50 µL of 0.5 N HCL was added followed by brief vortexing. To each well, 500 µL of MTBE was added, the plate covered and vortex-mixed for approximately 5 minutes. The sample was centrifuged at 3500 rpm (approximately 2000 x g) for 5 minutes at approximately 10°C. Approximately 400 µL of the organic layer was transferred to a separate 96-well 1 mL round-bottom plate. The organic layer was evaporated to dryness under nitrogen at 40 ºC. The sample residues were reconstituted with 300 µL of methanol:water:formic acid, (10:90:0.1, v/v/v). A 2 µL aliquot was injected on the LC-MS/MS system.	Frozen plasma or urine samples were thawed at room temperature, vortexed on a vortex mixer and centrifuged at 3000 rpm for 5 minutes at 10°C (set temperature) Using a Microlab STAR, a 0.1 mL aliquot of each sample was transferred to a disposable 1.4-mL insertion tube in a 96-well rack, following addition of a 50 µL aliquot of internal standard solution; 100 ng/mL (for plasma analysis) or 1000 ng/mL (for urine analysis) of [$^{13}C_4$]AHU377, [$^{13}C_4$]LBQ657 and [D_9]Valsartan in methanol / water (50:50, v/v). (For blank samples, 50 µL of methanol/water (50:50, v/v) was added instead of internal standard solution.) After addition of 50 µL of 0.5 mol/L hydrochloric acid to each tube using a Quadra 96, the tubes in a 96-well rack were vortexed for 30 seconds on a plate mixer. 550 µL of methyl tert-butyl ether was added to each tube using a Quadra 96, and the tubes were capped and shaken on a shaker for 5 minutes at 300 rpm. After centrifugation of the tubes for 5 minutes at 3000 rpm at 10°C (set temperature), 400 µL of the organic layer in each tube was transferred into each well of a 96-well collection plate followed by evaporation to dryness under nitrogen flow at 40°C in an evaporator. After complete evaporation, the sample in each well was reconstituted with 100 µL (for plasma analysis) or 300 µL (for urine analysis) of water/ methanol / formic acid (90:10:0.1, v/v/v) added by a Quadra 96. The sample plate was vortexed on a plate mixer for 30 seconds, sonicated for 1 minute and vortexed for 30 seconds followed by centrifugation at 3000 rpm for 5 minutes at 10°C (set temperature). The sample plate was placed in the autosampler set at 10°C and a 30 µL aliquot of the processed sample was injected onto the analytical	The sample preparation was performed using liquid-liquid extraction with incorporation of a Quadra 96 TomTec system for solvent transfers. A 25 µL aliquot of each plasma sample was added to a 96 well 2 mL V-bottom plate. A 50 µL aliquot of internal standard solution of approximately 500 ng/mL (475/495/500 ng/mL $^{13}C_4$-AHU377/$^{13}C_4$-LBQ657/D_9-Valsartan in methanol/water, 50/50,v/v) was added to each well, except for monkey plasma blanks, and the plate was vortexed for about 1 minute. To each well, 50 µL of 0.5 N HCL was added followed by vortexing for about 1 minute. To each well, 300 µL of MTBE was added followed by vortexing for 15 minutes. The sample was centrifuged at 3500 rpm (approximately 2000 x g) for 5 minutes at approximately 10 °C. Approximately 250 µL of the organic layer was transferred to a separate 96-well 1 mL round-bottom plate. The organic layer was evaporated to dryness under nitrogen at 40°C. The extract was reconstituted with 150 µL of methanol:water:formic acid, (10:90:0.1, v/v/v). A 15 µL aliquot was injected onto the LCMS/MS system.

	Human	Human	Monkey
		column.	
Results:			
LLOQ	1.00 ng/mL	1.0 ng/mL in plasma 10 ng/mL in urine	10 ng/mL
Stability	The stability of the reference standard(s) were assessed in the stock/spiking solutions, in QC matrix samples after storage under different conditions and in incurred samples following long term storage.	AHU377, LBQ657 and Valsartan were stable for 5 months at about - 20°C in human plasma and urine, respectively, and stable for 96 hours at about 10°C on the autosampler in plasma extracts and urine extracts.	The stability of the reference standard(s) were assessed in the stock/spiking solutions, in QC matrix samples after storage under different conditions and in incurred samples following long term storage.

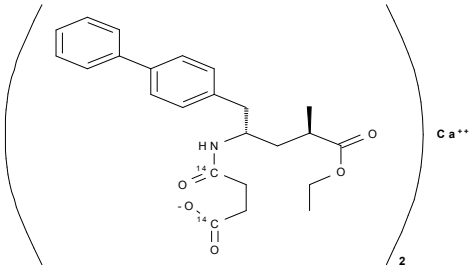
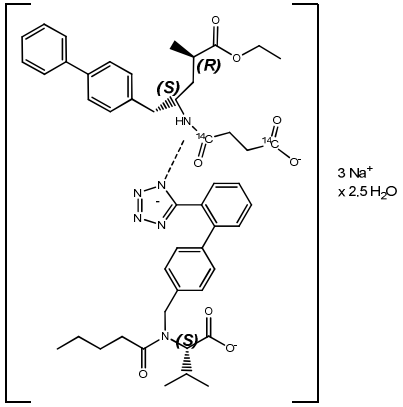
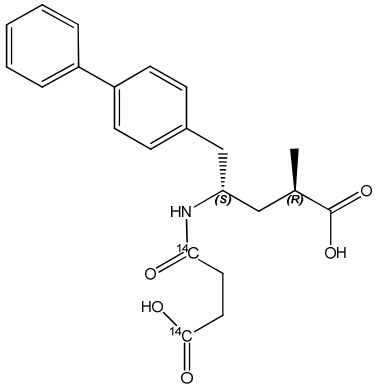
	Rat and Mouse	Rabbit
Study number:	DMPK R0600483	DMPK R0700176
Study title:	Quantitative determination of AHU377/LBQ657/Valsartan in rat and mouse plasma by LC-MS/MS	Quantitative determination of AHU377/LBQ657/Valsartan in rabbit plasma by LC-MS/MS
GLP	No	No
Test facility:	Novartis Pharmaceuticals Corporation Drug Metabolism and Pharmacokinetics, BA-US East Hanover, New Jersey, USA	Novartis Pharmaceuticals Corporation Drug Metabolism and Pharmacokinetics, BA-US East Hanover, New Jersey, USA
Analyte	AHU377 calcium salt (batch: 0824011),VAL489 (batch: C0115), LBQ657 (batch: 07/2)	AHU377 calcium salt (batch: 0824011),VAL489 (batch: C0115), LBQ657 (batch: 11/3)
Reference compound	AHU377 calcium salt (batch: 0824011: purity 99.2%), VAL489 (batch: C0115, purity 99.1%), LBQ657 (batch: 07/2, purity 98.7%)	AHU377 calcium salt (batch: 0824011: purity 99.2%), VAL489 (batch: C0115, purity 99.1%), LBQ657 (batch: 11/3, purity 98.4%)
Assay	Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)	Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)

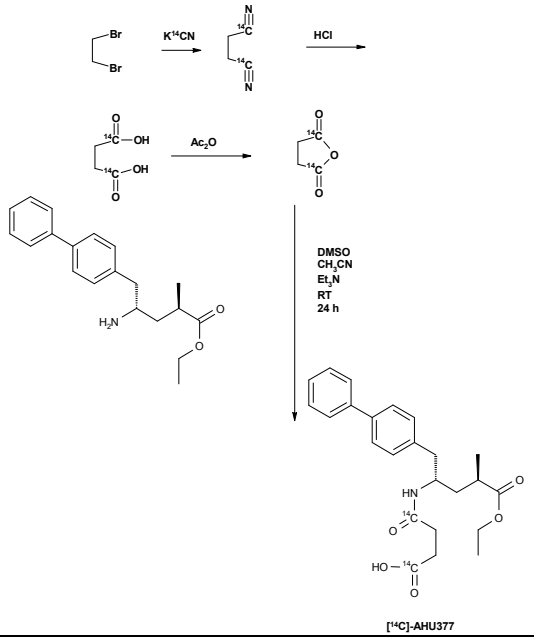
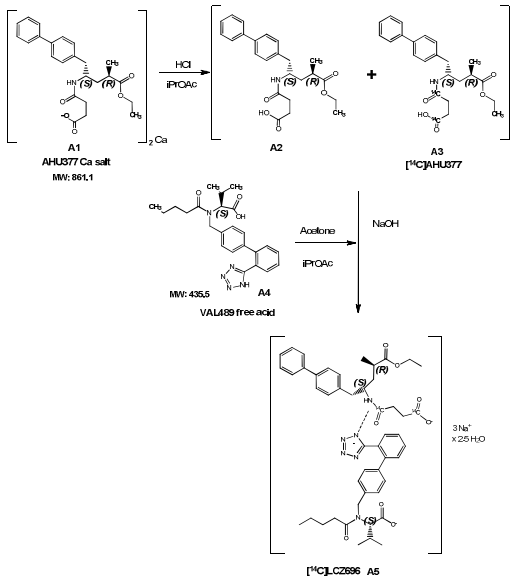
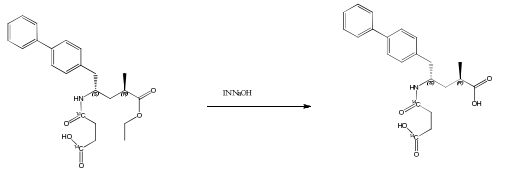
	Rat and Mouse	Rabbit
Method description	The objective of this study was to validate a sensitive and selective method for the quantitative determination of AHU377, Valsartan and LBQ657, the metabolite of AHU377, in rat and mouse plasma. The results of these investigations are presented in this report. The method used an MTBE liquid-liquid extraction, followed by evaporation of the supernatant to dryness, and analysis of the reconstituted sample residue by liquid chromatography – tandem mass spectrometry (LC-MS/MS) in SRM mode using ESI as the ionization technique. The rat and mouse plasma was pre-treated with sodium fluoride and phosphoric acid to prevent conversion of AHU377 to the metabolite LBQ657 in the rat and mouse plasma samples. The method is suitable for the determination of AHU377/LBQ657/Valsartan in rat and mouse plasma (K2 EDTA) over the range of 10.0 (LLOQ) to 10,000 ng/mL using 25 µL of plasma. 10-fold dilution of rat plasma and 100-fold dilution of mouse plasma samples have been validated.	The objective of this study was to validate a sensitive and selective method for the quantitative determination of AHU377, Valsartan and LBQ657, the metabolite of AHU377, in rabbit plasma and fetal tissue. The results of these investigations are presented in this report. The rabbit plasma method used an MTBE liquid-liquid extraction, followed by evaporation of the supernatant to dryness and reconstitution of the sample residue. For the rabbit fetal tissue method, blank rabbit fetal tissue was homogenized with PBS buffer using a 3-fold dilution. The fetal tissue homogenate samples were processed using protein precipitation, followed by evaporation of the supernatant to dryness and reconstitution of the sample residue. For both methods, the reconstituted samples were analyzed by liquid chromatography - tandem mass spectrometry (LC-MS/MS) in SRM mode using ESI as the ionization technique. The rabbit plasma method is suitable for the determination of AHU377/LBQ657/Valsartan in rabbit plasma (lithium heparin) over the range of 1.00 (LLOQ) to 5,000 ng/mL using 100 µL of plasma. The rabbit fetal tissue method is suitable for the determination of AHU377/LBQ657/Valsartan in rabbit fetal tissue over the range of 0.300 (LLOQ) to 300 ng/gram using 100 µL of fetal tissue homogenate. An additional method cross-validation in rabbit plasma was preformed using protein precipitation similar to that used for fetal tissue. This method is suitable for the determination of AHU377/LBQ657/Valsartan in rabbit plasma (sodium EDTA) over the range of 5.00 (LLOQ) to 20,000 ng/mL using 50 µL of plasma. 100-fold dilution of rabbit plasma has been validated

	Rat and Mouse	Rabbit
Sample preparation	Sample preparation was performed using liquid-liquid extraction with incorporation of a Quadra 96 TomTec system for solvent transfers. A 100 µL aliquot of each plasma sample was added to a 96 well 2 mL round-bottom plate. A 50 µL aliquot of internal standard solution of approximately 100 ng/mL (119/124/125 ng/mL 13C4-AHU377/13C4-LBQ657/D9-Valsartan in methanol/water, 50/50,v/v) was added to each well, except for human plasma blanks, and the plate was vortexed briefly. To each well, 50 µL of 0.5 N HCL was added followed by brief vortexing. To each well, 500 µL of MTBE was added, the plate covered and vortex-mixed for approximately 5 minutes. The sample was centrifuged at 3500 rpm (approximately 2000 x g) for 5 minutes at approximately 10°C. Approximately 400 µL of the organic layer was transferred to a separate 96-well 1 mL round-bottom plate. The organic layer was evaporated to dryness under nitrogen at 40 oC. The sample residues were reconstituted with 300 µL of methanol:water:formic acid, (10:90:0.1, v/v/v). A 2 µL aliquot was injected on the LC-MS/MS system.	Sample preparation was performed using liquid-liquid extraction with incorporation of a TomTec Quadra system for solvent transfers. A 100 µL aliquot of each plasma sample was added to a 2 mL 96-well V-bottom plate. A 50 µL aliquot of internal standard solution (475/495/500 ng/mL $^{13}C_4$-AHU377/$^{13}C_4$-LBQ657/D_9-VAL489 in methanol/water, 50/50,v/v) was added to each well, except for rat plasma blanks, and the plate was vortexed briefly. To each well was added 50 µL of 0.5 N HCL followed by mixing. To each well, 500 µL of MTBE was added followed by vortexing for approximately 5 minutes. The sample was centrifuged at 3500 rpm (approximately 2000 x g) for 5 minutes at approximately 10 °C. Approximately 400 µL of the organic layer was transferred to a separate 1 mL 96-well round-bottom plate. The organic layer was evaporated to dryness under nitrogen at 40 °C. The extract was reconstituted with 100 µL of methanol/water/formic acid, (10/90/0.1). A 15 µL aliquot was injected onto the LC-MS/MS system.
Results:		
LLOQ	10 ng/mL	1 – 5 ng/mL in plasma (lithium heparin) 5 ng/mL in plasma (Sodium EDTA) 300 ng/g fetal tissue homogenate
Stability	The stability of the reference standard(s) were assessed in the stock/spiking solutions, in QC matrix samples after storage under different conditions and in incurred samples following long term storage.	

2.6.5.2B 薬物動態試験：分析方法

Study number:	DMPK R0600259	DMPK R0900365	DMPK R1200840A
Study title:	[^{14}C]AHU377 Resynthesis and Analysis	[^{14}C]LBQ657 Synthesis and Analysis	[^{14}C]LBQ657 Synthesis and Analysis
GLP compliance:	No	No	No
Test facility:	IL, East Hanover, NJ (USA)		
Labeled compound:	[^{14}C] AHU377	[^{14}C] LCZ696	[^{14}C] LBQ657
Substance number:	AHU377	LCZ696	LBQ657
Synthesis number:	RSU5632	-	RSU6153-2012-7293-01
Chemical name:	1,1'-biphenyl-4-pentanoic acid, γ-{(3-carboxy-1-oxopropyl-carboxy,1-$^{14}C_2$)amino-a-methyl-a-ethylester, calcium salt, (α, γ S)-(2:1)	Trisodium [3-((1S,3R)-1-biphenyl-4-ylmethyl-3-ethoxycarbonyl-1-bultylcarbamoyl-[*carbamoyl-^{14}C*] propionate-[*carboxy-^{14}C*]-(S)-3'-methyl-2'-(pentanoyl{2"-(tetrazol-5-ylate)biphenyl-4'-ylmethyl}amino)butyrate] hemipentahydrate	(2R,4S)-5-([1,1'-biphenyl]-4-yl)-4-(3-carboxypropanamido-*1, 3-$^{14}C_2$*)-2-methylpentanoic acid

Study number:	DMPK R0600259	DMPK R0900365	DMPK R1200840A
Structural formula:	$(^{14}C_2C_{22}H_{28}NO_5)_2\ Ca^{++}$ 	$^{14}C_2C_{46}H_{55}N_6O_8 + 3Na^+ \cdot 2.5\ H_2O$ 	$^{14}C_2C_{20}H_{25}NO_5$ 

Study number:	DMPK R0600259	DMPK R0900365	DMPK R1200840A
Synthetic scheme::			
Confirmation of identity:	**By mass spectroscopy:** Mass spectra were obtained using LCQ-advantage coupled with Alliance HPLC system. The standard gave [M+H]+ = 412, while the sample gave [M+H]+ = 412, 414 and 416 with enriched peak at 416.	**By X-Ray Powder diffraction:** Spectrum taken on a Bruker DS Advance with a scan rate of 0.3s/step, a step size of 0.017º (2 Theta), a Soller slit of 2.5º, slits of (from left to right) of 2, 3, 0.3 and 0.2 mm using 20 mg of material. **By mass spectroscopy:** Spectrum taken on LCQ Fleet Ion trapass spectrometer in positive ion mode on sample and standard solutions from HPLC Assay and showed MH^+ ions of 412.3 and 436.3	**By HPLC:** UV detection at 254 nm with on-line radiodectection. **By mass spectroscopy:** Mass spectra of both standard and sample were run by LCQ fleet coupled with Waters UPLC system. Both standard and sample gave desired MW with matching retention time.

Study number:	DMPK R0600259	DMPK R0900365	DMPK R1200840A
Determination of specific activity:	By LC-MS with on-line radiodetection.	By liquid scintillation counting using a Packard Tri-Carb 3110 TR.	By LC-MS + ion mode and Single ion traces of 384.5 386.5 and 388.5 peak height
Radiochemical purity:	≥ 98% by HPLC-RA	98.6% by HPLC-RA	≥ 98% by HPLC-RA
Chemical purity:	≥ 98% by HPLC-UV	98.9% by HPLC-UV	≥ 98% by HPLC-UV

2.6.5.3A 薬物動態試験：単回投与後の吸収

Single dose – Radioactivity

	Mouse	
Study number:	DMPK(US)R1200673	
GLP compliance:	not required	
Location in CTD:	4.2.2.2-4	
Gender(M/F) / Number of animals:	M/28	
	CD-1	
Feeding condition:	Fed	
Vehicle / Formulation:	Sterile solution in 0.9% saline for injection	
Method of Administration:	Intravenous	
Dose (mg/kg):	30 = 78200 nmol/kg as [^{14}C]LBQ657	
Sample:	Plasma	Blood
Analyte:	Radioactivity – ^{14}C	Radioactivity – ^{14}C
Assay:	Liquid scintillation counting	Liquid scintillation counting
PK parameters:	mean	mean
Tmax (h)	0.083[a]	0.083[a]
Cmax (nM)	50300	36000
AUClast (nM▪h)	15600	11300
AUCinf (nM▪h)	15900	11400
AUCinf/Dose (nM▪h)/(nmol/kg)	0.203	0.146
T1/2 (h)	0.49	1.7

[a] Observed value; the first sampling time for the intravenous dose was 0.083 h

	Mouse	
Study number:	DMPK(US)R1200673	
GLP compliance:	not required	
Location in CTD:	4.2.2.2-4	
Gender(M/F) / Number of animals:	M/39	
	CD-1	
Feeding condition:	Fed	
Vehicle / Formulation:	Suspension in 0.5% aqueous KLUCEL	
Method of Administration:	Oral	
Dose (mg/kg):	150 = 365000 nmol/kg as [^{14}C]AHU377	
Sample:	Plasma	Blood
Analyte:	Radioactivity – ^{14}C	Radioactivity – ^{14}C
Assay:	Liquid scintillation counting	Liquid scintillation counting
PK parameters:	mean	mean
Tmax (h)	0.25	0.25
Cmax (nM)	57900	46700
AUClast (nM▪h)	66700	50700
AUCinf (nM▪h)	66900	50900
AUCinf/Dose (nM▪h)/(nmol/kg)	0.184	0.140
T1/2 (h)	1.2	1.3
% Absorption	90.6[a]	95.9[a]

[a] The absorption was ~91-96% based on blood and plasma radioactivity data, but it is considered to be 100% since the bioavailability was complete.

	Rat	
Study number:	ADME(US) R0300247-1	
GLP compliance:	not required	
Location in CTD:	4.2.2.2-1	
Gender(M/F) / Number of animals:	M/3	M/3
	(Han Wistar)	(Han Wistar)
Feeding condition:	Fed	Fed
Vehicle / Formulation:	Solution in 0.9% saline	Solution in 0.9% saline
Method of Administration:	Intravenous	Oral gavage
Dose (mg/kg):	15 = 34602 nmol/kg as [^{14}C]AHU377 sodium salt	45 = 103806 nmol/kg [^{14}C]AHU377 sodium salt
Sample:	Blood	Blood
Analyte:	Radioactivity – ^{14}C	Radioactivity – ^{14}C
Assay:	Liquid scintillation counting	Liquid scintillation counting
PK parameters:	mean ± SD	mean ± SD
Tmax (h)	0.083 ± 0 [b]	0.33 ± 0.14
Cmax (nM)	27600 ± 6860	5600 ± 4380
AUClast (nM•h)	9750	18600
AUCinf (nM•h)	9780	20700
T1/2 (h):	1.6	2.5
% Absorption	- [a]	71

[a] Not applicable or not determined

[b] Observed value; the first sampling time for the intravenous dose was 0.083 h

	Dog	
Study number:	DMPK(US) R0301244	
GLP compliance:	not required	
Location in CTD:	4.2.2.2-2	
Gender(M/F) / Number of animals:	M/2	M/3
	(Beagle)	(Beagle)
Feeding condition:	Fasted	Fasted
Vehicle / Formulation:	Solution in 0.9% saline	Solution in 0.9% saline
Method of Administration:	Intravenous	Oral gavage
Dose (mg/kg):	3 = 7823 nmol/kg [^{14}C]LBQ657	15 = 34602 nmol/kg [^{14}C]AHU377
Sample:	Blood	Blood
Analyte:	Radioactivity – ^{14}C	Radioactivity – ^{14}C
Assay:	Liquid scintillation counting	Liquid scintillation counting
PK parameters:	mean ± SD	mean ± SD
Tmax (h)	0.083 [b]	0.7 ± 0.3
Cmax (nM)	8610	13800 ± 7400
AUClast (nM•h)	3430	25100 ± 4200
AUCinf (nM•h)	3490	26500 ± 3500
T1/2 (h):	3.6	26 ± 10
% Absorption	- [a]	90[c]-complete

[a] Not applicable or not determined

[b] Observed value; the first sampling time for the intravenous dose was 0.083 h

[c] Absorption based on urinary excretion

	Monkey			
Study number:	DMPK(US) R1200729			
GLP compliance:	not required			
Location in CTD:	4.2.2.2-3			
Gender(M/F) / Number of animals:	M/2		M/3	
	Cynomolgus		Cynomolgus	
Feeding condition:	Fed		Fed	
Vehicle / Formulation:	Sterile solution in 0.9% saline for injection, USP.		Suspension in 0.5% (w/v) sodium carboxymethylcellulose (CMC) aqueous solution	
Method of Administration:	Intravenous		Oral gavage	
Dose (mg/kg):	10 as [^{14}C]LBQ657		30 as [^{14}C]LCZ696	
Sample:	Blood		Blood	
Analyte:	Radioactivity – ^{14}C		Radioactivity – ^{14}C	
Assay:	Liquid scintillation counting		Liquid scintillation counting	
PK parameters:	mean		mean ± SD	
Matrix	Blood	Plasma	Blood	Plasma
Tmax (h)	0.0833	0.0833	0.833 ± 0.289	0.833 ± 0.289
Cmax (nM)	163000	305000	24700 ± 5050	49700 ± 13500
AUClast (nM•h)	104000	203000	55600 ± 1460	112000 ± 10800
AUCinf (nM•h)	107000	209000	57600 ± 2500	114000 ± 11300
T1/2 (h)	24.1	39.5	5.24 ± 2.27	7.15 ± 2.90
% Absorption	- [a]	- [a]	65[b]	

[a] Not applicable or not determined

[b] 65% was estimated based urine recovery although it was estimated to be 40% based on blood and plasma AUC

2.6.5.3B 薬物動態試験：単回投与後の吸収

Single dose – AHU377

	Rat			
Study number:	ADME(US) R0300247-1			
GLP compliance:	Not required			
Location in CTD:	4.2.2.2-1			
Gender(M/F) / Number of animals:	M/3 (Han Wistar)	M/3 (Han Wistar)	M/3 (Han Wistar)	M/3 (Han Wistar)
Feeding condition:	Fed	Fed	Fed	Fed
Formulation:	Solution in 0.9% saline	Solution in 0.9% saline	Solution in 0.9% saline	Solution in 0.9% saline
Method of Administration:	Intravenous	Oral gavage	Intravenous	Oral gavage
Dose (mg/kg):	15 = 34602 nmol/kg [^{14}C]AHU377	45 = 103806 nmol/kg [^{14}C]AHU377	15 = 34602 nmol/kg [^{14}C]AHU377	45 = 103806 nmol/kg [^{14}C]AHU377
Sample:	Blood	Blood	Blood	Blood
Analyte:	AHU377	AHU377	LBQ657	LBQ657
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters:	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Cmax (nM)	1200 ± 378	128 ± 34	22500 ± 5550	6340 ± 5360
Tmax (h)	0.083 ± 0 [b]	0.58 ± 0.38	0.083 ± 0 [b]	0.5 ± 0
AUClast (nM•h)	320	233	8020	17400
AUCinf (nM•h)	320	238	8030	17400
T1/2 (h):	0.10	1.4	5.7	2.7
CL (L/h/kg):	108	- [a]	4.3	- [a]
Vss (L/kg	- [a]	- [a]	2.0	- [a]
Contribution to total AUC (%)[c]	4	1	96	99
% Bioavailability	- [a]	- [a]	- [a]	72

[a] Not applicable or not determined

[b] Observed value; the first sampling time for the intravenous dose was 0.083 h

[c] Total AUC represents the AUC for LBQ657 plus AHU377

Single dose – LBQ657 and AHU377

	Dog		
Study number:	DMPK(US) R0301244		
GLP compliance:	Not required		
Location in CTD:	4.2.2.2-2		
Gender(M/F) / Number of animals:	M/2	M/3	M/3
	(Beagle)	(Beagle)	(Beagle)
Feeding condition:	Fasted	Fasted	Fasted
Formulation:	Solution in 0.9% saline	Solution in 0.9% saline	Solution in 0.9% saline
Method of Administration:	Intravenous	Oral gavage	Oral gavage
Dose (mg/kg):	3 = 7823 nmol/kg [^{14}C]LBQ657	15 = 34602 nmol/kg [^{14}C]AHU377	15 = 34602 nmol/kg [^{14}C]AHU377
Sample:	Blood	Blood	Blood
Analyte:	LBQ657	LBQ657	AHU377
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters:	Mean	Mean ± SD	Mean ± SD
Cmax (nM)	6190	7230 ± 4230	7740 ± 4030
Tmax (h)	0.083 [b]	0.7 ± 0.3	0.6 ± 0.4
AUClast (nM•h)	2770	9520 ± 3530	5960 ± 1910
AUCinf (nM•h)	2810	9600 ± 3550	5980 ± 1910
T1/2 (h):	3.2	3.1 ± 1.0	2.8 ± 0.4
CL (L/h/kg)	2.9	[a]	[a]
Vss (L/kg)	2.5	[a]	[a]
Contribution to total AUC (%)[c]	[a]	61.0 ± 2.0	39.0 ± 2.0
% Bioavailability	[a]	77.0 ± 29.0	[a]

[a] Not applicable or not determined

[b] Observed value; the first sampling time for the intravenous dose was 0.083 h

[c] Total AUC represents the AUC for LBQ657 plus AHU377

Single dose – AHU377 and LBQ657

	Mouse		
Study number:	DMPK(US) R1200673		
GLP compliance:	Not required		
Location in CTD:	4.2.2.2-4		
Gender(M/F) / Number of animals:	M/28	M/39	M/39
	CD-1	CD-1	CD-1
Feeding condition:	Fed	Fed	Fed
Formulation:	Sterile solution in 0.9% saline for injection	Suspension in 0.5% aqueous KLUCEL	Suspension in 0.5% aqueous KLUCEL
Method of Administration:	Intravenous	Oral	Oral
Dose (mg/kg):	30 = 78200 nmol/kg as [^{14}C]LBQ657	150 = 365000 nmol/kg as [^{14}C]AHU377	150 = 365000 nmol/kg as [^{14}C]AHU377
Sample:	Plasma	Plasma	Plasma
Analyte:	LBQ657	AHU377	LBQ657
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters:	Mean	Mean	Mean
Cmax (nM)	32700	6250	51400
Tmax (h)	0.083[b]	0.25	0.25
AUClast (nM•h)	9950	2950	55600
AUCinf (nM•h)	10100	3140	55800
T1/2 (h):	0.17	1.6	1.1
CL (L/h/kg):	7.78	- [a]	- [a]
Vss (L/kg)	1.32	- [a]	- [a]
% Bioavailability	- [a]	- [a]	119 (complete)

[a] Not applicable or not determined

[b] Observed value; the first sampling time for the intravenous dose was 0.083 h

[c] Total AUC represents the AUC for LBQ657 plus AHU377

2.6.5.3C 薬物動態試験：単回投与後の吸収

Single dose PK

	Dog		
Study number:	DMPK(US) R0600309		
GLP compliance:	Not required		
Location in CTD:	4.2.2.2-5		
Gender(M/F) / Number of animals:	M/4		
	(beagle)		
Feeding condition:	Fasted		
Formulation:	Powder in capsule		
Method of Administration:	Oral capsule		
Dose (mg/kg):	11.3 [11.8 µmol/kg] LCZ696		
Sample:	Plasma		
Analyte:	VAL489, LBQ657, AHU377		
Assay:	LC-MS/MS		
PK parameters:	Mean ± SD		
	VAL489	LBQ657	AHU377
Cmax (ng/mL), [µM]	2530 ± 606 [5.81 ± 1.39]	927 ± 457 [2.42 ± 1.19]	1230 ± 906 [2.99 ± 2.20]
Tmax (h)	1.1 ± 0.63	0.75 ± 0.29	0.50 ± 0.35
AUClast (ng•h/mL), [µM•h]	9480 ± 2590 [21.8 ± 5.95]	1460 ± 680 [3.81 ± 1.77]	1050 ± 500 [2.55 ± 1.22]
AUCinf (ng•h/mL), [µM•h]	11600 ± 3400 [26.6 ± 7.80]	1910 ± 471 [4.98 ± 1.23]	1210 ± 639 [2.94 ± 1.56]
Relative exposure [a]	2.9	1.0	NC

Plasma concentration determined by LC-MS/MS; AUC was calculated using the linear trapezoidal rule; NC = not calculated since AHU377 is an intermediate product of LCZ696 and will be subsequently further converted to the active principal LBQ657

[a] The relative exposure was calculated using the mean AUC value of the combination dose form as reference

[b] Value from n =3

[c] Value from n =2

	Dog		
Study number:	DMPK(US) R0600309		
GLP compliance:	Not required		
Location in CTD:	4.2.2.2-5		
Gender(M/F) / Number of animals:	M/4		
	(beagle)		
Feeding condition:	Fasted		
Formulation:	Powder in capsule		
Method of Administration:	Oral capsule		
Dose (mg/kg):	5.14 [11.8 µmol/kg] VAL489		
	5.08 [11.8 µmol/kg] AHU377		
Sample:	Plasma		
Analyte:	VAL489, LBQ657, AHU377		
Assay:	LC-MS/MS		
PK parameters:	Mean ± SD		
	VAL489	LBQ657	AHU377
Cmax (ng/mL), [µM]	516 ± 250 [1.18 ± 0.574]	224 ± 90.6 [0.584 ± 0.236]	224 ± 61.6 [0.544 ± 0.150]
Tmax (h)	3.0 ± 3.4	2.6 ± 3.6	2.6 ± 3.6
AUClast (ng•h/mL), [µM•h]	3220 ± 1410 [7.39 ± 3.24]	1430 ± 1670 [3.73 ± 4.36]	518 ± 140 [1.26 ± 0.340]
AUCinf (ng•h/mL), [µM•h]	4050 ± 1280 [b] [9.30 ± 2.94] [b]	2240 [c] [5.84] [c]	679[c] [1.65] [c]
Relative exposure [a]	1	1	NC

Plasma concentration determined by LC-MS/MS; AUC was calculated using the linear trapezoidal rule; NC = not calculated since AHU377 is an intermediate product of LCZ696 and will be subsequently further converted to the active principal LBQ657

[a] The relative exposure was calculated using the mean AUC value of the combination dose form as reference

[b] Value from n =3

[c] Value from n =2

2.6.5.4A 薬物動態試験：反復投与後の吸収

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670221							
GLP compliance:	No							
Location in CTD:	4.2.3.2-1							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 400, 800							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377		AHU377		AHU377		AHU377	
Dose (mg/kg/day)	50		200		400		800	
Day	14		14		14		14	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	18.1	100	964	2930	9080	22000	69000	145000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	0.363 ± 0.148	2.00 ± 0.51	4.82 ± 2.22	14.6 ± 5.84	22.7 ± 2.78	55.1 ± 41.4	86.2 ± 43.1	181 ± 33.8
Cmax (ng/mL)	14.9	103	664	3730	14800	39400	88200	80800
Cmax /Dose (ng/mL)/(mg/kg/day)	0.298	2.06	3.32	18.7	37.0	98.5	110	101
Tmax (h)	1.0	0.5	1.0	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670221							
GLP compliance:	No							
Location in CTD:	4.2.3.2-1							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 400, 800							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	LBQ657		LBQ657		LBQ657		LBQ657	
Dose (mg/kg/day)	50		200		400		800	
Day	14		14		14		14	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	1330	1660	22300	38400	225000	160000	3780000	2160000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	26.6 ± 8.57	33.3 ± 9.86	111 ± 31.1	192 ± 87.8	562 ± 255	399 ± 74.4	4720 ± 4410	2700 ± 176
Cmax (ng/mL)	734	1080	21500	41300	181000	227000	330000	682000
Cmax /Dose (ng/mL)/(mg/kg/day)	14.7	21.6	108	207	453	568	413	853
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	2.0

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670221							
GLP compliance:	No							
Location in CTD:	4.2.3.2-1							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 400, 800							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	VAL489		VAL489		VAL489		VAL489	
Dose (mg/kg/day)	50		200		400		800	
Day	14		14		14		14	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	811	2440	5930	10300	18300	20800	681000	740000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	16.2 ± 5.82	48.7 ± 25.5	29.6 ± 7.22	51.3 ± 13.5	45.7 ± 8.98	52.1 ± 13.1	852 ± 779	925 ± 46.9
Cmax (ng/mL)	168	493	2690	7500	13400	15300	69800	252000
Cmax /Dose (ng/mL)/(mg/kg/day)	3.36	9.86	13.5	37.5	33.5	38.3	87.3	315
Tmax (h)	1.0	0.5	0.5	0.5	0.5	0.5	0.5	2.0

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670524							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-2							
Gender(M/F) / Number of animals:	20 animals/sex/group							
Duration:	13-weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 100, 200, 400							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377				AHU377			
Dose (mg/kg/day)	50				100			
Day	1		75		1		75	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	105 ± 62.0	425 ± 161	67.9 ± 15.5	388 ± 56.9	197 ± 25.0	758 ± 161	223 ± 47.7	462 ± 196
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	2.10 ± 1.24	8.50 ± 3.22	1.36 ± 0.310	7.77 ± 1.14	1.97 ± 0.250	7.58 ± 1.61	2.23 ± 0.477	4.62 ± 1.96
Cmax (ng/mL)	51.7	115	58.9	94.1	81.6	166	86.4	644
Cmax /Dose (ng/mL)/(mg/kg/day)	1.03	2.30	1.18	1.88	0.816	1.66	0.864	6.44
Tmax (h)	0.5	1.0	0.5	0.5	0.5	0.5	1.0	0.5

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670524							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-2							
Gender(M/F) / Number of animals:	20 animals/sex/group							
Duration:	13-weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 100, 200, 400							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377				AHU377			
Dose (mg/kg/day)	200				400			
Day	1		75		1		75	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	490 ± 106	932 ± 99.1	224 ± 40.8	3630 ± 2620	1560 ± 323	6500 ± 474	1050	14500
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	2.45 ± 0.530	4.66 ± 0.496	1.12 ± 0.204	18.1 ± 13.1	3.90 ± 0.808	16.3 ± 1.19	2.62	36.2
Cmax (ng/mL)	179	282	164	1660	748	628	1330	14200
Cmax /Dose (ng/mL)/(mg/kg/day)	0.895	1.41	0.820	8.30	1.87	1.57	3.33	35.5
Tmax (h)	1.0	0.5	0.5	0.5	0.5	1.0	0.5	0.5

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	[R0670524]							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-2							
Gender(M/F) / Number of animals:	20 animals/sex/group							
Duration:	13-weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 100, 200, 400							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	LBQ657				LBQ657			
Dose (mg/kg/day)	50				100			
Day	1		75		1		75	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	1920 ± 404	20100 ± 13200	1560 ± 127	54500 ± 9160	3860 ± 471	38900 ± 14800	4170 ± 411	12100 ± 5810
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	38.5 ± 8.08	402 ± 264	31.1 ± 2.54	1090 ± 183	38.6 ± 4.71	389 ± 148	41.7 ± 4.11	121 ± 58.1
Cmax (ng/mL)	929	6800	1330	4440	2820	5870	1560	6790
Cmax /Dose (ng/mL)/(mg/kg/day)	18.6	136	26.6	88.8	28.2	58.7	15.6	67.9
Tmax (h)	1.0	1.0	0.5	6.0	0.5	2.0	0.5	0.5

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670524							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-2							
Gender(M/F) / Number of animals:	20 animals/sex/group							
Duration:	13-weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 100, 200, 400							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	LBQ657				LBQ657			
Dose (mg/kg/day)	200				400			
Day	1		75		1		75	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	14900 ± 2530	36900 ± 5470	8660 ± 3200	363000 ± 62700	34100 ± 9760	295000 ± 45000	56100	78300
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	74.4 ± 12.7	184 ± 27.4	43.3 ± 16.0	1810 ± 314	85.2 ± 24.4	738 ± 113	140	196
Cmax (ng/mL)	9680	8060	4690	31100	23100	25500	52700	62300
Cmax /Dose (ng/mL)/(mg/kg/day)	48.4	40.3	23.5	156	57.8	63.8	132	156
Tmax (h)	0.5	0.5	0.5	6.0	0.5	6.0	1.0	0.5

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670524							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-2							
Gender(M/F) / Number of animals:	20 animals/sex/group							
Duration:	13-weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 100, 200, 400							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	VAL489				VAL489			
Dose (mg/kg/day)	50				100			
Day	1		75		1		75	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	558 ± 61.1	1510 ± 286	684 ± 83.7	2690 ± 214	1350 ± 29.0	3840 ± 606	1670 ± 104	2800 ± 652
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	11.2 ± 1.22	30.3 ± 5.72	13.7 ± 1.67	53.9 ± 4.28	13.5 ± 0.290	38.4 ± 3.06	16.7 ± 1.04	28.0 ± 6.52
Cmax (ng/mL)	133	259	338	535	439	665	454	2210
Cmax /Dose (ng/mL)/(mg/kg/day)	2.66	5.18	6.76	10.7	4.39	6.65	4.54	22.1
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR							
Study number:	R0670524							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-2							
Gender(M/F) / Number of animals:	20 animals/sex/group							
Duration:	13-weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 100, 200, 400							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	VAL489				VAL489			
Dose (mg/kg/day)	200				400			
Day	1		75		1		75	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	3120 ± 238	5480 ± 336	3620 ± 589	14800 ± 8490	4320 ± 583	20600 ± 1520	7850	16800
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	15.6 ± 1.19	27.4 ± 1.68	18.1 ± 2.95	73.9 ± 42.5	10.8 ± 1.46	51.5 ± 3.80	19.6	42.1
Cmax (ng/mL)	548	1630	1470	4020	1600	2520	3120	7180
Cmax /Dose (ng/mL)/(mg/kg/day)	2.74	8.15	7.35	20.1	4.00	6.30	7.80	18.0
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR					
Study number:	R0770896					
GLP compliance:	Yes					
Location in CTD:	4.2.3.4.1-4					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	13-weeks					
Vehicle / Formulation:	AHU377 solution in 0.5% HPC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 400, 800, 1200					
Sample:	Plasma					
Analyte:	AHU377, LBQ657					
Assay:	LC-MS/MS					
PK parameters:	AHU377					
Week	11					
Dose (mg/kg/day)	400		800		1200	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	3380 ± 1250	5410 ± 1350	8000 ± 5200	31300 ± 8410	55000	33800 ± 10300
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	8.46 ± 3.13	13.5 ± 3.38	10.0 ± 6.50	39.1 ± 10.5	45.8	28.2 ± 8.58
Cmax (ng/mL)	2810	4730	2480	28100	17200	17900
Cmax /Dose (ng/mL)/(mg/kg/day)	7.03	11.8	3.10	35.1	14.3	14.9
Tmax (h)	1.00	0.50	2.00	0.50	0.50	0.50

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR					
Study number:	R0770896					
GLP compliance:	Yes					
Location in CTD:	4.2.3.4.1-4					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	13-weeks					
Vehicle / Formulation:	AHU377 solution in 0.5% HPC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 400, 800, 1200					
Sample:	Plasma					
Analyte:	AHU377, LBQ657					
Assay:	LC-MS/MS					
PK parameters:	LBQ657					
Week	11					
Dose (mg/kg/day)	400		800		1200	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	106000 ± 60400	111000 ± 40100	178000 ± 96500	337000 ± 96000	759000	510000 ± 125000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	265 ± 151	277 ± 100	223 ± 121	422 ± 120	632	425 ± 104
Cmax (ng/mL)	100000	82700	44600	261000	230000	268000
Cmax /Dose (ng/mL)/(mg/kg/day)	250	207	55.8	326	192	223
Tmax (h)	1.00	1.00	2.00	0.50	0.50	1.00

* mean ± SEM

Toxicokinetic data	Mouse Crl:CD1® (ICR)BR
Study number:	R0870374
GLP compliance:	Yes
Location in CTD:	4.2.3.4.1-5
Gender(M/F) / Number of animals:	8 animals/sex/group
Duration:	104-weeks
Vehicle / Formulation:	AHU377 solution in 0.5% HPC
Method of Administration:	Oral gavage
Dose (mg/kg/day):	0, 150, 400, 1200
Sample:	Plasma
Analyte:	AHU377, LBQ657
Assay:	LC-MS/MS

Mean (n = 2) AHU377/LBQ657 concentrations in mouse plasma:

	0.5 h post-dose			1 h post dose		
Daosage/Day/Gender	AHU377 (ng/mL)	LBQ657 (ng/mL)	AHU377/ LBQ657 (%)	AHU377 (ng/mL)	LBQ657 (ng/mL)	AHU377/ LBQ657 (%)
150 mg/kg/day / Day 28/Male	211	8610	2.5	107	4740	2.3
150 mg/kg/day / Day 28/Female	784	7720	10.2	108	1540	7.0
400 mg/kg/day / Day 28/Male	4830	55500	8.7	683	31200	2.2
400 mg/kg/day / Day 28/Female	13400	111000	12.1	1760	32200	5.5
1200 mg/kg/day / Day 28/Male	25800	154000	16.8	5930	110000	5.4
1200 mg/kg/day / Day 28/Female	66400	301000	22.1	13000	211000	6.2
150 mg/kg/day / Day 182/Male	1660	24600	6.7	70.1	1530	4.6
150 mg/kg/day / Day 182/Female	2170	35700	6.1	276	3600	7.7
400 mg/kg/day / Day 182/Male	3350	80600	4.2	1080	50700	2.1
400 mg/kg/day / Day 182/Female	38700	306000	12.6	4530[a]	100000[a]	4.5
1200 mg/kg/day / Day 182/Male	33700	264000	12.8	981[a]	81700[a]	1.2
1200 mg/kg/day / Day 182/Female	111000[a]	380000[a]	29.2	16700	199000	8.4

[a] n = 1

2.6.5.4B 薬物動態試験：反復投与後の吸収

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670220							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-3							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 600, 1200							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377				AHU377			
Dose (mg/kg/day)	50				200			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	2070	2200	811	594	3940	1610	1410	1910
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	41.4 ± 5.43	43.9 ± 8.73	16.2 ± 3.68	11.9 ± 2.15	19.7 ± 6.04	8.07 ± 1.43	7.03 ± 1.30	9.56 ± 1.86
Cmax (ng/mL)	206	162	163	306	418	772	786	1510
Cmax /Dose (ng/mL)/(mg/kg/day)	4.12	3.24	3.26	6.12	2.09	3.86	3.93	7.55
Tmax (h)	1.0	2.0	0.5	0.5	2.0	1.0	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670220							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-3							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 600, 1200							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377				AHU377			
Dose (mg/kg/day)	600				1200			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	4550	8580	5600	4720	22100	12800	9450	12200
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	7.58 ± 2.22	14.3 ± 2.49	9.33 ± 0.880	7.87 ± 0.700	18.4 ± 4.63	10.7 ± 2.86	7.88 ± 0.200	10.2 ± 0.110
Cmax (ng/mL)	572	1110	2330	1270	2080	2610	1810	1400
Cmax /Dose (ng/mL)/(mg/kg/day)	0.953	1.85	3.88	2.12	1.73	2.18	1.51	1.17
Tmax (h)	2.0	1.0	0.5	0.5	2.0	0.5	1.0	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670220							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-3							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 600, 1200							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	LBQ657				LBQ657			
Dose (mg/kg/day)	50				200			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	13400	11700	6070	10500	41200	29400	41100	54400
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	268 ± 43.2	235 ± 18.3	121 ± 33.3	209 ± 30.3	206 ± 86.9	147 ± 41.6	206 ± 33.1	272 ± 57.6
Cmax (ng/mL)	2780	3270	4230	6140	6520	8350	14300	20500
Cmax /Dose (ng/mL)/(mg/kg/day)	55.6	65.4	84.6	123	32.6	41.8	71.5	103
Tmax (h)	0.5	0.5	0.5	0.5	1.0	0.5	1.0	0.5

* mean ± SEM

Toxicokinetic data	Rat Wistar Hannover							
Study number:	R0670220							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-3							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 600, 1200							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	LBQ657				LBQ657			
Dose (mg/kg/day)	600				1200			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	70200	122000	112000	193000	203000	126000	315000	222000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	117 ± 14.3	203 ± 57.2	187 ± 21.5	322 ± 43.6	169 ± 67.5	105 ± 28.2	262 ± 13.1	185 ± 3.98
Cmax (ng/mL)	11800	14200	36700	27900	19600	29200	35300	28500
Cmax /Dose (ng/mL)/(mg/kg/day)	19.7	23.7	61.2	46.5	16.3	24.3	29.4	23.8
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat Wistar Hannover							
Study number:	R0670220							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-3							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 600, 1200							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	VAL489				VAL489			
Dose (mg/kg/day)	50				200			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	13000	13200	12100	20100	62500	52000	90100	74700
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	260 ± 26.7	264 ± 34.9	243 ± 23.7	402 ± 109	312 ± 99.7	260 ± 57.5	450 ± 125	373 ± 68.0
Cmax (ng/mL)	3550	3930	4930	5990	12000	8690	19600	12900
Cmax /Dose (ng/mL)/(mg/kg/day)	71.0	78.6	98.6	120	60.0	43.5	98.0	64.5
Tmax (h)	0.5	0.5	0.5	0.5	1.0	0.5	1.0	0.5

* mean ± SEM

Toxicokinetic data	Rat Wistar Hannover							
Study number:	R0670220							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-3							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 50, 200, 600, 1200							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	VAL489				VAL489			
Dose (mg/kg/day)	600				1200			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	160000	186000	247000	299000	390000	342000	562000	379000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	267 ± 58.7	310 ± 62.6	412 ± 79.9	499 ± 75.2	325 ± 56.7	285 ± 57.5	468 ± 12.2	315 ± 2.02
Cmax (ng/mL)	18800	26900	32800	28500	49100	94300	43100	36000
Cmax /Dose (ng/mL)/(mg/kg/day)	31.3	44.8	54.7	47.5	40.9	78.6	35.9	30.0
Tmax (h)	0.5	0.5	0.5	2.0	0.5	0.5	1.0	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670468							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-4							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 10, 30							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377				AHU377			
Dose (mg/kg/day)	10				30			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	NA	NA	NA	NA	71.9	57.3	56.3	48.6
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	NA	NA	NA	NA	2.40 ± 0.863	1.91 ± 0.654	1.88 ± 0.661	1.62 ± 0.589
Cmax (ng/mL)	NA	NA	NA	NA	58.6	35.6	88.5	49.1
Cmax /Dose (ng/mL)/(mg/kg/day)	NA	NA	NA	NA	1.95	1.19	2.95	1.64
Tmax (h)	NA	NA	NA	NA	0.5	0.5	0.5	0.5

NA: not available. TK parameters not calculated due to lack of sufficient measurable AHU377 concentrations.

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670468							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-4							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 10, 30							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	LBQ657				LBQ657			
Dose (mg/kg/day)	10				30			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	3440	2390	2350	2490	7120	8070	6310	5420
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	344 ± 58.9	239 ± 53.6	235 ± 54.3	249 ± 125	237 ± 17.2	269 ± 136	210 ± 37.1	181 ± 61.2
Cmax (ng/mL)	1050	1020	1410	1150	3760	2300	3760	4550
Cmax /Dose (ng/mL)/(mg/kg/day)	105	102	141	115	125	76.7	125	152
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670468							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-4							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	2 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 10, 30							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	VAL489				VAL489			
Dose (mg/kg/day)	10				30			
Day	1		13		1		13	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	5440	4020	4950	3950	13200	11800	10700	11500
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	544 ± 42.7	402 ± 89.5	495 ± 118	395 ± 137	440 ± 30.2	393 ± 181	357 ± 32.5	383 ± 120
Cmax (ng/mL)	1170	939	1750	1250	3940	2190	3710	3580
Cmax /Dose (ng/mL)/(mg/kg/day)	117	93.9	175	125	131	73.0	124	119
Tmax (h)	0.5	0.5	0.5	0.5	0.5	1.0	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670283							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-5							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	13 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 10, 30, 100							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377				AHU377			
Dose (mg/kg/day)	10				30			
Day	1		76		1		76	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	NA	NA	NA	NA	NA	NA	NA	NA
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	NA	NA	NA	NA	NA	NA	NA	NA
Cmax (ng/mL)	16.5	16.7	10.0	15.9	41.7	38.2	19.0	11.9
Cmax /Dose (ng/mL)/(mg/kg/day)	1.65	1.67	1.00	1.59	1.39	1.27	0.633	0.397
Tmax (h)	24.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670283							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-5							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	13 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 10, 30, 100							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	AHU377				LBQ657			
Dose (mg/kg/day)	100				10			
Day	1		76		1		76	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	363 ± 105	351 ± 122	345 ± 56.9	291 ± 89.0	2670 ± 691	1830 ± 442	2110 ± 239	1470 ± 393
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	3.63 ± 1.05	3.51 ± 1.22	3.45 ± 0.569	2.91 ± 0.890	267 ± 69.1	183 ± 44.2	211 ± 23.9	147 ± 39.3
Cmax (ng/mL)	239	143	219	126	1130	1330	1650	1480
Cmax /Dose (ng/mL)/(mg/kg/day)	2.39	1.43	2.19	1.26	113	133	165	148
Tmax (h)	0.5	0.5	0.5	0.5	1.0	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670283							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-5							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	13 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 10, 30, 100							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	LBQ657				LBQ657			
Dose (mg/kg/day)	30				100			
Day	1		76		1		76	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	6290 ± 2040	4490 ± 851	5160 ± 1380	4800 ± 739	22800 ± 2720	23600 ± 5720	36300 ± 3630	19300 ± 3230
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	210 ± 68.0	150 ± 28.4	172 ± 46.0	160 ± 24.6	228 ± 27.2	236 ± 57.2	363 ± 36.3	193 ± 32.3
Cmax (ng/mL)	2840	1430	1900	1520	9710	4670	12800	5740
Cmax /Dose (ng/mL)/(mg/kg/day)	94.7	47.7	63.3	50.7	97.1	46.7	128	57.4
Tmax (h)	1.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670283							
GLP compliance:	Yes							
Location in CTD:	4.2.3.2-5							
Gender(M/F) / Number of animals:	10 animals/sex/group							
Duration:	13 weeks							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Oral gavage							
Dose (mg/kg/day):	0, 10, 30, 100							
Sample:	Plasma							
Analyte:	AHU377, LBQ657 or VAL489							
Assay:	LC-MS/MS							
PK parameters:	VAL489				VAL489			
Dose (mg/kg/day)	10				30			
Day	1		76		1		76	
Gender	M	F	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	6210 ± 1050	3950 ± 1060	4840 ± 726	3170 ± 431	17000 ± 4400	12600 ± 2980	11500 ± 3090	10300 ± 978
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	621 ± 105	395 ± 106	484 ± 72.6	317 ± 43.1	565 ± 147	421 ± 99.3	382 ± 103	344 ± 32.6
Cmax (ng/mL)	1690	1820	1990	1290	3440	2030	3440	1900
Cmax /Dose (ng/mL)/(mg/kg/day)	169	182	199	129	115	67.7	115	63.3
Tmax (h)	1.0	0.5	0.5	0.5	1.0	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover			
Study number:	R0670283			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-5			
Gender(M/F) / Number of animals:	10 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 10, 30, 100			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	VAL489			
Dose (mg/kg/day)	100			
Day	1		76	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	47800 ± 6430	46600 ± 4610	53500 ± 6620	32600 ± 9390
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	478 ± 64.3	466 ± 46.1	535 ± 66.2	326 ± 93.9
Cmax (ng/mL)	12300	9080	8660	5640
Cmax /Dose (ng/mL)/(mg/kg/day)	123	90.8	86.6	56.4
Tmax (h)	0.5	1.0	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	AHU377					
Dose (mg/kg/day)	10					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	18.8 ± 11.6	12.4 ± 9.14	NA	NA	NA	NA
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	1.88 ± 1.16	1.24 ± 0.914	NA	NA	NA	NA
Cmax (ng/mL)	15.4	10.8	24.6	16.2	7.70	7.25
Cmax /Dose (ng/mL)/(mg/kg/day)	1.54	1.08	2.46	1.62	0.770	0.725
Tmax (h)	1.0	1.0	0.5	0.5	0.5	0.5

NA: AUC were not calculated due to lack of sufficient measurable AHU377 concentrations.

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	AHU377					
Dose (mg/kg/day)	30					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	29.9 ± 21.5	NA	33.2 ± 4.93	51.5 ± 25.6	15.3 ± 9.08	74.7 ± 25.9
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	0.995 ± 0.717	NA	1.11 ± 0.164	1.72 ± 0.853	0.510 ± 0.303	2.48 ± 0.863
Cmax (ng/mL)	28.6	41.2	37.3	47.3	12.6	61.7
Cmax /Dose (ng/mL)/(mg/kg/day)	0.953	1.37	1.24	1.58	0.420	2.06
Tmax (h)	1.0	0.5	0.5	0.5	0.5	0.5

NA: AUC were not calculated due to lack of sufficient measurable AHU377 concentrations.

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	AHU377					
Dose (mg/kg/day)	100					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	374 ± 174	184 ± 77.0	163	483 ± 273	253 ± 134	239 ± 59.4
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	3.74 ± 1.74	1.84 ± 0.770	1.63	4.83 ± 2.73	2.53 ± 1.34	2.39 ± 0.594
Cmax (ng/mL)	202	128	155	312	87.2	206
Cmax /Dose (ng/mL)/(mg/kg/day)	2.02	1.28	1.55	3.12	0.872	2.06
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5

NA: AUC were not calculated due to lack of sufficient measurable AHU377 concentrations.

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	LBQ657					
Dose (mg/kg/day)	10					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	1630 ± 346	1170 ± 305	1470 ± 678	1580 ± 556	1620 ± 496	1730 ± 452
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	163 ± 34.6	117 ± 30.5	147 ± 67.8	158 ± 55.6	162 ± 49.6	173 ± 45.2
Cmax (ng/mL)	1190	1160	1040	1550	1360	1830
Cmax /Dose (ng/mL)/(mg/kg/day)	119	116	104	155	136	183
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	LBQ657					
Dose (mg/kg/day)	30					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	5300 ± 2160	3410 ± 557	3830 ± 867	3310 ± 486	3620 ± 670	6720 ± 1980
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	177 ± 72.0	114 ± 18.6	128 ± 28.9	110 ± 16.2	121 ± 22.3	224 ± 66.0
Cmax (ng/mL)	1770	3540	2000	2260	1600	4140
Cmax /Dose (ng/mL)/(mg/kg/day)	59.0	118	66.7	75.3	53.3	138
Tmax (h)	1.0	0.5	1.0	0.5	1.0	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	LBQ657					
Dose (mg/kg/day)	100					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	24700 ± 6760	11200 ± 3900	21100	10900 ± 2400	14700 ± 5280	15700 ± 5590
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	247 ± 67.6	112 ± 39.0	211	109 ± 24.0	147 ± 52.8	157 ± 55.9
Cmax (ng/mL)	9400	4250	15700	8090	4930	10600
Cmax /Dose (ng/mL)/(mg/kg/day)	94.0	42.5	157	80.9	49.3	106
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	VAL489					
Dose (mg/kg/day)	10					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	3210 ± 743	2710 ± 451	2410 ± 619	2740 ± 474	2710 ± 584	2240 ± 345
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	321 ± 74.3	271 ± 45.1	241 ± 61.9	274 ± 47.4	271 ± 58.4	224 ± 34.5
Cmax (ng/mL)	1250	1430	1360	1340	1110	1440
Cmax /Dose (ng/mL)/(mg/kg/day)	125	143	136	134	111	144
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	VAL489					
Dose (mg/kg/day)	30					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	12400 ± 2730	6220 ± 664	8660 ± 1240	5580 ± 967	8650 ± 1980	7560 ± 2450
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	413 ± 91.0	207 ± 22.1	289 ± 41.3	186 ± 32.2	288 ± 66.0	252 ± 81.7
Cmax (ng/mL)	2610	4210	2530	2470	1240	3080
Cmax /Dose (ng/mL)/(mg/kg/day)	87.0	140	84.3	82.3	41.3	103
Tmax (h)	1.0	0.5	0.5	0.5	1.0	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670620					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-6					
Gender(M/F) / Number of animals:	10 animals/sex/group					
Duration:	26 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 10, 30, 100					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	VAL489					
Dose (mg/kg/day)	100					
Day	1		24		150	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)*	47200 ± 7760	31200 ± 7410	47400	50800 ± 27800	28400 ± 5680	25900 ± 4850
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	472 ± 77.6	312 ± 74.1	474	508 ± 278	284 ± 56.8	259 ± 48.5
Cmax (ng/mL)	9600	6250	11200	6970	4660	5060
Cmax /Dose (ng/mL)/(mg/kg/day)	96.0	62.5	112	69.7	46.6	50.6
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5

* mean ± SEM

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0670624					
GLP compliance:	No					
Location in CTD:	4.2.3.6-2					
Gender(M/F) / Number of animals:	M/10 animals/group					
Duration:	5 days					
Vehicle / Formulation:	Oral: LCZ696 suspension in 0.5% CMC					
	Subcutaneous: LCZ696 solutions in 0.9% saline					
Method of Administration:	Oral gavage					
	Subcutaneous injection					
Dose (mg/kg/day):	200					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
	Plasma concentration (ng/mL)					
PK parameters:	AHU377		LBQ657		VAL489	
Day	5 (2 hours post-dose)		5 (2 hours post-dose)		5 (2 hours post-dose)	
Dose Route	p.o.	s.c.	p.o.	s.c.	p.o.	s.c.
	125	984	7430	24800	15200	88100
	359	1370	9560	23500	14300	96200
	19.9	1380	2160	22700	2880	82300
	123	1230	7050	19900	10700	71000
	70.4	1660	1000	18200	4720	74600
	20.3	3490	438	22600	985	93700
	45.9	527	1650	14900	3930	55500
	32.1	1980	1320	27700	3920	98900
	11.0	1780	407	18000	572	63700
	186	1210	5690	19200	11800	69500

Toxicokinetic data	Rat, Wistar Hannover						
Study number:	R0770711						
GLP compliance:	Yes						
Location in CTD:	4.2.3.2-18						
Gender(M/F) / Number of animals:	13 week: 10 animals/sex/group 4 week: 6 animals/sex/group						
Duration:	4 and 13 weeks						
Vehicle / Formulation:	AHU377 solution in 0.5% Klucel						
Method of Administration:	Oral gavage						
Dose (mg/kg/day):	0, 400, 800, 1200						
Sample:	Plasma						
Analyte:	AHU377, LBQ657						
Assay:	LC-MS/MS						
PK parameters:	AHU377 (4-week subgroup)						
Dose (mg/kg/day)	Study Day	Gender	AUC0-24 h (ng•h/mL)	AUC/Dose (ng•h/mL)/(mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL)/(mg/kg/day)	Tmax (h)
400	1	Male	5370	13.4	380	0.950	6.00
		Female	4810	12.0	872	2.18	0.500
	27	Male	2330	5.83	936	2.34	1.00
		Female	4010	10.0	1500	3.75	0.500
800	1	Male	10900	13.7	1960	2.45	0.500
		Female	15100	18.9	1120	1.40	6.00
	27	Male	13800	17.3	4660	5.83	0.500
		Female	8810	11.0	3090	3.86	0.500
1200	1	Male	10400	8.65	1880	1.57	0.500
		Female	5050	4.21	590	0.492	1.00
	27	Male	35100	29.3	4300	3.58	0.500
		Female	27800	23.1	3640	3.03	1.00

Toxicokinetic data	Rat, Wistar Hannover						
PK parameters:	LBQ657 (4-week subgroup)						
Dose (mg/kg/day)	Study Day	Gender	AUC0-24 h (ng•h/mL)	AUC/Dose (ng•h/mL)/(mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL)/(mg/kg/day)	Tmax (h)
400	1	Male	186000	464	19300	48.3	1.00
		Female	150000	375	20500	51.3	0.500
	27	Male	113000	284	54100	135	1.00
		Female	166000	414	34900	87.3	1.00
800	1	Male	379000	474	27300	34.1	6.00
		Female	344000	430	24000	30.0	2.00
	27	Male	401000	501	64000	80.0	0.500
		Female	250000	312	56300	70.4	0.500
1200	1	Male	292000	243	22500	18.8	0.500
		Female	104000	86.5	15900	13.3	1.00
	27	Male	1110000	928	84100	70.1	6.00
		Female	916000	763	98600	82.2	1.00
PK parameters:	AHU377 (13-week subgroup)						
Dose (mg/kg/day)	Study Day	Gender	AUC0-24 h (ng•h/mL)	AUC/Dose (ng•h/mL)/(mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL)/(mg/kg/day)	Tmax (h)
400	1	Male	6310	15.8	597	1.49	0.500
		Female	4220	10.5	377	0.943	2.00
	72	Male	9710	24.3	2370	5.93	0.500
		Female	5610	14.0	1850	4.63	0.500
800	1	Male	9570	12.0	804	1.01	0.500
		Female	7260	9.08	1260	1.58	1.00
	72	Male	11900	14.9	2510	3.14	0.500
		Female	24500	30.6	3320	4.15	0.500
1200	1	Male	13100	10.9	1680	1.40	0.500
		Female	10500	8.75	1440	1.20	0.500
	72	Male	39500	32.9	4350	3.63	0.500
		Female	24600	20.5	2390	1.99	1.00

Toxicokinetic data	Rat, Wistar Hannover						
PK parameters:	LBQ657 (13-week subgroup)						
Dose (mg/kg/day)	Study Day	Gender	AUC0-24 h (ng•h/mL)	AUC/Dose (ng•h/mL)/(mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL)/(mg/kg/day)	Tmax (h)
400	1	Male	170000	424	20300	50.8	0.500
		Female	132000	331	10800	27.0	2.00
	72	Male	266000	666	53900	135	0.500
		Female	214000	534	74500	186	0.500
800	1	Male	246000	307	26100	32.6	0.500
		Female	161000	201	26600	33.3	1.00
	72	Male	304000	380	46800	58.5	0.500
		Female	475000	593	71200	89.0	0.500
1200	1	Male	422000	351	33800	28.2	0.500
		Female	356000	296	26900	22.4	0.500
	72	Male	807000	673	78700	65.6	1.00
		Female	739000	615	115000	95.8	1.00

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R0870373					
GLP compliance:	Yes					
Location in CTD:	4.2.3.4.1-6					
Gender(M/F) / Number of animals:	4 animals/sex/group					
Duration:	104 weeks					
Vehicle / Formulation:	AHU377 solution in 0.5% HPC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 50, 150, 400					
Sample:	Plasma					
Analyte:	AHU377, LBQ657					
Assay:	LC-MS/MS					
Mean plasma concentration (n = 2)	0.5 h post -dose			1 h post-dose		
	AHU377 (ng/mL)	LBQ657 (ng/mL)	AHU377/ LBQ657 (%)	AHU377 (ng/mL)	LBQ657 (ng/mL)	AHU377/ LBQ657 (%)
50 mg/kg/day / Day 24/Male	105	5230	2.0	70.1	5380	1.3
50 mg/kg/day / Day 24/Female	63.4	3610	1.8	66.2	5020	1.3
150 mg/kg/day / Day 24/Male	234	11500	2.0	272	9540	2.9
150 mg/kg/day / Day 24/Female	231	9730	2.4	259	14000	1.9
400 mg/kg/day / Day 24/Male	912	29100	3.1	676	29800	2.3
400 mg/kg/day / Day 24/Female	214	12800	1.7	687	23800	2.9
50 mg/kg/day / Day 178/Male	124	11900	1.0	87.4	5690	1.5
50 mg/kg/day / Day 178/Female	88.9	5840	1.5	91.5	13200	0.7
150 mg/kg/day / Day 178/Male	411	15600	2.6	132	8500	1.6
150 mg/kg/day / Day 178/Female	598	28700	2.1	457	30500	1.5
400 mg/kg/day / Day 178/Male	1510	25300	6.0	531	27900	1.9
400 mg/kg/day / Day 178/Female	2110	58100	3.6	1000	54200	1.8

Toxicokinetic data	Rat, Wistar Hannover					
Study number:	R1270139					
GLP compliance:	Yes					
Location in CTD:	4.2.3.7.3-3					
Gender(M/F) / Number of animals:	F / 10 animals/ group					
Duration:	13 weeks					
Vehicle / Formulation:	AHU377 solution in 0.5% HPC					
Method of Administration:	Oral gavage					
Dose (mg/kg/day):	0, 100, 400					
Sample:	Plasma					
Analyte:	AHU377, LBQ657					
Assay:	LC-MS/MS					
PK Parameters:	AHU377					
Dose (mg/kg/day)	Study Day	AUC0-24 h (ng•h/mL)*	AUC/Dose (ng•h/mL)/(mg/kg/day)*	Cmax (ng/mL)	Cmax/Dose (ng/mL)/(mg/kg/day)	Tmax (h)
100	1	806 ± 99.3	8.06 ± 0.993	394	3.94	1.0
	78	1020 ± 148	10.2 ± 1.48	508	5.08	1.0
400	1	4460 ± 2200	11.2 ± 5.50	745	1.86	0.5
	78	4130 ± 1690	10.3 ± 4.23	1080	2.70	0.5
	LBQ657					
100	1	38600 ± 5430	386 ± 54.3	17000	170	0.5
	78	71200 ± 17500	712 ± 175	24500	245	0.5
400	1	152000 ± 63700	381 ± 159	27600	69.0	0.5
	78	263000 ± 38200	657 ± 95.5	70600	177	0.5

*mean ± SEM

2.6.5.4C 薬物動態試験：反復投与後の吸収

Toxicokinetic data	Monkey, cyno			
Study number:	R0670259			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-7			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	1 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	AHU377		AHU377	
Dose (mg/kg/day)	25 (mean ± SD)		100 (mean ± SD)	
Day	1		1	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	6440 ± 2930	3710 ± 972	27500 ± 9690	31000 ± 11000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	257 ± 117	148 ± 38.9	275 ± 96.9	310 ± 110
Cmax (ng/mL)	2810 ± 1940	2030 ± 690	13600 ± 8370	12700 ± 9140
Cmax /Dose (ng/mL)/(mg/kg/day)	112 ± 77.4	81.1 ± 27.5	136 ± 83.7	127 ± 91.4
Tmax (h)	1.00 ± 0.00	0.667 ± 0.289	1.17 ± 0.764	1.00 ± 0.866

Toxicokinetic data	Monkey, cyno			
Study number:	R0670259			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-7			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	1 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	LBQ657		LBQ657	
Dose (mg/kg/day)	25 (mean ± SD)		100 (mean ± SD)	
Day	1		1	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	31700 ± 4320	29800 ± 2310	180000 ± 9170	147000 ± 28300
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	1270 ± 173	1190 ± 96.4	1800 ± 91.7	1470 ± 283
Cmax (ng/mL)	8750 ± 1840	16300 ± 6240	45100 ± 2610	45000 ± 23200
Cmax /Dose (ng/mL)/(mg/kg/day)	350 ± 73.5	653 ± 250	451 ± 26.1	450 ± 232
Tmax (h)	1.00 ± 0.00	0.833 ± 0.289	1.33 ± 0.577	1.33 ± 0.577

Toxicokinetic data	Monkey, cyno			
Study number:	R0670259			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-7			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	1 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	VAL489		VAL489	
Dose (mg/kg/day)	25 (mean ± SD)		100 (mean ± SD)	
Day	1		1	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	5700 ±1960	5300 ± 2400	16900 ± 1190	17100 ± 7140
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	228 ± 77.8	212 ± 96.0	169 ± 11.9	171 ± 71.4
Cmax (ng/mL)	1160 ± 50.0	876 ± 309	2750 ± 1680	2180 ± 737
Cmax /Dose (ng/mL)/(mg/kg/day)	46.4 ± 2.00	35.0 ± 12.4	27.5 ± 16.8	21.8 ± 7.37
Tmax (h)	0.667 ± 0.289	0.833 ± 0.289	0.667 ± 0.289	0.667 ± 0.289

Toxicokinetic data	Monkey, cyno			
Study number:	R0670276			
GLP compliance:	No			
Location in CTD:	4.2.3.2-8			
Gender(M/F) / Number of animals:	1-2 animals/sex/group			
Duration:	2 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 50, 100, 300, 600			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n=1)		AHU377 (n=1)	
Dose (mg/kg/day)	50		100	
Week	2		2	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	7950	15000	38300	50100
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	159	300	383	501
Cmax (ng/mL)	2010	2980	13500	23400
Cmax /Dose (ng/mL)/(mg/kg/day)	40.2	59.6	135	234
Tmax (h)	0.5	1.0	0.5	0.5

Toxicokinetic data	Monkey, cyno			
Study number:	R0670276			
GLP compliance:	No			
Location in CTD:	4.2.3.2-8			
Gender(M/F) / Number of animals:	1-2 animals/sex/group			
Duration:	2 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 50, 100, 300, 600			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n=1)		AHU377 (n=2)	
Dose (mg/kg/day)	300		600	
Week	2		2	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	258000	181000	778000	269000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	861	602	1300	448
Cmax (ng/mL)	105000	42500	174000	37100
Cmax /Dose (ng/mL)/(mg/kg/day)	350	142	290	61.8
Tmax (h)	1.0	2.0	0.5	1.5

Toxicokinetic data	Monkey, cyno			
Study number:	R0670276			
GLP compliance:	No			
Location in CTD:	4.2.3.2-8			
Gender(M/F) / Number of animals:	1-2 animals/sex/group			
Duration:	2 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 50, 100, 300, 600			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n=1)		LBQ657 (n=1)	
Dose (mg/kg/day)	50		100	
Week	2		2	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	70000	88800	233000	284000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	1400	1780	2330	2840
Cmax (ng/mL)	16700	19600	51700	68900
Cmax /Dose (ng/mL)/(mg/kg/day)	334	392	517	689
Tmax (h)	2.0	2.0	2.0	2.0

Toxicokinetic data	Monkey, cyno			
Study number:	R0670276			
GLP compliance:	No			
Location in CTD:	4.2.3.2-8			
Gender(M/F) / Number of animals:	1-2 animals/sex/group			
Duration:	2 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 50, 100, 300, 600			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n=1)		LBQ657 (n=2)	
Dose (mg/kg/day)	300		600	
Week	2		2	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	1100000	1220000	4010000	2990000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	3660	4060	6680	4980
Cmax (ng/mL)	299000	162000	355000	215000
Cmax /Dose (ng/mL)/(mg/kg/day)	997	540	592	358
Tmax (h)	2.0	2.0	4.0	4.0

Toxicokinetic data	Monkey, cyno			
Study number:	R0670276			
GLP compliance:	No			
Location in CTD:	4.2.3.2-8			
Gender(M/F) / Number of animals:	1-2 animals/sex/group			
Duration:	2 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 50, 100, 300, 600			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	VAL489 (n=1)		VAL489 (n=1)	
Dose (mg/kg/day)	50		100	
Week	2		2	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	8110	8980	35200	14200
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	162	180	352	142
Cmax (ng/mL)	1090	1260	2300	2440
Cmax /Dose (ng/mL)/(mg/kg/day)	21.8	25.2	23.0	24.4
Tmax (h)	1.0	1.0	1.0	2.0

Toxicokinetic data	Monkey, cyno			
Study number:	R0670276			
GLP compliance:	No			
Location in CTD:	4.2.3.2-8			
Gender(M/F) / Number of animals:	1-2 animals/sex/group			
Duration:	2 week			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 50, 100, 300, 600			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	VAL489 (n=1)		VAL489 (n=2)	
Dose (mg/kg/day)	300		600	
Week	2		2	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	157000	98100	488000	274000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	522	327	814	457
Cmax (ng/mL)	46900	16500	136000	43200
Cmax /Dose (ng/mL)/(mg/kg/day)	156	55.0	227	72.0
Tmax (h)	1.0	0.5	1.0	0.5

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	AHU377			
Dose (mg/kg/day)	30			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	6590 ± 1650	6450 ± 2850	5990 ± 728	5760 ± 2700
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	219 ± 55.2	215 ± 94.9	200 ± 24.2	192 ± 90.0
Cmax (ng/mL)	3710	5800	4920	4020
Cmax /Dose (ng/mL)/(mg/kg/day)	124	193	164	134
Tmax (h)	0.8	0.5	0.5	0.5

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	AHU377			
Dose (mg/kg/day)	100			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	24500 ± 13900	40100 ± 19800	24600 ± 16800	34500 ± 22800
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	245 ± 139	401 ± 198	246 ± 168	345 ± 228
Cmax (ng/mL)	8300	16000	11900	15500
Cmax /Dose (ng/mL)/(mg/kg/day)	83.0	160	119	155
Tmax (h)	1.2	0.8	1.2	0.5

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	AHU377			
Dose (mg/kg/day)	300			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	126000 ± 67600	134000 ± 61600	147000 ± 21500	154000 ± 29400
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	419 ± 226	445 ± 204	491 ± 73.0	515 ± 98.5
Cmax (ng/mL)	42400	49200	96000	71200
Cmax /Dose (ng/mL)/(mg/kg/day)	142	164	320	237
Tmax (h)	1.2	1.5	0.5	1.0

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	LBQ657			
Dose (mg/kg/day)	30			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	47700 ± 723	35800 ± 8150	37800 ± 529	34000 ± 6550
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	1590 ± 26.5	1190 ± 272	1260 ± 17.3	1130 ± 219
Cmax (ng/mL)	18000	17200	17600	17700
Cmax /Dose (ng/mL)/(mg/kg/day)	600	573	587	591
Tmax (h)	1.2	0.7	0.8	0.5

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	LBQ657			
Dose (mg/kg/day)	100			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	189000 ± 27100	146000 ± 32400	176000 ± 35900	146000 ± 34200
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	1890 ± 271	1460 ± 324	1760 ± 359	1460 ± 342
Cmax (ng/mL)	53900	40300	47400	48300
Cmax /Dose (ng/mL)/(mg/kg/day)	539	403	474	483
Tmax (h)	1.7	1.0	1.7	0.5

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	LBQ657			
Dose (mg/kg/day)	300			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	687000 ± 322000	551000 ± 206000	748000 ± 137000	894000 ± 482000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	2290 ± 1080	1840 ± 688	2490 ± 458	2990 ± 1620
Cmax (ng/mL)	129000	126000	183000	186000
Cmax /Dose (ng/mL)/(mg/kg/day)	430	419	610	620
Tmax (h)	2.0	1.7	1.3	1.7

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	VAL489			
Dose (mg/kg/day)	30			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	7900 ± 1360	8540 ± 455	5650 ± 1500	4920 ± 1310
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	263 ± 45.2	285 ± 15.0	188 ± 50.2	164 ± 43.7
Cmax (ng/mL)	1310	1700	1170	1110
Cmax /Dose (ng/mL)/(mg/kg/day)	43.8	56.8	38.9	36.9
Tmax (h)	1.0	1.5	0.5	0.5

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	VAL489			
Dose (mg/kg/day)	100			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	29200 ± 8190	15200 ± 4120	20500 ± 3610	12500 ± 1810
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	292 ± 81.9	152 ± 41.2	205 ± 36.1	125 ± 18.1
Cmax (ng/mL)	6250	3260	2990	3140
Cmax /Dose (ng/mL)/(mg/kg/day)	62.5	32.6	29.9	31.4
Tmax (h)	2.3	1.0	1.5	0.5

* mean ± SD

Toxicokinetic data	Monkey, cyno			
Study number:	R0670282			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-9			
Gender(M/F) / Number of animals:	3 animals/sex/group			
Duration:	13 weeks			
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 30, 100, 300			
Sample:	Plasma			
Analyte:	AHU377, LBQ657 or VAL489			
Assay:	LC-MS/MS			
PK parameters:	VAL489			
Dose (mg/kg/day)	300			
Day	1		73	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)*	97700 ± 31700	78300 ± 32100	74900 ± 14100	70300 ± 36400
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)*	326 ± 107	262 ± 108	250 ± 46.6	234 ± 121
Cmax (ng/mL)	30000	21700	33400	16200
Cmax /Dose (ng/mL)/(mg/kg/day)	100	72.4	111	54.1
Tmax (h)	0.7	1.7	0.5	0.7

* mean ± SD

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	AHU377 (n = 4)					
Dose (mg/kg/day)	30 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	4650 ± 2500	3130 ± 1200	5110 ± 3270	4160 ± 1530	2640 ± 1010	2000 ± 594
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	155 ± 83.1	104 ± 40.1	170 ± 109	138 ± 50.8	87.9 ± 33.9	66.5 ± 19.8
Cmax (ng/mL)	2310 ± 1370	1340 ± 516	4470 ± 3670	1880 ± 1060	1270 ± 996	1020 ± 742
Cmax /Dose (ng/mL)/(mg/kg/day)	77.1 ± 45.7	44.6 ± 17.2	149 ± 122	62.5 ± 35.3	42.5 ± 33.2	34.1 ± 24.7
Tmax (h)	0.750	0.500	0.500	0.625	0.750	0.625

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	AHU377 (n = 4)					
Dose (mg/kg/day)	100 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	19100 ± 4550	23000 ± 12100	24500 ± 8490	28300 ± 10400	20800 ± 4630	20100 ± 10800
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	191 ± 45.5	230 ± 121	245 ± 84.9	283 ± 104	208 ± 46.3	201 ± 108
Cmax (ng/mL)	11000 ± 4970	14800 ± 7900	12500 ± 10900	16400 ± 10800	13100 ± 3680	11700 ± 6990
Cmax /Dose (ng/mL)/(mg/kg/day)	110 ± 49.7	148 ± 79.0	125 ± 109	164 ± 108	131 ± 36.8	117 ± 69.9
Tmax (h)	0.750	0.750	0.750	0.625	0.625	0.625

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	AHU377 (n = 6)					
Dose (mg/kg/day)	300 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M (n = 5)	F
AUC0-24 h (ng•h/mL)	123000 ± 45400	121000 ± 9430	119000 ± 26100	130000 ± 23100	93400 ± 54200	111000 ± 41000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	409 ± 151	402 ± 30.8	397 ± 87.6	433 ± 76.7	311 ± 180	371 ± 136
Cmax (ng/mL)	51800 ± 24200	46400 ± 29200	41500 ± 12400	56800 ± 20400	36900 ± 35100	38300 ± 20000
Cmax /Dose (ng/mL)/(mg/kg/day)	173 ± 80.7	155 ± 97.2	139 ± 41.3	190 ± 67.9	123 ± 117	128 ± 66.6
Tmax (h)	1.00	1.00	1.42	0.667	1.60	1.08

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	LBQ657 (n = 4)					
Dose (mg/kg/day)	30 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	29300 ± 4570	26500 ± 6560	27900 ± 4210	28800 ± 8820	30300 ± 7180	24100 ± 10700
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	978 ± 154	882 ± 217	931 ± 142	960 ± 296	1010 ± 239	802 ± 356
Cmax (ng/mL)	11400 ± 1830	7940 ± 2400	11200 ± 2670	13400 ± 6920	8680 ± 3310	6680 ± 3720
Cmax /Dose (ng/mL)/(mg/kg/day)	381 ± 61.0	264 ± 80.1	374 ± 89.0	447 ± 230	289 ± 110	223 ± 124
Tmax (h)	0.875	0.750	0.500	0.625	1.00	0.875

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	LBQ657 (n = 4)					
Dose (mg/kg/day)	100 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	117000 ± 37700	140000 ± 37200	129000 ± 31100	135000 ± 27400	148000 ± 50700	156000 ± 22100
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	1170 ± 377	1400 ± 372	1290 ± 311	1350 ± 274	1480 ± 507	1560 ± 221
Cmax (ng/mL)	38900 ± 23400	55300 ± 33200	37400 ± 21500	45800 ± 15400	44600 ± 30400	48500 ± 15000
Cmax /Dose (ng/mL)/(mg/kg/day)	389 ± 234	553 ± 332	374 ± 215	458 ± 154	446 ± 304	485 ± 150
Tmax (h)	1.13	1.13	1.13	0.750	0.750	1.00

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	LBQ657 (n = 6)					
Dose (mg/kg/day)	300 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M (n = 5)	F
AUC0-24 h (ng•h/mL)	673000 ± 176000	551000 ± 119000	884000 ± 676000	564000 ± 68700	659000 ± 110000	569000 ± 154000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	2240 ± 585	1840 ± 394	2950 ± 2250	1880 ± 230	2200 ± 365	1900 ± 513
Cmax (ng/mL)	117000 ± 32100	102000 ± 19000	152000 ± 18400	129000 ± 26400	141000 ± 51500	146000 ± 60900
Cmax /Dose (ng/mL)/(mg/kg/day)	389 ± 107	340 ± 63.4	505 ± 61.3	429 ± 87.9	468 ± 172	486 ± 203
Tmax (h)	1.75	1.75	1.83	1.67	2.00	2.00

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	VAL489 (n = 4)					
Dose (mg/kg/day)	30 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	4970 ± 2350	4820 ± 3330	3740 ± 965	4330 ± 1640	9990 ± 4650	7010 ± 3050
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	166 ± 78.6	161 ± 111	125 ± 32.2	144 ± 54.9	333 ± 155	234 ± 102
Cmax (ng/mL)	781 ± 245	1450 ± 1150	1070 ± 234	1280 ± 761	813 ± 252	825 ± 442
Cmax /Dose (ng/mL)/(mg/kg/day)	26.1 ± 8.17	48.5 ± 38.4	35.7 ± 7.81	42.5 ± 25.4	27.1 ± 8.39	27.5 ± 14.7
Tmax (h)	0.750	0.625	0.500	0.625	4.63	2.00

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	VAL489 (n = 4)					
Dose (mg/kg/day)	100 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M	F
AUC0-24 h (ng•h/mL)	13900 ± 2870	15600 ± 10700	16900 ± 3400	15500 ± 4850	29100 ± 16700	46200 ± 27500
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	139 ± 28.7	156 ± 107	169 ± 34.0	155 ± 48.5	291 ± 167	462 ± 275
Cmax (ng/mL)	4980 ± 2560	5580 ± 4740	3410 ± 2350	3710 ± 1770	2900 ± 1360	4270 ± 1700
Cmax /Dose (ng/mL)/(mg/kg/day)	49.8 ± 25.6	55.8 ± 47.4	34.1 ± 23.5	37.1 ± 17.7	29.0 ± 13.6	42.7 ± 17.0
Tmax (h)	0.625	0.625	0.750	0.750	2.25	3.50

Toxicokinetic data	Monkey, cyno					
Study number:	R0670621					
GLP compliance:	Yes					
Location in CTD:	4.2.3.2-10					
Gender(M/F) / Number of animals:	4-6 animals/sex/group					
Duration:	39 weeks					
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC					
Method of Administration:	Nasal gavage					
Dose (mg/kg/day):	0, 30, 100, 300					
Sample:	Plasma					
Analyte:	AHU377, LBQ657 or VAL489					
Assay:	LC-MS/MS					
PK parameters:	VAL489 (n = 6)					
Dose (mg/kg/day)	300 (mean ± SD)					
Day	1		29		272	
Gender	M	F	M	F	M (n = 5)	F
AUC0-24 h (ng•h/mL)	84900 ± 25200	77100 ± 16500	83800 ± 59400	64500 ± 13900	69800 ± 27400	65200 ± 15700
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	283 ± 83.2	257 ± 54.8	280 ± 198	215 ± 46.5	233 ± 91.6	217 ± 52.2
Cmax (ng/mL)	34000 ± 23800	28100 ± 24200	18700 ± 12900	16400 ± 4120	9330 ± 6360	10600 ± 6340
Cmax /Dose (ng/mL)/(mg/kg/day)	113 ± 79.3	93.7 ± 80.9	62.3 ± 43.1	54.7 ± 13.7	31.1 ± 21.2	35.2 ± 21.1
Tmax (h)	1.00	0.625	0.750	1.00	2.40	1.58

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n = 5)			
Dose (mg/kg/day)	25			
Day	1		50	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	57600	74100	69100	59500
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	2300	2960	2760	2380
Cmax (ng/mL)	29100	37800	25100	30300
Cmax /Dose (ng/mL)/(mg/kg/day)	1160	1510	1000	1210
Tmax (h)	1.0	1.0	1.0	1.0

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n = 5)			
Dose (mg/kg/day)	25			
Day	273		346	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	61200	51100	75200	36000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	2450	2040	3010	1440
Cmax (ng/mL)	33100	27400	40700	9990
Cmax /Dose (ng/mL)/(mg/kg/day)	1320	1100	1630	400
Tmax (h)	1.0	1.0	1.0	1.0

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n = 5)			
Dose (mg/kg/day)	100			
Day	1		50	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	345000	294000	341000	365000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	3450	2940	3410	3650
Cmax (ng/mL)	153000	131000	127000	192000
Cmax /Dose (ng/mL)/(mg/kg/day)	1530	1310	1270	1920
Tmax (h)	1.0	1.0	1.0	1.0

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n = 5)			
Dose (mg/kg/day)	100			
Day	273		346	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	300000	342000	266000	332000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	3000	3420	2660	3320
Cmax (ng/mL)	88100	185000	53500	198000
Cmax /Dose (ng/mL)/(mg/kg/day)	881	1850	535	1980
Tmax (h)	2.0	1.0	2.0	1.0

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n = 8)			
Dose (mg/kg/day)	200			
Day	1		50	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL) *	920000 ± 85300	599000 ± 200000	1470000 ± 198000	1100000 ± 471000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day) *	4600 ± 427	3000 ± 1000	7330 ± 990	5500 ± 2360
Cmax (ng/mL)	270000	193000	327000	220000
Cmax /Dose (ng/mL)/(mg/kg/day)	1350	965	1640	1100
Tmax (h)	2.0	1.0	2.0	1.0

* mean ± SEM

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	AHU377 (n = 8)			
Dose (mg/kg/day)	200			
Day	273		346	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL) *	900000 ± 53500	891000 ± 124000	556000 ± 102000	694000 ± 163000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day) *	4500 ± 268	4460 ± 620	2780 ± 510	3470 ± 815
Cmax (ng/mL)	341000	333000	236000	269000
Cmax /Dose (ng/mL)/(mg/kg/day)	1710	1670	1180	1350
Tmax (h)	1.0	1.0	1.0	1.0

* mean ± SEM

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n = 5)			
Dose (mg/kg/day)	25			
Day	1		50	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	32700	59600	42700	33000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	1310	2380	1710	1320
Cmax (ng/mL)	19300	26000	25200	17200
Cmax /Dose (ng/mL)/(mg/kg/day)	772	1040	1010	688
Tmax (h)	1.00	1.00	1.00	1.00

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n = 5)			
Dose (mg/kg/day)	25			
Day	273		346	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	35800	34400	34200	26600
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	1430	1370	1370	1060
Cmax (ng/mL)	19800	12500	17100	8440
Cmax /Dose (ng/mL)/(mg/kg/day)	792	500	684	338
Tmax (h)	1.00	1.00	1.00	2.00

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n = 5)			
Dose (mg/kg/day)	100			
Day	1		50	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	221000	208000	195000	186000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	2210	2080	1950	1860
Cmax (ng/mL)	67400	62400	53000	58400
Cmax /Dose (ng/mL)/(mg/kg/day)	674	624	530	584
Tmax (h)	2.00	2.00	1.00	1.00

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n = 5)			
Dose (mg/kg/day)	100			
Day	273		346	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL)	225000	189000	247000	193000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	2250	1890	2470	1930
Cmax (ng/mL)	66300	57200	66400	59600
Cmax /Dose (ng/mL)/(mg/kg/day)	663	572	664	596
Tmax (h)	2.00	1.00	2.00	1.00

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n = 8)			
Dose (mg/kg/day)	200			
Day	1		50	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL) *	685000 ± 88200	355000 ± 121000	997000 ± 183000	741000 ± 398000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day) *	3420 ± 441	1780 ± 605	4980 ± 915	3700 ± 1990
Cmax (ng/mL)	211000	109000	244000	114000
Cmax /Dose (ng/mL)/(mg/kg/day)	1060	545	1220	570
Tmax (h)	2.00	2.00	2.00	2.00

* mean ± SEM

Toxicokinetic data	Monkey, marmoset			
Study number:	R0570307			
GLP compliance:	Yes			
Location in CTD:	4.2.3.2-20			
Gender(M/F) / Number of animals:	8 animals/sex/group (control and 200 mg/kg/day) 5 animals/sex/group (25 and 100 mg/kg/day)			
Duration:	52 week			
Vehicle / Formulation:	AHU377 suspension in 0.5% CMC			
Method of Administration:	Oral gavage			
Dose (mg/kg/day):	0, 25, 100, 200			
Sample:	Plasma			
Analyte:	AHU377, LBQ657			
Assay:	LC-MS/MS			
PK parameters:	LBQ657 (n = 8)			
Dose (mg/kg/day)	200			
Day	273		346	
Gender	M	F	M	F
AUC0-24 h (ng•h/mL) *	602000 ± 37300	508000 ± 56800	401000 ± 45500	482000 ± 111000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day) *	3010 ± 187	2540 ± 284	2000 ± 228	2410 ± 555
Cmax (ng/mL)	175000	138000	122000	137000
Cmax /Dose (ng/mL)/(mg/kg/day)	875	690	610	685
Tmax (h)	2.00	2.00	2.00	2.00

* mean ± SEM

2.6.5.5 薬物動態試験：分布

Study title:	Tissue distribution following an intravenous or oral dose of [^{14}C]AHU377 in the rat				
GLP compliance:	not required				
Study Number:	ADME (US) R0300247-2				
Location in CTD:	4.2.2.3-4				
Species:	Rat (Long Evans Hooded, pigmented and/or Han Wistar)				
Gender(M/F) / number of animals:	M/ 11 LEH, 2 HW (i.v.: 4 LEH, 1 HW, p.o.: 7 LEH, 1 HW)				
Feeding condition:	Water and food available ad libitum				
Vehicle / formulation:	Solution in 0.9% saline				
Method of administration:	Intravenous bolus and/or oral gavage				
Dose (mg/kg):	15 intravenous and 45 oral gavage				
Radionuclide:	[^{14}C]AHU377				
Specific activity (μCi/mg):	7.5 intravenous; 2.4 oral gavage				
Analyte / assay (unit):	Radioactivity, ngEq/g				
Sampling time:	Intravenous: 0.083, 1, 24 and 168 h				
	Oral: 0.25, 0.5, 1, 2, 4, 24 and 168 h				
	Concentration (ngEq/g) after 15 mg/kg intravenous dose				
	Radioactivity concentration (ngEq/g)				
Sample	0.083 h	1 h	24 h	168 h	168 h (HW)
Adrenal Cortex	14700	445	NM	NM	NM
Adrenal Medulla	15900	489	NM	NM	NM
Bile	178000	11500	NM	NM	NM
Blood	29100	890	NM	NM	NM
Bone Marrow	5080	150	NM	NM	NM
Bone mineral	4090	1030	NM	NM	NM
Brain	470	57.2	NM	NM	NM
Epididymis	5610	NM[d]	NM	NM	NM

Study title:	Tissue distribution following an intravenous or oral dose of [^{14}C]AHU377 in the rat				
	Concentration (ngEq/g) after 15 mg/kg intravenous dose Radioactivity concentration (ngEq/g)				
Sample	0.083 h	1 h	24 h	168 h	168 h (HW)
Esophagus	11800	838	NM	NM	NM
Eye	241	86.8	NM	NM	NM
Fat (brown)	11400	NM	NM	NM	NM
Fat (white)	2730	78.1	NM	NM	NM
Harderian gland	9390	303	NM	NM	NM
Heart	15600	392	NM	NM	NM
Intestine (large)	6560	NM	NM	NM	NM
Intestine (small)	12900	907	470	NM	NM
Kidney Cortex	57200	1880	234	65.8	68.9
Kidney Medulla	90500	4330	879	147	226
Kidney pelvis	117000	4730	19.0	16.2	33.5
Lachrymal gland	NS[c]	NS	NM	NM	NM
Liver	107000	3260	NM	NM	NM
Lung	17300	781	NM	NM	NM
Lymph Nodes	6990	NM	NM	NM	NM
Muscle	5280	138	NM	NM	NM
Pancreas	6100	261	NM	NM	NM
Pituitary gland	7780	NM	NM	NM	NM
Salivary gland	9670	257	NM	NM	NM
Seminal vesicles	459	278	NM	NM	NM
Skin	7720	780	NM	NM	NM
Spinal cord	532	18.9	NM	NM	NM
Spleen	4520	184	NM	NM	NM
Stomach glandular	3880	247	NM	NM	NM

Study title:	Tissue distribution following an intravenous or oral dose of [^{14}C]AHU377 in the rat				
	Concentration (ngEq/g) after 15 mg/kg intravenous dose Radioactivity concentration (ngEq/g)				
Sample	0.083 h	1 h	24 h	168 h	168 h (HW)
Stomach nonglandular	3230	875	NM	NM	NM
Testis	1590	482	NM	NM	NM
Thymus	5850	183	NM	NM	NM
Thyroid gland	5490	NM	NM	NM	NM
Trachea	5590	NM	NM	NM	NM
Urine	NS	NS	NS	NS	NS
Uveal tract	3360	251	NM	NM	NM
Quality Control[a]	17000	18400	16000	17400	20500
LOD[b] (ngEq/g)	2.11	7.28	2.30	0.00	16.3
LOQ[b] (ngEq/g)	4.22	14.6	4.61	0.00	32.6

[a] Theoretical QC concentration was 15900 and 9960 ngEq/g for the IV and oral study, respectively

[b] LOD =limit of detection; LOQ = limit of quantification

[c] NS = tissue not sectioned, [d] NM = not measured, radioactivity is too low and not distinguishable from surrounding tissues or content

	Concentration (ngEq/g) after 45 mg/kg oral dose Radioactivity concentration (ngEq/g)							
Sample	0.25 h	0.50 h	1 h	2 h	4 h	24 h	168 h	168 h (HW)
Adrenal Cortex	2430	2500	4320	1350	593	NM	NM	NM
Adrenal Medulla	NM[d]	NM	NM	NM	NM	NM	NM	NM
Bile	190000	104000	246000	265000	71900	NM	NM	NM
Blood	5430	4250	6830	3000	1180	NM	NM	NM
Bone Marrow	625	687	3050	454	NM	NM	NM	NM
Bone mineral	907	570	559	275	NM	NM	NM	NM
Brain	123	369	216	Trace[f]	<LOD[b]	NM	NM	NM
Epididymis	545	628	NM	NM	NM	NM	NM	NM

Study title:	Tissue distribution following an intravenous or oral dose of [^{14}C]AHU377 in the rat							
	Concentration (ngEq/g) after 45 mg/kg oral dose Radioactivity concentration (ngEq/g)							
Sample	0.25 h	0.50 h	1 h	2 h	4 h	24 h	168 h	168 h (HW)
Esophagus	NM	4770	992	NM	NM	NM	NM	NM
Eye	105	<LOD	Trace	148	NM	NM	NM	NM
Fat (brown)	NM	NM	1690	752	NM	NM	NM	NM
Fat (white)	227	327	492	246	NM	NM	NM	NM
Harderian gland	NM	933	1810	841	NM	NM	NM	NM
Heart	2340	1510	3490	1560	638	NM	NM	NM
Intestine (large)	841	854	1010	NM	11400	16500	NM	NM
Intestine (small)	623	892	172000	128000	200000	NM	NM	815
Kidney Cortex	8220	7340	15200	4970	2670	Trace	Trace	Trace
Kidney Medulla	16000	21400	35400	10900	7940	1910	654	677
Kidney pelvis	17000	21800	34500	6430	3460	NM	<LOD	<LOD
Lachrymal gland	NM	604	2250	609	NM	NM	NS[e]	NM
Liver	26500	35000	25400	16300	3310	<LOD	<LOD	<LOD
Lung	3930	2950	5460	2330	796	NM	NM	NM
Lymph Nodes	1310	NM	NM	NM	NM	NM	NM	NM
Muscle	402	447	906	359	Trace	NM	NM	NM
Pancreas	1840	1730	NM	773	647	NM	NM	NM
Pituitary gland	1380	NM	3140	NM	NM	NM	NM	NM
Salivary gland	1780	1410	2610	919	195	NM	NM	NM
Seminal vesicles	462	448	949	259	NM	NM	NM	NM
Skin	833	1090	2340	1060	333	NM	NM	NM
Spinal cord	416	Trace	116	<LOD	NM	NM	NM	NM
Spleen	880	584	1930	635	573	NM	NM	NM
Stomach glandular	TH[c]	TH	16000	10500	1530	NM	NM	NM

Study title:	Tissue distribution following an intravenous or oral dose of [^{14}C]AHU377 in the rat							
	Concentration (ngEq/g) after 45 mg/kg oral dose Radioactivity concentration (ngEq/g)							
Sample	0.25 h	0.50 h	1 h	2 h	4 h	24 h	168 h	168 h (HW)
Stomach nonglandular	TH	TH	TH	TH	80900	7990	NM	NM
Testis	104	241	623	527	200	NM	NM	NM
Thymus	379	517	914	403	168	NM	NM	NM
Thyroid gland	NM	3540	NM	NM	NM	NM	NM	NM
Trachea	NM	NM	NM	NM	NM	NM	NM	NM
Urine	NS	NS	NS	NS	NS	NS	NS	NS
Uveal tract	NM	Trace	566	913	NM	NM	NM	NM
Quality Control[a]	10500	10200	10700	9670	10500	9250	8800	9450
LOD[b] (ngEq/g)	24.3	88.3	56.8	68.4	70.0	85.2	51.8	129
LOQ[b] (ngEq/g)	48.5	177	114	137	140	170	104	259

[a] Theoretical QC concentration was 15900 and 9960 ngEq/g for the IV and oral study, respectively

[b] LOD =limit of detection; LOQ = limit of quantification

[c] TH = not measured, radioactivity is too high and not distinguishable from surrounding tissues or content

[d] NM = not measured, radioactivity is too low and not distinguishable from surrounding tissues or content

[e] NS = tissue not sectioned

[f] Trace = concentration below the limit of quantification and greater than the limit of detection

2.6.5.6A 薬物動態試験：血球移行性及び血漿たん白結合

Test article: [^{14}C]LBQ657

Study title:	In vitro blood distribution and binding of ^{14}C-labeled LBQ657 to plasma and/or serum proteins in the rat, dog, monkey and human			
Study no.:	R0301324			
GLP compliance:	not required			
Location in CTD:	4.2.2.3-1			
Study system:	In vitro			
Radionuclide:	[^{14}C]LBQ657			
Specific activity (µCi/mg):	135.5			
Method:	Ultracentrifugation			
Analyte / Assay:	[^{14}C] radioactivity, liquid scintillation counting			
Species:	Concentration tested (µg/mL)	fBC (mean ± SD)	Cb/Cp (mean ± SD)	% Bound in plasma (mean ± SD)
Rat (pooled, n=3)	0.02	-0.63 ± 0.80	0.47 ± 0.30	82.4 ± 1.6
Hematocrit: 0.4	0.1	-0.22 ± 0.12	0.49 ± 0.04	88.3 ± 1.2
	1	-0.06 ± 0.06	0.57 ± 0.03	94.0 ± 0.5
	10	-0.22 ± 0.35	0.52 ±0.13	94.3 ± 0.3
	100	-0.22 ± 0.14	0.50 ± 0.06	90.7 ± 0.8
	Overall average	-0.270 ± 0.389	0.51 ± 0.13	89.9 ± 4.6
Dog (pooled, n=3)	0.02	-0.16 ± 0.22	0.44 ± 0.08	83.5 ± 2.8
Hematocrit: 0.5	0.1	-0.14 ± 0.06	0.44 ± 0.02	85.8 ± 1.7
	1	-0.06 ± 0.10	0.47 ± 0.04	79.2 ± 5.1
	10	-0.04 ± 0.05	0.48 ± 0.03	78.5 ± 1.1
	100	-0.07 ± 0.05	0.47 ± 0.02	72.3 ± 5.1
	Overall average	-0.096 ± 0.110	0.46 ± 0.04	79.9 ± 5.7
Monkey (pooled, n=3)	0.02	0.16 ± 0.24	0.76 ± 0.23	91.5 ± 2.7
Hematocrit: 0.4	0.1	-0.11 ± 0.09	0.55 ± 0.04	94.2 ± 0.8
	1	-0.14 ± 0.08	0.53 ± 0.04	93.5 ± 0.1

Study title:	In vitro blood distribution and binding of ^{14}C-labeled LBQ657 to plasma and/or serum proteins in the rat, dog, monkey and human			
Species:	Concentration tested (µg/mL)	fBC (mean ± SD)	Cb/Cp (mean ± SD)	% Bound in plasma (mean ± SD)
Monkey	10	-0.19 ± 0.08	0.50 ± 0.03	94.0 ± 0.5
	100	-0.13 ± 0.06	0.53 ± 0.03	89.2 ± 0.3
	Overall average	-0.081 ± 0.168	0.57 ± 0.13	92.5 ± 2.3
	Concentration tested (µg/mL)	fBC	Cb/Cp	% Bound in plasma
Human subject 1	0.02	0.024	0.53	91.6
Hematocrit: 0.5	0.1	-0.087	0.48	97.8
	1	0.040	0.54	99.1
	10	-0.084	0.48	98.8
	100	-0.068	0.49	98.2
Human subject 2	0.02	0.061	0.60	91.3
Hematocrit: 0.4	0.1	0.091	0.62	98.5
	1	-0.286	0.44	99.1
	10	-0.024	0.55	98.9
	100	-0.014	0.55	-
Human subject 3	0.02	0.069	0.58	93.9
Hematocrit: 0.5	0.1	-0.274	0.42	98.1
	1	-0.054	0.51	98.6
	10	-0.073	0.50	98.5
	100	-0.003	0.54	97.8
	Overall average (mean ± SD)	-0.045 ± 0.111	0.52 ± 0.06	97.1 ± 2.8

fBC = 1-[(1-H)(Cp/Cb)]

Cp = concentration of radioactivity in plasma.

Cb = concentration of radioactivity in blood.Triplicate values are from matrix harvested from three drug, caffeine and alcohol free male volunteers.

- sample lost due to contamination.

2.6.5.6B 薬物動態試験：血球移行性及び血漿たん白結合

Test article: [^{14}C]AHU377

Study title:	In vitro blood distribution and plasma protein binding of [^{14}C]AHU377 in the monkey and human			
Study no.:	R1200657			
GLP compliance:	not required			
Location in CTD:	4.2.2.3-2			
Study system:	In vitro			
Radionuclide:	[^{14}C]AHU377			
Specific activity (µCi/mg):	202			
Method:	Ultracentrifugation			
Analyte / Assay:	[^{14}C] radioactivity, liquid scintillation counting			
Species:	Concentration tested (µg/mL)	fBC (mean ± SD)	Cb/Cp (mean ± SD)	% Bound to protein (mean ± SD)
Monkey plasma	0.02	0.177 ± 0.045	0.722 ± 0.041	91.1 ± 2.9
(pooled, n = 3)	0.1	0.135 ± 0.048	0.687 ± 0.038	90.4 ± 0.9
Hematocrit: 0.41	1	0.168 ± 0.077	0.717 ± 0.068	91.7 ± 0.2
	10	0.123 ± 0.027	0.677 ± 0.021	92.4 ± 0.5
	100	0.124 ± 0.015	0.678 ± 0.012	90.9 ± 0.1
	Overall average	0.145 ± 0.025	0.696 ± 0.022	91.3 ± 0.8
Human plasma [a]	0.02	-0.011 ± 0.036	0.576 ± 0.088	96.0 ± 2.0
(n = 3)	0.1	0.008 ± 0.092	0.585 ± 0.046	96.4 ± 1.2
	1	0.025 ± 0.152	0.597 ± 0.022	96.6 ± 0.8
	10	0.010 ± 0.219	0.594 ± 0.050	97.4 ± 0.2
	100	-0.043 ± 0.196	0.561 ± 0.025	97.0 ± 0.6
	Overall average	-0.002 ± 0.026	0.583 ± 0.015	96.7 ± 0.5
Human serum [a]	0.02	-	-	96.8*
(n = 3)	100	-	-	97.4 ± 0.5
			Overall average (mean)	97.1

fBC = 1-[(1-H)(Cp/Cb)]
Cp = concentration of radioactivity in plasma.
Cb = concentration of radioactivity in blood.
*One values below LOD, therefore not used in mean calculation (n=2).
[a] Average hematocrit values from triplicate measurements of 3 individual human subjects were 0.48, 0.30 and 0.47.

2.6.5.6C 薬物動態試験：血球移行性及び血漿たん白結合

Test article: [^{14}C]LBQ657 and [^{14}C]AHU377

Study title:	In vitro protein binding of [^{14}C]LBQ657 and [^{14}C]AHU377 to human albumin and α1-acid glycoprotein
Study no.:	R1200658
GLP compliance:	not required
Location in CTD:	4.2.2.3-3
Study system:	In vitro
Radionuclide:	[^{14}C]
Specific activity (μCi/mg):	[^{14}C]LBQ657 = 128 [^{14}C]AHU377 = 202
Method:	Ultracentrifugation
Analyte / Assay:	[^{14}C] radioactivity, liquid scintillation counting

HSA (μM)	[^{14}C]LBQ657 β (%)	[^{14}C]AHU377 β (%)	α1-AGP (μM)	[^{14}C]LBQ657 β (%)	[^{14}C]AHU377 β (%)
600	98.8 ± 0.215	98.9 ± 0.431	125	11.3 ± 2.60	55.1 ± 16.5
60	46.0 – 95.6*	58.4 – 94.6*	25	13.3 ± 5.60	30.2 ± 7.30
6	9.00 – 68.8*	17.4 – 62.0*	5	6.10 ± 4.10	14.5 ± 4.00

Mean ± SD, * range of the mean values.

β: fraction bound to human serum albumin (HSA) and α1-acid glycoprotein (α1-AGP).

Binding to HSA and AGP was determined at concentrations of 0.02, 0.1, 1, 10, or 100 μg/mL [^{14}C]LBQ657 and/or [^{14}C]AHU377 in PBS (pH 7.4) containing various concentrations of HSA (6, 60 and 600 μM) or AGP (5, 25, and 125 μM).

Method: ultracentrifugation at 37˚C.

- There were no significant differences in the binding of LBQ657 and AHU377 to human serum albumin (HSA).
- Both LBQ657 and AHU377 preferentially bind HSA rather than α_1-acid glycoprotein (AGP). Overall, AHU377 binding to AGP was greater than LBQ657.
- Both LBQ657 and AHU377 binding was high (99%) at a physiologically relevant HSA concentration (600 μM).
- The binding of LBQ657 and AHU377 to AGP at a physiologically relevant concentration (25 μM) can be characterized as low, 13.3 and 30.2%, respectively.

2.6.5.7A 薬物動態試験：妊娠動物における試験

Toxicokinetics: embryo-fetal development

Toxicokinetic data	Rat, Wistar Hannover							
Study number:	R0670390							
GLP compliance:	Yes							
Location in CTD:	4.2.3.5.2-1							
Gender(M/F) / Number of animals:	F / 5 animals/group							
Study conditions:	Dosed between gestation day 6 and 17							
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC							
Method of Administration:	Once daily by oral gavage							
Dose (mg/kg/day):	0, 30, 100 and 200							
Sample	Plasma							
Analyte:	AHU377, LBQ657 and VAL489							
Assay:	LC-MS/MS							
PK parameters (gestation day 17):	AHU377		LBQ657			VAL489		
Dose (mg/kg/day)	100 (n = 3)	200 (n = 3)	30 (n = 4)	100 (n = 5)	200 (n = 3)	30 (n = 4)	100 (n = 5)	200 (n = 3)
Tmax (h)	0.50	2.33	0.50	1.60	2.33	0.50	1.60	1.00
Cmax (ng/mL)	157	214	1970	3290	5200	3020	7770	12100
Cmax /Dose (ng/mL)/(mg/kg/day) *	1.57 ± 0.48	1.07 ± 0.51	65.8 ± 10.8	32.9 ± 23.9	26.0 ± 13.5	101 ± 15.6	77.7 ± 68.9	60.5 ± 43.1
AUC0-24 h (ng•h/mL)	509	821	5950	19200	29400	13400	60000	119000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day) *	5.09 ± 3.19	4.11 ± 3.39	198 ± 65.4	192 ± 155	147 ± 45.0	448 ± 106	600 ± 584	593 ± 233

* mean ± SD

Toxicokinetics: embryo-fetal development

Toxicokinetic data	Rat, Wistar Hannover
Study number:	R0570301
GLP compliance:	Yes
Location in CTD:	4.2.3.5.2-4
Gender(M/F) / Number of animals:	F / 3 animals/ control group F / 5 animals/ each dose group
Study conditions:	Dosed between gestation day 6 and 17
Vehicle / Formulation:	AHU377 in 50 mM phosphate buffer, pH 8.8
Method of Administration:	Once daily by oral gavage
Dose (mg/kg/day):	0, 75, 250 and 750
Sample	Plasma
Analyte:	AHU377, LBQ657
Assay:	LC-MS/MS

PK parameters (gestation day 17): AHU377

Dose (mg/kg/day)	AUC0-24h (ng•h/mL)*	AUC0-24h/Dose (ng•h/mL)/(mg/kg/day)*	Cmax (ng/mL)	Cmax/Dose (ng/mL)/(mg/kg/day)	Tmax (h)
75	2460 ± 785	32.8 ± 10.5	289	3.85	8.5
250	56800 ± 122000	227 ± 488	6600	26.4	0.8
750	38000 ± 52000	50.7 ± 69.3	24600	32.8	1.0

PK parameters(gestation day 17): LBQ657

Dose (mg/kg/day)	$AUC_{0\text{-}24h}$ ± SD (ng•h/mL)*	$AUC_{0\text{-}24h}$/Dose ± SD (ng•h/mL)/(mg/kg/day)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL)/(mg/kg/day)	T_{max} (h)
75	60300 ± 34600	804 ± 461	10500	140	0.5
250	58700 ± 55000	235 ± 220	13400	53.6	0.5
750	659000 ± 896000	879 ± 1200	67000	89.3	0.9

N=3, 5 and 4 for 75, 250 and 750 mg/kg/day dose groups, respectively.

* mean ± SD

Toxicokinetics: embryo-fetal development

Toxicokinetic data	Rat, Wistar Hannover						
Study number:	R0870734						
GLP compliance:	Yes						
Location in CTD:	4.2.3.5.4-2						
Gender(M/F) / Number of animals:	5 pups / sex / group						
Study conditions:	Dosed between post partum day 7 and 66						
Vehicle / Formulation:	AHU377 solution in 0.5% HPC						
Method of Administration:	Once daily by oral gavage						
Dose (mg/kg/day):	0, 100, 400, 800						
Sample	Plasma						
Analyte:	AHU377, LBQ657						
Assay:	LC-MS/MS						
PK parameters:	AHU377						
Dose (mg/kg/day)	post partum day	Gender	AUC0-24h (ng•h/mL) *	AUC0-24h/Dose (ng•h/mL)/ (mg/kg/day)*	Cmax (ng/mL)	Cmax/Dose (ng/mL)/ (mg/kg/day)*	Tmax (h)
100	64	Male	874 ± 198	8.74 ± 1.98	252	2.52	0.500
		Female	402 ± 125	4.02 ± 1.25	226	2.26	1.00
400	64	Male	4590 ± 1320	11.5 ± 3.30	610	1.53	0.500
		Female	5360 ± 1260	13.4 ± 3.15	1010	2.53	0.500
800	64	Male	16300 ± 6140	20.4 ± 7.68	5440	6.80	0.500
		Female	12100 ± 2890	15.1 ± 3.61	1340	1.68	0.500
PK parameters:				LBQ657			
100	7	Male	435000	4350	51000	510	0.500
		Female	309000	3090	43900	439	0.500
	64	Male	46600 ± 19700	466 ± 197	10400	104	0.500
		Female	37300 ± 15800	373 ± 158	12200	122	1.00
400	7	Male	1780000	4450	145000	363	1.00

Toxicokinetic data	Rat, Wistar Hannover						
		Female	1990000	4980	202000	505	2.00
Dose (mg/kg/day)	Study Day	Gender	AUC_{0-24h} (ng•h/mL) *	AUC_{0-24h}/Dose (ng•h/mL)/ (mg/kg/day) *	C_{max} (ng/mL)	C_{max}/Dose (ng/mL)/ (mg/kg/day)	T_{max} (h)
400	64	Male	157000 ± 44200	393 ± 111	19300	48.3	0.500
		Female	207000 ± 52500	518 ± 131	27000	67.5	0.500
800	7	Male	3240000	4060	248000	310	2.00
		Female	3200000	4000	213000	266	6.00
	64	Male	465000 ± 131000	581± 164	75000	93.8	1.00
		Female	802000± 273000	1000± 341	77500	96.9	0.500

* mean ± SEM

2.6.5.7B 薬物動態試験：妊娠動物における試験

Toxicokinetics: embryo-fetal development

Toxicokinetic data	Rabbit, NZW								
Study number:	R0670280								
GLP compliance:	Yes								
Location in CTD:	4.2.3.5.2-3								
Gender(M/F) / Number of animals:	F / 5/treatment group + 3 control (18 total)								
Study conditions:	Dosed between gestation day 7 and 20								
Vehicle / Formulation:	LCZ696 suspension in 0.5% CMC								
Method of Administration:	Once daily by oral gavage								
Dose (mg/kg/day):	0, 3, 10 and 30								
Sample	Plasma and fetal tissue								
Analyte:	AHU377, LBQ657 and VAL489								
Assay:	LC-MS/MS								
PK parameters (gestation day 20):	AHU377 (mean ± SD)			LBQ657 (mean ± SD)			VAL489 (mean ± SD)		
Dose (mg/kg/day)	3	10	30	3	10	30	3	10	30
Tmax (h)	1.00 ± 0	1.00 ± 0	2.00 ± 1.15	1.00 ± 0	1.00 ± 0	2.00 ± 1.15	3.80 ± 1.79	6.00 ± 2.00	5.00 ± 2.31
Cmax (ng/mL)	15.0 ± 3.71	56.2 ± 28.5	143 ± 16.2	491 ± 56.0	1810 ± 620	10500 ± 11600	2850 ± 332	10200 ± 2560	20100 ± 8200
Cmax /Dose (ng/mL)/(mg/kg/day)	4.99 ± 1.24	5.62 ± 2.85	4.75 ± 0.54	163 ± 18.6	181 ± 62.0	349 ± 387	949 ± 110	1020 ± 256	670 ± 275
AUC0-24 h (ng•h/mL)	52.9 ± 18.2	303 ± 132	878 ± 287	1930 ± 269	8920 ± 1690	102000 ± 158000	41100 ± 6960	169000 ± 31600	400000 ± 221000
AUC0-24 h/Dose (ng•h/mL)/(mg/kg/day)	17.6 ± 6.06	30.3 ± 13.2	29.3 ± 9.57	642 ± 89.7	892 ± 169	3400 ± 5270	13700 ± 2310	16900 ± 3160	13400 ± 7370
Ctissue/Cplasma x 100 (%)	-	-	-	20.6 ± 15.8	8.95 ± 4.25	6.44 ± 3.93	0.96 ± 0.27	1.01 ± 0.21	1.04 ± 0.11

C_{tissue}/C_{plasma} = the ratio fetal tissue concentration to maternal plasma concentration at 24 hours post-dose.
- not measured

2.6.5.8 薬物動態試験：その他の分布試験

Test article: [^{14}C]LCZ696

Study title:	Tissue distribution of radioactivity following an oral dose of [^{14}C]LCZ696 in the pregnant rat							
GLP compliance:	not required							
Study Number:	R1200674							
Location in CTD:	4.2.2.3-5							
Species:	Rat (Han Wistar)							
Gender(M/F) / number of animals:	F/8 animals							
Feeding condition:	Water and food available ad libitum							
Vehicle / formulation:	Solution in 0.5% (w/v) sodium carboxymethylcellulose (CMC)							
Method of administration:	Oral gavage							
Dose (mg/kg):	25							
Radionuclide:	[^{14}C]LCZ696							
Specific activity (µCi/mg):	5.0							
Analyte / assay (unit):	Radioactivity, ngEq/g							
Sampling time:	Gestation days 12 and 17; 1, 3, 6 and 24 h							
	Gestation day 12							
	Concentration (ngEq/g)				Concentration ratio (tissue/blood)			
Tissues / organs:	Rat #1 (1 h)	Rat #2 (3 h)	Rat #3 (6 h)	Rat #4 (24 h)	Rat #1 (1 h)	Rat #2 (3 h)	Rat #3 (6 h)	Rat #4 (24 h)
Blood	7780	873	512	NM	1	1	1	NA
Amnion	NM	NM	NM	NM	NA	NA	NA	NA
Amnionic fluid	269	55.4	111	NM	0.0346	0.0635	0.217	NA
Brain	109	T	T	NM	0.014	NA	NA	NA
Fetal blood	NM	NM	NM	NM	NA	NA	NA	NA
Fetal brain	NM	NM	NM	NM	NA	NA	NA	NA
Fetal heart	NM	NM	NM	NM	NA	NA	NA	NA
Fetal liver	NM	NM	NM	NM	NA	NA	NA	NA
Heart	2620	376	246	NM	0.337	0.431	0.48	NA

Study title:	Tissue distribution of radioactivity following an oral dose of [^{14}C]LCZ696 in the pregnant rat							
	Gestation day 12							
	Concentration (ngEq/g)				Concentration ratio (tissue/blood)			
Tissues / organs:	Rat #1 (1 h)	Rat #2 (3 h)	Rat #3 (6 h)	Rat #4 (24 h)	Rat #1 (1 h)	Rat #2 (3 h)	Rat #3 (6 h)	Rat #4 (24 h)
Implant/whole fetus	3960	613	479	NM	0.509	0.702	0.936	NA
Kidney cortex	16000	1920	1490	2150	2.06	2.2	2.91	NA
Kidney medulla	27800	7110	5350	339	3.57	8.14	10.4	NA
Kidney pelvis	112000	8670	1830	58	14.4	9.93	3.57	NA
Liver	26100	5280	6820	NM	3.35	6.05	13.3	NA
Lung	5690	702	490	NM	0.731	0.804	0.957	NA
Mammary gland	4080	390	274	NM	0.524	0.447	0.535	NA
Muscle	554	77.1	62.4	NM	0.0712	0.0883	0.122	NA
Placenta	4760	730	463	NM	0.612	0.836	0.904	NA
Spleen	865	237	248	NM	0.111	0.271	0.484	NA
Uterus	5110	1240	601	NM	0.657	1.42	1.17	NA
White fat	213	52.9	30.7	NM	0.0274	0.0606	0.06	NA
Quality control [a]	10600	11200	10200	10200				
LOD (ngEq/g)	14.8	11.5	11.4	9.47				
LOQ (ngEq/g)	29.8	22.9	22.9	18.9				
	Gestation day 17							
	Concentration (ngEq/g)				Concentration ratio (tissue/blood)			
Tissues / organs:	Rat #5 (1 h)	Rat #6 (3 h)	Rat #7 (6 h)	Rat #8 (24 h)	Rat #5 (1 h)	Rat #6 (3 h)	Rat #7 (6 h)	Rat #8 (24 h)
Blood	1580	1610	128	NM	1	1	1	NA
Amnion	NM	NM	NM	NM	NA	NA	NA	NA
Amnionic fluid	47.4	30.1	NM	NM	0.03	0.0187	NA	NA
Brain	17.0	59.8	NM	NM	0.0108	0.0371	NA	NA
Fetal blood	NM	NM	NM	NM	NA	NA	NA	NA
Fetal brain	NM	NM	NM	NM	NA	NA	NA	NA
Fetal heart	NM	NM	NM	NM	NA	NA	NA	NA

Study title:	Tissue distribution of radioactivity following an oral dose of [^{14}C]LCZ696 in the pregnant rat							
	Gestation day 17							
	Concentration (ngEq/g)				Concentration ratio (tissue/blood)			
Tissues / organs:	Rat #5 (1 h)	Rat #6 (3 h)	Rat #7 (6 h)	Rat #8 (24 h)	Rat #5 (1 h)	Rat #6 (3 h)	Rat #7 (6 h)	Rat #8 (24 h)
Fetal liver	NM	NM	NM	NM	NA	NA	NA	NA
Heart	664	756	56.5	NM	0.42	0.47	0.441	NA
Implant/whole fetus	285	396	74.9	NM	0.18	0.246	0.585	NA
Kidney cortex	2930	2810	4360	3420	1.85	1.75	34.1	NA
Kidney medulla	7580	10200	1260	479	4.8	6.34	9.84	NA
Kidney pelvis	8150	8020	2620	<LOD	5.16	4.98	20.5	NA
Liver	13200	11800	2580	NM	8.35	7.33	20.2	NA
Lung	1220	1400	137	NM	0.772	0.87	1.07	NA
Mammary gland	590	675	NM	NM	0.373	0.419	NA	NA
Muscle	118	159	NM	NM	0.0747	0.0988	NA	NA
Placenta	878	1210	NM	NM	0.556	0.752	NA	NA
Spleen	345	196	NM	NM	0.218	0.122	NA	NA
Uterus	812	1200	627	NM	0.514	0.745	4.9	NA
White fat	48.5	108	NM	NM	0.0307	0.671	NA	NA
Quality control [a]	10300	10800	10100	10800				
LOD (ngEq/g)	8.47	11.6	10.8	16.8				
LOQ (ngEq/g)	16.9	23.3	21.7	33.7				

[a] Theoretical QC concentration was 91900 ngEq/g for this study

<LOD = below limit of detection; T = Trace, between LOD and LOQ

NA = not applicable

NM = not measured, tissue concentration too low and not distinguishable from surrounding tissues

2.6.5.9A 薬物動態試験：*in vivo* における代謝

Test article: [^{14}C]AHU377

Study title: Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse		
Study Number:	DMPK R1200673	
GLP compliance:	Not required	
Location in CTD:	4.2.2.2-4	
Gender(M/F) / Number of animals per time-point:	M / 2 animals (IV) and 3 animals (PO), pooled	
Feeding condition:	Fed	
Formulation:	Solution in 0.9% saline	Suspension in 0.5% HPC (KLUCEL)
Method of Administration:	Intravenous injection	Oral gavage
Dose (mg/kg):	30 = 78200 nmol/kg [^{14}C]LBQ657	150 = 36500 nmol/kg [^{14}C]AHU377
Radionuclide:	[^{14}C]	
Specific activity / purity:	5.0 (free acid) µCi/mg / >98% radiochemical purity	2.0 µCi/mg (calcium salt) / >98% radiochemical purity
Analyte/Assay (unit):	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS	

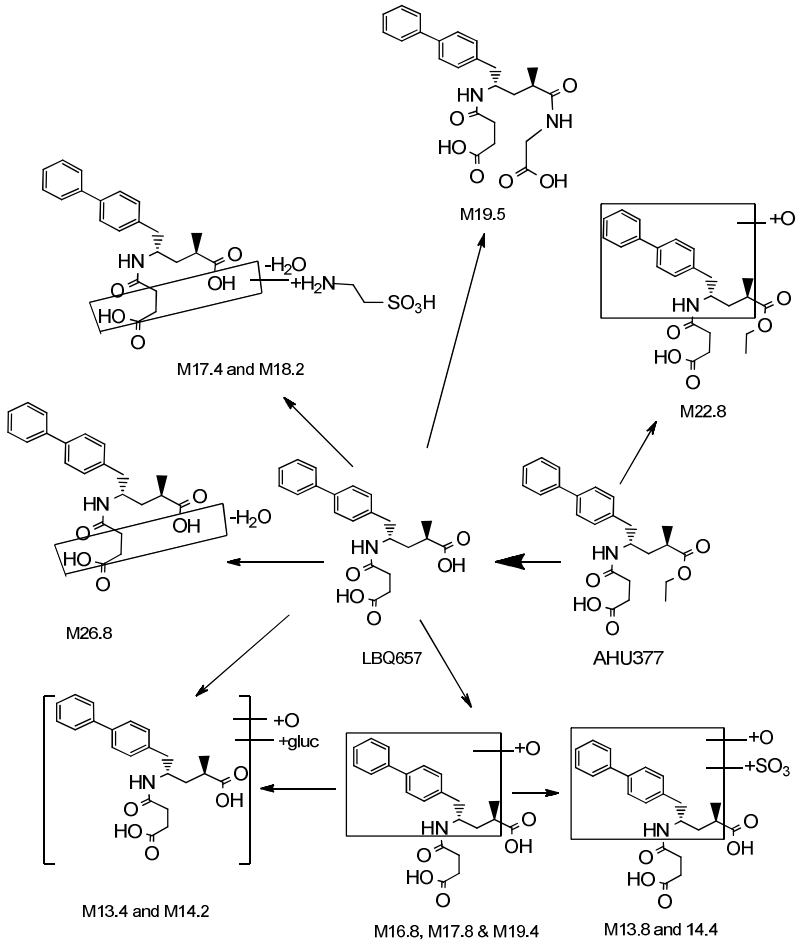
Metabolite	mouse plasma (i.v.) (nM)					Tmax (h)	Cmax (nM)	AUC0-2h (nM•h)	%AUC
	0.083 h	0.25 h	0.5 h	1 h	2 h				
M16.8	946	1632	482	271	257	0.25	946	971	8.51
M17.8	0	0	0	0	0			0	0
M19.4	317	124	0	0	0	0.083	317	65.5	0.574
M22.8	237	0	0	0	0	0.083	237	29.6	0.258
M26.8	0	0	0	0	0			0	0
LBQ657	48615	10515	4107	542	188	0.083	48615	10310	90.4
Total	50326	12269	4589	812	445			11401	100

Study title: Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse

Metabolite	mouse plasma (p.o.) (nM)						T_{max} (h)	C_{max} (nM)	$AUC_{0\text{-}6h}$ (nM•h)	%AUC
	0.25 h	0.5 h	1 h	2 h	4 h	6 h				
M16.8	1537	4644	3721	1507	230	117	0.5	4644	7225	12.0
M17.8	208	0	0	0	44.7	20.4	0.25	208	11463	0.25
M19.4	272	227	221	0	89.1	31.4	0.25	272	13450	0.81
M22.8	3404	1641	1146	440	215	59.1	0.25	3404	15350	5.36
M26.8	185	210	209	377	59.3	0	2	377	13460	1.49
LBQ657	46604	25934	13532	7344	1806	951	0.25	46600	3930	72.6
AHU377	4948	1054	378	754	149	22.9	0.25	4950	7225	5.19
Total	57800	33900	19900	10800	2660	1270			64878	100

Study title: Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse

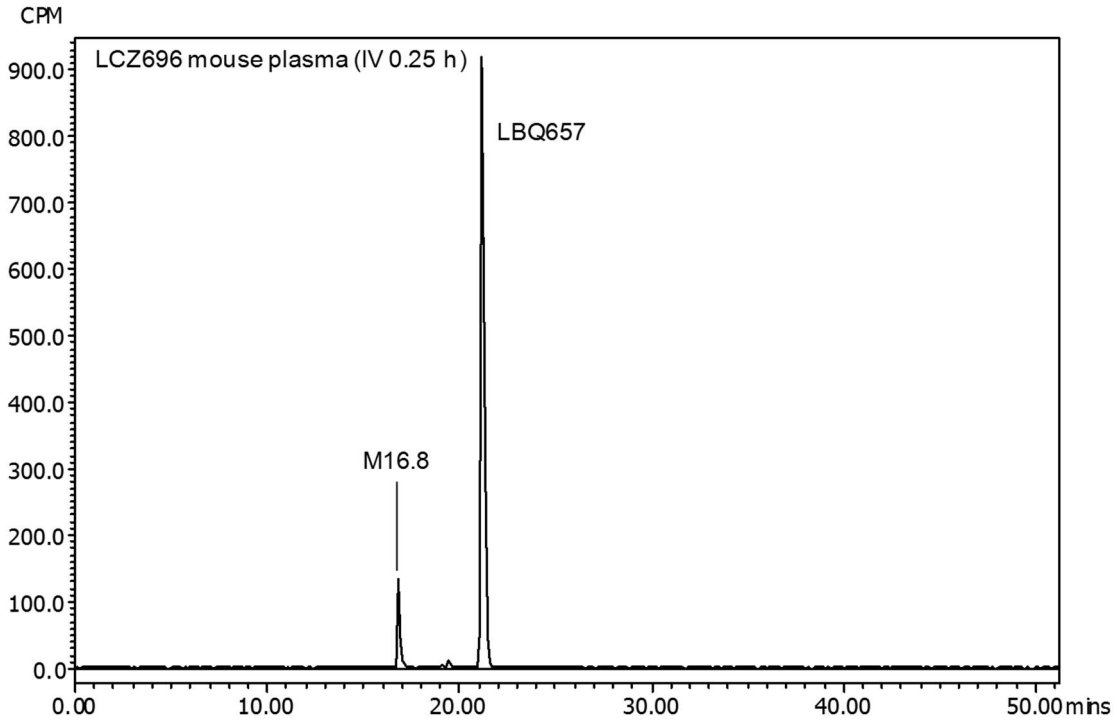
Proposed metabolic pathways of AHU377 in mouse



Study title: Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse

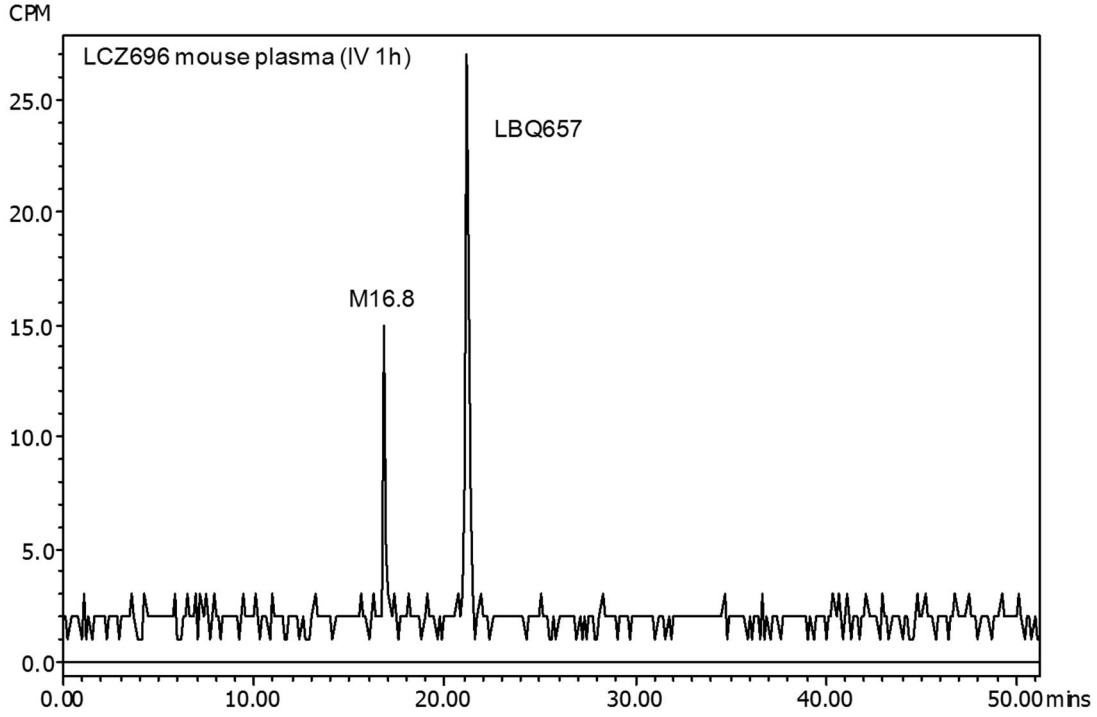
Plasma: Radio-chromatograms

Intravenous - 0.25 h post dose



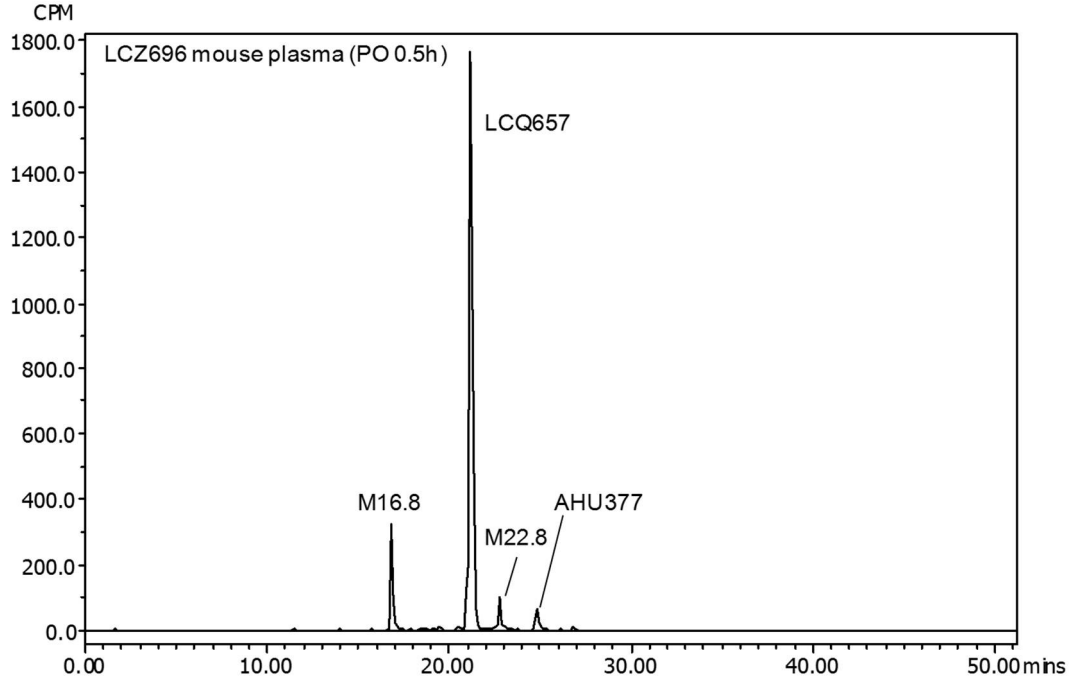
Study title: Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse

Intravenous - 1 h post dose



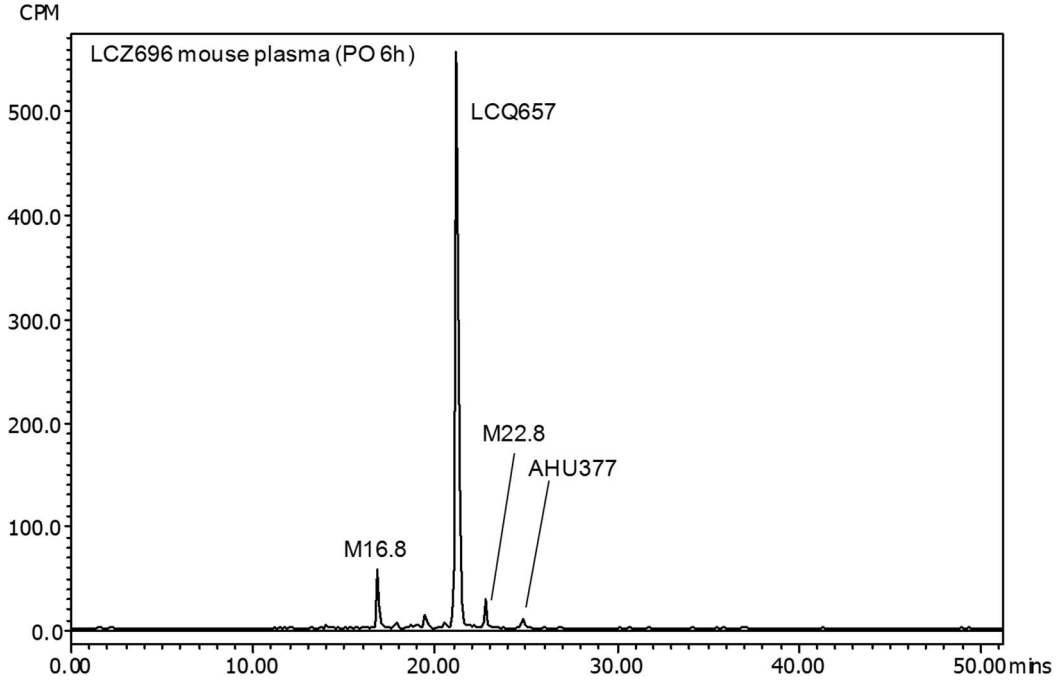
Study title: Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse

Oral – 0.5 h post dose



Study title: Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse

Oral – 6 h post dose



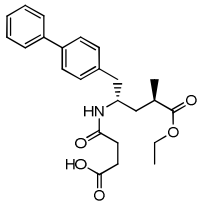
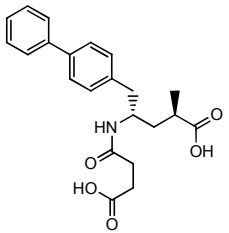
2.6.5.9B 薬物動態試験：*in vivo* における代謝

Test article: [^{14}C]AHU377

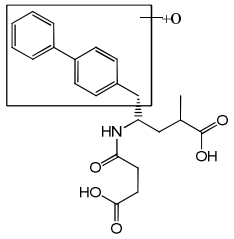
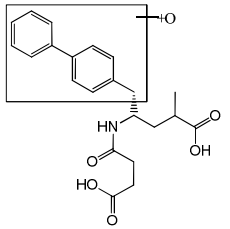
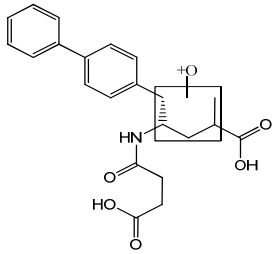
Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse		
Study Number:	DMPK R1200673	
GLP compliance:	Not required	
Location in CTD:	4.2.2.2-4	
Gender(M/F) / Number of animals per time-point:	M / 2 animals (IV) and 3 animals (PO), pooled	
Feeding condition:	Fed	
Formulation:	Solution in 0.9% saline	Suspension in 0.5% HPC (KLUCEL)
Method of Administration:	Intravenous injection	Oral gavage
Dose (mg/kg):	30 = 78200 nmol/kg [^{14}C]LBQ657	150 = 36500 nmol/kg [^{14}C]AHU377
Radionuclide:	[^{14}C]	
Specific activity / purity:	5.0 (free acid) µCi/mg / >98% radiochemical purity	2.0 µCi/mg (calcium salt) / >98% radiochemical purity
Analyte/Assay (unit):	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS	

Metabolite	Mouse Urine % of dose		Mouse Feces % of dose	
	i.v. dose (0-24 h)	p.o. dose (0-48 h)	i.v. dose (0-48 h)	p.o. dose (0-48 h)
M16.8	0.06	0.33	0.22	0.18
M17.8	0.00	0.00	0.52	0.39
M19.4	0.00	0.03	0.00	0.50
M19.5	0.00	0.00	0.00	0.30
M13.8	0.00	0.01	0.00	0.13
M14.4	0.00	0.00	0.00	0.16
M17.4	0.00	0.02	0.41	0.24
M18.2	0.00	0.00	0.00	0.38
M13.4	0.00	0.03	0.00	0.41
M14.2	0.00	0.02	0.00	0.25

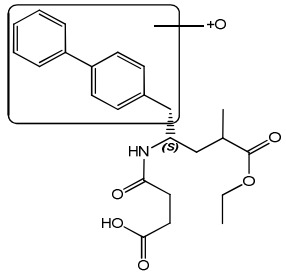
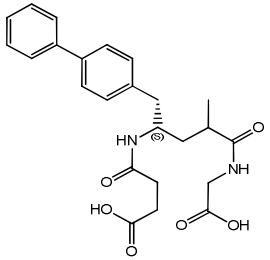
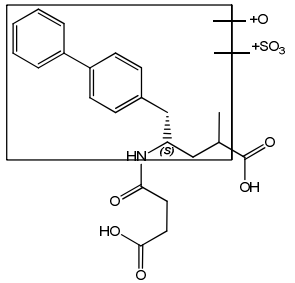
Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse					
M22.8	0.00		0.02	0.00	0.72
Metabolite	Mouse Urine % of dose			Mouse Feces % of dose	
	i.v. dose (0-24 h)		p.o. dose (0-48 h)	i.v. dose (0-48 h)	p.o. dose (0-48 h)
M26.8	0.00		0.00	0.00	0.00
LBQ657	4.48		7.89	95.8	95.9
AHU377	0.00		0.00	0.00	0.48

Peak ID	**Measured mass MH^+**	**Accurate Mass MH^+**	**t_R (min)**	**Matrix**	**Proposed structures**
AHU377	412.2122 (0.850 ppm)	412.2118	25.1	Urine Feces Plasma	
LBQ657	384.1809 (0.913 ppm)	384.1905	21.6	Urine Feces Plasma	

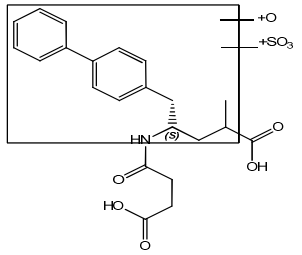
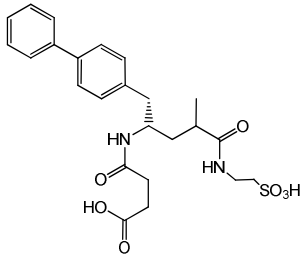
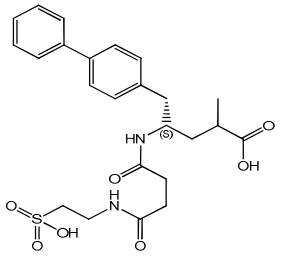
Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

Peak ID	Measured mass MH^+	Accurate Mass MH^+	t_R (min)	Matrix	Proposed structures
M16.8	400.1759 (1.090 ppm)	400.1755	17.1	Feces	
M17.8	400.1760 (1.339 ppm)	400.1755	17.8	Feces	
M19.4	400.1761 (1.589 ppm)	400.1755	19.4	Feces	

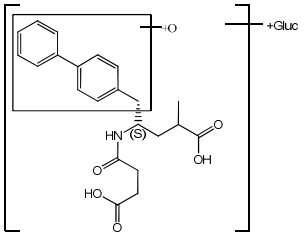
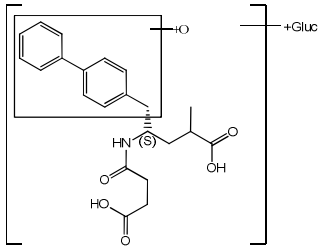
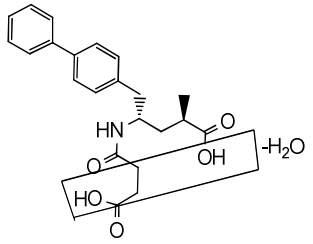
Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

Peak ID	Measured mass MH^+	Accurate Mass MH^+	t_R (min)	Matrix	Proposed structures
M22.8	428.2071 (0.784 ppm)	428.2068	22.8	Feces	
M19.5	441.2024 (0.877 ppm)	441.2020	19.5	Feces	
M13.8	480.1328 (1.086 ppm)	480.1323	13.9	Feces	

Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

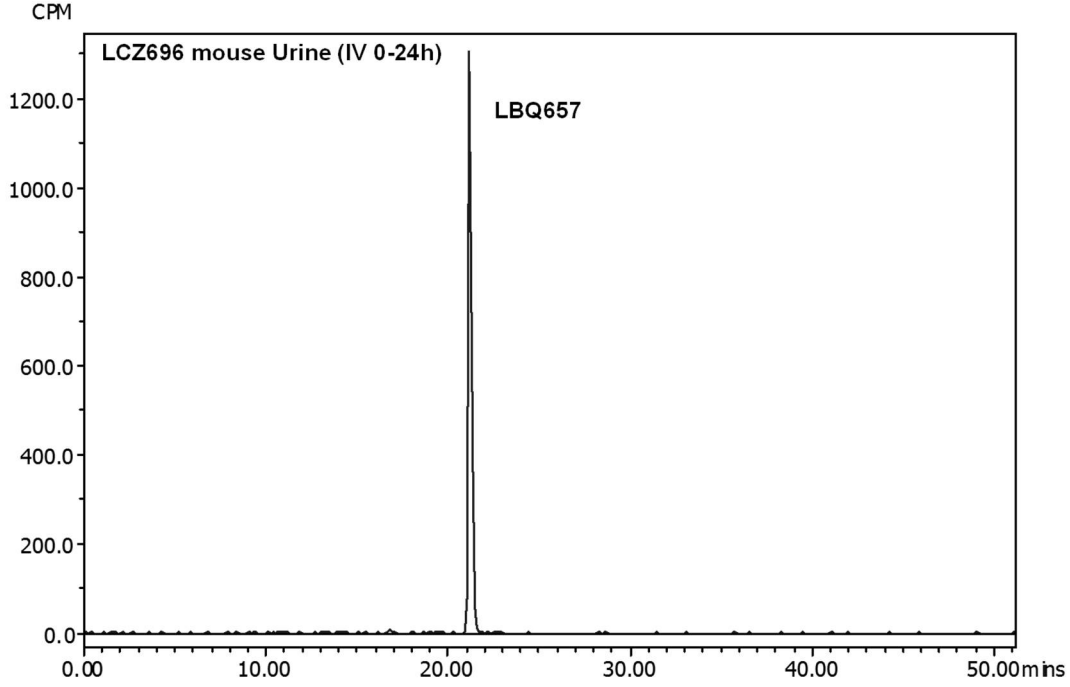
Peak ID	Measured mass MH^+	Accurate Mass MH^+	t_R (min)	Matrix	Proposed structures
M14.4	480.1328 (1.086 ppm)	480.1323	14.4	Feces	
M17.4	491.1853 (1.327 ppm)	491.1846	17.4	Feces	
M18.2	491.1853 (1.327 ppm)	491.1846	18.2	Feces	

Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

Peak ID	Measured mass MH^+	Accurate Mass MH^+	t_R (min)	Matrix	Proposed structures
M13.4	576.2073 (-0.437 ppm)	576.2076	13.3	Urine	
M14.2	576.2076 (0.084 ppm)	576.2076	14.2	Urine	
M26.8	366.1703 (0.752 ppm)	366.1700	27.08	plasma	

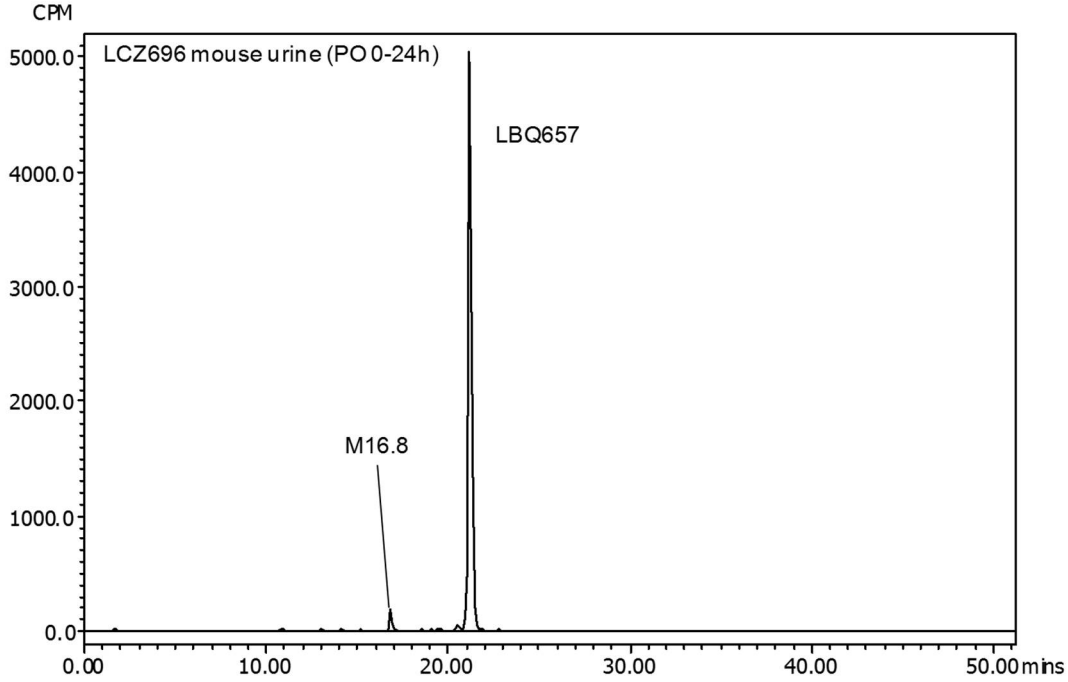
Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

Radio-chromatograms
Intravenous – urine 24h post-dose



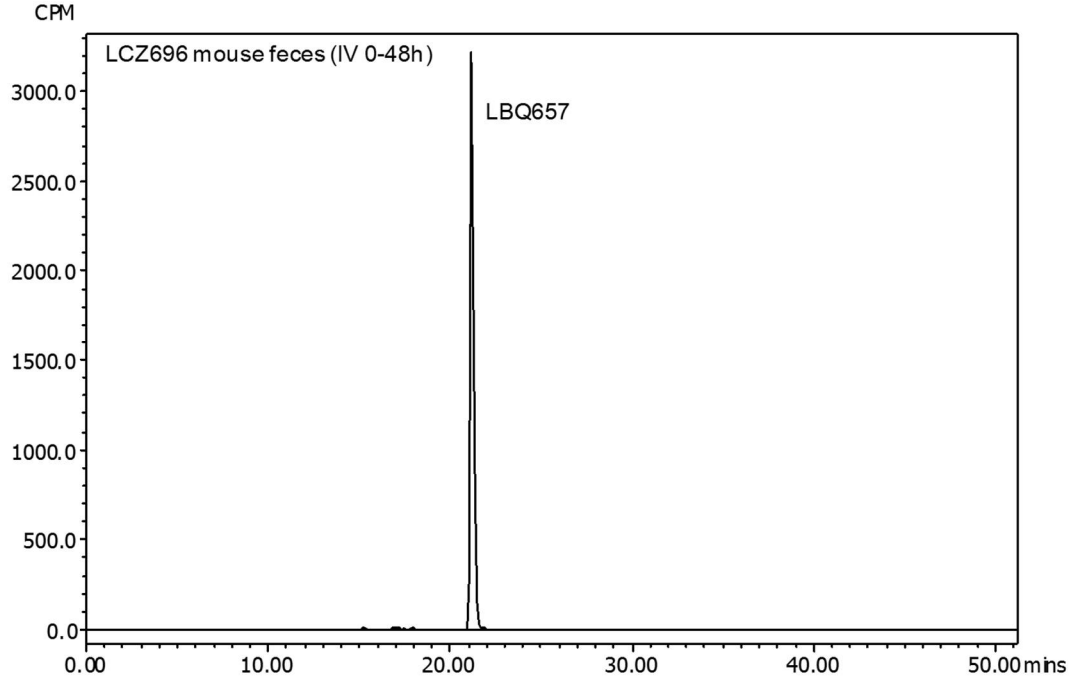
Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

Oral – urine 24h post-dose



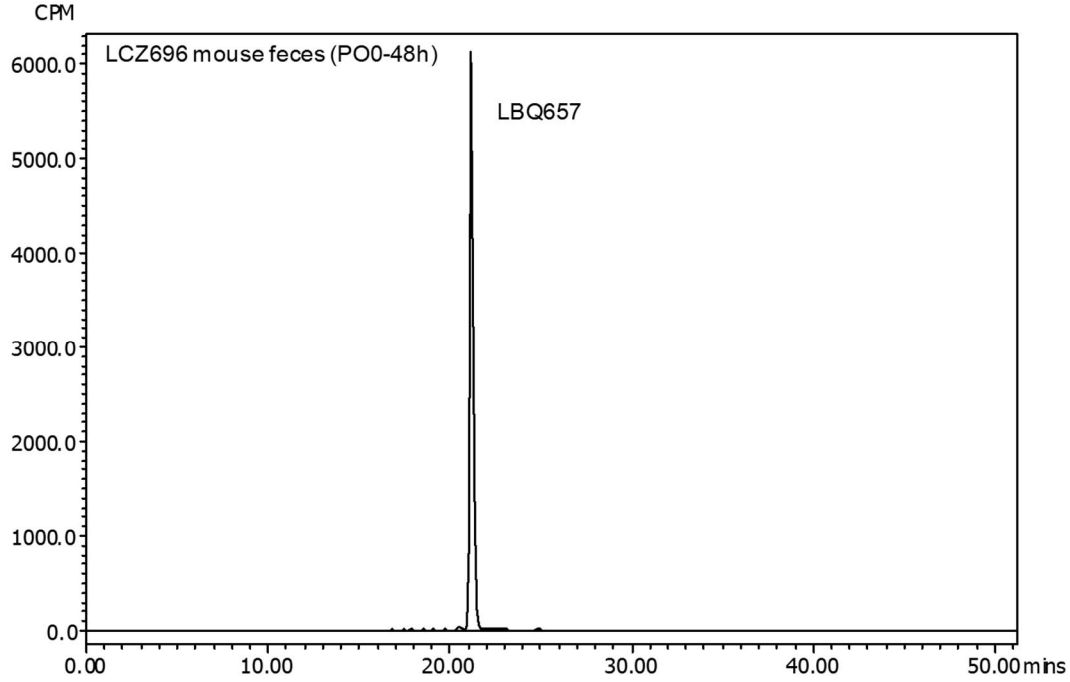
Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

Intravenous – feces 48h post-dose



Study title: Absorption, metabolism and excretion following an intravenous dose of [14C]LBQ657 or oral dose of [14C]AHU377 in the mouse

Oral – feces 48h post-dose



2.6.5.9C 薬物動態試験：*in vivo* における代謝

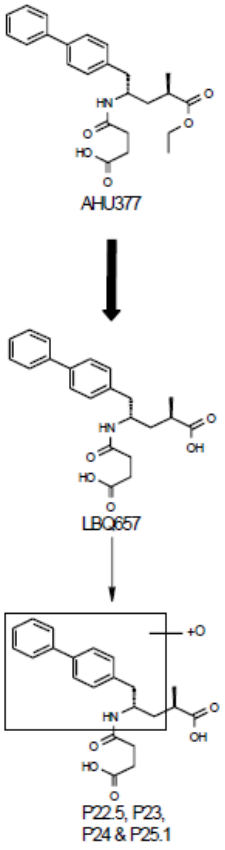
Test article: [^{14}C]AHU377

Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats				
Study Number:	ADME (US) R0300247-1			
GLP compliance:	Not required			
Location in CTD:	4.2.2.2-1			
Gender(M/F) / Number of animals:	M / 3 animals (pooled)			
Feeding condition:	Fed			
Formulation:	Solution in 0.9% saline		Solution in 0.9% saline	
Method of Administration:	Intravenous injection		Oral gavage	
Dose (mg/kg):	15 = 34602 nmol/kg [^{14}C]AHU377		45 = 103806 nmol/kg [^{14}C]AHU377	
Radionuclide:	[^{14}C]			
Specific activity / purity:	7.5 µCi/mg / >98% radiochemical purity		2.4 µCi/mg / >98% radiochemical purity	
Analyte/Assay (unit):	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS			
	Blood concentrations (nM)[b]			
	Intravenous		Oral	
Sampling time (h)	LBQ657	AHU377	LBQ657	AHU377
0.083	13000	1950	-[c]	-
0.25	4040	324	4100	468
0.5	541	23	5190	279
1.0	400	n.d.[a]	2540	178
2.0	-	-	1480	179
4.0	-	-	715	111
Cmax (nM)	13000	1950	5190	468
Tmax (h)	0.083	0.083	0.5	0.25
AUClast (nM· h)	2770	320	7810	734
Tlast (h)	1.0	0.5	4.0	4.0
% AUC	89.6	10.4	79.9	7.51

[a] not detected
[b] Values are rounded to three significant figures or to the nearest nM; pooled samples from 3 animals per time-point; metabolite and parent drug concentrations were determined by HPLC with on-line radioactivity monitoring
[c] not determined

Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats

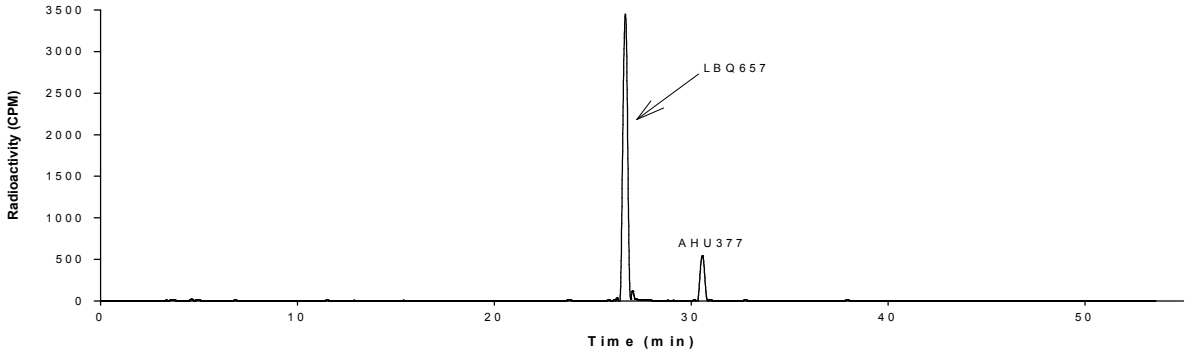
Proposed metabolic pathways of AHU377 in rat



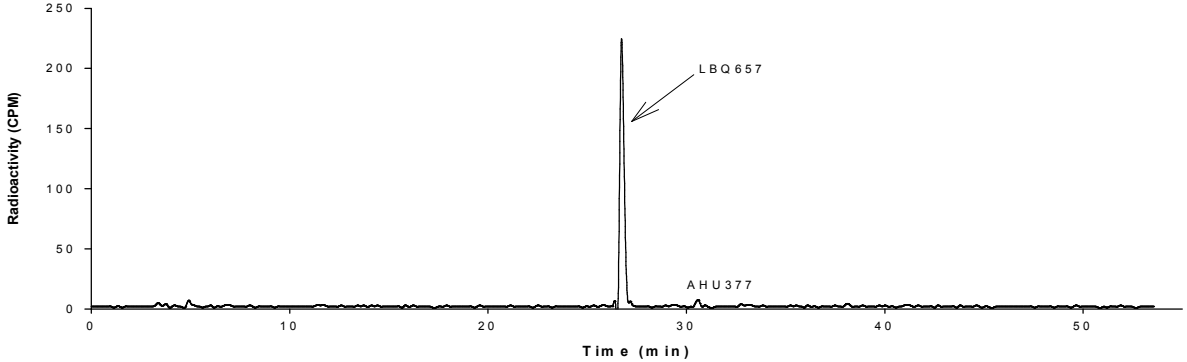
Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats

Blood: Radio-chromatograms

Intravenous - 0.083 h post dose

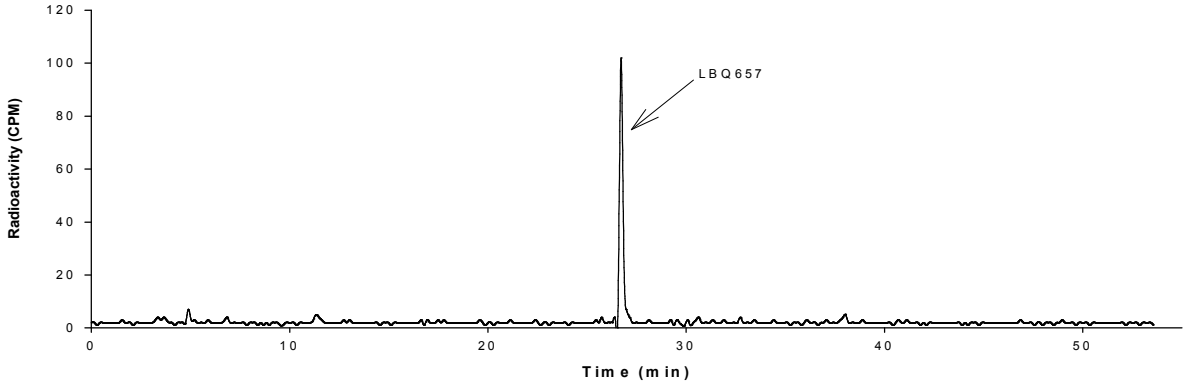


Intravenous - 0.5 h post dose

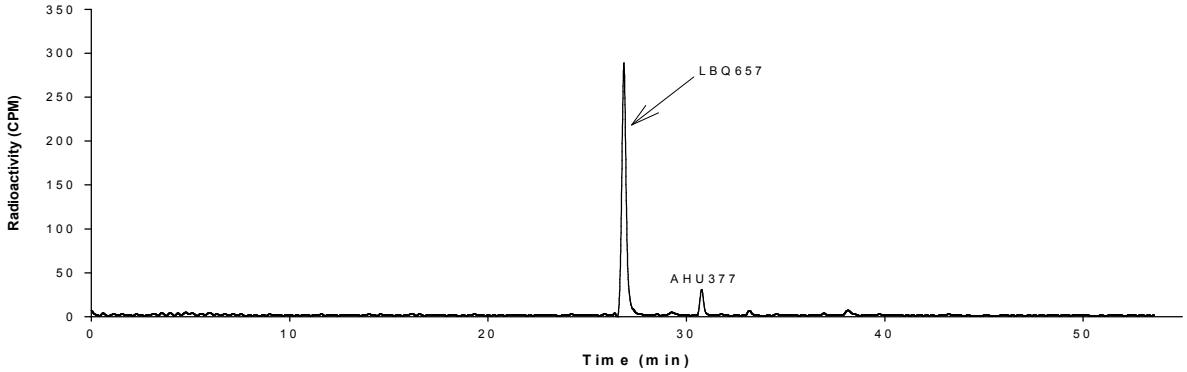


Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats

Intravenous - 1 h post dose

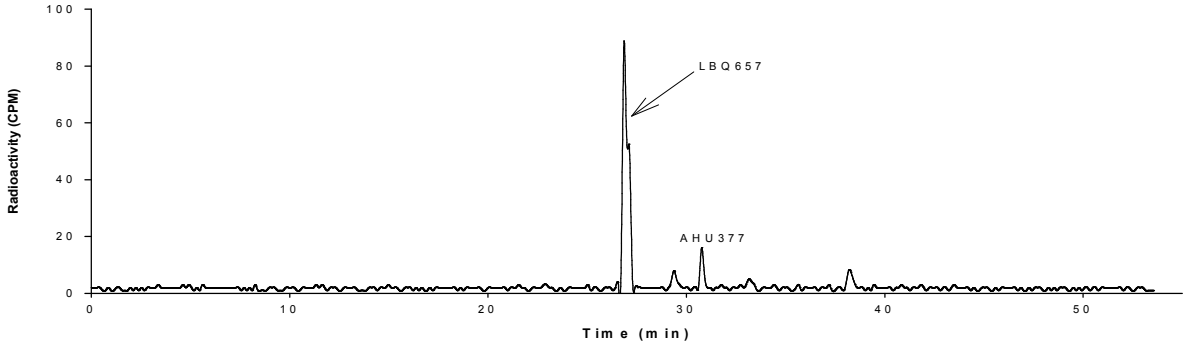


Oral – 0.25 h post dose

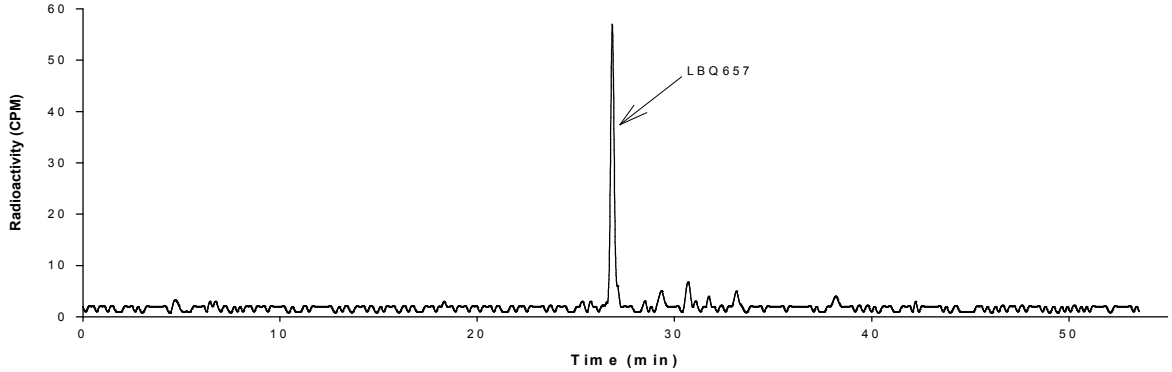


Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats

Oral – 2 h post dose



Oral – 4 h post dose



2.6.5.9D 薬物動態試験：*in vivo* における代謝

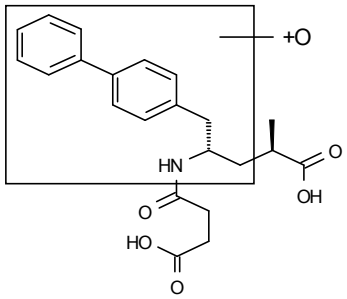
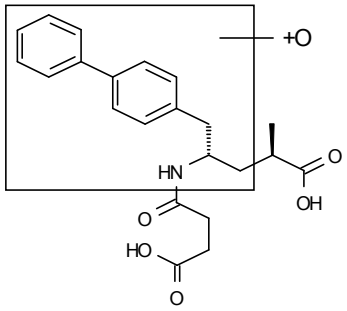
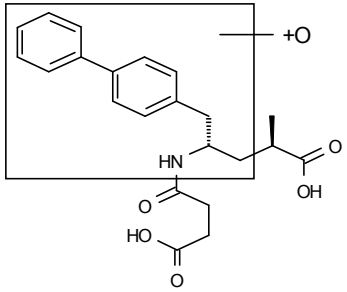
Test article: [^{14}C]AHU377

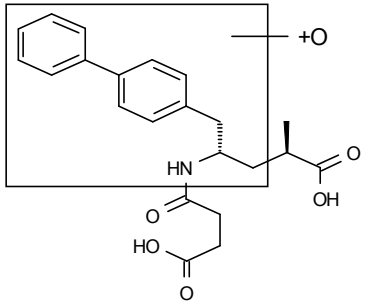
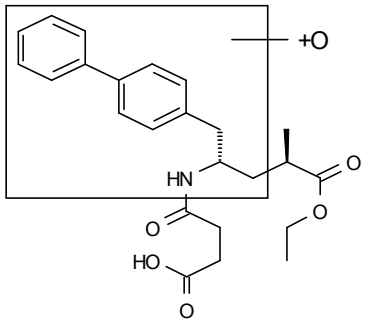
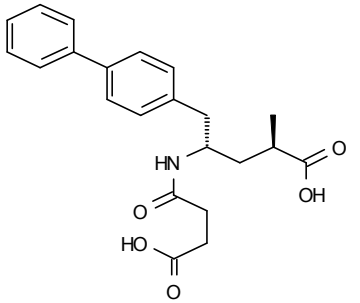
Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats						
Study Number:	ADME(US) R0300247-1					
GLP compliance:	Not required					
Location in CTD:	4.2.2.2-1					
Gender(M/F) / Number of animals:	M / 3 animals (pooled)					
Feeding condition:	Fed					
Method of Administration:	Intravenous injection			Oral gavage		
Dose (mg/kg):	15 = 34602 nmol/kg [^{14}C]AHU377			45 = 103806 nmol/kg [^{14}C]AHU377		
Formulation:	Solution in 0.9% saline			Solution in 0.9% saline		
Radionuclide:	[^{14}C]					
Specific activity:	7.5 µCi/mg / >98% radiochemical purity			2.4 µCi/mg / >98% radiochemical purity		
Analyte/Assay:	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS					
	Intravenous			Oral		
Metabolite	Urinary (%, 0-24 h)	Fecal (%, 0-48 h)	Total (%, 0-48 h)	Urinary (%, 0-24 h)	Fecal (%, 0-48 h)	Total (%, 0-48 h)
P22.5	[b]	0.49	0.49	[b]	0.33	0.33
P23	[b]	0.54	0.54	[b]	0.79	0.79
P24	[b]	0.40	0.40	[b]	0.43	0.43
P25.1	[b]	0.17	0.17	[b]	0.25	0.25
LBQ657	23.0	69.6	92.6	7.37	87.0	94.4
AHU377	[b]	0.28	0.28	[b]	0.33	0.33
Sum of trace peaks	[b]	0.24	0.24	[b]	1.18	1.18
Total analyzed pool	23.0	71.7	94.6	7.37	90.3	97.7
Total excretion	94.6			97.7		

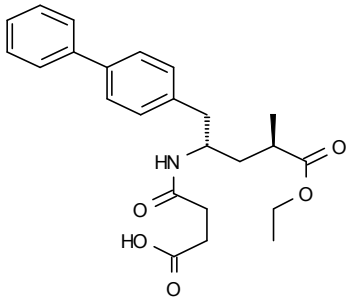
[a] Relative proportions of metabolites were based on metabolite profiles from pooled samples (n=3) 0-48 h.

[b] Not detected or not quantifiable

Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats

Peak Designation	~Retention time (min)	Molecular ion MH^+ (*m/z*)	Species	Proposed structure
P22.5	22.6	400	Feces	
P23	23	400	Urine (trace) Feces	
P24	24	400	Feces (trace)	

Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats				
P25.1	25.1	400	Feces	
P26	26.3	428	Feces (trace)	
LBQ657	28	384	Blood, urine and feces	

Study title: Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats				
AHU377	31	412	Blood and urine (trace)	

2.6.5.9E 薬物動態試験：*in vivo* における代謝

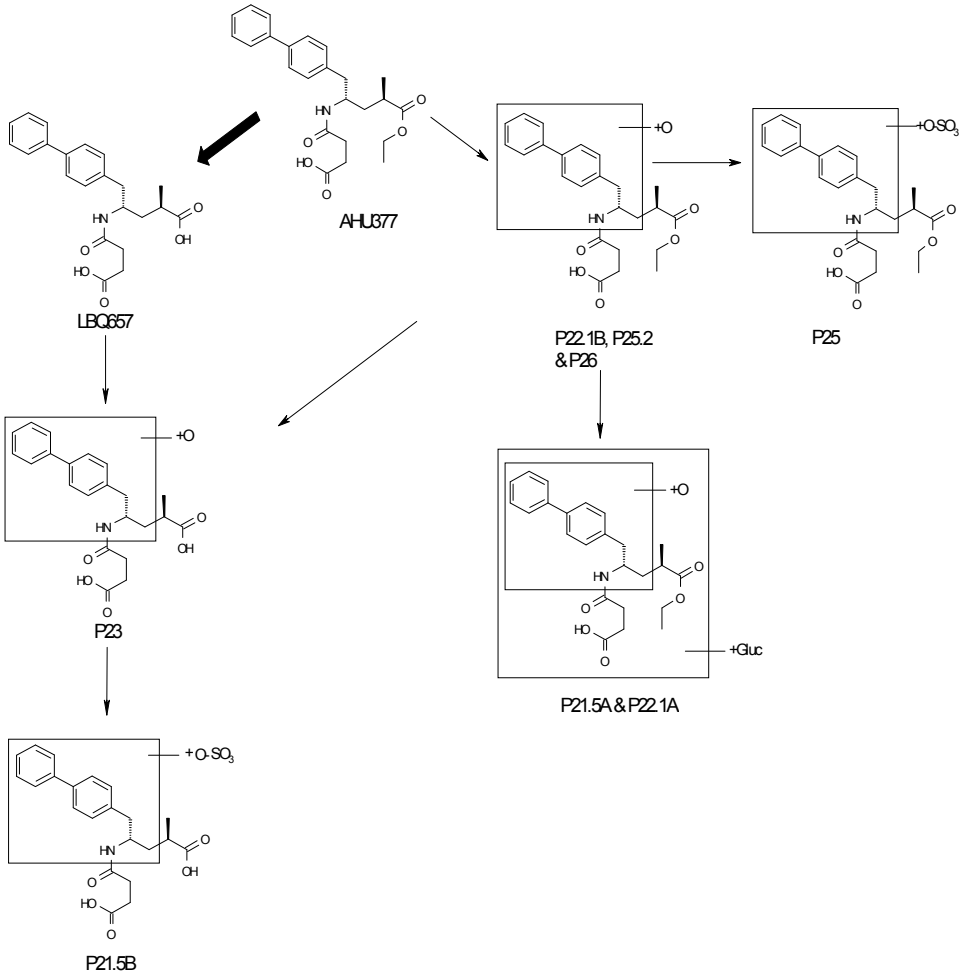
Test article: [^{14}C]LBQ657 and [^{14}C]AHU377

Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog						
Study Number:	ADME(US) R0301244					
GLP compliance:	Not required					
Location in CTD:	4.2.2.2-2					
Gender(M/F) / Number of animals:	M / 2 animals for i.v. and 3 animals for p.o. (pooled)					
Feeding condition:	Fasted					
Formulation:	Solution in sterile saline		Solution in sterile saline			
Method of Administration:	Intravenous injection		Oral gavage			
Dose (mg/kg):	3 mg/kg = 7823 nmol/kg as [^{14}C]LBQ657		15 mg/kg = 34602 nmol/kg [14C]AHU377			
Radionuclide:	[^{14}C]					
Specific activity / purity:	9.0 µCi/mg / >98% radiochemical purity		1.94 µCi/mg / >98% radiochemical purity			
Analyte/Assay (unit):	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS					
	Blood radioactivity concentrations (nM)[b]					
	Intravenous		Oral			
Sampling time (h)	LBQ657	Other	P26	LBQ657	AHU377	Other
0.083	7630	-[a]	-	-	-	-
0.25	1950	-	595	787	3600	767
0.5	1020	-	639	4510	5450	972
1	472	-	642	4050	1470	757
2	-	-	611	1330	302	234
C_{max} (nM)	7630	-	642	4510	5450	-
t_{max} (h)	0.083	-	1	0.5	0.5	-
AUC_{last} (nM· h)	1860	-	1180	5600	4190	-
T_{last} (h)	1	-	2	2	2	2
% AUC	100	-	9.6	45.8	34.4	-

[a] Not detected or not calculated
[b] Values are rounded to three significant figures or to the nearest nM; pooled samples from 3 animals per time-point; metabolite and parent drug concentrations were determined by HPLC with on-line radioactivity monitoring

Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

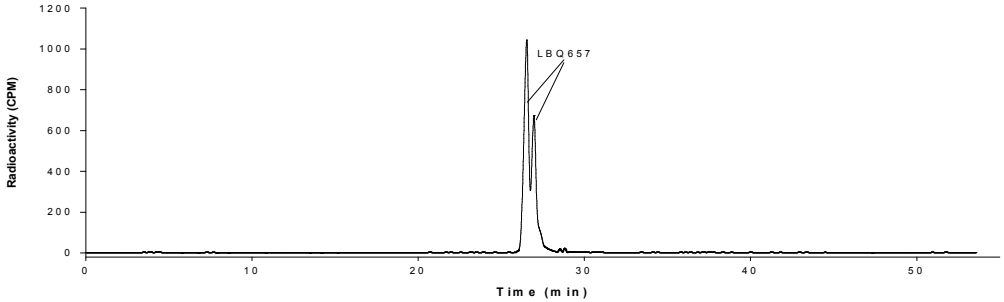
Proposed metabolic pathways of AHU377 in dog



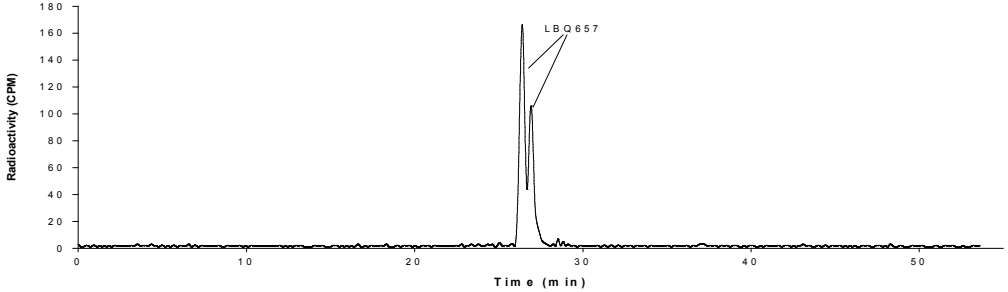
Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Blood: Radio-chromatograms

Intravenous - 0.083 h post dose

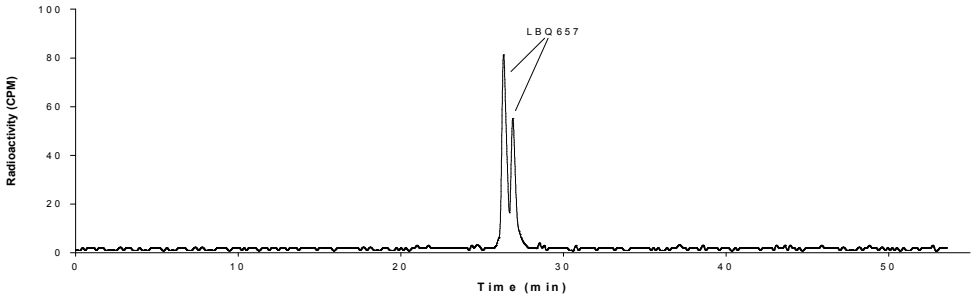


Intravenous - 0.5 h post dose

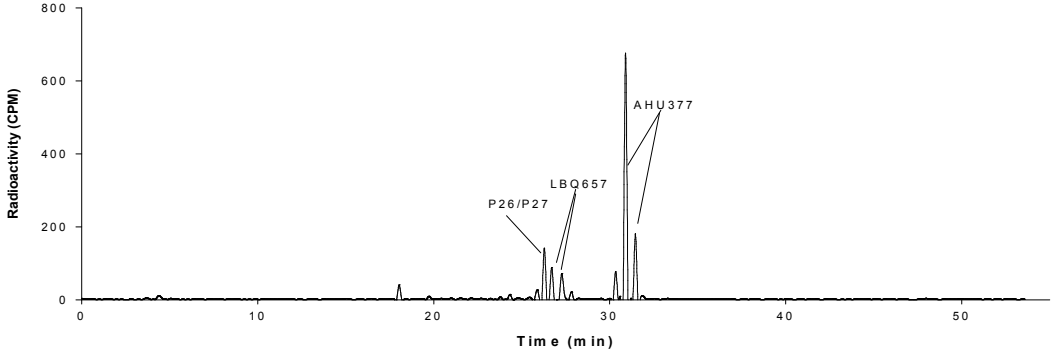


Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Intravenous - 1 h post dose

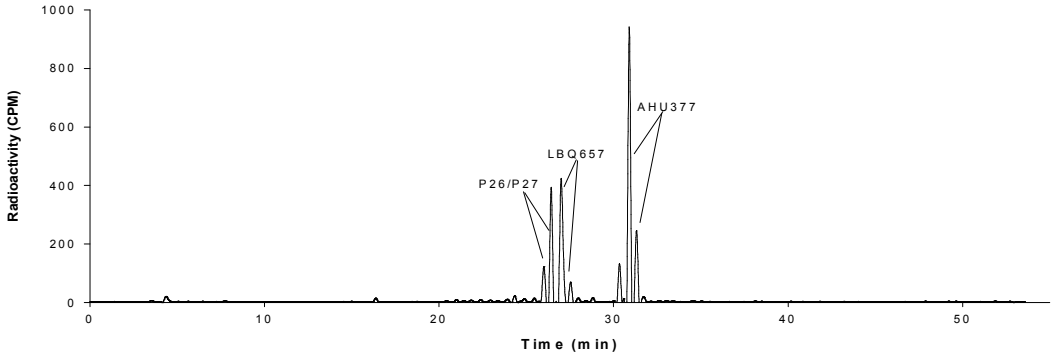


Oral – 0.25 h post dose

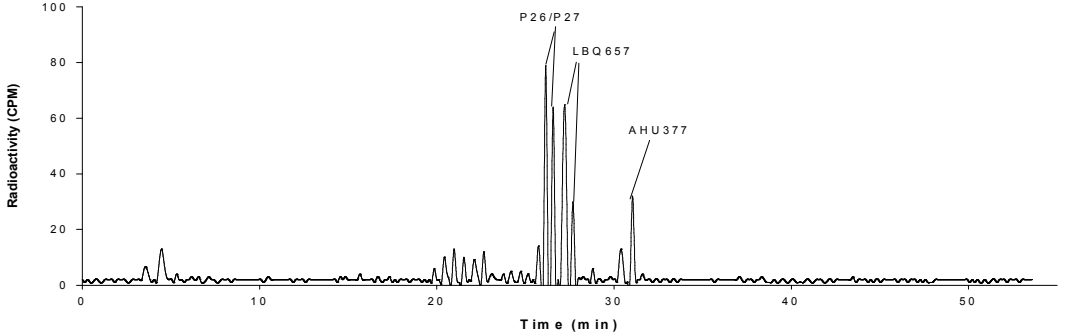


Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Oral – 0.5 h post dose



Oral – 2 h post dose



Note: Each peak pair for AHU377, LBQ657 and P26 showed the same particular transition monitored (unique for each compound), indicating peak pair contained the same compound.

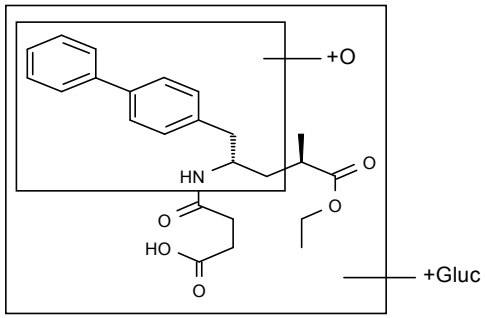
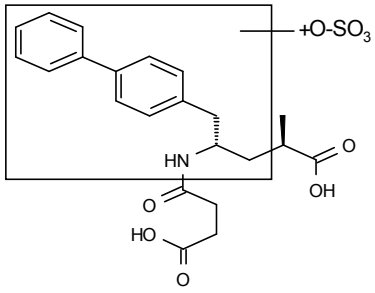
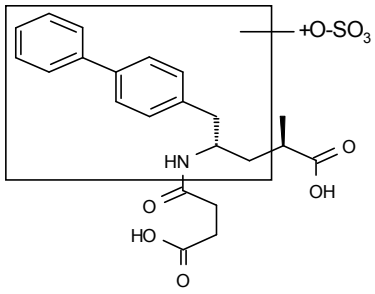
2.6.5.9F 薬物動態試験：*in vivo* における代謝

Test article: [^{14}C]LBQ657 and [^{14}C]AHU377

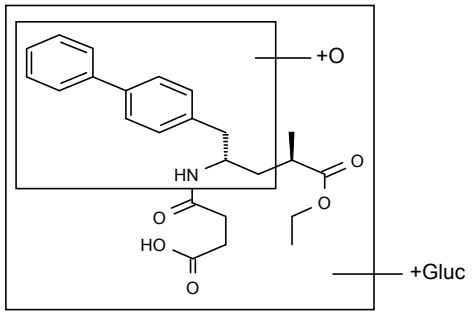
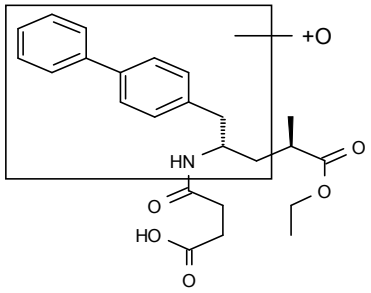
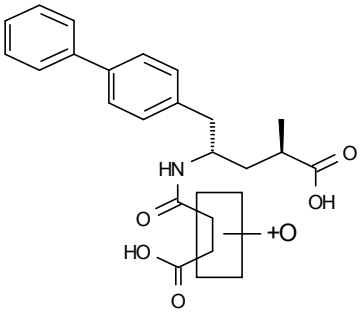
Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog						
Study Number:	ADME(US) R0301244					
GLP compliance:	Not required					
Location in CTD:	4.2.2.2-2					
Gender(M/F) / Number of animals:	M / 2 i.v. and 3 p.o. (pooled)					
Feeding condition:	Fasted					
Method of Administration:	Intravenous injection			Oral gavage		
Dose (mg/kg):	3 mg/kg = 7823 nmol/kg as [^{14}C]LBQ657			15 mg/kg = 34602 nmol/kg [^{14}C]AHU377		
Formulation:	Solution in 0.9% saline			Solution in 0.9% saline		
Radionuclide:	[^{14}C]					
Specific activity:	9.0 µCi/mg / >98% radiochemical purity			1.94 µCi/mg / >98% radiochemical purity		
Analyte/Assay:	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS					
	Intravenous			Oral		
Metabolite	Urinary (%, 0-24 h)	Fecal (%, 0-48 h)	Total (%, 0-48 h)	Urinary (%, 0-24 h)	Fecal (%, 0-48 h)	Total (%, 0-48 h)
P21.5	-	-	-	0.10	-	0.10
P22.1	-	-	-	0.22	-	0.22
P23	-	-	-	0.49	1.07	1.56
P25/P25.2	-	-	-	0.17	-	0.17
P26	-	-	-	0.26	1.26	1.52
LBQ657	10.5	86.2	96.7	6.73	71.1	77.8
AHU377	na	na	na	0.30	13.2	13.5
Other [a]	-	-	-	0.34	1.75	2.09
Total analyzed pool	10.5	86.2	96.7	8.62	88.3	96.9
Total excretion	96.7			96.9		

[a] Summation of remaining components not characterized and tabulated
- Not detected
na Not applicable

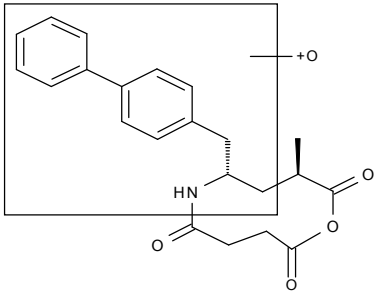
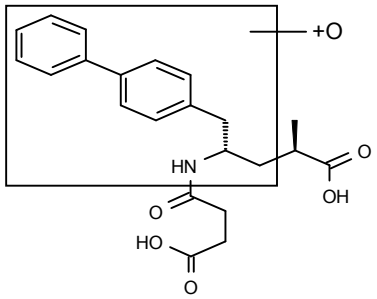
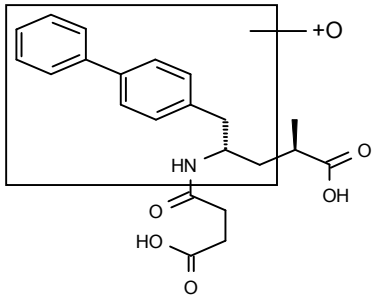
Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Peak ID	Retention time (min)	Molecular ion (MH^+)	Matrix	Proposed structures
P21.5A	21.5	604	Urine	
P21.5B	21.5	480	Urine	
P22	22	480	Urine (trace)	

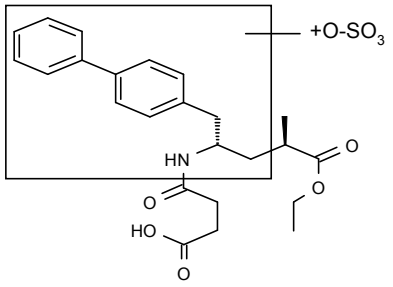
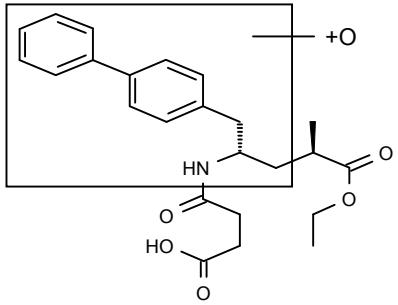
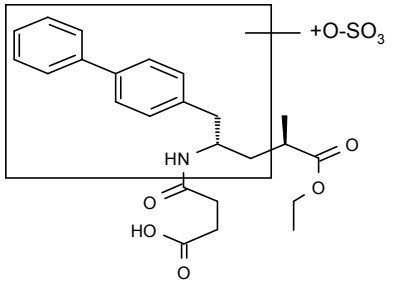
Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Peak ID	Retention time (min)	Molecular ion (MH^+)	Matrix	Proposed structures
P22.1A	22.1	604	Urine	
P22.1B	22.1	428	Urine	
P22.4	22.4	400	Urine (trace)	

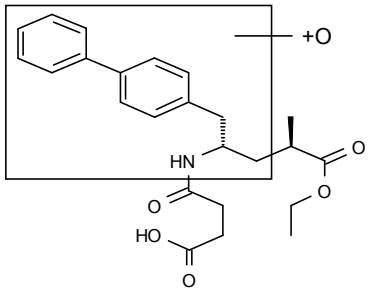
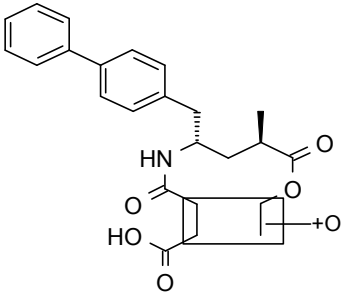
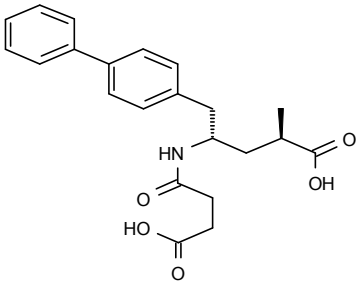
Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Peak ID	Retention time (min)	Molecular ion (MH^+)	Matrix	Proposed structures
P22.7	22.7	382	Urine (trace)	
P23	22.8	400	Urine, feces	
P24	24.3	400	Urine (trace)	

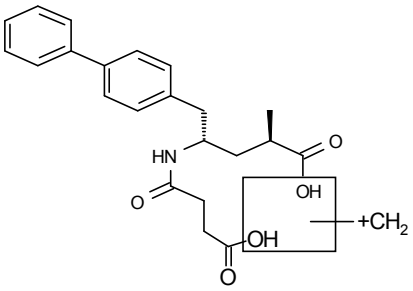
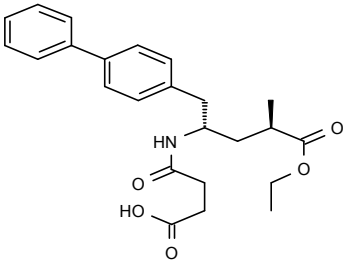
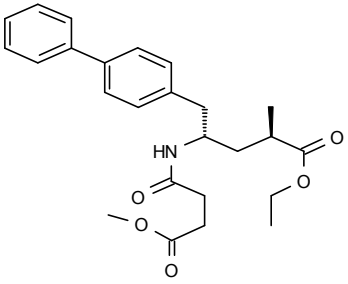
Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Peak ID	Retention time (min)	Molecular ion (MH^+)	Matrix	Proposed structures
P25	25	508	Urine	
P25.2	25.2	428	Urine	
P25.4	25.4	508	Urine (trace)	

Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Peak ID	Retention time (min)	Molecular ion (MH^+)	Matrix	Proposed structures
P26	26.3	428	Blood and urine	
P27	27.4	428	Urine (trace)	
LBQ657	28.5	384	Blood, urine and feces	

Study title: Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog

Peak ID	Retention time (min)	Molecular ion (MH^+)	Matrix	Proposed structures
P29	30	398	Urine (trace), feces (trace)	
AHU377	32.5	412	Blood, urine and feces	
P33	34	426	Urine (trace), feces (trace)	

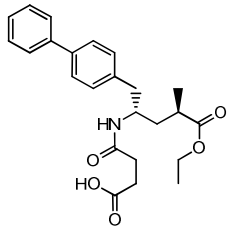
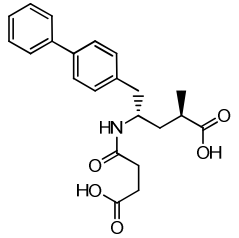
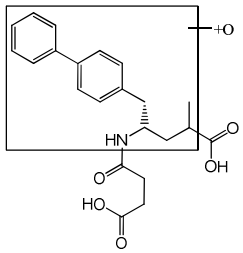
2.6.5.9G 薬物動態試験：*in vivo* における代謝

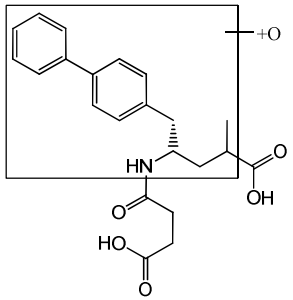
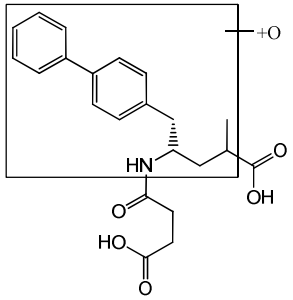
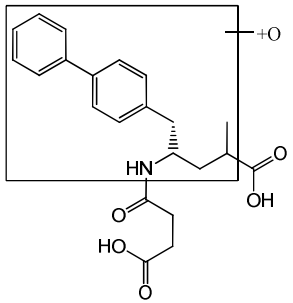
Test article: [^{14}C]LBQ657 and [^{14}C]LCZ696

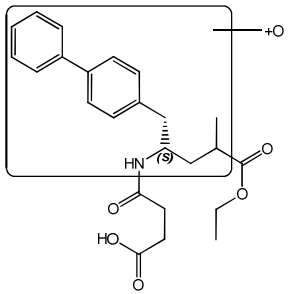
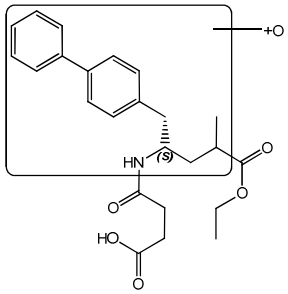
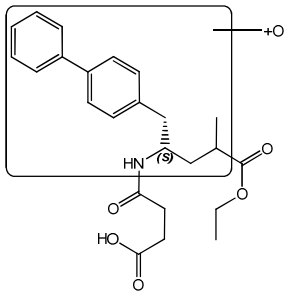
Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey		
Study Number:	DMPK(US) R1200729	
GLP compliance:	Not required	
Location in CTD:	4.2.2.2-3	
Gender(M/F) / Number of animals:	M / 2 animals for i.v. and 3 animals for p.o. (pooled)	
Feeding condition:	Fed	
Formulation:	Sterile solution in 0.9% saline for injection, USP	Suspension in 0.5% (w/v) sodium carboxymethylcellulose (CMC)
Method of Administration:	Intravenous injection	Oral gavage
Dose (mg/kg):	10 as [^{14}C]LBQ657	30 as [^{14}C]LCZ696
Radionuclide:	[^{14}C]	
Specific activity / purity:	7.5 µCi/mg / >98% radiochemical purity	2.5 µCi/mg / >98% radiochemical purity
Analyte/Assay (unit):	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS	

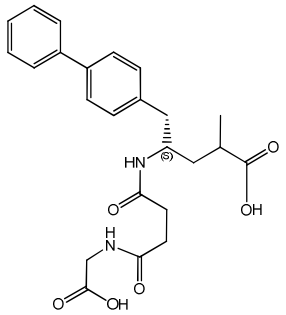
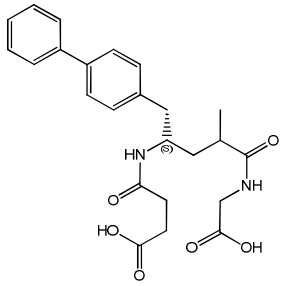
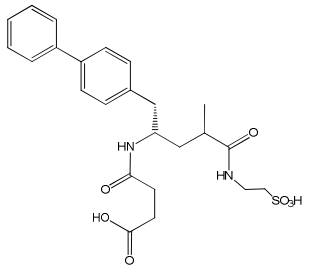
Metabolite	Monkey urine		Monkey feces	
	% of dose		% of dose	
	i.v. dose (0-48h)	p.o. dose (0-48h)	i.v. dose (0-48h)	p.o. dose (0-48h)
M16.8	0.04	0.37	0.05	1.08
M17.8	0.00	0.07	0.03	0.65
M18.7	0.00	0.00	0.06	0.27
M19.4	0.00	0.04	0.05	0.24
M16.2	0.00	0.00	0.00	0.00
M19.5	0.00	0.04	0.00	0.00
M17.4	0.00	0.00	0.00	0.00
M18.2	0.00	0.09	0.00	0.00
M20.5	0.00	0.14	0.10	0.60
M21.5	0.00	0.00	0.00	0.25
M22.8	0.00	0.11	0.00	0.55
LBQ657	55.1	29.0	21.2	40.6
AHU377	0.00	1.00	0.00	3.22

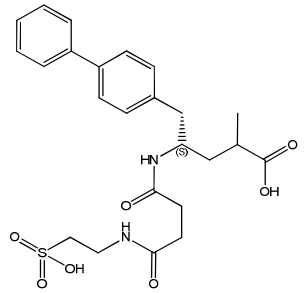
Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey

Peak ID	Measured mass MH+	Accurate Mass MH+	tR (min)	Matrix	Proposed structures
AHU377	412.2113 (-1.333 ppm)	412.2118	25.1	Urine Feces Plasma	
LBQ657	384.1795 (-2.731 ppm)	384.1905	21.4	Urine Feces Plasma	
M16.8	400.1753 (-0.410 ppm)	400.1755	17.0	Urine Feces	

Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey					
M17.8	400.1750 (-1.159 ppm)	400.1755	17.8	Feces	
M18.7	400.1748 (-1.659 ppm)	400.1755	18.7	Feces	
M19.4	400.1749 (-1.409 ppm)	400.1755	19.6	Feces	

Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey					
M20.5	428.2060 (-1.784 ppm)	428.2068	20.6	Feces	
M21.2	428.2060 (-1.784 ppm)	428.2068	21.2	Feces	
M22.8	428.2060 (-1.784 ppm)	428.2068	22.9	Feces	

Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey					
M16.2	441.2021 (0.197 ppm)	441.2020	16.2	Feces	
M19.5	441.2014 (-1.390 ppm)	441.2020	19.5	Feces	
M17.4	491.1830 (-3.356 ppm)	491.1846	17.5	Feces	

Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey					
M18.2	491.1839 (-1.524 ppm)	491.1846	18.3	Feces	

2.6.5.9H 薬物動態試験：*in vivo* における代謝

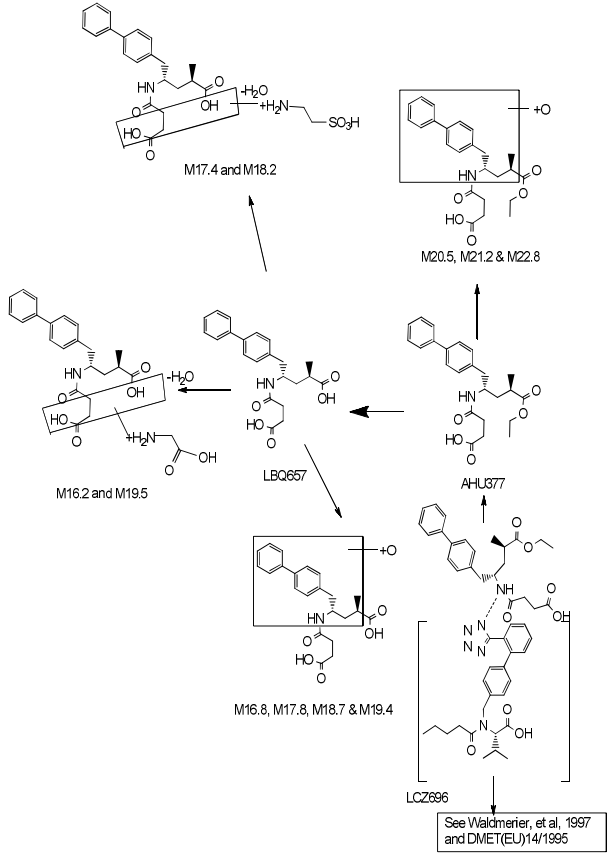
Test article: [^{14}C]LBQ657 and [^{14}C]LCZ696

Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey					
Study Number:	DMPK(US) R1200729				
GLP compliance:	Not required				
Location in CTD:	4.2.2.2-3				
Gender(M/F) / Number of animals:	M / 2 animals for i.v. and 3 animals for p.o. (pooled)				
Feeding condition:	Fed				
Formulation:	Sterile solution in 0.9% saline for injection, USP		Suspension in 0.5% (w/v) sodium carboxymethylcellulose (CMC)		
Method of Administration:	Intravenous injection		Oral gavage		
Dose (mg/kg):	10 as [^{14}C]LBQ657		30 as [^{14}C]LCZ696		
Radionuclide:	[^{14}C]				
Specific activity / purity:	7.5 µCi/mg / >98% radiochemical purity		2.5 µCi/mg / >98% radiochemical purity		
Analyte/Assay (unit):	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS				
	Intravenous plasma concentrations (nM)		Oral plasma concentrations (nM)		
Sampling time (h)	LBQ657	Total radioactivity	LBQ657	AHU377	Total
0.083	296000	305000	-	-	-
0.25	203000	209000	367	1890	2323
0.5	93000	108000	9550	12800	22800
2	6340	8300	20100	11400	32000
4	1520	2090	19700	6040	26000
8	366	632	6130	5600	11700
10	-[a]	-	2250	667	2920
24	-	-	9730	1660	11400
T_{max} (h)	0.083	-	2	1	-
C_{max} (nM)	296000	-	20100	12800	-
$AUC_{0\text{-}8h}$ or $AUC_{0\text{-}24h}$ (nM·h)	177000	198000	67800	40000	109000
% AUC	89.5	100	62.1	36.6	100

[a] Not detected or not calculated

Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey

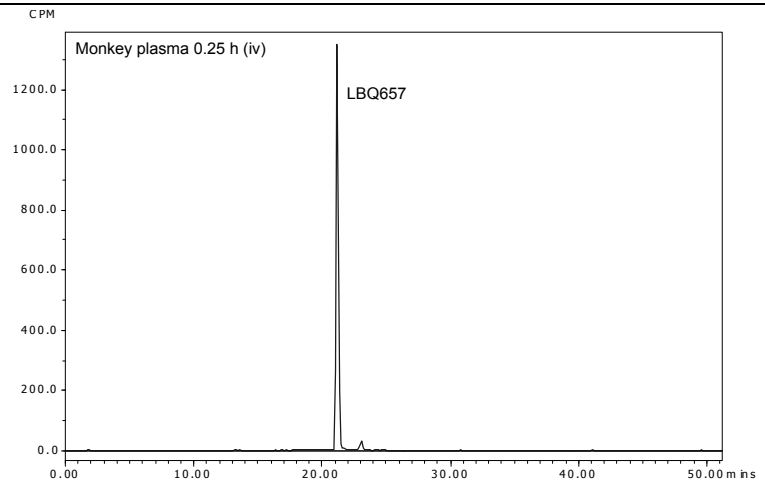
Proposed metabolic pathway for LCZ696 in the monkey



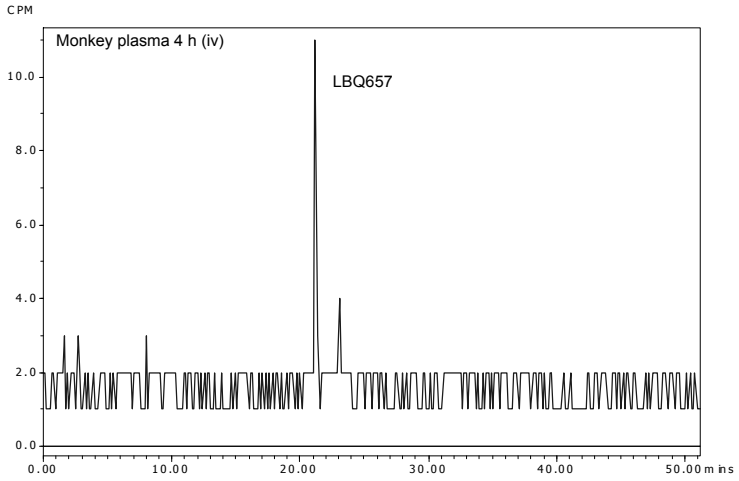
Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey

Plasma: Radio-chromatograms

Intravenous - 0.25 h post dose



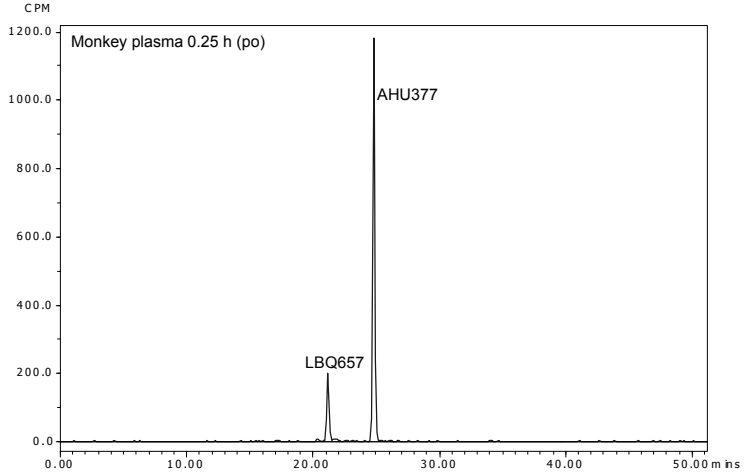
Intravenous - 4 h post dose



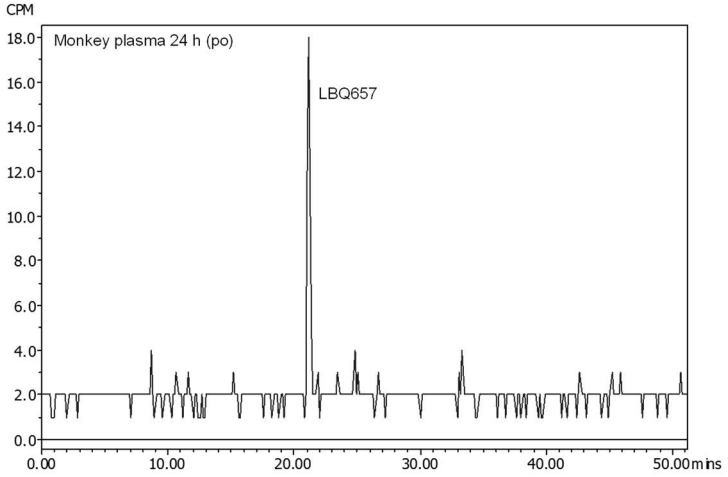
Study title: Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey

Plasma: Radio-chromatograms

Oral – 0.25 h post dose



Oral – 24 h post dose

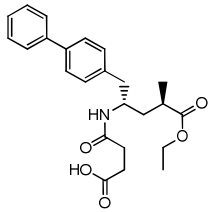
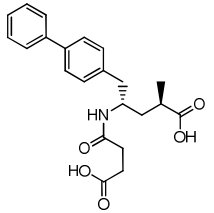
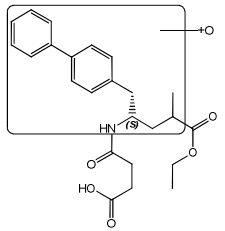


2.6.5.9I 薬物動態試験：*in vivo* における代謝

Test article: [14C]LCZ696

Study title: Excretion in milk after a single oral dose of [^{14}C]LCZ696 in the rat		
Study Number:	DMPK(US) R1200622	
GLP compliance:	Not required	
Location in CTD:	4.2.2.5-1	
Gender(M/F) / Number of animals:	F / 15 animals (3 per timepoint); lactating, 8-9 days post-parturition; HanWistar	
Feeding condition:	fed	
Formulation:	0.5% (w/v) sodium carboxymethylcellulose (CMC)	
Method of Administration:	Oral gavage	
Dose (mg/kg):	30	
Radionuclide:	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS	
Specific activity:	5.0 µCi/mg / >98% radiochemical purity	
Analyte/Assay:	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS	
Radioactivity in matrix	Plasma	Milk
Tmax (h):	1	4
Cmax (ngEq/mL):	1770 (4300 nM)	262 (637 nM)
AUC0-24h (ngEq•h/mL):	3770 (9160 nM•h)	2870 (6970 nM•h)
AUCinf (ngEq•h/mL):	3810 (9260 nM•h)	3470 (8430 nM•h)
T1/2 (h):	4.67	8.92
LBQ657 in matrix	Plasma	Milk
Tmax (h):	1	4
Cmax (ngEq/mL):	1610 (3910 nM)	262 (637 nM)
AUC0-24h (ngEq•h/mL):	3050 (7410 nM•h)	2870 (6970 nM•h)
AHU377 in matrix	Plasma	Milk
Tmax (h):	0	0
Cmax (ngEq/mL):	0	0
AUC0-24h (ngEq•h/mL):	0	0

Study title: Excretion in milk after a single oral dose of [^{14}C]LCZ696 in the rat

Peak ID	Measured mass MH+	Accurate Mass MH+	tR (min)	Matrix	Proposed structures
AHU377	412.2116 (-0.605 ppm)	412.2118	25.1	Plasma	
LBQ657	384.1800 (-1.43 ppm)	384.1905	21.6	Plasma	
M22.8	428.20832 (3.587ppm)	428.2068	22.8	Plasma	

Conclusions: After a single 30 mg/kg [^{14}C]LCZ696 dose to lactating rats, the transfer of LBQ657 (active metabolite) into milk was observed. The overall milk:plasma (M/P) concentration ratio of total radioactivity was 0.76 and 0.91, respectively, based on $AUC_{0\text{-}24h}$ and AUC_{inf} values. Projecting the rat data to humans, it is estimated that the maximum amount of AHU377 and/or its metabolites, that a breast-fed infant could be exposed to by ingesting 1 L of milk daily, is 0.889% of a 400 mg (or 200 md BID) LCZ696 adult dose LBQ657 was the only AHU377 related metabolite detected in rat milk. AHU377 underwent ester hydrolysis to generate a major metabolite (LBQ657) and a very minor hydroxylated metabolite (M22.8) in rat plasma.

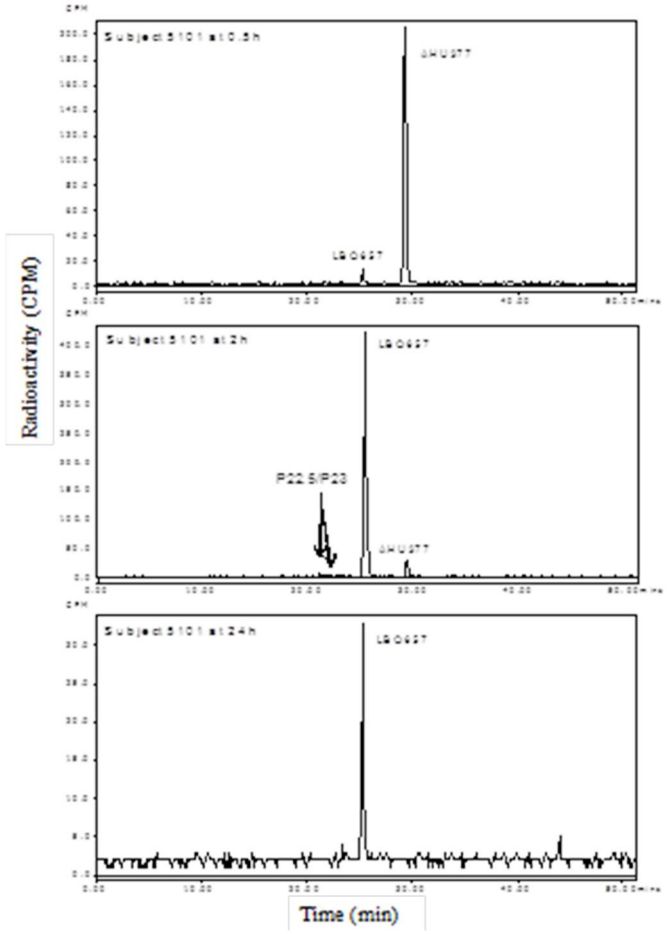
2.6.5.9J 薬物動態試験：*in vivo* における代謝

Test article: [^{14}C]LCZ696

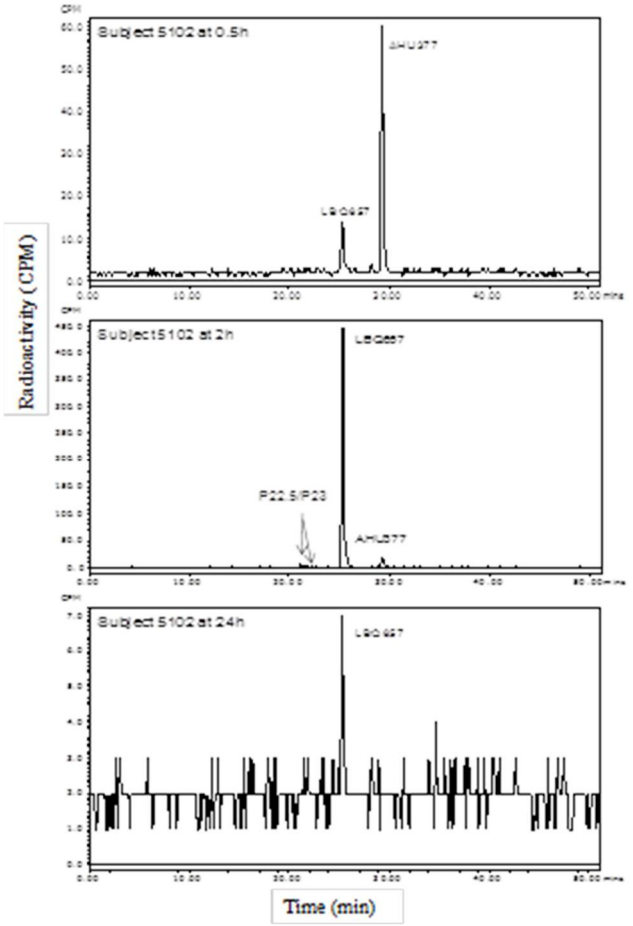
Study title: An open-label, single dose study to investigate the absorption, distribution, metabolism and elimination of 200 mg [^{14}C]LCZ696 and its metabolites in healthy male subjects					
Study Number:	CLCZ696B2105				
GLP compliance:	Yes, GCP				
Location in CTD:	5.3.3.1-3				
Gender(M/F) / Age range / Number of subjects:	M / 23-30 years / 4 subjects				
Formulation:	100 mg gelatin capsules (batch number AEUS/2009-0225) for oral use.				
Method of Administration:	Oral				
Dose (mg / subject):	200				
Radionuclide:	[^{14}C]				
Analyte/Assay (unit):	Total radioactivity in blood, plasma, urine and feces (and vomitus) by liquid scintillation counting. Valsartan, AHU377 and LBQ657 in plasma by LC/MS/MS (LOQ: 1 ng/mL for all three analytes). Metabolites of AHU377 in plasma, urine and feces by HPLC with radioactivity detection. Characterization of AHU377 metabolites by LC/MS.				
	Ratio of plasma AHU377 or LBQ657 to plasma radioactivity				
Parameter	Subject 5101	Subject 5102	Subject 5103	Subject 5104	Mean
Plasma AHU377					
AUCinf (ng•h/mL)	5093	2038	1472	1641	2561
Plasma LBQ657					
AUCinf (ng•h/mL)	82122	70629	66726	66204	71420
Plasma radioactivity					
AUCinf (ngEq•h/mL)	117837	90620	84706	81972	93784
AUC ratio					
(AHU377/radioactivity)	0.043	0.022	0.017	0.020	0.026
AUC ratio					
(LBQ657/radioactivity)	0.697	0.779	0.788	0.808	0.768

Study title: An open-label, single dose study to investigate the absorption, distribution, metabolism and elimination of 200 mg [^{14}C]LCZ696 and its metabolites in healthy male subjects

Metabolite profile in plasma (0.5, 2, 24 h) in subject 5101

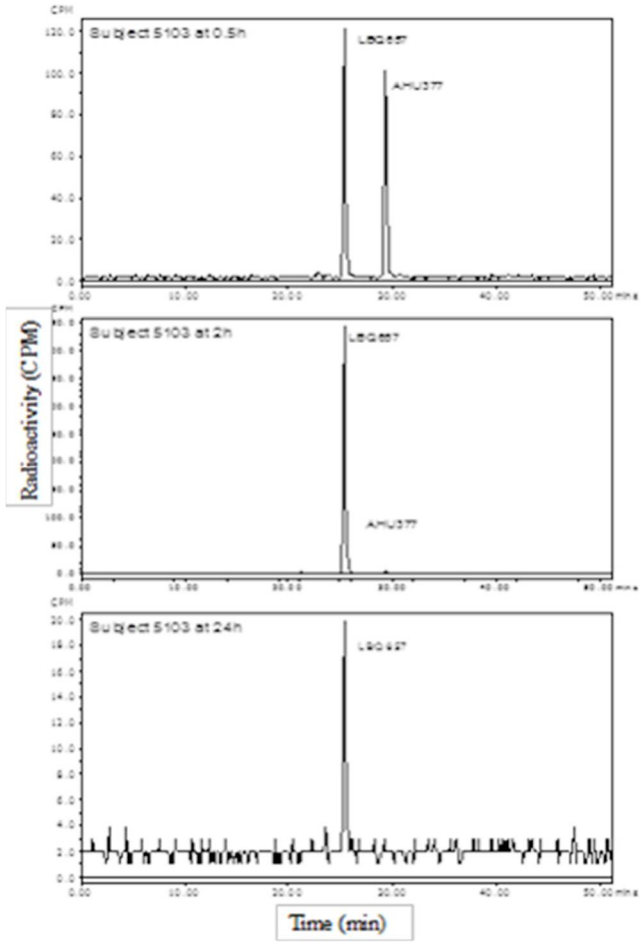


Metabolite profile in plasma (0.5, 2, 24 h) in subject 5102

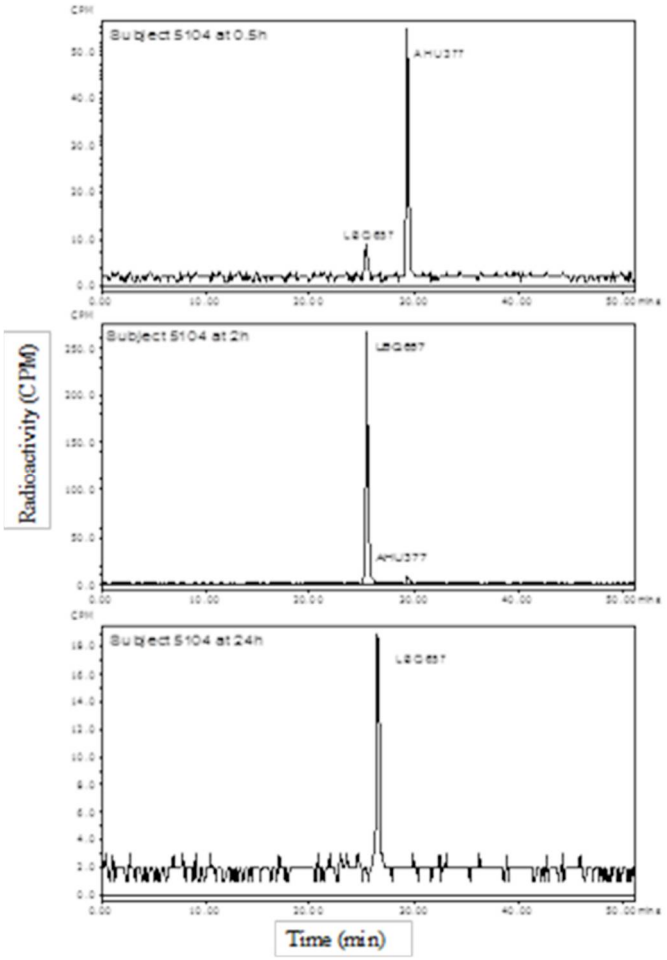


Study title: An open-label, single dose study to investigate the absorption, distribution, metabolism and elimination of 200 mg [^{14}C]LCZ696 and its metabolites in healthy male subjects

Metabolite profile in plasma (0.5, 2, 24 h) in subject 5103



Metabolite profile in plasma (0.5, 2, 24 h) in subject 5104



2.6.5.9K 薬物動態試験：*in vivo* における代謝

Test article: [^{14}C] LCZ696

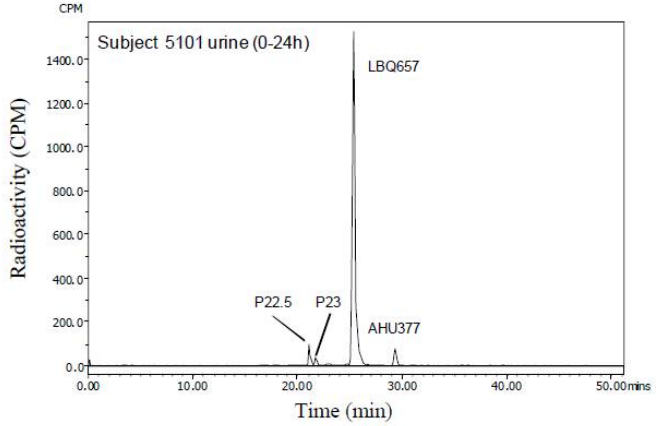
Study title: An open-label, single dose study to investigate the absorption, distribution, metabolism and elimination of 200 mg [^{14}C]LCZ696 and its metabolites in healthy male subjects

Study Number:	CLCZ696B2105
GLP compliance:	Yes, GCP
Location in CTD:	5.3.3.1-3
Gender(M/F) / Number of subjects:	M / 23-30 years / 4 subjects
Formulation:	100 mg gelatin capsules (batch number AEUS/2009-0225) for oral use.
Method of Administration:	Oral
Dose (mg / subject):	200
Radionuclide:	[^{14}C]
Analyte/Assay:	Total radioactivity in blood, plasma, urine and feces (and vomitus) by liquid scintillation counting. Valsartan, AHU377 and LBQ657 in plasma by LC/MS/MS (LOQ: 1 ng/mL for all three analytes). Metabolites of AHU377 in plasma, urine and feces by HPLC with radioactivity detection. Characterization of AHU377 metabolites by LC/MS.

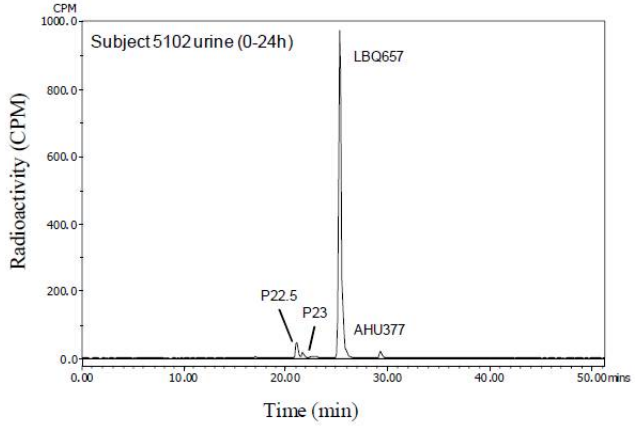
Urine pooled (0-24 h)	Total % of dose in pooled sample	P22.5	P23	LBQ657	AHU377
5101	60.8	2.82	1.45	53.8	2.82
5102	57.0	2.66	0.91	52.3	1.16
5103	47.5	1.52	0.82	44.0	1.17
5104	53.8	2.44	1.12	48.4	0.82
Mean ± SD	54.8 ± 5.6	2.4 ± 0.6	1.1 ± 0.3	49.6 ± 4.4	1.5 ± 0.9
Feces pooled					
5101 (24-96 h)	38.8	2.33	1.88	34.1	0.45
5102 (0-48 h)	36.0	2.09	1.71	31.6	0.57
5103 (0-120 h)	44.2	1.56	0.81	41.5	0.31
5104 (0-96 h)	41.3	2.12	1.78	36.4	0.96
Mean ± SD	40.1 ± 3.5	2.0 ± 0.3	1.5 ± 0.5	35.9 ± 4.2	0.57 ± 0.28

Study title: An open-label, single dose study to investigate the absorption, distribution, metabolism and elimination of 200 mg [^{14}C]LCZ696 and its metabolites in healthy male subjects

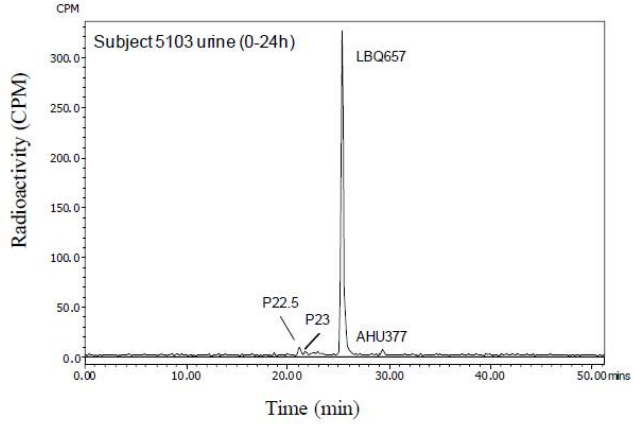
Metabolite profiles in pooled human urine (subject 5101 from 0-24 h) after oral dose of 200 mg [^{14}C]LCZ696



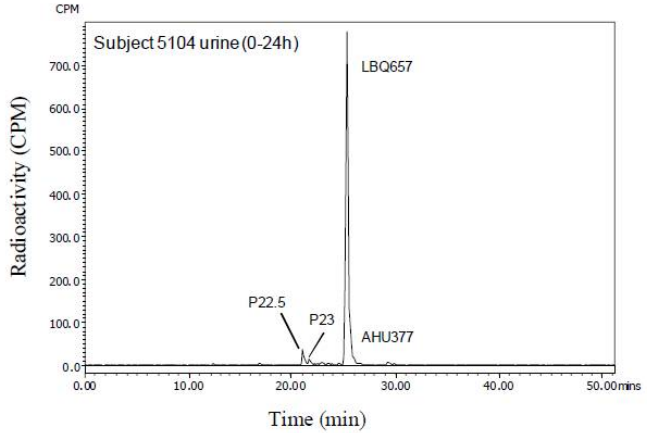
Metabolite profiles in pooled human urine (subject 5102 from 0-24 h) after oral dose of 200 mg [^{14}C]LCZ696



Metabolite profiles in pooled human urine (subject 5103 from 0-24 h) after oral dose of 200 mg [^{14}C]LCZ696

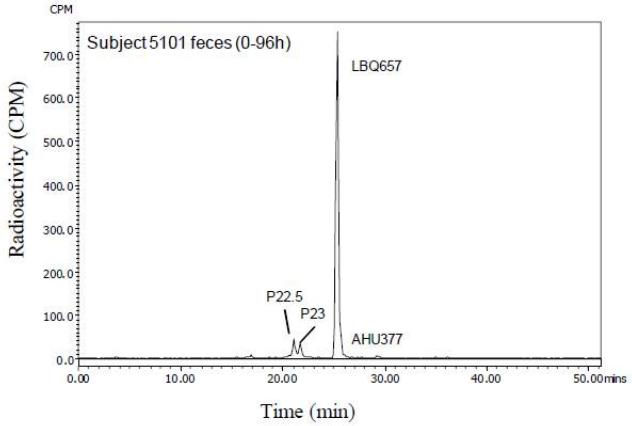


Metabolite profiles in pooled human urine (subject 5104 from 0-24 h) after oral dose of 200 mg [^{14}C]LCZ696

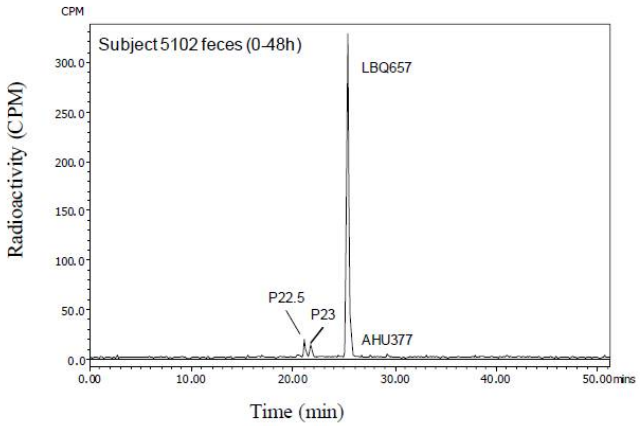


Study title: An open-label, single dose study to investigate the absorption, distribution, metabolism and elimination of 200 mg [^{14}C]LCZ696 and its metabolites in healthy male subjects

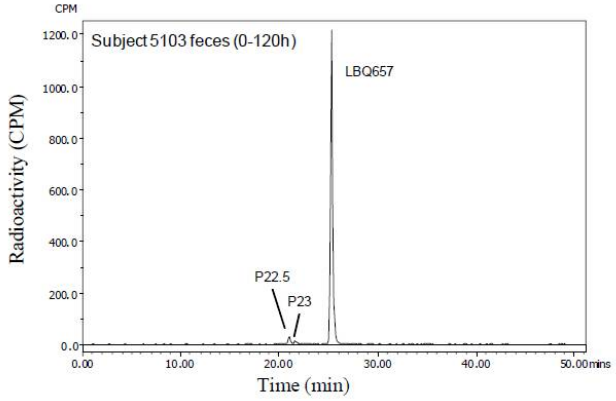
Metabolite profiles in pooled human feces (subject 5101 from 0-96 h) after a single oral dose of 200 mg [^{14}C]LCZ696



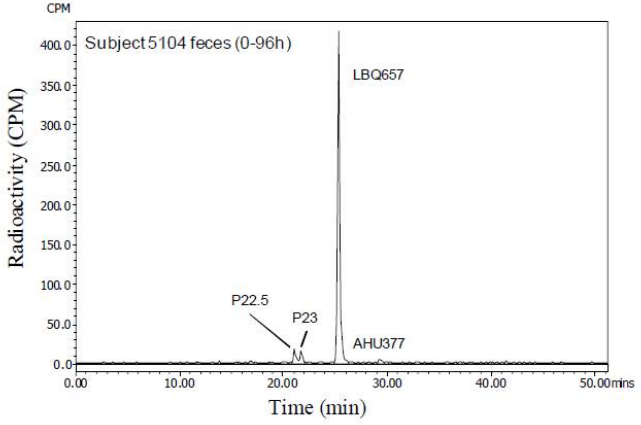
Metabolite profiles in pooled human feces (subject 5102 from 0-48 h) after a single oral dose of 200 mg [^{14}C]LCZ696



Metabolite profiles in pooled human feces (subject 5103 from 0-120 h) after a single oral dose of 200 mg [^{14}C]LCZ696



Metabolite profiles in pooled human feces (subject 5104 from 0-96 h) after a single oral dose of 200 mg [^{14}C]LCZ696



2.6.5.10A　薬物動態試験：*in vitro* における代謝

Test article: [^{14}C]AHU377

Study Title: In vitro metabolism of [^{14}C]AHU377 in rat, dog and human liver slices	
Study Number:	ADME(US) R0300249
GLP compliance:	Not required
Location in CTD:	4.2.2.4-1
Study System:	Incubations of with rat, dog and human liver slices.
	Tissue source: Association of Human Tissue Users (Tuscon, AZ). All human liver manipulations (slicing and in vitro incubation with test compounds) were performed by Vitron Inc. (Tuscon, AZ).
Initial Concentration(s):	5-20 µMol/L [^{14}C]AHU377
Radionuclide:	^{14}C (50.1 mCi/mmol, radiochemical purity ~ 98%)
Analyte/Assay:	Radioactivity / HPLC with radioactivity detection.
	Metabolite structure elucidation by LC-MS-MS.

Conclusions: In the rat, dog and human liver slices predominantly metabolite LBQ657 was observed. In liver slices from all species, AHU377 was almost completely metabolized after a 24-h incubation period.

Generally, AHU377 was metabolized relatively quickly at both concentrations in the liver slices from all species (initial metabolic rate rank order at 20 µM AHU377 concentration: rat > dog ~ human). The dog liver slice radioprofiles were most similar to the human liver profiles.

Proposed metabolic pathway of AHU377 in rat, dog and human liver slices



Study Title: In vitro metabolism of [^{14}C]AHU377 in rat, dog and human liver slices

Average radioactivity percent recovery for [^{14}C]AHU377 rat liver slice incubations

Conc. (μM)	Incubation Duration	DPM Medium	DPM Slice	DPM MeOH Wash	Total DPM of Incubate	% Recovery[a,b]
5.2	1 h	845221	39500	163443	1048163	107.3
5.2	2 h	795797	50739	114175	960711	98.4
5.2	4 h	760698	70700	134176	965574	98.9
5.2	8 h	753807	33703	110296	897806	91.9
5.2	18 h	813077	42199	138282	993558	101.7
5.2	24 h	814279	40330	88625	943234	96.6
5.2	24 h Dead Slice	1335224	177055	160131	1672409	171.3
5.2	24 h No Slice	965330	-	119461	1084791	111.1
21.2	1 h	3289478	131669	591657	4012804	101.1
21.2	2 h	3225262	137150	623758	3986170	100.4
21.2	4 h	3129762	185711	450381	3765854	94.9
21.2	8 h	2845741	312478	450050	3608269	90.9
21.2	18 h	3193819	208644	740675	4143138	104.4
21.2	24 h	3390741	69292	497621	3957654	99.7
21.2	24 h Dead Slice	3138083	93074	425878	3657036	92.1
21.2	24 h No Slice	3984180	-	703191	4687371	118.1

[a] Recovery was calculated by dividing the total radioactivity recovered following sample workup by the total expected DPM counts.

[b] Total expected DPM counts were 975600 and 3969457 for the 5.2 and 21.2 μM solutions, respectively.

Study Title: In vitro metabolism of [^{14}C]AHU377 in rat, dog and human liver slices

Average radioactivity percent recovery for [^{14}C]AHU377 dog liver slice incubations

Conc. (μM)	Incubation Duration	DPM Medium	DPM Slice	DPM MeOH Wash	Total DPM of Incubate	% Recovery[a,b]
5.3	1 h	721821	81440	105478	908739	91.9
5.3	2 h	756013	33506	155382	944901	95.6
5.3	4 h	620708	57626	83638	761972	77.1
5.3	8 h	593517	56887	81094	731499	74.0
5.3	18 h	722144	61613	92610	876367	88.6
5.3	24 h	789542	35608	113657	938808	95.0
5.3	24 h Dead Slice	646974	86113	158814	891902	90.2
5.3	24 h No Slice	789955	-	106306	896260	90.7
21.4	1 h	3125151	119670	581929	3826750	95.4
21.4	2 h	2739464	54259	580213	3373937	84.1
21.4	4 h	2742451	203009	411994	3357453	83.7
21.4	8 h	3036631	196451	440397	3673479	91.6
21.4	18 h	2929759	200917	412913	3543589	88.4
21.4	24 h	3251332	121748	421302	3794382	94.6
21.4	24 h Dead Slice	2930588	288034	596341	3814962	95.1
21.4	24 h No Slice	3246346	-	493606	3739951	93.3

[a] Recovery was calculated by dividing the total radioactivity recovered following sample workup by that in a comparable volume of medium used in the same study.

[b] Total DPM counts used in this study were 988620 and 4009717 for the 5.3 and 21.4 μM solutions, respectively.

Study Title: In vitro metabolism of [^{14}C]AHU377 in rat, dog and human liver slices

Average radioactivity percent recovery for [^{14}C]AHU377 human liver slice incubations

Conc (μ□)	Incubation Duration	DPM Medium	DPM Slice	DPM [a] MeOH Wash	Total DPM of incubate	% Recovery [b]
4.9	1 h	784089	18908	-	802996	88.2
4.9	2 h	821051	15947	-	836998	92.0
4.9	4 h	779243	14869	-	794112	87.3
4.9	8 h	781720	15246	-	796966	87.6
4.9	18 h	768607	15472	-	784080	86.1
4.9	24 h	820612	12570	-	833182	91.5
4.9	24 h Dead Slice	698652	79303	-	777955	85.5
4.9	24 h No slice	926400	-	-	926400	102
20.2	1 h	2968292	63909	-	3032201	78.7
20.2	2 h	3208909	68931	-	3277839	85.1
20.2	4 h	3219778	71222	-	3291000	85.5
20.2	8 h	3114858	70607	-	3185464	82.7
20.2	18 h	3099188	54560	-	3153748	81.9
20.2	24 h	3288609	42633	-	3331242	86.5
20.2	24 h Dead Slice	3009975	288443	-	3298418	85.6
20.2	24 h No slice	3978971	-	-	3978971	103.3

[a] Radioactivity associated with methanol wash was not included in recovery calculations. Methanol wash fraction is expected to contain approximately 10% of the rafioactivity based upon rat and dog sample processing

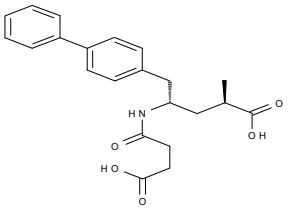
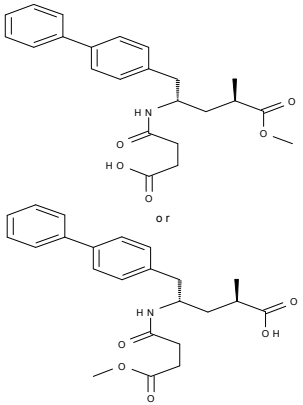
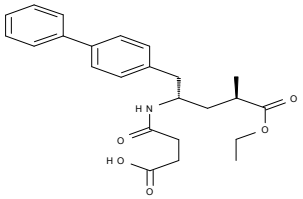
[b] Recovery was based on radioactivity recovered in the media and slice fractions only. Total expected DPM counts were 910141 and 3851281 for the 4.9 and 20.2 μM solutions, respectively.

Study Title: In vitro metabolism of [^{14}C]AHU377 in rat, dog and human liver slices			
Rate of Cyclosporin A (CsA) total metabolism in rat, dog and human liver slice incubations			
Sample	CsA μM	Incubation Duration, h	Total Metabolism pmol formed /hr/mg wet weight of tissue
Rat	1	2	4.4
Rat	1	24	0.37
Dog	1	2	2.0
Dog	1	24	1.5
Human	1	1	ND
Human	1	24	ND
Rate of [^{14}C]diclofenac total metabolism by rat, dog and human slices			
Sample	Concentration (μM)	Incubation time (h)	Total Metabolism (pmol/mg tissue/hr)
Rat	20	2	88.0
Rat	20	24	89.0
Dog	20	2	27.6
Dog	20	24	10.5
Human	20	1	ND
Human	20	24	ND

ND: not determined

Study Title: In vitro metabolism of [^{14}C]AHU377 in rat, dog and human liver slices

Structures of AHU377 and proposed metabolites formed by rat, dog and human liver slice incubation with [^{14}C]AHU377

Peak designation (retention time in min)	**Theoretical mass of molecular ion MH^+ (*m/z*)[1]**	**Measured mass of molecular ion MH^+ (*m/z*)[1]**	**Mass error (ppm)[a]**	**Species**	**Proposed structure**
LBQ657 (28.5)	384.1824	384.1823	-0.4	Rat, dog and human liver	
P33 (31)	398.1967	398.1970	0.7	Rat and human liver	
AHU377 (32.5)	412.2137	412.2132	-1.2	Rat, dog and human liver	

[a]Determined in human liver slices at 20 µM AHU377 concentration after one hour incubation .

2.6.5.10B　薬物動態試験：*in vitro* における代謝

Test article: [^{14}C]AHU377 and [^{14}C]LBQ657

Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9

Study Number:	ADME(US) R0300251
GLP compliance:	Not required
Location in CTD:	4.2.2.4-2
Study System:	Pooled human liver microsomes (n = 46 donors, 20 male and 26 female) and pooled human liver S9 (n = 29 donors, 16 male and 13 female) were purchased from Gentest, BD Biosciences Co. (Woburn, Massachusetts). The metabolism of [^{14}C]AHU377 and [^{14}C]LBQ657 was examined in pooled human liver microsomes in the presence (or absence) of NADPH. Human liver microsomes at varying concentrations (1 mg protein·mL^{-1}, 0.75 mg protein·mL^{-1}, or 0.5 mg protein·mL^{-1}) were pre-incubated at 37ºC for 3 min in an Eppendorf Thermomixer 5436 (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) in potassium phosphate buffer (100 mM, pH 7.4) with 5 mM $MgCl_2$ and 1 mM NADPH, final concentrations. The reactions were initiated by the addition of 42 µM [^{14}C]AHU377 or 41 µM [^{14}C]LBQ657 (final concentrations) and the samples were incubated for 10, 20, or 30 min at 37°C. Control incubations (30 min only) for [^{14}C]AHU377 and [^{14}C]LBQ657 contained all reaction components with the exception of human liver microsomes or NADPH (incubations containing 1 mg microsomal protein·mL^{-1}).
Radionuclide:	[^{14}C]AHU377 = 49.03 mCi·mmol^{-1}, 98% radiochemical purity; [^{14}C]LBQ657 = 49.20 mCi·mmol^{-1}, 98% radiochemical purity
Analyte/Assay:	Radioactivity / HPLC with radioactivity detection Metabolite structure elucidation by LC-MS-MS

Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9

Metabolism of [^{14}C]AHU377 in human liver microsomes[a]

[^{14}C]AHU377

Protein concentration (mg microsomal protein·mL^{-1})	Incubation time (min)	% metabolite formed (LBQ657)	Rate of metabolite formation (nmol·mg protein^{-1}·h^{-1})
0.5	10	14.1	71.8
	20	33.2	84.5
	30	42.2	71.6
0.75	10	24.6	83.5
	20	39.4	67.0
	30	50.8	57.5
1.0	10	31.2	79.3
	20	49.0	62.4
	30	54.8	46.6
1.0 (no NADPH)	30	78.1	66.3

[a] No metabolism of [^{14}C]LBQ657 was apparent under these same reaction conditions

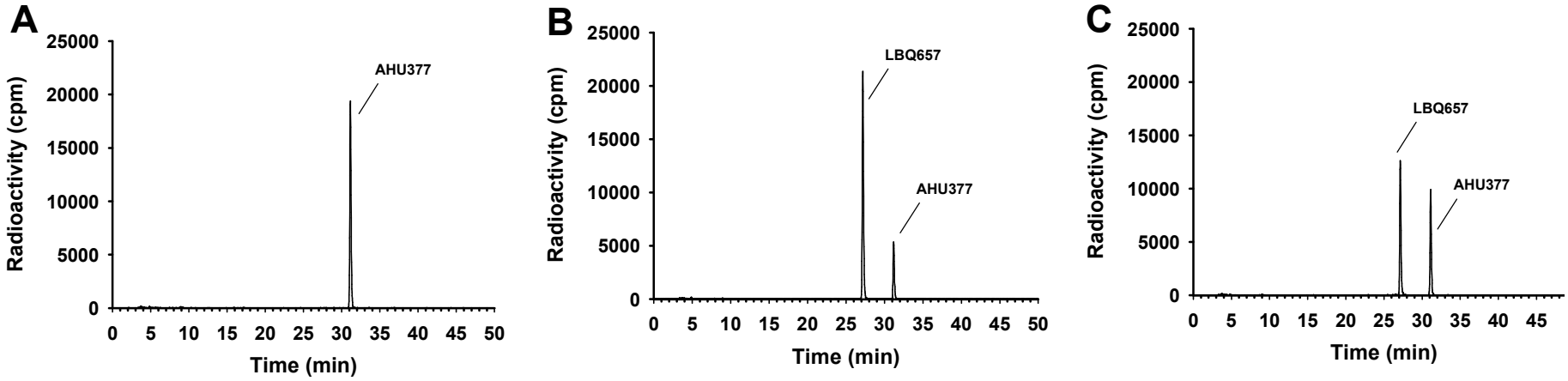
Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9			
Metabolism of [^{14}C]AHU377 in human liver S9[a]			
[^{14}C]AHU377			
Protein concentration (mg microsomal protein·mL^{-1})	Incubation time (min)	% metabolite formed (LBQ657)	Rate of metabolite formation (nmol·mg protein^{-1}·h^{-1})
0.5	10	14.4	73.3
	20	25.8	65.5
	30	35.2	59.5
0.75	10	20.1	68.0
	20	34.2	57.8
	30	42.9	48.4
1.0	10	23.8	60.3
	20	37.5	47.5
	30	50.4	42.6
1.0 (no NADPH)	30	54.1	45.7

[a] No metabolism of [^{14}C]LBQ657 was apparent under these same reaction conditions

Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9

Metabolism of [^{14}C]AHU377 by human liver microsomes

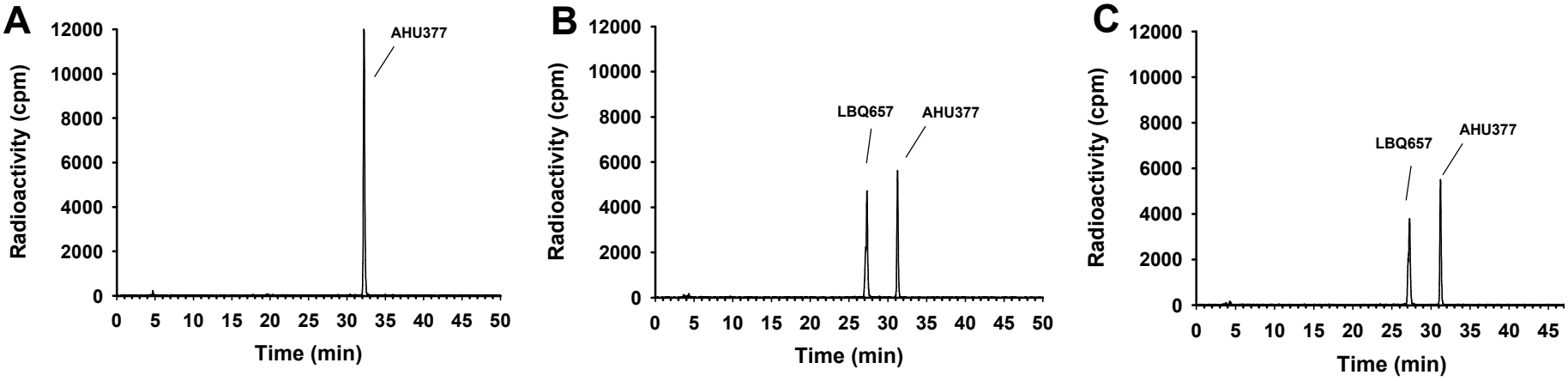
[^{14}C]AHU377 (42 µM) was incubated in potassium phosphate buffer with $MgCl_2$ in the absence (**A**) or presence (**B & C**) of human liver microsomal protein (1 mg protein·mL^{-1}) for 30 min at 37°C in the presence (**A &C**) or absence (**B**) of NADPH. Reactions were quenched with an equal volume of cold acetonitrile and products analyzed by HPLC.



Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9

Metabolism of [14C]AHU377 by human liver S9

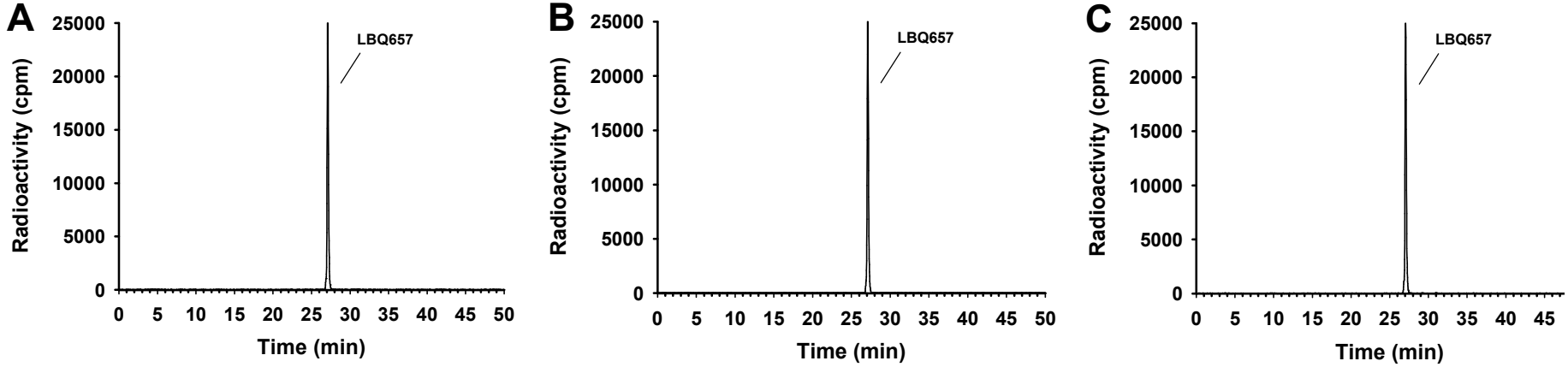
[^{14}C]AHU377 (42 µM) was incubated in potassium phosphate buffer with $MgCl_2$ in the absence (**A**) or presence (**B & C**) of human liver S9 (1 mg protein·mL^{-1}) for 30 min at 37°C in the presence (**A &C**) or absence (**B**) of NADPH. Reactions were quenched with an equal volume of cold acetonitrile and products analyzed by HPLC.



Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9

Metabolism of [^{14}C]LBQ657 by human liver microsomes

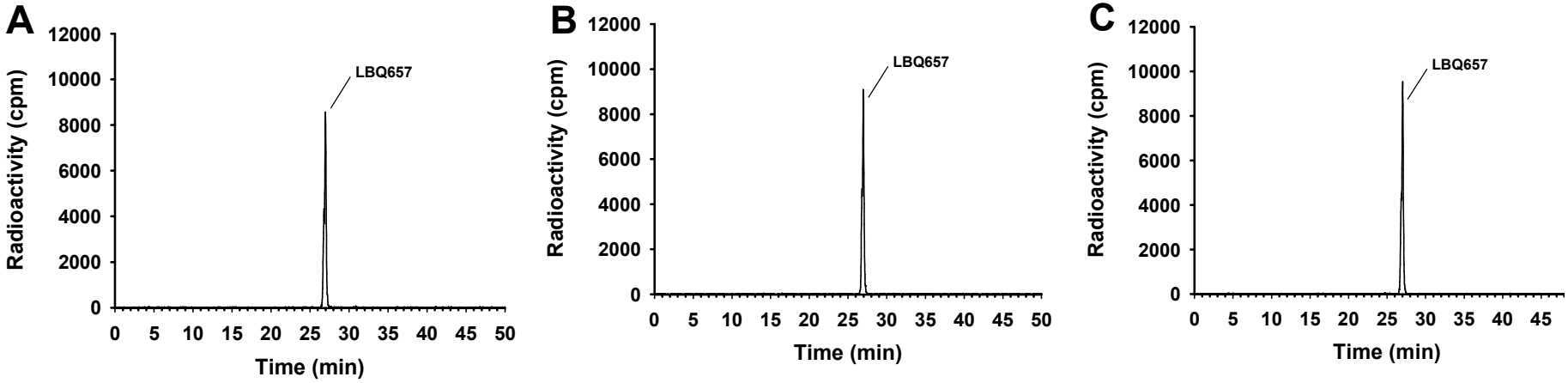
[^{14}C]LBQ657 (41 µM) was incubated in potassium phosphate buffer with $MgCl_2$ in the absence (**A**) or presence (**B & C**) of human liver microsomal protein (1 mg protein·mL^{-1}) for 30 min at 37°C in the presence (**A &C**) or absence (**B**) of NADPH. Reactions were quenched with an equal volume of cold acetonitrile and products analyzed by HPLC.



Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9

Metabolism of [^{14}C]LBQ657 by human liver S9

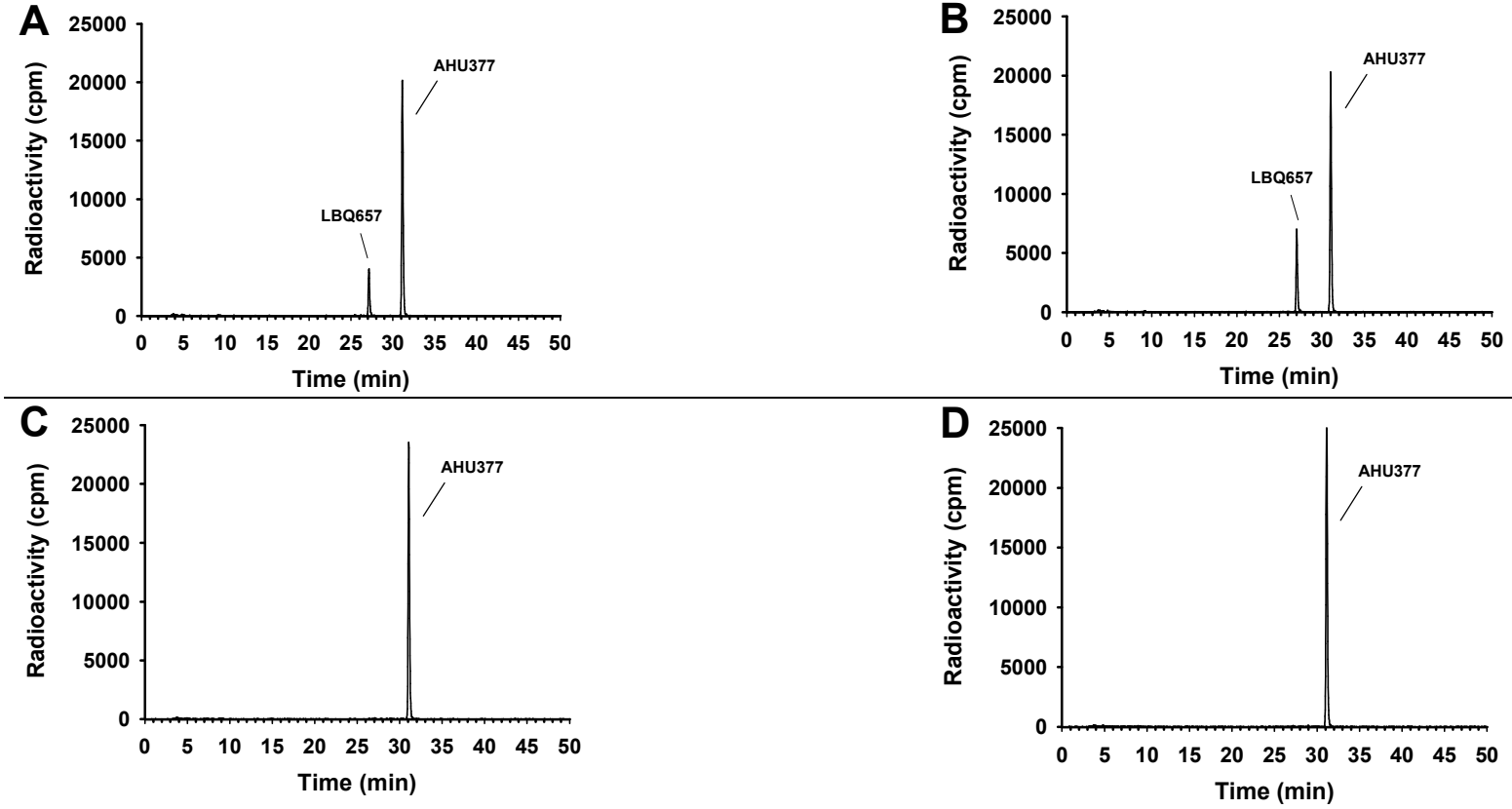
[^{14}C]LBQ657 (41 µM) was incubated in potassium phosphate buffer with $MgCl_2$ in the absence (**A**) or presence (**B & C**) of human liver S9 (1 mg protein·mL^{-1}) for 30 min at 37°C in the presence (**A &C**) or absence (**B**) of NADPH. Reactions were quenched with an equal volume of cold acetonitrile and products analyzed by HPLC.



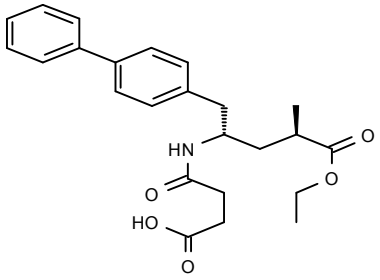
Study Title: Metabolism of AHU377 and its active metabolite, LBQ657, in human liver microsomes and S9

Inhibition of [^{14}C]AHU377 metabolism in human liver microsomes by the esterase inhibitor, KF

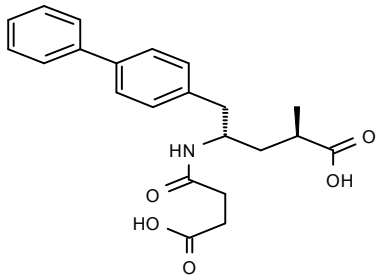
(A & B) Human liver microsomal protein (0.5 mg protein·mL-1) was pre-incubated in potassium phosphate buffer with MgCl2 in the presence (A) or absence (B) of NADPH for 3 min at 37°C. (C & D) Human liver microsomal protein (0.5 mg protein·mL-1) was pre-incubated in potassium phosphate buffer with MgCl2 and 100 mM KF for 5 min at 37°C. Samples were then pre-incubated for an additional 3 min in the presence (C) or absence (D) of NADPH. (A-D) After pre-incubations, samples were initiated by the addition of [^{14}C]AHU377 (42 μM) and were quenched after 30 min with an equal volume of cold acetonitrile and products analyzed by HPLC.



2.6.5.11 薬物動態試験：推定代謝経路



AHU377



LBQ657

2.6.5.12A　薬物動態試験：薬物代謝酵素の誘導／阻害

Test article: AHU377 and LBQ657

Study Title(s):	In vitro assessment of cytochrome P450 enzyme inhibition by NVP-AHU377 and its metabolite NVP-LBQ657		
Study Number:	ADME(US) R0300252		
GLP compliance:	Not required		
Location in CTD:	4.2.2.6-1		
Study System:	Pooled human liver microsomes (source: Xenotech LLC)		
Probe substrate/Assay:	Phenacetin, Paclitaxel, Diclofenac, S-mephenytoin, Bufuralol, Chlorzoxazone, Midazolam, testosterone; LC-MS/MS analysis of specific metabolite formations from probe substrates by cytochrome P450		
CYP Enzyme	Probe reaction(s)	IC_{50} (μM)[a]	
		AHU377	LBQ657
1A2	phenacetin *O*-deethylation	> 100	> 100
2C8	paclitaxel 6α-hydroxylation	~ 15	> 100
2C9	diclofenac 4′-hydroxylation	> 100	~ 40
2C19	*S*-mephenytoin 4′-hydroxylation	~ 20	> 100
2D6	bufuralol 1′-hydroxylation	> 100	> 100
2E1	chlorzoxazone 6-hydroxylation	> 100	> 100
3A4/5	midazolam 1′-hydroxylation	> 100	> 100
3A4/5	testosterone 6β-hydroxylation	>100	> 100

[a] Concentration producing 50% inhibition of the probe substrate reaction

AHU377 was found to be relatively stable under the incubation conditions with >60% of the unchanged drug remaining at the end of the incubation period. Little or no inhibition of CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4/5 by AHU377 was observed when AHU377 was tested at concentrations of up to 100 μM. In one instance, AHU377 appeared to produce a small increase in the probe substrate metabolic reaction with CYP2D6 (bufuralol 1′-hydroxylation). The relevance, if any, of this to in vivo effects is not known. AHU377 was found to be a weak inhibitor of CYP2C8 (IC_{50} ~15 μM) and CYP2C19 (IC_{50} ~20 μM), with no time-dependent inhibition of either activity observed. LBQ657 was also found to be stable under the incubation conditions. LBQ657 showed little or no inhibition of CYP1A2, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5 when LBQ657 was tested at concentrations of up to 100 μM. LBQ657 was found to be an very weak inhibitor of CYP2C9 (IC_{50} ~40 μM). Like AHU377, LBQ657 appeared to produce a slight increase in CYP2D6 activity.

2.6.5.12B　薬物動態試験：薬物代謝酵素の誘導／阻害

Test articles: AHU377 and LBQ657

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

Study Number:	DMPK R1200084
GLP compliance:	Not required
Location in CTD:	4.2.2.6-2
Study System:	Primary human hepatocytes (Celsis / In Vitro Technologies)
Analyte/Assay:	Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in enzyme activities were assessed after the induction period by quantitative LC-MS/MS analysis of enzyme-selective probe substrate metabolism

AHU377 was examined for its potential to induce cytochrome P450 (CYP) enzymes mRNA and activities in primary human hepatocytes of three individual donors after 48h of treatment.

Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in enzyme activities were assessed after the induction period by quantitative LC-MS/MS analysis of enzyme-selective probe substrate metabolism.

Treatment of human hepatocytes with AHU377 (10-100 µM) did not result in induction of CYP1A2, CYP2B6, CYP2C9, or CYP3A4 mRNA or activity (induction levels were < 2-fold and/or < 40% of the positive control response) in the three donor hepatocytes. AHU377 was converted to its active metabolite, LBQ657 by > 50% at 4h incubation time point and by 100% at 24h incubation time point.

Based upon this in vitro study, both AHU377 and LBQ657 are unlikely to induce the expression and/or catalytic activities of CYP1A2, CYP2B6, CYP2C9, or CYP3A in vivo.

mRNA fold induction[a] (Liver 1)

48h treatment of human hepatocytes

(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
DMSO (0.1%)[b]	1.00	1.00	1.00	1.00
AHU377, 10 µM	1.20 ± 0.15	1.35 ± 0.11	0.873 ± 0.053	1.44 ± 0.13
AHU377, 50 µM	0.785 ± 0.073	1.08 ± 0.086	1.06 ± 0.19	1.36 ± 0.11
AHU377, 100 µM	0.825 ± 0.14	1.27 ± 0.040	1.09 ± 0.29	0.642 ± 0.079
RIF, 10 µM	0.813 ± 0.034	6.47 ± 0.56	1.72 ± 0.36	28.1 ± 3.7
PB, 1000 µM	1.28 ± 0.15	7.43 ± 0.27	1.60 ± 0.073	28.0 ± 1.3
Omep, 50 µM	56.7 ± 6.7	4.74 ± 0.44	1.11 ± 0.23	10.9 ± 0.91

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

mRNA fold induction[a] (Liver 2)

48h treatment of human hepatocytes

(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
DMSO (0.1%)[b]	1.00	1.00	1.00	1.00
AHU377, 10 µM	0.734 ± 0.025	0.724 ± 0.11	1.10 ± 0.21	0.611 ± 0.11
AHU377, 50 µM	0.666 ± 0.053	0.685 ± 0.11	1.01 ± 0.17	0.731 ± 0.20
AHU377, 100 µM	0.580 ± 0.080	0.667 ± 0.070	1.25 ± 0.17	0.939 ± 0.14
RIF, 10 µM	0.407 ± 0.058	6.95 ± 0.31	1.74 ± 0.25	36.0 ± 9.6
PB, 1000 µM	0.922 ± 0.063	7.75 ± 1.1	3.69 ± 1.3	29.8 ± 4.8
Omep, 50 µM	18.4 ± 0.60	2.63 ± 0.42	1.17 ± 0.21	8.06 ± 1.1

mRNA fold induction[a] (Liver 3)

48h treatment of human hepatocytes

(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
DMSO (0.1%)[b]	1.00	1.00	1.00	1.00
AHU377, 10 µM	0.819 ± 0.047	1.03 ± 0.084	0.869 ± 0.11	0.947 ± 0.043
AHU377, 50 µM	0.787 ± 0.16	1.22 ± 0.20	1.18 ± 0.13	1.10 ± 0.17
AHU377, 100 µM	0.553 ± 0.031	0.891 ± 0.1	1.07 ± 0.14	0.750 ± 0.058
RIF, 10 µM	0.543 ± 0.046	6.66 ± 0.30	2.16 ± 0.28	20.8 ± 1.7
PB, 1000 µM	0.739 ± 0.14	8.86 ± 0.45	2.05 ± 0.035	18.4 ± 1.3
Omep, 50 µM	49.0 ± 8.5	5.95 ± 0.69	1.94 ± 0.25	9.98 ± 1.8

[a] Calculated from quadruplicate real-time PCR reactions

[b] Vehicle control

RIF: rifampicin

PB: phenobarbital

Omep: omeprazole

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP1A2 activity (phenacetin *O*-deethylation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP1A2 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0167 ± 0.00013	0.0333 ± 0.00026	0.333 ± 0.0055	1.7	1.00 ± 0.023	0
AHU377, 10 µM	0.0265 ± 0.0014	0.0530 ± 0.0029	0.476 ± 0.018	3.8	1.43 ± 0.059	3.07 ± 0.26
AHU377, 50 µM	0.0219 ± 0.0027	0.0438 ± 0.0055	0.368 ± 0.024	6.5	1.11 ± 0.074	0.754 ± 0.065
AHU377, 100 µM	0.0235 ± 0.0024	0.0471 ± 0.0049	0.453 ± 0.034	7.6	1.36 ± 0.11	2.58 ± 0.22
RIF, 10 µM	0.0227 ± 0.0018	0.0454 ± 0.0035	0.326 ± 0.014	4.1	0.981 ± 0.044	-0.135 ± -0.012
PB, 1000 µM	0.0264 ± 0.0033	0.0529 ± 0.0067	0.507 ± 0.042	8.3	1.52 ± 0.13	3.74 ± 0.32
Omep, 50 µM	0.317 ± 0.023	0.633 ± 0.047	4.99 ± 0.40	8.0	15.0 ± 1.2	100 ± 8.6

CYP1A2 activity (phenacetin O-deethylation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP1A2 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0858 ± 0.0052	0.172 ± 0.010	1.10 ± 0.053	4.8	1.00 ± 0.068	0
AHU377, 10 µM	0.0661 ± 0.0061	0.132 ± 0.012	0.778 ± 0.057	7.3	0.705 ± 0.062	-3.91 ± -0.38
AHU377, 50 µM	0.0725 ± 0.0053	0.145 ± 0.011	0.912 ± 0.034	3.7	0.827 ± 0.050	-2.29 ± -0.23
AHU377, 100 µM	0.0615 ± 0.0057	0.123 ± 0.011	0.824 ± 0.065	7.9	0.747 ± 0.069	-3.36 ± -0.33
RIF, 10 µM	0.0873 ± 0.0041	0.175 ± 0.0082	1.07 ± 0.021	2.0	0.972 ± 0.051	-0.367 ± -0.037
PB, 1000 µM	0.0726 ± 0.0027	0.145 ± 0.0054	0.914 ± 0.038	4.2	0.829 ± 0.053	-2.27 ± -0.22
Omep, 50 µM	0.725 ± 0.031	1.45 ± 0.062	9.42 ± 0.82	8.7	8.54 ± 0.85	100 ± 9.9

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP1A2 activity (phenacetin O-deethylation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (μM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP1A2 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0320 ± 0.0021	0.0640 ± 0.0041	0.479 ± 0.036	7.4	1.00 ± 0.11	0
AHU377, 10 μM	0.0285 ± 0.0015	0.0569 ± 0.0029	0.394 ± 0.024	6.1	0.823 ± 0.079	-1.33 ± -0.071
AHU377, 50 μM	0.0251 ± 0.0017	0.0503 ± 0.0034	0.375 ± 0.013	3.4	0.782 ± 0.064	-1.63 ± -0.087
AHU377, 100 μM	0.0256 ± 0.0031	0.0512 ± 0.0062	0.468 ± 0.018	3.8	0.976 ± 0.081	-0.178 ± -0.011
RIF, 10 μM	0.0507 ± 0.0054	0.101 ± 0.011	0.742 ± 0.043	5.8	1.55 ± 0.15	4.11 ± 0.22
PB, 1000 μM	0.0521 ± 0.0024	0.104 ± 0.0048	0.728 ± 0.041	5.6	1.52 ± 0.14	3.89 ± 0.21
Omep, 50 μM	0.466 ± 0.019	0.931 ± 0.038	6.88 ± 0.34	4.9	14.4 ± 1.3	100 ± 5.3

[a]percent turnover of phenacetin (200 μM) to acetaminophen during the 0.5 hour incubation

[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells

[c]calculated mean activity (total nmol acetaminophen formed/mg protein in the well/h) ± standard deviation of quadruplicate wells

[d]Fold increase in phenacetin *O*-deethylation activity with respect to vehicle control treated cells ± standard deviation.

[e]percent of maximal positive control induction

[f]vehicle control

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP2B6 activity (OH-bupropion formation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2B6 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.00372 ± 0.00049	0.0149 ± 0.0020	0.128 ± 0.011	9.0	1.00 ± 0.13	0
AHU377, 10 µM	0.00393 ± 0.00038	0.0157 ± 0.0015	0.137 ± 0.0092	6.8	1.07 ± 0.12	2.80 ± 0.21
AHU377, 50 µM	0.00363 ± 0.00014	0.0145 ± 0.00057	0.113 ± 0.0063	5.6	0.885 ± 0.094	-4.56 ± -0.34
AHU377, 100 µM	0.00313 ± 0.00040	0.0125 ± 0.0016	0.114 ± 0.0081	7.1	0.895 ± 0.10	-4.14 ± -0.31
RIF, 10 µM	0.0131 ± 0.00061	0.0522 ± 0.0024	0.427 ± 0.019	4.4	3.35 ± 0.34	93.0 ± 6.8
PB, 1000 µM	0.0132 ± 0.00079	0.0529 ± 0.0031	0.450 ± 0.021	4.6	3.53 ± 0.36	100 ± 7.3
Omep, 50 µM	0.00519 ± 0.00054	0.0208 ± 0.0021	0.174 ± 0.016	9.0	1.36 ± 0.17	14.4 ± 1.1

CYP2B6 activity (OH-bupropion formation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2B6 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0167 ± 0.00094	0.0668 ± 0.0038	0.407 ± 0.034	8.3	1.00 ± 0.12	0
AHU377, 10 µM	0.0173 ± 0.0016	0.0692 ± 0.0066	0.406 ± 0.030	7.3	0.998 ± 0.11	-0.0873 ± -0.061
AHU377, 50 µM	0.0172 ± 0.0015	0.0688 ± 0.0059	0.414 ± 0.027	6.5	1.02 ± 0.11	0.968 ± 0.12
AHU377, 100 µM	0.0153 ± 0.00092	0.0614 ± 0.0037	0.376 ± 0.036	9.6	0.925 ± 0.12	-4.13 ± -0.46
RIF, 10 µM	0.0499 ± 0.0034	0.200 ± 0.014	1.15 ± 0.074	6.4	2.82 ± 0.30	100 ± 11
PB, 1000 µM	0.0413 ± 0.0020	0.165 ± 0.0082	0.973 ± 0.019	2.0	2.39 ± 0.20	76.3 ± 8.4
Omep, 50 µM	0.0208 ± 0.0011	0.0832 ± 0.0044	0.538 ± 0.026	4.8	1.32 ± 0.13	17.7 ± 1.9

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP2B6 activity (OH-bupropion formation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (μM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2B6 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0123 ± 0.0013	0.0492 ± 0.0051	0.358 ± 0.031	8.6	1.00 ± 0.12	0
AHU377, 10 μM	0.0137 ± 0.00044	0.0549 ± 0.0018	0.391 ± 0.020	5.2	1.09 ± 0.11	2.62 ± 0.27
AHU377, 50 μM	0.0110 ± 0.00070	0.0441 ± 0.0028	0.337 ± 0.030	8.8	0.943 ± 0.12	-1.62 ± -0.17
AHU377, 100 μM	0.00791 ± 0.00090	0.0317 ± 0.0036	0.269 ± 0.018	6.5	0.751 ± 0.081	-7.07 ± -0.73
RIF, 10 μM	0.0524 ± 0.0037	0.210 ± 0.015	1.44 ± 0.072	5.0	4.02 ± 0.40	85.7 ± 8.9
PB, 1000 μM	0.0568 ± 0.0061	0.227 ± 0.024	1.62 ± 0.13	7.9	4.52 ± 0.53	100 ± 10
Omep, 50 μM	0.0227 ± 0.0031	0.0908 ± 0.013	0.718 ± 0.082	11	2.01 ± 0.29	28.6 ± 3.0

[a]percent turnover of bupropion (400 μM) to OH-bupropion during the 0.5 hour incubation

[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells

[c]calculated mean activity (total nmol OH-bupropion formed/mg protein in the well/h) ± standard deviation of quadruplicate wells

[d]Fold increase in bupropion hydroxylation activity with respect to vehicle control treated cells ± standard deviation

[e]percent of maximal positive control induction

[f]vehicle control

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP2C9 activity (4' OH-diclofenac formation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2C9 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.387 ± 0.053	0.387 ± 0.053	3.31 ± 0.31	9.4	1.00 ± 0.13	0
AHU377, 10 µM	0.381 ± 0.021	0.381 ± 0.021	3.15 ± 0.25	7.9	0.953 ± 0.12	-9.33 ± -2.6
AHU377, 50 µM	0.356 ± 0.033	0.356 ± 0.033	3.43 ± 0.16	4.7	1.04 ± 0.11	7.62 ± 2.1
AHU377, 100 µM	0.408 ± 0.069	0.408 ± 0.069	4.30 ± 0.37	8.6	1.30 ± 0.17	60.3 ± 17
RIF, 10 µM	0.547 ± 0.053	0.547 ± 0.053	4.96 ± 0.34	6.9	1.50 ± 0.17	100 ± 28
PB, 1000 µM	0.460 ± 0.025	0.460 ± 0.025	3.85 ± 0.37	9.5	1.16 ± 0.16	33.0 ± 9.2
Omep, 50 µM	0.386 ± 0.034	0.386 ± 0.034	3.52 ± 0.35	9.9	1.06 ± 0.14	12.8 ± 3.6

CYP2C9 activity (4' OH-diclofenac formation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2C9 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.397 ± 0.037	0.397 ± 0.037	2.54 ± 0.15	5.9	1.00 ± 0.083	0
AHU377, 10 µM	0.388 ± 0.028	0.388 ± 0.028	2.50 ± 0.14	5.6	0.987 ± 0.080	-1.10 ± -0.11
AHU377, 50 µM	0.443 ± 0.037	0.443 ± 0.037	2.94 ± 0.26	8.9	1.16 ± 0.12	13.7 ± 1.1
AHU377, 100 µM	0.344 ± 0.016	0.344 ± 0.016	2.23 ± 0.17	7.5	0.881 ± 0.084	-10.0 ± -0.83
RIF, 10 µM	0.888 ± 0.026	0.888 ± 0.026	5.53 ± 0.20	3.6	2.18 ± 0.15	100 ± 8.3
PB, 1000 µM	0.774 ± 0.056	0.774 ± 0.056	5.10 ± 0.38	7.5	2.01 ± 0.19	85.7 ± 7.1
Omep, 50 µM	0.417 ± 0.031	0.417 ± 0.031	2.74 ± 0.20	7.4	1.08 ± 0.10	6.71 ± 0.56

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP2C9 activity (4' OH-diclofenac formation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (μM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2C9 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.505 ± 0.033	0.505 ± 0.033	3.92 ± 0.33	8.5	1.00 ± 0.12	0
AHU377, 10 μM	0.585 ± 0.066	0.585 ± 0.066	4.46 ± 0.43	9.6	1.14 ± 0.15	10.3 ± 1.6
AHU377, 50 μM	0.579 ± 0.043	0.579 ± 0.043	4.89 ± 0.45	9.1	1.25 ± 0.16	18.6 ± 2.9
AHU377, 100 μM	0.432 ± 0.044	0.432 ± 0.045	4.05 ± 0.29	7.1	1.03 ± 0.11	2.39 ± 0.38
RIF, 10 μM	1.20 ± 0.097	1.20 ± 0.097	9.10 ± 0.72	7.9	2.32 ± 0.27	100 ± 15
PB, 1000 μM	1.07 ± 0.10	1.07 ± 0.10	7.79 ± 0.65	8.3	1.99 ± 0.24	74.7 ± 12
Omep, 50 μM	0.682 ± 0.063	0.682 ± 0.063	5.33 ± 0.50	9.3	1.36 ± 0.17	27.2 ± 4.2

[a]percent turnover of diclofenac (100 μM) to 4' OH-diclofenac during the 0.5 hour incubation

[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells

[c]calculated mean activity (total nmol 4' OH-diclofenac formed/mg protein in the well/h) ± standard deviation of quadruplicate wells

[d]Fold increase in diclofenac 4' hydroxylation activity with respect to vehicle control treated cells ± standard deviation.

[e]percent of maximal positive control induction

[f]vehicle control

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP3A activity (1' OH-midazolam formation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP3A activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0355 ± 0.0021	0.0355 ± 0.0021	0.299 ± 0.021	7.1	1.00 ± 0.1	0
AHU377, 10 µM	0.0380 ± 0.0054	0.0380 ± 0.0054	0.316 ± 0.025	7.9	1.06 ± 0.11	1.75 ± 0.18
AHU377, 50 µM	0.0309 ± 0.0030	0.0309 ± 0.0030	0.308 ± 0.027	8.7	1.03 ± 0.12	0.942 ± 0.10
AHU377, 100 µM	0.0268 ± 0.0045	0.0268 ± 0.0045	0.293 ± 0.025	8.5	0.981 ± 0.11	-0.552 ± -0.066
RIF, 10 µM	0.168 ± 0.0093	0.168 ± 0.0093	1.31 ± 0.10	7.8	4.38 ± 0.46	100 ± 10
PB, 1000 µM	0.151 ± 0.016	0.151 ± 0.016	1.27 ± 0.095	7.5	4.27 ± 0.44	96.6 ± 10
Omep, 50 µM	0.0321 ± 0.0015	0.0321 ± 0.0015	0.255 ± 0.0091	3.5	0.855 ± 0.068	-4.27 ± -0.44

CYP3A activity (1' OH-midazolam formation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP3A activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0846 ± 0.0039	0.0846 ± 0.0039	0.724 ± 0.016	2.2	1.00 ± 0.031	0
AHU377, 10 µM	0.0750 ± 0.0086	0.0750 ± 0.0086	0.614 ± 0.051	8.4	0.848 ± 0.073	-3.04 ± -0.16
AHU377, 50 µM	0.0713 ± 0.0038	0.0713 ± 0.0038	0.489 ± 0.042	8.6	0.676 ± 0.060	-6.48 ± -0.33
AHU377, 100 µM	0.0597 ± 0.0045	0.0597 ± 0.0045	0.371 ± 0.026	7.1	0.513 ± 0.038	-9.74 ± -0.50
RIF, 10 µM	0.680 ± 0.034	0.680 ± 0.034	4.34 ± 0.19	4.3	6.00 ± 0.29	100 ± 5.2
PB, 1000 µM	0.692 ± 0.055	0.692 ± 0.055	4.29 ± 0.40	9.4	5.93 ± 0.57	98.5 ± 5.1
Omep, 50 µM	0.0738 ± 0.0048	0.0738 ± 0.0048	0.527 ± 0.044	8.3	0.728 ± 0.063	-5.43 ± -0.28

Study title: Evaluation of AHU377 as an inducer of cytochrome P450s in human hepatocytes

CYP3A activity (1' OH-midazolam formation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (μM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP3A activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0613 ± 0.0020	0.0613 ± 0.0020	0.483 ± 0.040	8.2	1.00 ± 0.12	0
AHU377, 10 μM	0.0704 ± 0.0063	0.0704 ± 0.0063	0.557 ± 0.028	5.0	1.15 ± 0.11	2.39 ± 0.25
AHU377, 50 μM	0.0491 ± 0.00082	0.0491 ± 0.00082	0.417 ± 0.0077	1.8	0.864 ± 0.072	-2.13 ± -0.23
AHU377, 100 μM	0.0313 ± 0.0037	0.0313 ± 0.0037	0.293 ± 0.021	7.2	0.606 ± 0.066	-6.16 ± -0.65
RIF, 10 μM	0.471 ± 0.046	0.471 ± 0.046	3.57 ± 0.33	9.1	7.39 ± 0.91	100 ± 11
PB, 1000 μM	0.424 ± 0.039	0.424 ± 0.039	2.99 ± 0.24	7.9	6.19 ± 0.70	81.1 ± 8.6
Omep, 50 μM	0.0623 ± 0.0038	0.0623 ± 0.0038	0.415 ± 0.030	7.1	0.860 ± 0.093	-2.19 ± -0.23

[a]percent turnover of midazolam (100 μM) to 1' OH-midazolam during the 0.5 hour incubation

[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells

[c]calculated mean activity (total nmol 1' OH-midazolam formed/mg protein in the well/h) ± standard deviation of quadruplicate wells

[d]Fold increase in midazolam 1' hydroxylation activity with respect to vehicle control treated cells ± standard deviation.

[e]percent of maximal positive control induction

[f]vehicle control

2.6.5.12C 薬物動態試験：薬物代謝酵素の誘導／阻害

Test articles: VAL489 (Valsartan)

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

Study Number:	DMPK R1200239
GLP compliance:	Not required
Location in CTD:	4.2.2.6-3
Study System:	Primary human hepatocytes (Celsis / In Vitro Technologies)
Analyte/Assay:	Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in enzyme activities were assessed after the induction period by quantitative LC-MS/MS analysis of enzyme-selective probe substrate metabolism

- Valsartan was examined for its potential to induce cytochrome P450 (CYP) enzymes mRNA and activities in primary human hepatocytes of three individual donors after 48h of treatment.
- Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in enzyme activities were assessed after the induction period by quantitative LC-MS/MS analysis of enzyme-selective probe substrate metabolism.
- Treatment of human hepatocytes with valsartan (1-100 µM) did not result in induction of CYP1A2, CYP2B6, CYP2C9, or CYP3A4 mRNA or CYP2B6, CYP2C9, or CYP3A4 activities in the three donors. The levels of expression and activity with respect to the vehicle control were < 2-fold and/or < 40% of the positive control levels. No induction of CYP1A2 activity was observed in all three donors except for one outlier in Liver 1 (no induction at CYP1A2 mRNA level with the same treatment).
- Based upon this in vitro study, valsartan is unlikely to induce the expression and/or catalytic activities of CYP1A2, CYP2B6, CYP2C9, or CYP3A in vivo.

mRNA fold induction[a] (Liver 1)

48h treatment of human hepatocytes

(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
DMSO (0.1%)[b]	1.00	1.00	1.00	1.00
valsartan, 1 µM	0.751 ± 0.19	0.835 ± 0.070	0.747 ± 0.16	0.699 ± 0.091
valsartan, 10 µM	0.835 ± 0.056	0.881 ± 0.099	0.674 ± 0.091	0.695 ± 0.088
valsartan, 25 µM	0.690 ± 0.15	0.957 ± 0.057	0.742 ± 0.063	0.854 ± 0.099
valsartan, 50 µM	0.947 ± 0.12	0.903 ± 0.14	0.761 ± 0.13	0.698 ± 0.064
valsartan, 100 µM	n.a.[c]	n.a.[c]	n.a.[c]	n.a.[c]

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

mRNA fold induction[a] (Liver 1)
48h treatment of human hepatocytes
(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
RIF, 10 µM	0.763 ± 0.17	5.61 ± 0.95	1.53 ± 0.25	13.8 ± 2.6
PB, 1000 µM	1.29 ± 0.20	9.13 ± 0.84	1.98 ± 0.41	18.9 ± 2.0
BNF, 10 µM	19.8 ± 3.1	3.01 ± 0.26	0.660 ± 0.078	0.212 ± 0.056
Omep, 50 µM	n.a.	n.a.	n.a.	n.a.

mRNA fold induction[a] (Liver 2)
48h treatment of human hepatocytes
(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
DMSO (0.1%)[b]	1.00	1.00	1.00	1.00
valsartan, 1 µM	1.14 ± 0.12	1.08 ± 0.13	0.702 ± 0.078	0.864 ± 0.14
valsartan, 10 µM	1.31 ± 0.11	1.77 ± 0.25	0.795 ± 0.18	0.892 ± 0.13
valsartan, 25 µM	1.10 ± 0.058	0.829 ± 0.058	0.921 ± 0.12	0.646 ± 0.10
valsartan, 50 µM	1.16 ± 0.049	0.765 ± 0.066	0.718 ± 0.16	0.567 ± 0.046
valsartan, 100 µM	1.03 ± 0.14	0.663 ± 0.070	0.738 ± 0.089	0.697 ± 0.18
RIF, 10 µM	0.682 ± 0.050	7.50 ± 0.48	1.61 ± 0.21	64.6 ± 2.0
PB, 1000 µM	1.38 ± 0.089	7.82 ± 0.98	1.77 ± 0.21	110 ± 16
BNF, 10 µM	n.a.	n.a.	n.a.	n.a.
Omep, 50 µM	33.2 ± 10	3.75 ± 0.39	0.882 ± 0.14	17.1 ± 3.2

mRNA fold induction[a] (Liver 3)
48h treatment of human hepatocytes
(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
DMSO (0.1%)[b]	1.00	1.00	1.00	1.00
valsartan, 1 µM	0.999 ± 0.17	1.14 ± 0.088	0.978 ± 0.13	1.12 ± 0.19
valsartan, 10 µM	1.12 ± 0.051	1.29 ± 0.095	0.908 ± 0.059	0.989 ± 0.067

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

mRNA fold induction[a] (Liver 3)

48h treatment of human hepatocytes

(mean ± SD)

Treatment	CYP1A2	CYP2B6	CYP2C9	CYP3A4
valsartan, 25 µM	1.14 ± 0.16	1.14 ± 0.18	1.01 ± 0.15	0.926 ± 0.055
valsartan, 50 µM	0.857 ± 0.088	0.759 ± 0.096	0.829 ± 0.23	0.851 ± 0.12
valsartan, 100 µM	0.719 ± 0.11	0.659 ± 0.024	0.551 ± 0.12	0.596 ± 0.032
RIF, 10 µM	0.687 ± 0.057	7.68 ± 0.54	2.27 ± 0.37	24.1 ± 2.7
PB, 1000 µM	1.52 ± 0.33	12.1 ± 1.1	2.73 ± 0.53	30.3 ± 2.8
BNF, 10 µM	n.a.	n.a.	n.a.	n.a.
Omep, 50 µM	50.5 ± 2.7	6.39 ± 0.24	1.47 ± 0.25	9.95 ± 0.32

[a] Calculated from quadruplicate real-time PCR reactions

[b] Vehicle control

[c] n.a not available

[c] RIF, rifampicin

[d] PB, phenobarbital

[e] Omep, omeprazole

[f] BNF, β-napthoflavone

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP1A2 activity (phenacetin *O*-deethylation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP1A2 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0338 ± 0.0061	0.135 ± 0.024	0.529 ± 0.026	4.9	1.00 ± 0.069	0
valsartan, 1 µM	0.0408 ± 0.0042	0.163 ± 0.017	0.740 ± 0.072	9.8	1.40 ± 0.15	18.4 ± 1.7
valsartan, 10 µM	0.133 ± 0.0086	0.531 ± 0.034	2.49 ± 0.22	8.7	4.71 ± 0.47	171 ± 16
valsartan, 25 µM	0.0457 ± 0.0031	0.183 ± 0.012	0.883 ± 0.079	9.0	1.67 ± 0.17	30.8 ± 2.8
valsartan, 50 µM	0.0397 ± 0.0019	0.159 ± 0.0077	0.736 ± 0.011	1.5	1.39 ± 0.071	18.0 ± 1.7

CYP1A2 activity (phenacetin *O*-deethylation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP1A2 activity ± SD[d]	% Max ± SD[e]
valsartan, 100 µM	0.0233 ± 0.0022	0.0932 ± 0.0088	0.468 ± 0.041	8.8	0.885 ± 0.089	-5.33 ± -0.49
RIF, 10 µM	0.0431 ± 0.0013	0.173 ± 0.0052	0.805 ± 0.039	4.8	1.52 ± 0.10	24.1 ± 2.2
PB, 1000 µM	0.0273 ± 0.0013	0.109 ± 0.0051	0.504 ± 0.038	7.6	0.953 ± 0.086	-2.18 ± -0.20
BNF, 10 µM	0.117 ± 0.0074	0.467 ± 0.030	1.68 ± 0.10	6.1	3.17 ± 0.25	100 ± 9.2

CYP1A2 activity (phenacetin O-deethylation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP1A2 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0633 ± 0.0056	0.127 ± 0.011	0.799 ± 0.050	6.2	1.00 ± 0.088	0
valsartan, 1 µM	0.0838 ± 0.0075	0.168 ± 0.015	0.901 ± 0.079	8.7	1.13 ± 0.12	0.974 ± 0.085
valsartan, 10 µM	0.0824 ± 0.0031	0.165 ± 0.0062	0.913 ± 0.058	6.3	1.14 ± 0.10	1.10 ± 0.095
valsartan, 25 µM	0.106 ± 0.0099	0.212 ± 0.020	1.22 ± 0.10	8.2	1.53 ± 0.16	4.08 ± 0.35
valsartan, 50 µM	0.0969 ± 0.0057	0.194 ± 0.011	1.10 ± 0.085	7.8	1.38 ± 0.14	2.89 ± 0.25
valsartan, 100 µM	0.110 ± 0.0071	0.220 ± 0.014	1.25 ± 0.055	4.4	1.56 ± 0.12	4.30 ± 0.37
RIF, 10 µM	0.149 ± 0.012	0.298 ± 0.023	1.47 ± 0.069	4.7	1.84 ± 0.14	6.44 ± 0.56
PB, 1000 µM	0.273 ± 0.032	0.547 ± 0.063	2.85 ± 0.13	4.7	3.56 ± 0.28	19.7 ± 1.7
Omep, 50 µM	1.03 ± 0.086	2.05 ± 0.17	11.2 ± 0.90	8.0	14.0 ± 1.4	100 ± 8.7

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP1A2 activity (phenacetin O-deethylation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (μM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP1A2 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0625 ± 0.0032	0.125 ± 0.0064	0.714 ± 0.053	7.4	1.00 ± 0.10	0
valsartan, 1 μM	0.0654 ± 0.0031	0.131 ± 0.0062	0.747 ± 0.033	4.4	1.05 ± 0.090	0.392 ± 0.020
valsartan, 10 μM	0.0500 ± 0.0071	0.0999 ± 0.014	0.633 ± 0.056	8.8	0.887 ± 0.10	-0.945 ± -0.046
valsartan, 25 μM	0.0486 ± 0.0026	0.0972 ± 0.0053	0.628 ± 0.049	7.9	0.880 ± 0.095	-1.00 ± -0.048
valsartan, 50 μM	0.0520 ± 0.0033	0.104 ± 0.0066	0.635 ± 0.035	5.5	0.890 ± 0.082	-0.920 ± -0.044
valsartan, 100 μM	0.0494 ± 0.0054	0.0987 ± 0.011	0.638 ± 0.029	4.6	0.893 ± 0.078	-0.890 ± -0.043
RIF, 10 μM	0.0708 ± 0.0035	0.142 ± 0.0070	0.914 ± 0.049	5.3	1.28 ± 0.12	2.34 ± 0.11
PB, 1000 μM	0.0990 ± 0.0033	0.198 ± 0.0067	1.19 ± 0.046	3.8	1.67 ± 0.14	5.59 ± 0.27
Omep, 50 μM	0.811 ± 0.029	1.62 ± 0.057	9.27 ± 0.40	4.3	13.0 ± 1.1	100 ± 4.8

[a]percent turnover of phenacetin (200 μM) to acetaminophen during the 0.5 hour incubation
[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells
[c]calculated mean activity (total nmol acetaminophen formed/mg protein in the well/h) ± standard deviation of quadruplicate wells
[d]Fold increase in phenacetin *O*-deethylation activity with respect to vehicle control treated cells ± standard deviation.
[e]percent of maximal positive control induction
[f]vehicle control

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP2B6 activity (OH-bupropion formation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2B6 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.00347 ± 0.00031	0.0278 ± 0.0025	0.119 ± 0.011	9.3	1.00 ± 0.13	0
valsartan, 1 µM	0.00254 ± 0.00021	0.0204 ± 0.0017	0.0907 ± 0.0077	8.5	0.764 ± 0.097	-83.7 ± -41
valsartan, 10 µM	0.00232 ± 0.00023	0.0185 ± 0.0019	0.0825 ± 0.0046	5.6	0.695 ± 0.076	-108 ± -53
valsartan, 25 µM	0.00191 ± 0.00020	0.0153 ± 0.0016	0.0799 ± 0.0065	8.1	0.674 ± 0.083	-116 ± -56
valsartan, 50 µM	0.00225 ± 0.00015	0.0180 ± 0.0012	0.0835 ± 0.0065	7.8	0.704 ± 0.086	-105 ± -51
valsartan, 100 µM	0.00233 ± 0.00011	0.0187 ± 0.00090	0.0869 ± 0.00095	1.1	0.732 ± 0.069	-95.2 ± -46
RIF, 10 µM	0.00405 ± 0.00058	0.0324 ± 0.0046	0.152 ± 0.012	7.8	1.28 ± 0.16	100 ± 49
PB, 1000 µM	0.00433 ± 0.00024	0.0347 ± 0.0019	0.141 ± 0.0034	2.4	1.19 ± 0.11	65.8 ± 32
Omep, 50 µM	0.00215 ± 0.00025	0.0172 ± 0.0020	0.0643 ± 0.0050	7.7	0.542 ± 0.066	-163 ± -79

CYP2B6 activity (OH-bupropion formation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2B6 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0267 ± 0.0022	0.107 ± 0.0089	0.628 ± 0.061	9.7	1.00 ± 0.14	0
valsartan, 1 µM	0.0257 ± 0.00096	0.103 ± 0.0039	0.588 ± 0.047	7.9	0.936 ± 0.12	-1.64 ± -0.14
valsartan, 10 µM	0.0263 ± 0.0030	0.105 ± 0.012	0.560 ± 0.052	9.2	0.892 ± 0.12	-2.78 ± -0.23
valsartan, 25 µM	0.0311 ± 0.0021	0.125 ± 0.0083	0.710 ± 0.048	6.8	1.13 ± 0.13	3.37 ± 0.28
valsartan, 50 µM	0.0322 ± 0.0017	0.129 ± 0.0069	0.673 ± 0.028	4.2	1.07 ± 0.11	1.84 ± 0.15
valsartan, 100 µM	0.0334 ± 0.0014	0.134 ± 0.0057	0.686 ± 0.016	2.3	1.09 ± 0.11	2.40 ± 0.20
RIF, 10 µM	0.146 ± 0.0094	0.583 ± 0.038	3.08 ± 0.19	6.2	4.91 ± 0.56	100 ± 8.2
PB, 1000 µM	0.121 ± 0.0038	0.483 ± 0.015	2.64 ± 0.031	1.2	4.21 ± 0.41	82.2 ± 6.7
Omep, 50 µM	0.0458 ± 0.0036	0.183 ± 0.014	1.03 ± 0.032	3.1	1.65 ± 0.17	16.6 ± 1.4

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP2B6 activity (OH-bupropion formation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2B6 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0346 ± 0.00029	0.138 ± 0.0012	0.784 ± 0.022	2.8	1.00 ± 0.039	0
valsartan, 1 µM	0.0372 ± 0.0020	0.149 ± 0.0078	0.844 ± 0.052	6.2	1.08 ± 0.073	1.67 ± 0.20
valsartan, 10 µM	0.0303 ± 0.00054	0.121 ± 0.0022	0.786 ± 0.066	8.4	1.00 ± 0.089	0.0663 ± 0.021
valsartan, 25 µM	0.0259 ± 0.0029	0.104 ± 0.012	0.721 ± 0.071	9.8	0.920 ± 0.094	-1.74 ± -0.21
valsartan, 50 µM	0.0287 ± 0.0022	0.115 ± 0.0088	0.718 ± 0.042	5.8	0.916 ± 0.059	-1.82 ± -0.21
valsartan, 100 µM	0.0237 ± 0.0011	0.0949 ± 0.0045	0.588 ± 0.045	7.7	0.750 ± 0.062	-5.46 ± -0.64
RIF, 10 µM	0.128 ± 0.012	0.513 ± 0.050	3.46 ± 0.22	6.5	4.41 ± 0.31	74.4 ± 8.7
PB, 1000 µM	0.152 ± 0.022	0.609 ± 0.090	4.37 ± 0.42	9.6	5.58 ± 0.56	100 ± 12
Omep, 50 µM	0.0721 ± 0.0038	0.289 ± 0.015	1.93 ± 0.12	6.4	2.46 ± 0.17	31.8 ± 3.7

[a]percent turnover of bupropion (400 µM) to OH-bupropion during the 0.5 hour incubation

[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells

[c]calculated mean activity (total nmol OH-bupropion formed/mg protein in the well/h) ± standard deviation of quadruplicate wells

[d]Fold increase in bupropion hydroxylation activity with respect to vehicle control treated cells ± standard deviation

[e]percent of maximal positive control induction

[f]vehicle control

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP2C9 activity (4' OH-diclofenac formation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2C9 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0993 ± 0.0067	0.199 ± 0.013	0.811 ± 0.061	7.5	1.00 ± 0.11	0
valsartan, 1 µM	0.107 ± 0.0099	0.214 ± 0.020	1.01 ± 0.086	8.6	1.25 ± 0.14	11.6 ± 1.1
valsartan, 10 µM	0.113 ± 0.0040	0.226 ± 0.0079	1.06 ± 0.099	9.4	1.30 ± 0.16	14.3 ± 1.3
valsartan, 25 µM	0.111 ± 0.0079	0.221 ± 0.016	1.12 ± 0.089	8.0	1.38 ± 0.15	17.8 ± 1.7
valsartan, 50 µM	0.110 ± 0.0031	0.220 ± 0.0062	1.00 ± 0.068	6.8	1.23 ± 0.12	11.0 ± 1.0
valsartan, 100 µM	0.0973 ± 0.0042	0.195 ± 0.0083	0.935 ± 0.017	1.8	1.15 ± 0.089	7.20 ± 0.68
RIF, 10 µM	0.268 ± 0.016	0.536 ± 0.032	2.42 ± 0.21	8.7	2.98 ± 0.34	93.2 ± 8.7
PB, 1000 µM	0.285 ± 0.017	0.570 ± 0.034	2.54 ± 0.15	5.9	3.13 ± 0.30	100 ± 9.4
BNF, 10 µM	0.184 ± 0.017	0.368 ± 0.034	1.59 ± 0.099	6.2	1.96 ± 0.19	45.3 ± 4.2

CYP2C9 activity (4' OH-diclofenac formation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2C9 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.413 ± 0.047	0.413 ± 0.047	2.51 ± 0.23	9.1	1.00 ± 0.13	0
valsartan, 1 µM	0.364 ± 0.024	0.364 ± 0.024	2.10 ± 0.17	8.1	0.838 ± 0.10	-7.96 ± -0.98
valsartan, 10 µM	0.355 ± 0.023	0.355 ± 0.023	2.08 ± 0.14	6.9	0.831 ± 0.095	-8.33 ± -1.0
valsartan, 25 µM	0.477 ± 0.029	0.477 ± 0.029	2.88 ± 0.14	4.8	1.15 ± 0.12	7.29 ± 0.90
valsartan, 50 µM	0.452 ± 0.032	0.452 ± 0.032	2.44 ± 0.19	7.6	0.974 ± 0.12	-1.27 ± -0.17
valsartan, 100 µM	0.461 ± 0.047	0.461 ± 0.047	2.57 ± 0.21	8.4	1.02 ± 0.13	1.17 ± 0.16
RIF, 10 µM	1.43 ± 0.097	1.43 ± 0.097	7.60 ± 0.58	7.7	3.03 ± 0.36	100 ± 12
PB, 1000 µM	1.31 ± 0.17	1.31 ± 0.17	6.65 ± 0.62	9.3	2.65 ± 0.35	81.4 ± 10
Omep, 50 µM	0.831 ± 0.047	0.831 ± 0.047	5.19 ± 0.33	6.4	2.07 ± 0.23	52.8 ± 6.5

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP2C9 activity (4' OH-diclofenac formation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP2C9 activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	1.12 ± 0.087	1.12 ± 0.087	6.00 ± 0.23	3.9	1.00 ± 0.055	0
valsartan, 1 µM	0.994 ± 0.092	0.994 ± 0.092	6.30 ± 0.21	3.4	1.05 ± 0.054	2.72 ± 0.23
valsartan, 10 µM	1.13 ± 0.039	1.13 ± 0.039	6.71 ± 0.44	6.5	1.12 ± 0.084	6.40 ± 0.54
valsartan, 25 µM	1.16 ± 0.086	1.16 ± 0.086	8.19 ± 0.57	7.0	1.37 ± 0.11	19.7 ± 1.7
valsartan, 50 µM	1.12 ± 0.055	1.12 ± 0.055	8.30 ± 0.22	2.6	1.38 ± 0.064	20.7 ± 1.8
valsartan, 100 µM	1.06 ± 0.11	1.06 ± 0.11	8.29 ± 0.59	7.1	1.38 ± 0.11	20.6 ± 1.7
RIF, 10 µM	2.42 ± 0.20	2.42 ± 0.20	17.1 ± 0.92	5.3	2.86 ± 0.19	100 ± 8.5
PB, 1000 µM	2.41 ± 0.19	2.41 ± 0.19	15.3 ± 1.5	9.6	2.56 ± 0.26	83.7 ± 7.1
Omep, 50 µM	1.80 ± 0.074	1.80 ± 0.074	11.9 ± 0.86	7.3	1.98 ± 0.16	52.7 ± 4.5

[a]percent turnover of diclofenac (100 µM) to 4' OH-diclofenac during the 0.5 hour incubation

[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells

[c]calculated mean activity (total nmol 4' OH-diclofenac formed/mg protein in the well/h) ± standard deviation of quadruplicate wells

[d]Fold increase in diclofenac 4' hydroxylation activity with respect to vehicle control treated cells ± standard deviation.

[e]percent of maximal positive control induction

[f]vehicle control

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP3A activity (1' OH-midazolam formation) in cultured primary human hepatocytes after treatment for 48h (Liver 1)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP3A activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0106 ± 0.00099	0.0211 ± 0.0020	0.0981 ± 0.0071	7.3	1.00 ± 0.10	0
valsartan, 1 µM	0.0120 ± 0.00064	0.0240 ± 0.0013	0.107 ± 0.0070	6.5	1.09 ± 0.11	0.879 ± 0.086
valsartan, 10 µM	0.0139 ± 0.00089	0.0278 ± 0.0018	0.128 ± 0.0089	7.0	1.30 ± 0.13	2.82 ± 0.28
valsartan, 25 µM	0.0116 ± 0.00079	0.0232 ± 0.0016	0.115 ± 0.0086	7.4	1.17 ± 0.12	1.62 ± 0.16
valsartan, 50 µM	0.0126 ± 0.00050	0.0251 ± 0.0010	0.124 ± 0.0094	7.6	1.27 ± 0.13	2.51 ± 0.25
valsartan, 100 µM	0.0124 ± 0.00051	0.0248 ± 0.0010	0.114 ± 0.0050	4.4	1.16 ± 0.098	1.52 ± 0.15
RIF, 10 µM	0.114 ± 0.012	0.227 ± 0.024	1.07 ± 0.093	8.7	10.9 ± 1.2	92.6 ± 9.0
PB, 1000 µM	0.126 ± 0.0034	0.253 ± 0.0068	1.15 ± 0.10	8.9	11.7 ± 1.3	100 ± 9.8
BNF, 10 µM	0.00829 ± 0.00077	0.0166 ± 0.0015	0.0653 ± 0.0057	8.8	0.665 ± 0.076	-3.13 ± -0.31

CYP3A activity (1' OH-midazolam formation) in cultured primary human hepatocytes after treatment for 48h (Liver 2)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP3A activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.0978 ± 0.0031	0.0978 ± 0.0031	0.531 ± 0.022	4.2	1.00 ± 0.059	0
valsartan, 1 µM	0.0951 ± 0.0049	0.0951 ± 0.0049	0.522 ± 0.016	3.1	0.984 ± 0.051	-0.127 ± -0.0081
valsartan, 10 µM	0.0950 ± 0.0036	0.0950 ± 0.0036	0.545 ± 0.038	7.0	1.03 ± 0.083	0.209 ± 0.013
valsartan, 25 µM	0.115 ± 0.0039	0.115 ± 0.0039	0.658 ± 0.027	4.1	1.24 ± 0.072	1.87 ± 0.10
valsartan, 50 µM	0.121 ± 0.0091	0.121 ± 0.0091	0.657 ± 0.044	6.7	1.24 ± 0.098	1.85 ± 0.10
valsartan, 100 µM	0.131 ± 0.0095	0.131 ± 0.0095	0.720 ± 0.034	4.8	1.36 ± 0.086	2.77 ± 0.15
RIF, 10 µM	1.36 ± 0.058	1.36 ± 0.058	7.34 ± 0.38	5.1	13.8 ± 0.91	100 ± 5.5
PB, 1000 µM	1.21 ± 0.075	1.21 ± 0.075	6.80 ± 0.52	7.7	12.8 ± 1.1	92.0 ± 5.1
Omep, 50 µM	0.138 ± 0.0099	0.138 ± 0.0099	0.829 ± 0.046	5.5	1.56 ± 0.11	4.38 ± 0.24

Study title: Evaluation of valsartan as an inducer of cytochrome P450s in human hepatocytes

CYP3A activity (1' OH-midazolam formation) in cultured primary human hepatocytes after treatment for 48h (Liver 3)

Treatment	% Turnover[a]	Mean [metabolite] ± SD[b] (µM)	Mean activity ± SD[c] (nmol/mg protein/h)	% CV	Fold increase in CYP3A activity ± SD[d]	% Max ± SD[e]
DMSO (0.1%)[f]	0.139 ± 0.0049	0.139 ± 0.0049	0.933 ± 0.079	8.4	1.00 ± 0.12	0
valsartan, 1 µM	0.156 ± 0.010	0.156 ± 0.010	1.01 ± 0.069	6.8	1.08 ± 0.12	1.34 ± 0.11
valsartan, 10 µM	0.135 ± 0.0092	0.135 ± 0.0092	0.970 ± 0.065	6.7	1.04 ± 0.11	0.630 ± 0.054
valsartan, 25 µM	0.123 ± 0.0085	0.123 ± 0.0085	0.838 ± 0.054	6.5	0.898 ± 0.095	-1.62 ± -0.13
valsartan, 50 µM	0.132 ± 0.0090	0.132 ± 0.0090	0.891 ± 0.052	5.9	0.954 ± 0.098	-0.725 ± -0.061
valsartan, 100 µM	0.119 ± 0.0092	0.119 ± 0.0092	0.816 ± 0.060	7.3	0.874 ± 0.097	-2.00 ± -0.16
RIF, 10 µM	0.867 ± 0.066	0.867 ± 0.066	6.46 ± 0.57	8.8	6.93 ± 0.84	94.3 ± 7.6
PB, 1000 µM	0.886 ± 0.096	0.886 ± 0.096	6.80 ± 0.47	6.9	7.28 ± 0.79	100 ± 8.1
Omep, 50 µM	0.160 ± 0.017	0.160 ± 0.017	1.17 ± 0.051	4.4	1.25 ± 0.12	4.03 ± 0.33

[a]percent turnover of midazolam (100 µM) to 1' OH-midazolam during the 0.5 hour incubation

[b]mean metabolite concentration in the MS sample ± standard deviation of quadruplicate wells

[c]calculated mean activity (total nmol 1' OH-midazolam formed/mg protein in the well/h) ± standard deviation of quadruplicate wells

[d]Fold increase in midazolam 1' hydroxylation activity with respect to vehicle control treated cells ± standard deviation.

[e]percent of maximal positive control induction

[f]vehicle control

2.6.5.13A 薬物動態試験：排泄

Test article: [^{14}C]AHU377 and [^{14}C]LBQ657

Study Title		Absorption, metabolism and excretion following an intravenous dose of [^{14}C]LBQ657 or oral dose of [^{14}C]AHU377 in the mouse					
Study number:		DMPK(US) R1200673					
GLP compliance:		not required					
Location in CTD		4.2.2.2-4					
Gender(M/F) / Number of animals:		M / 3 animals for urine and feces collection (CD-1)					
Feeding condition:		Fed					
Analyte / Radionuclide:		Radioactivity / ^{14}C					
Assay:		Liquid scintillation counting (LSC) or LSC after solubilization					
Route / Duration of administration:		Intravenous injection, single dose			Oral gavage, single dose		
Vehicle / Formulation		Solution in 0.9% saline			suspension in 0.5% (w/v) hydroxypropylcellulose (Klucel)		
Dose (mg/kg):		30 = 78200 nmol/kg as [^{14}C]LBQ657			150 = 365000 nmol/kg as [^{14}C]AHU377		
Specific activity (µCi/mg) :		5.0, >98% radiochemical purity			2.0, >98% radiochemical purity		
		Intravenous			Oral		
Excretion route:		Urine (%, mean ± SD)	Feces (%, mean ± SD)	Cage wash (%, mean ± SD)	Urine (%, mean ± SD)	Feces (%, mean ± SD)	Cage wash (%, mean ± SD)
Interval (h)	0 – 24	4.57	89.2	-	8.30	99.1	-
	24 - 48	0.165	8.17	-	0.295	3.65	-
	48 - 72	0.071	0.41	-	0.0392	0.246	-
	72 - 96	0.0318	0.174	-	0.0534	0.126	-
	96 - 168	0.063	0.223	-	0.0851	0.145	-
Subtotal (h)	0 - 168	4.90 (4.8)*	98.2 (95.2)*	-	8.77 (7.8)*	103 (92.2)*	-
Total Recovery (% of dose)		103 (100)*			112 (100)*		

* Value was normalized to 100% recovery

-: not determined or not detected

2.6.5.13B　薬物動態試験：排泄

Test article: [^{14}C]AHU377

Study Title		Absorption, metabolism, and excretion following an intravenous or oral dose of [^{14}C]AHU377 in rats					
Study number:		ADME(US) R0300247-1					
GLP compliance:		not required					
Location in CTD		4.2.2.2-1					
Gender(M/F) / Number of animals:		M / 3 animals for urine and feces collection (Han Wistar)					
Feeding condition:		Fed					
Analyte / Radionuclide:		Radioactivity / ^{14}C					
Assay:		Liquid scintillation counting (LSC) or LSC after solubilization					
Route / Duration of administration:		Intravenous injection, single dose			Oral gavage, single dose		
Vehicle / Formulation		Solution in 0.9% saline			Solution in 0.9% saline		
Dose (mg/kg):		15			45		
Specific activity (µCi/mg) :		7.5, >98% radiochemical purity			2.4, >98% radiochemical purity		
		Intravenous			Oral		
Excretion route:		Urine (%, mean ± SD)	Feces (%, mean ± SD)	Cage wash (%, mean ± SD)	Urine (%, mean ± SD)	Feces (%, mean ± SD)	Cage wash (%, mean ± SD)
Interval (h)	0 – 24	23.0 ± 12.0	47.6 ± 11.4	-	7.37 ± 3.40	78.8 ± 7.3	-
	24 - 48	0.043 ± 0.074	24.0 ± 15.5	-	0.825 ± 0.715	11.5 ± 6.8	-
	48 - 72	0.117 ± 0.065	0.914 ± 0.635	-	0.237 ± 0.238	0.388 ± 0.112	-
	72 - 96	0.422 ± 0.682	0.766 ± 0.663	-	0.107 ± 0.094	0.421 ± 0.462	-
	96 - 168	0.112 ± 0.146	1.07 ± 0.88	-	0.221 ± 0.139	0.235 ± 0.296	-
	0 - 168	23.7 ± 12.2	74.4 ± 17.3	2.75 ± 1.26	8.75 ± 2.68	91.4 ± 1.1	0.057 ± 0.046
Total Recovery (% of dose)		101 ± 8			100 ± 4		

2.6.5.13C 薬物動態試験：排泄

Test article: [^{14}C]LCZ696

Study title: Excretion in milk after a single oral dose of [^{14}C]LCZ696 in the rat		
Study number:	DMPK R1200622	
GLP compliance:	Not required	
Location in CTD:	4.2.2.5-1	
Gender(M/F) / Number of animals:	F / 15 animals (3 per timepoint); lactating, 8-9 days post-parturition; HanWistar	
Feeding condition:	fed	
Vehicle / Formulation	0.5% (w/v) sodium carboxymethylcellulose (CMC) aqeous	
Method / Route:	oral	
Dose (mg/kg):	30	
Specific activity / radiochemical purity:	5 µCi/mg, >98% radiochemical purity	
Analyte / Radionuclide:	Radioactivity / ^{14}C	
Assay:	Liquid scintillation counting; metabolite profiles by HPLC with off-line radioactivity detection; metabolite identification by LC-MS/MS	
Radioactivity	Plasma	Milk
Cmax (ngEq/mL)	1770 (4300 nM)	262 (637 nM)
Tmax (h)	1	4
AUC0-24h (ngEq•h/mL):	3770 (9160 nM*h)	2870 (6970 nM*h)
AUCinf (ngEq•h/mL):	3810 (9260 nM*h)	3470 (8430 nM*h)
LBQ657 in matrix		
Cmax (ngEq/mL)	1610 (3910 nM*h)	262 (637 nM)
Tmax (h)	1	4
AUC0-24h (ngEq•h/mL):	3050 (7410 nM*h)	2870 (6970 nM*h)
AHU377 in matrix		
Cmax (ngEq/mL)	0	0

2.6.5.13D 薬物動態試験：排泄

Test article: [^{14}C]AHU377 and [^{14}C]LBQ657

Study Title		Absorption, metabolism, and excretion following an intravenous dose of [^{14}C]LBQ657 and oral dose of [^{14}C]AHU377 in the dog					
Study number:		ADME(US) R0301244					
GLP compliance:		not required					
Location in CTD		4.2.2.2-2					
Gender(M/F) / Number of animals:		M /2 animals for iv, 3 animals for po					
Feeding condition:		Fasted					
Analyte / Radionuclide:		Radioactivity / ^{14}C					
Assay:		Liquid scintillation counting (LSC) or LSC after solubilization					
Route / Duration of administration:		Intravenous injection, single dose			Oral gavage, single dose		
Vehicle / Formulation		Solution in saline			Solution in saline		
Dose (mg/kg):		3 mg/kg = 7823 nmol/kg as [^{14}C]LBQ657			15 mg/kg = 34602 nmol/kg as [^{14}C]AHU377		
Specific activity (µCi/mg) :		9.0, >98% radiochemical purity			1.94, >98% radiochemical purity		
		Intravenous			Oral		
Excretion route:		Urine (%, mean)	Feces (%, mean)	Cage wash (%, mean ± SD)	Urine (%, mean ± SD)	Feces (%, mean ± SD)	Cage wash (%, mean ± SD)
Interval (h)	0-24	10.5	42.3	-	8.61 ± 1.70	10.7 ± 18.5	-
	24-48	0.348	37.6	-	0.829 ± 0.328	72.6 ± 14.7	-
	48-72	0.086	6.45	-	0.176 ± 0.058	4.05 ± 5.45	-
	72-96	0.0396	1.80	-	0.0867 ± 0.0472	2.96 ± 3.84	-
	96-120	0.0132	0.384	-	0.111 ± 0.119	0.0648 ± 0.0252	-
	120-144	0.0221	0.154	-	0.0345 ± 0.0095	0.075 ± 0.052	-
	144-168	0.0131	0.092	-	0.0920 ± 0.0526	0.138 ± 0.164	-
	0-168	11.0	88.8	0.321	9.94 ± 1.92	89.2 ± 1.7	2.55 ± 2.91
Total Recovery (% of dose)		100			102 ± 4		

2.6.5.13E 薬物動態試験：排泄

Test article: [^{14}C]LBQ657 and [^{14}C]LCZ696

Study Title		Absorption, metabolism and excretion following an intravenous (10 mg/kg) dose of [^{14}C]LBQ657 or oral (30 mg/kg) dose of [^{14}C]LCZ696 in the monkey			
Study number:		DMPK R1200729			
GLP compliance:		not required			
Location in CTD		4.2.2.2-3			
Gender(M/F) / Number of animals:		M/2		M/3	
		cynomolgus		cynomolgus	
Feeding condition:		Fed		Fed	
Vehicle / Formulation		Sterile solution in 0.9% saline for injection, USP		Suspension in 0.5% (w/v) sodium carboxymethylcellulose (CMC) aqueous solution	
Method/ Route / Duration of administration:		Intravenous		Oral gavage	
Dose (mg/kg):		10 as [^{14}C]LBQ657		30 as [^{14}C]LCZ696	
Specific activity /radiochemical purity:		7.5 / >98%		2.5 / >98%	
Analyte / Radionuclide:		Radioactivity / [^{14}C]		Radioactivity / [^{14}C]	
Assay:		Liquid scintillation counting (LSC) or LSC after solubilization		Liquid scintillation counting (LSC) or LSC after solubilization	
		mean		mean ± SD	
Excretion route:		Urine (% dose)	Feces (% dose)	Urine (% dose)	Feces (% dose)
Interval (h)	0 - 24	50.3	15.4	26.1 ± 10.9	36.7 ± 6.23
	0 - 168	56.1	23.0	31.1 ± 11.7	47.9 ± 8.54
	LBQ657/AHU377	55.1 (LBQ657)	21.2 (LBQ657)	29.0 (LBQ657), 1.0 (AHU377)	40.6 (LBQ657), 3.22 (AHU377)
Cage wash	0-168	9.1		11.0	
Total	0-168	88.2		90.0 ± 12.3	
Absorption (%)		- [a]		~ 40 – 65[b]	
Bioavailability (%)		- [a]		40.8 ± 5.07	

[a] Not applicable or not determined

[b] 65% was estimated based urine recovery although it was estimated to be 40% based on blood and plasma AUC

2.6.5.13F 薬物動態試験：排泄

Test article : [^{14}C]LCZ696

Study Title	An open-label, single dose study to investigate the absorption, distribution, metabolism and elimination of 200 mg [^{14}C]LCZ696 and its metabolites in healthy male subjects				
Study number:	CLCZ696B2105				
GLP compliance:	Yes, GCP				
Location in CTD	5.3.3.1-3				
Gender(M/F) / Age range / Number of subjects:	M / 23-30 years / 4 subjects				
Vehicle / Formulation	100 mg gelatin capsules (batch number AEUS/2009-0225) for oral use.				
Route of administration:	Oral				
Dose (µg/kg):	200				
Analyte / Radionuclide:	[^{14}C]				
Assay:	Total radioactivity in blood, plasma, urine and feces (and vomitus) by liquid scintillation counting. Valsartan, AHU377 and LBQ657 in plasma by LC/MS/MS (LOQ: 1 ng/mL for all three analytes). Metabolites of AHU377 in plasma, urine and feces by HPLC with radioactivity detection. Characterization of AHU377 metabolites by LC/MS.				
Excretion route:	Subject 5101	Subject 5102	Subject 5103	Subject 5104	Mean ± SD
Urine (0-168 hours)	67.8	61.5	51.7	61.7	60.7 ± 6.66
Feces (0-168 hours)	40.0	36.9	48.3	41.9	41.8 ± 4.81
Dose recovery (%)	107.8	98.4	100	103.6	102.5 ± 4.30

2.6.5.14 薬物動態試験：胆汁中排泄

Not applicable

2.6.5.15 薬物動態試験：薬物相互作用

Not applicable

2.6.5.16A 薬物動態試験：その他

Test article: AHU377 and LBQ657

Study Title:	In vitro permeability and transporter interaction assessment of AHU377 and LBQ657 across Caco-2 cell monolayers
Study nos.:	R0301243
GLP compliance:	not required
Location in CTD:	4.2.2.6-4
Study System:	Human intestinal cell line Caco-2 grown on permeable filter support
Analyte/Assay:	~5.0 and ~23 µM, [^{14}C]AHU377 and [^{14}C]LBQ657, radiolabeled specific activity [^{14}C]AHU377 (50.1 mCi/mmol) and [^{14}C]LBQ657 (53.0 mCi/mmol); radiochemical purity 98%)/ liquid scintillation counting

Compound	Conc. [µM]	Inhibitor [a]	Caco-2 permeability Papp (x 10-5 cm/min) [b] Apical to Basolateral	Caco-2 permeability Papp (x 10-5 cm/min) [b] Basolateral to Apical
[^{14}C]AHU377	4.9	-	23.2 ± 2.8	56.7 ± 4.1
		PSC833	43.6 ± 5.3	17.0 ± 1.5
		indomethacin	20.1 ± 2.2	67.4 ± 4.3
	22	-	28.0 ± 3.0	60.2 ± 4.9
		PSC833	50.9 ± 3.1	23.4 ± 1.1
		indomethacin	22.0 ± 3.4	69.3 ± 6.1
[^{14}C]LBQ657	4.9	-	0.75 ± 0.51	4.17± 1.8
		PSC833	0.69 ± 0.12	2.80± 1.3
		indomethacin	0.52 ± 0.06	3.59± 2.3
	24	-	0.67 ± 0.10	2.78 ± 1.8
		PSC833	0.99 ± 0.23	3.52 ± 1.6
		indomethacin	0.90 ± 0.28	3.45 ± 1.3
[^{14}C]mannitol	4.1	-	3.40 ± 0.7	7.10 ± 1.4
[^{3}H]propranolol	5.6	-	130 ± 15	147 ± 2.5

[a] Final concentrations for PSC833 and indomethacin were 1 µM and 50 µM, respectively.

[b] Values calculated represent the mean ± standard deviation of quadruple measurements.

Study Title: In vitro permeability and transporter interaction assessment of AHU377 and LBQ657 across Caco-2 cell monolayers

Compound (μM)	Inhibitor (μM)	Papp Ratio [a] (-Inhibitor)	Papp ratio [a] (+Inhibitor)	Ratio A-B [b] (+Inhibitor/control)	Ratio B-A [c] (+Inhibitor/control)
[^{14}C]AHU377 (4.9)	PSC833 (1.0)	2.4 ± 0.3	0.39 ± 0.1	1.9 ± 0.3	0.3 ± 0.03
[^{14}C]AHU377 (22)		2.1 ± 0.3	0.46 ± 0.0	1.8 ± 0.2	0.4 ± 0.04
[^{14}C]AHU377 (4.9)	Indomethacin (50)	2.4 ± 0.3	3.4 ± 0.4	0.9 ± 0.1	1.2 ± 0.1
[^{14}C]AHU377 (22)		2.1 ± 0.3	3.2 ± 0.6	0.8 ± 0.1	1.2 ± 0.1
[^{14}C]LBQ657 (4.9)	PSC833 (1.0)	5.6 ± 5	4.1 ± 0.9	0.9 ± 0.7	0.7 ± 0.4
[^{14}C]LBQ657 (24)		4.1 ± 3	3.6 ± 2	1.5 ± 0.4	1.3 ± 1
[^{14}C]LBQ657 (4.9)	Indomethacin (50)	5.6 ± 5	6.9 ± 5	0.7 ± 0.5	0.9 ± 0.7
[^{14}C]LBQ657 (24)		4.1 ± 3	3.9 ± 1.9	1.3 ± 0.5	1.2 ± 0.9

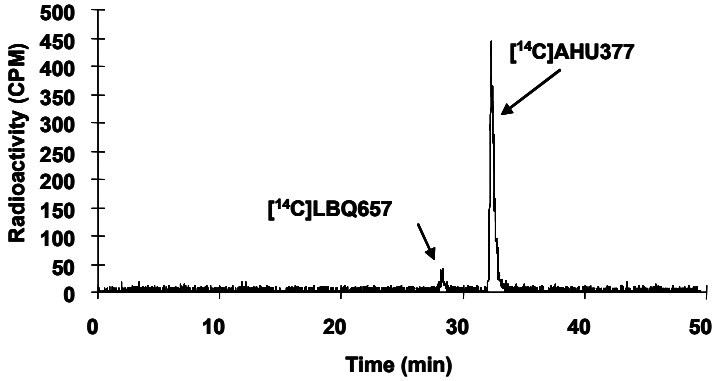
a Standard deviation(quotient)= Bl→Ap/Ap→Bl* SQRT ((Stdev Bl→Ap/ Bl→Ap)^2 + Stdev Ap→Bl/ Ap→Bl)^2)

b Standard deviation(quotient)= Ap→Bl(+inhibitor) /Ap→Bl* SQRT ((Stdev Ap→Bl(+inhibitor)/ Ap→Bl(+inhibitor))^2 + Stdev Ap→Bl/ Ap→Bl)^2)

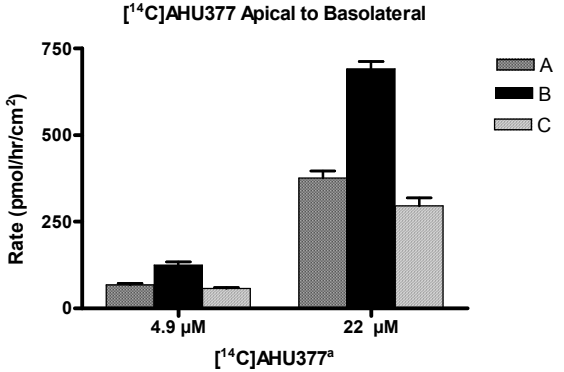
c Standard deviation(quotient)= Bl→Ap(+inhibitor) /Bl→Ap* SQRT ((Stdev Bl→Ap(+inhibitor)/ Bl→Ap(+inhibitor))^2 + Stdev Bl→Ap/ Bl→Ap)^2)

Study Title: In vitro permeability and transporter interaction assessment of AHU377 and LBQ657 across Caco-2 cell monolayers

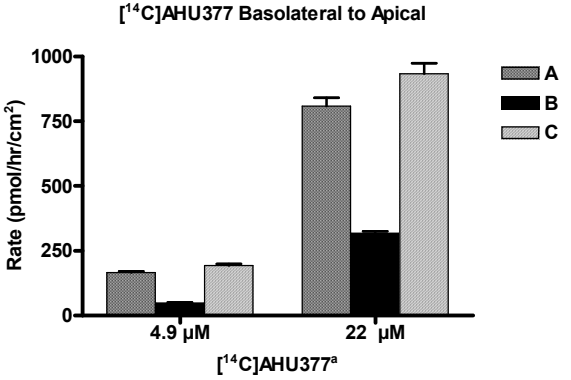
Stability of [^{14}C]AHU377 following movement through Caco-2 cell monolayers.



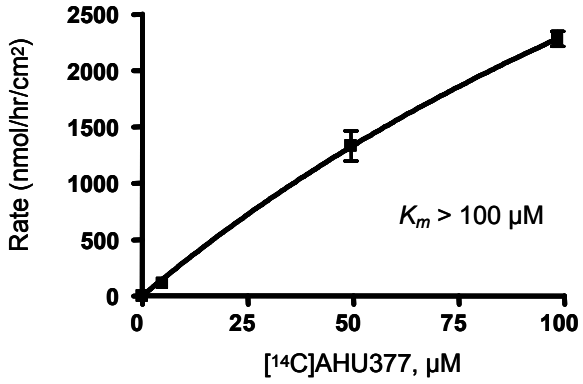
Steady-state [^{14}C]AHU377 Ap→Bl transport rates across Caco-2 cell monolayers (A) and in the presence of PSC833 (1.0 µM, B) or indomethacin (50 µM, C).



Steady-state [^{14}C]AHU377 Bl→Ap transport rates across Caco-2 cell monolayers (A) and in the presence of PSC833 (1.0 µM, B) or indomethacin (50 µM, C)



Active transport of [^{14}C]AHU377 across Caco-2 monolayers in the basolateral to apical direction.



[a] approximate concentration.

The estimated in vitro permeability values classify AHU377 as a moderately high permeable drug substance, whereas LBQ657 as poorly permeable.

Conclusions: AHU377 exhibited P-gp–mediated efflux based upon Caco-2 data. However, this low affinity (Km > 100 µM) interaction combined with the moderately high permeability is not predicted to have a significant effect on the net absorption of AHU377.

2.6.5.16B　薬物動態試験：その他

Test article: LBQ657

Study Title:	Assessment of LBQ657 as an inhibitor of human P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)
Study nos.:	R1300267
GLP compliance:	not required
Location in CTD:	4.2.2.6-13
Study System:	Inside out membrane vesicles containing the human P-gp or BCRP transporters
Analyte/Assay:	LBQ657 [^{3}H]NMQ or [^{3}H] E_3S / radioactivity

Effect of LBQ657 and the model inhibitor CsA on P-gp-mediated [^{3}H]NMQ transport

Substrate, concentration	**Inhibitor**	**Inhibitor concentration, µM**	**P-gp-mediated transport (pmol/mg protein/min)[a]**	**% inhibition**
[^{3}H]NMQ, 2 µM	None	-	12.3 ± 0.26	0
	CsA	10	3.45 ± 0.11	72.0
	LBQ657	2.5	12.2 ± 0.66	1.04
		5.0	11.8 ± 0.91	4.12
		10	12.6 ± 1.1	NI[b]
		25	13.2 ± 2.2	NI[b]
		50	13.1 ± 0.60	NI[b]

[a] P-gp mediated activity was calculated as the average difference in [^{3}H]NMQ accumulation in vesicles in the presence ATP from that in the absence ATP
[b] NI = no inhibition

Study Title:	Assessment of LBQ657 as an inhibitor of human P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)			
Effect of LBQ657 and the model inhibitor FTC on BCRP-mediated [^{3}H]E_3S transport				
Substrate, concentration	**Inhibitor**	**Inhibitor concentration, µM**	**BCRP mediated transport (pmol/mg protein/min) [a]**	**% Inhibition**
[^{3}H] E_3S,	None	-	15.0 ± 2.0	0
1 µM	FTC	10	0.248 ± 0.15	98.3
	LBQ657	2.5	15.8 ± 2.1	NI[b]
		5.0	14.8 ± 1.6	1.04
		10	15.5 ± 0.46	NI[b]
		25	14.1 ± 1.4	5.53
		50	15.3 ± 1.5	NI[b]

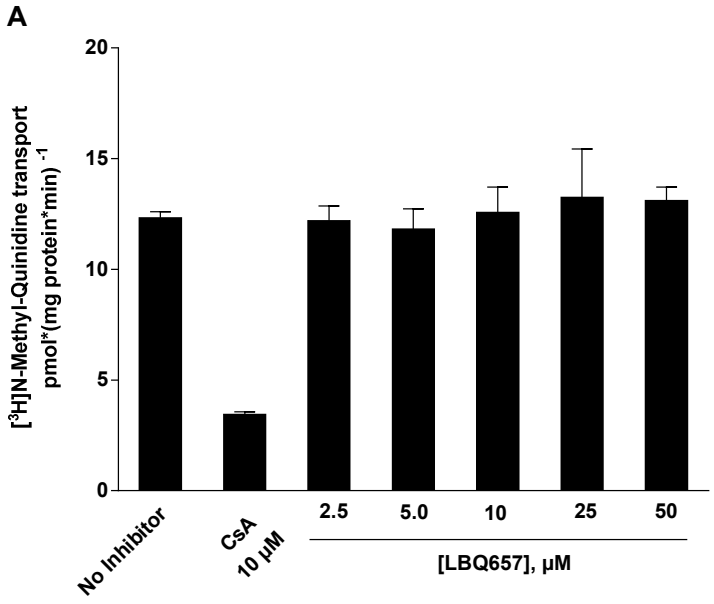
[a] BCRP-mediated activity was calculated as the average difference in [^{3}H] E_3S accumulation in vesicles in the presence ATP from that in the presence AMP
[b] NI = no inhibition

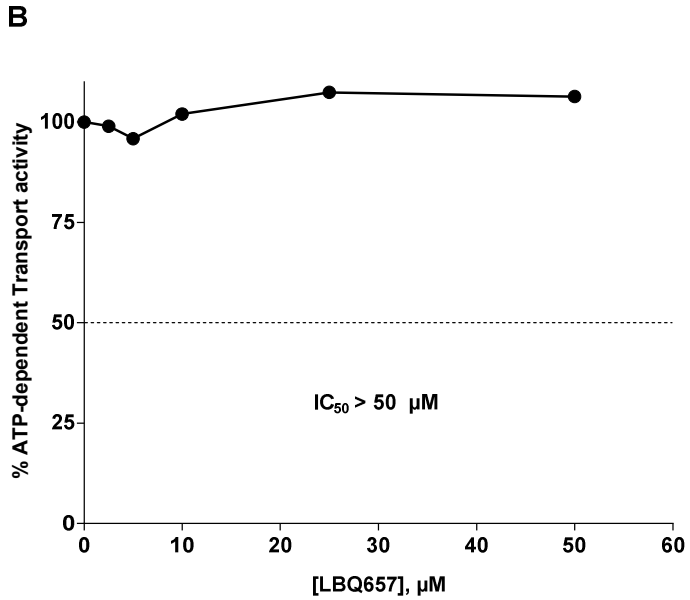
Study Title:	Assessment of LBQ657 as an inhibitor of human P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)

Assessment of LBQ657 as an inhibitor of P-gp

Inside-out membrane vesicles expressing P-gp were incubated with [^{3}H]*N*-methyl quinidine (2 µM) in the presence or absence of CsA (10 µM; nominal concentration, positive control inhibitor) or LBQ657 as indicated in the figure below. Each bar in panel A represents the average pmol [^{3}H]NMQ ATP-dependent accumulation per mg cellular protein/min ± standard deviation of four separate samples.

The IC_{50} was >50 µM **(Panel B).**



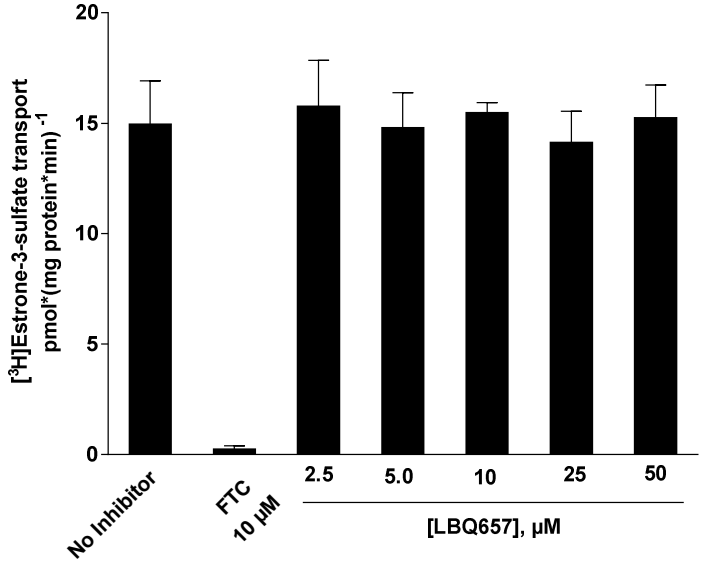


Study Title: Assessment of LBQ657 as an inhibitor of human P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)

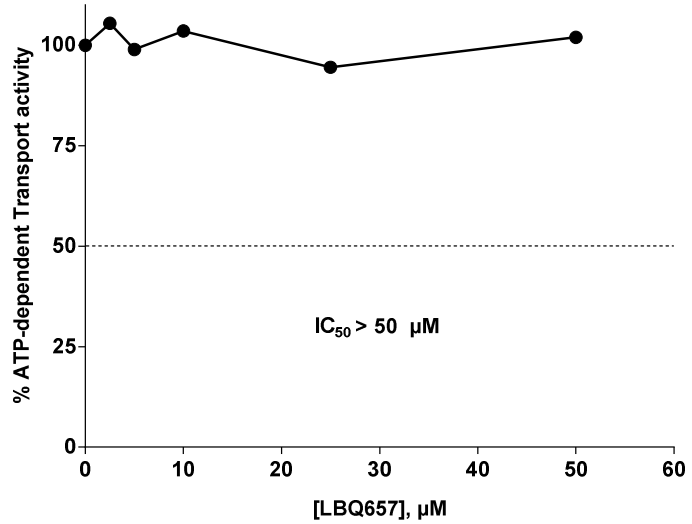
Assessment of LBQ657 as an inhibitor of BCRP

Inside-out membrane vesicles expressing BCRP were incubated with [^{3}H]E_3S (1 µM) in the presence or absence of FTC (10 µM; positive control inhibitor) or LBQ657 as indicated in the figure below. Each bar in panel A represents the average pmol [^{3}H]E_3S ATP-dependent accumulation per mg cellular protein/min ± standard deviation of four separate samples. The IC_{50} was >50 µM **(Panel B).**

A



B



Conclusion: LBQ657 at the highest concentration tested (50 µM), did not appreciably reduce the ATP-dependent accumulation in P-gp or BCRP vesicles. The IC_{50} values were estimated to be greater than 50 µM for both P-gp and BCRP. The observation of P-gp or BCRP inhibition *in vivo* will depend on the dose and the actual concentrations achieved at the active site of either transporter.

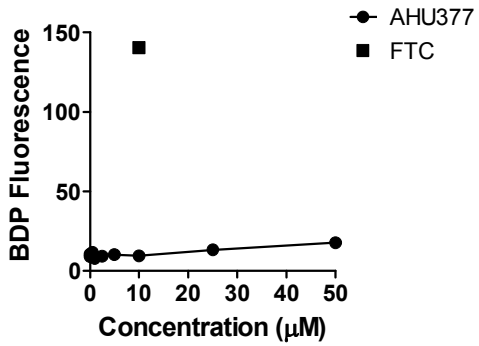
2.6.5.16C　薬物動態試験：その他

Test article: AHU377

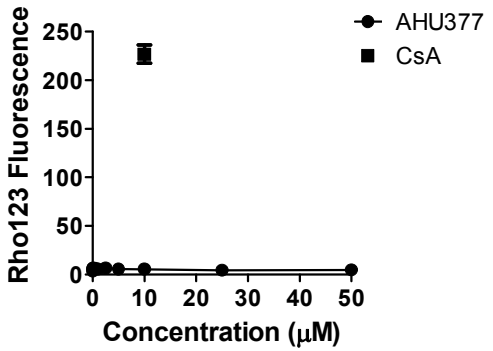
Study Titles:	Assessment of AHU377 as an inhibitor of human breast cancer resistance protein (BCRP), P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 (MRP2)
Study nos.:	R0900054
GLP compliance:	not required
Location in CTD:	4.2.2.6-5
Study System:	Human orthologs of BCRP (T8 cells), P-gp (MDA435 T0.3 cells) and MRP2 (MDCKII cells).
Analyte/Assay:	Bodipy FL prazosin (BDP) and Rhodamine 123 (Rho123) by BCRP and P-gp, respectively. [^{14}C]Valsartan was used as a probe substrate of MRP2./ fluorescence by flow cytometry

[AHU377] (µM)	BDP fluorescence (geometric mean)	recorded events (out of 10,000)
0	8.92	6989
0.01	10.36	7031
0.1	10.27	7179
0.5	11.70	7190
1	7.84	7050
2.5	9.24	6978
5	10.22	7245
10	9.47	6716
25	13.21	6764
50	17.73	6766
FTC (10 µM)	140.65	6592
FTC (10 µM)	140.48	6299

Effect of AHU377 on BCRP-mediated BDP efflux from T8 cells



BCRP inhibitor - Fumitremorgin C (FTC)

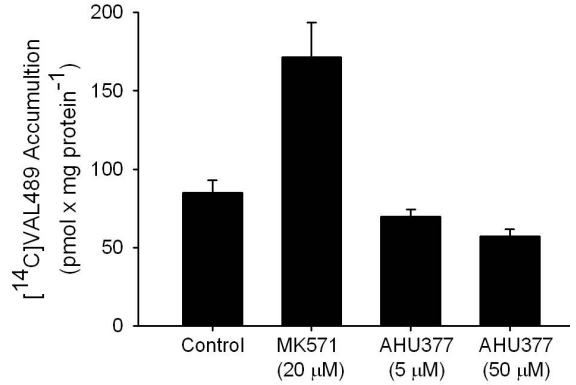
Study Titles:	Assessment of AHU377 as an inhibitor of human breast cancer resistance protein (BCRP), P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 (MRP2)		
[AHU377] (µM)	Rho123 fluorescence (geometric mean)	recorded events (out of 10,000)	Effect of AHU377 on P-gp-mediated Rho123 efflux from MDA435 T0.3 cells
0	5.98	9300	
0.01	3.60	9362	
0.1	6.71	9240	
0.5	6.15	9327	
1	5.71	9516	
2.5	6.55	9124	
5	5.76	9392	
10	5.56	9373	
25	4.57	9357	
50	4.70	9410	
CsA (10 µM)	231.41	9531	
CsA (10 µM)	222.05	9464	

PgP inhibitor - Cyclosporin A (CsA)

Study Titles:	Assessment of AHU377 as an inhibitor of human breast cancer resistance protein (BCRP), P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 (MRP2)

Effect of AHU377 on MRP2-mediated efflux from MDCKII cells

Effect of 5 μM or 50 μM AHU377 on the efflux of [^{14}C]VAL489 (10 μM) was examined after a 40 min accumulation phase in MDCKII-MRP2 cells. The effect of the positive control MRP2 inhibitor, MK571 (20 μM), on intracellular accumulation of [^{14}C]VAL489 was also conducted in parallel. The data below represent the mean ± standard deviation (quadruplicate measurements) of intracellular [^{14}C]VAL489 accumulation normalized to protein concentration.



Conclusion: At 50 μM AHU377, BDP levels in BCRP-expressing T8 cells were elevated 1.9-fold (Imax), but this level of inhibition was only 6.7% (%max) of that observed with the positive control inhibitor of BCRP, FTC (10 μM). Rho123 efflux from P-gp-expressing MDA435 T0.3 cells and [^{14}C]VAL489 efflux from MRP2-expressing MDCKII cells was not inhibited by AHU377 up to a concentration of 50 μM. Thus, it is unlikely that AHU377 will act as an inhibitor of BCRP, P-gp or MRP2. The relevance of these *in vitro* findings to *in vivo* drug interaction potential would be dependent on the dose and actual AHU377 concentrations achieved therapeutically.

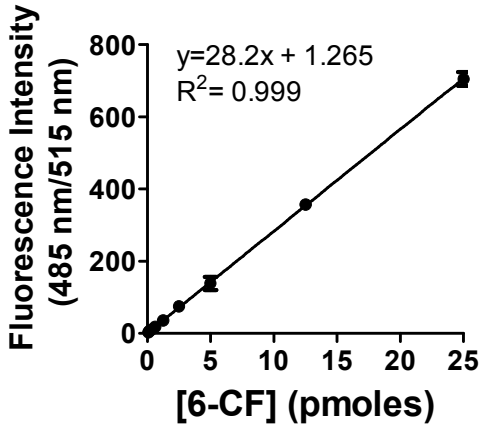
2.6.5.16D　薬物動態試験：その他

Test article: LBQ657

Study Title:	Assessment of LBQ657 (the active metabolite of AHU377) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)
Study no.:	R0900341
GLP compliance:	not required
Location in CTD:	4.2.2.6-6
Study System:	Human orthologs of OAT1 (hOAT1) and OAT3 (hOAT3) stably expressed in human embryonic kidney (HEK) cells
Analyte/Assay:	6CF in the presence or absence of LBQ657/ Fluorescence

6CF standard curve

The standard curve was generated by diluting 6-CF serially in lysis buffer. Fifty microliters of each sample was plated in a 96-well plate, and fluorescence intensity was determined with a SpectraMax fluorescence plate reader at excitation and emission wavelengths of 485 nm and 515 nm, respectively. The concentrations of 6CF used were 0, 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 250 and 500 nM. The data are means ± SEM of quadruplicate measures. All of the experimental samples were well within the boundaries of the standard curve.



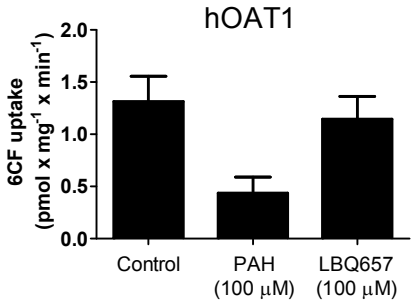
Study Title: Assessment of LBQ657 (the active metabolite of AHU377) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)

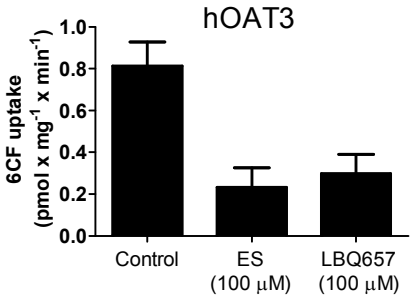
Effect of LBQ657 on 6CF transport by hOAT1 and hOAT3

HEK cells stably expressing hOAT1 or hOAT3 were grown to confluence in 96-well plates. Uptake of 0.5 μM 6CF (6CF) was conducted for 25 min at room temperature in the presence or absence of LBQ657, PAH, a substrate and inhibitor of OAT1, or ES, a substrate and inhibitor of OAT3. Fluorescence intensity was measured with a fluorescence plate reader using excitation and emission wavelengths of 485 nm and 515 nm, respectively. Intracellular 6-CF content was extrapolated from a standard curve. The data are means ± SEM of quadruplicate measures.

PAH – para-aminohippuric acid

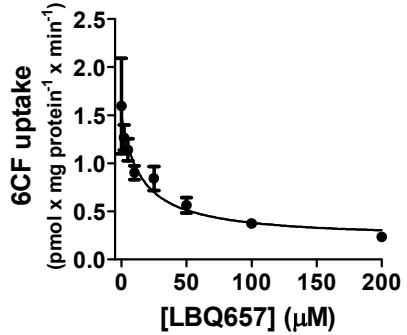
ES – Estrone-3-sulfate





IC_{50} determination for LBQ657 inhibition of hOAT3

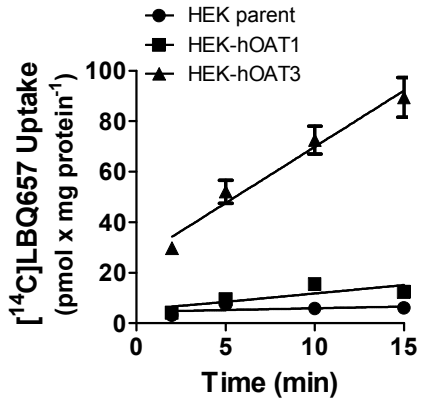
HEK cells stably expressing hOAT3 were grown to confluence in 96-well plates. Uptake of 3 μM 6CF (6CF) was conducted for 20 min at room temperature in the absence or presence of increasing concentrations of LBQ657. Fluorescence intensity was measured with a fluorescence plate reader using excitation and emission wavelengths of 485 nm and 515 nm, respectively. Intracellular 6-CF content was extrapolated from a standard curve. The data are means ± SEM of quadruplicate measures. The IC_{50} value for LBQ657 inhibition of hOAT2-mediated 6CF transport was 15.2 ± 5.60 μM.



Study Title: Assessment of LBQ657 (the active metabolite of AHU377) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)

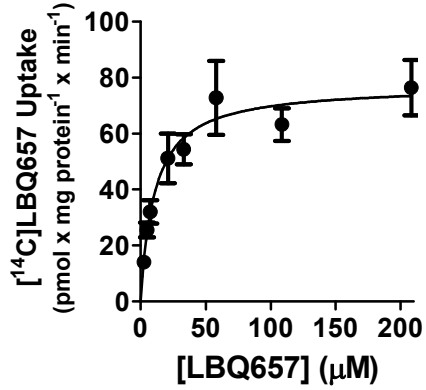
Time course of [^{14}C]LBQ657 uptake by cells expressing hOAT3

HEK Flp-In cells (HEK parent) or HEK Flp-In cells stably expressing hOAT1 or hOAT3 were grown to confluence in 24-well plates. Uptake of 10.8 µM [^{14}C]LBQ657 was conducted at room temperature for 2, 5, 10 or 15 minutes. The data are means ± SEM of quadruplicate measures. Uptake of 10.8 µM [^{14}C]LBQ657 by hOAT3 appeared to be linear for 15 min.



Kinetics of LBQ657 transport by hOAT3

HEK Flp-In cells stably expressing hOAT3 were grown to confluence in 24-well plates. Uptake of [^{14}C]LBQ657 was conducted at room temperature for 10 minutes. [^{14}C]LBQ657 concentration was 2.4 µM, 4.9 µM or 7.5 µM. Concentrations of total LBQ657 ([^{14}C]LBQ657 + LBQ657) above 7.5 µM were achieved by adding unlabeled LBQ657 to the buffer. Uptake of LBQ657 was a saturable process that could be described using Michealis-Menten kinetics. The J_{max} and K_m were 77.1 ± 3.69 pmol · mg protein^{-1} · min^{-1} and 10.6 ± 2.02 µM, respectively. The data are means ± SEM of quadruplicate measures.



Conclusion: LBQ657 was found to be an *in vitro* inhibitor and substrate of hOAT3. The IC_{50} value for LBQ657 inhibition of hOAT3-mediated 6CF transport was 15.2 ± 5.60 µM. Compared to HEK parental cells, the cellular accumulation of [^{14}C]LBQ657 (10.8 µM) was stimulated 14.5-fold by the expression of hOAT3. The maximal rate of [^{14}C]LBQ657 transport (J_{max}) and Michaelis constant (K_m) were 77.1 ± 3.69 pmol·mg protein^{-1}·min^{-1} and 10.6 ± 2.02 µM, respectively. In contrast, little accumulation of LBQ657 was observed in hOAT1-expressing cells and LBQ657 did not appear to inhibit 6CF transport mediated by hOAT1. In conclusion, there is a potential for inhibitors of hOAT3 to effect the distribution and elimination of LBQ657 *in vivo*. The effect of the inhibition of hOAT3 *in vivo* would be dependent on the dose and the actual concentrations of LBQ657 achieved therapeutically.

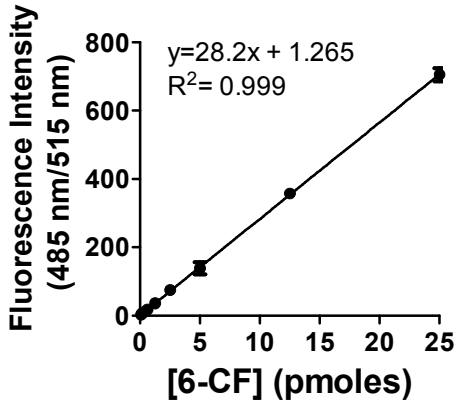
2.6.5.16E　薬物動態試験：その他

Test article: VAL489

Study Title:	Assessment of VAL489 (an active component of LCZ696) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)
Study no.:	R0900652
GLP compliance:	not required
Location in CTD:	4.2.2.6-7
Study System:	Human orthologs of OAT1 (hOAT1) and OAT3 (hOAT3) stably expressed in human embryonic kidney (HEK) cells
Analyte/Assay:	6CF in the presence or absence of VAL489/Fluorescence

6CF standard curve

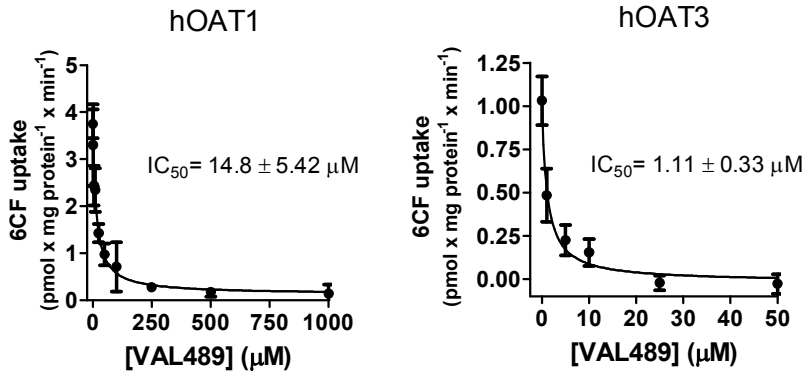
The standard curve was generated by diluting 6-CF serially in lysis buffer. Fifty microliters of each sample was plated in a 96-well plate, and fluorescence intensity was determined with a SpectraMax fluorescence plate reader at excitation and emission wavelengths of 485 nm and 515 nm, respectively. The concentrations of 6CF used were 0, 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 250 and 500 nM. The data are means ± SEM of quadruplicate measures. All of the experimental samples were well within the boundaries of the standard curve.



Study Title: Assessment of VAL489 (an active component of LCZ696) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)

IC_{50} determination for VAL489 inhibition of hOAT1 and hOAT3

HEK cells stably expressing hOAT1 or hOAT3 were grown to confluence in 96-well plates. Uptake of 0.5 μM (hOAT1) or 3 μM (hOAT3) 6CF was conducted for 20 min at room temperature in the absence or presence of increasing concentrations of VAL489. Fluorescence intensity was measured with a fluorescence plate reader using excitation and emission wavelengths of 485 nm and 515 nm, respectively. Intracellular 6-CF content was determined from a standard curve. The data are means ± SD of quadruplicate measures.



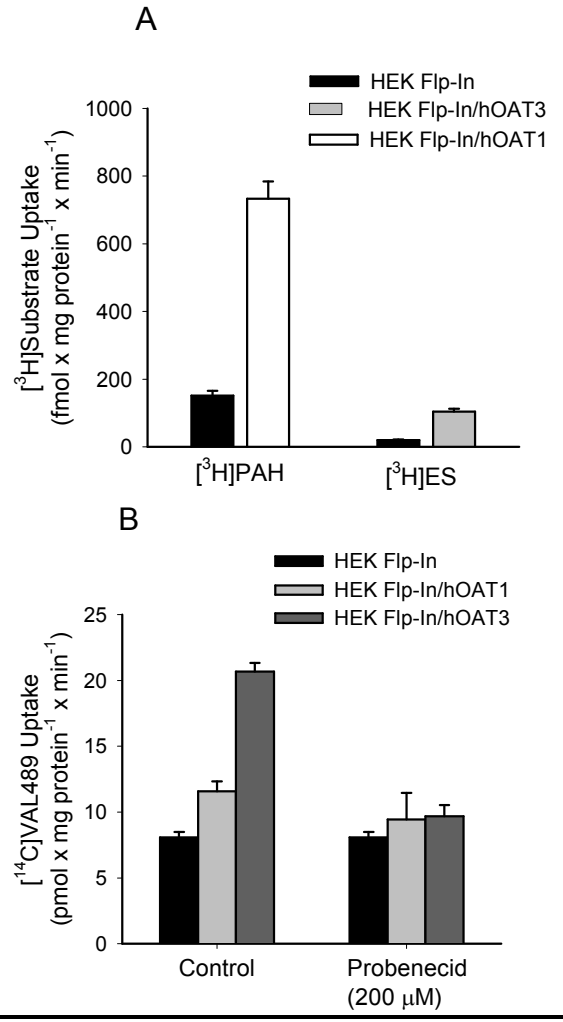
Study Title: Assessment of VAL489 (an active component of LCZ696) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)

Transport of [^{14}C]VAL489 and the positive control substrates [^{3}H]PAH and [^{3}H]ES by hOAT1 and hOAT3

HEK Flp-In cells (HEK parent) or HEK Flp-In cells stably expressing hOAT1 or hOAT3 were grown to confluence in 24-well plates. Panel A: The uptake of ~0.2 μM [^{3}H]PAH or ~0.02 μM [^{3}H]ES was conducted at room temperature for 10 minutes. Panel B: The uptake of 10 μM [^{14}C]VAL489 was conducted for 10 min at room temperature. The data are means ± SD of quadruplicate measures.

PAH – para-aminohippuric acid

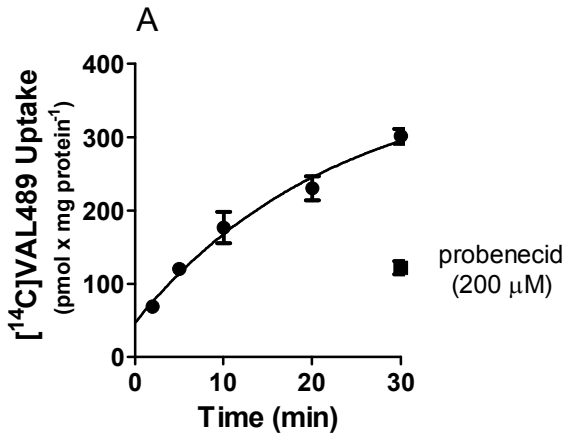
ES – Estrone-3-sulfate

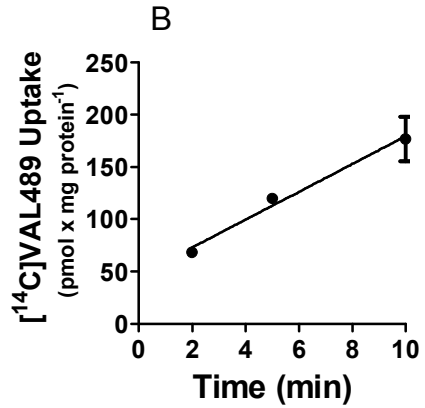


Study Title: Assessment of VAL489 (an active component of LCZ696) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)

Time course of [^{14}C]VAL489 uptake by cells expressing hOAT3

HEK Flp-In cells stably expressing hOAT3 were grown to confluence in 24-well plates. The uptake of 10 μM [^{14}C]VAL489 was conducted at room temperature for 2, 5, 10, 20 or 30 minutes. Uptake was also conducted for 30 min in the presence of 200 μM probenecid. The data are means ± SD of quadruplicate measures. A line was fit to the data (panel A) or linear regression analysis (panel B). Uptake of ~10 μM [^{14}C]VAL489 by hOAT3 was approximately linear for 10 min.

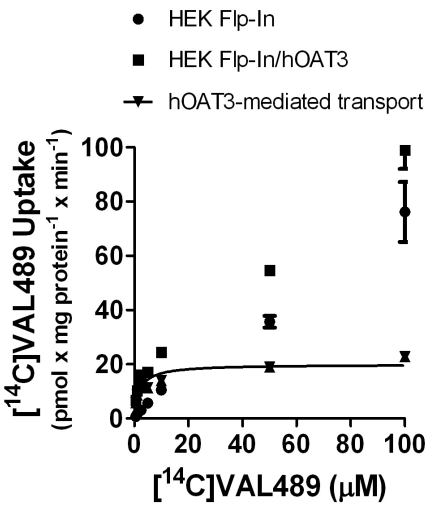




Study Title: Assessment of VAL489 (an active component of LCZ696) as an inhibitor and substrate of human organic anion transporters 1 (hOAT1) and 3 (hOAT3)

Kinetics of VAL489 transport by hOAT3

HEK Flp-In cells or HEK Flp-In cells stably expressing hOAT3 were grown to confluence in 24-well plates. Uptake of [^{14}C]VAL489 was conducted at room temperature for 10 minutes. The uptake of [^{14}C]VAL489 was found to be a saturable process after subtracting the uptake in control HEK Flp-In cells from the uptake in HEK Flp-In/hOAT3 cells (hOAT3-mediated transport). The kinetics of this process were determined using the Michealis-Menten equation. The J_{max} and K_m were 19.9 ± 1.86 pmol·mg protein^{-1}·min^{-1} and 1.9 ± 0.77 µM, respectively. The data are means ± SD of quadruplicate measures.



Conclusion: VAL489 was found to be an *in vitro* inhibitor and substrate of hOAT1 and hOAT3. The IC_{50} values for VAL489 inhibition of hOAT1- and hOAT3-mediated 6CF transport were 14.8 ± 5.42 µM and 1.11 ± 0.33 µM, respectively. The J_{max} and K_m values for hOAT3-mediated VAL489 transport were 19.9 ± 1.86 pmol·mg protein^{-1}·min^{-1} and 1.93 ± 0.77 µM, respectively. The kinetics of VAL489 transport by hOAT1 was not assessed, as VAL489 was poorly transported by hOAT1. There is a potential for inhibitors of hOAT1 and hOAT3 to effect the distribution and elimination of VAL489 *in vivo*. Additionally, there is the possibility that VAL489 could effect the renal excretion of co-administered drugs that are substrates of hOAT1 and/or hOAT3. The effect of hOAT3 inhibition *in vivo* would be dependent on the dose and the actual concentrations of VAL489 achieved therapeutically.

2.6.5.16F　薬物動態試験：その他

Test article: LBQ657

Study Titles:	Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)
Study nos.:	R1200043
GLP compliance:	not required
Location in CTD:	4.2.2.6-8
Study System:	Human embryonic kidney (HEK293) cells stably expressing OATP1B1 or OATP1B3.
Analyte/Assay:	[^{14}C]LBQ657 and/or ([^{3}H]Estradiol 17-beta glucuronide / radioactivity

Uptake of [^{14}C]LBQ657 or the positive control substrate ([^{3}H]Estradiol 17-beta Glucuronide) into control or OATP1B1-expressing cells.

Compound, concentration, µM	Inhibitor, concentration, µM	Cellular accumulation, (pmol·mg protein-1·min-1)		Accumulation ratio OATP1B1-expressing versus Control [a]
		OATP1B1 cells	Control cells	
[^{14}C]LBQ657, 12.0	-	5.17 ± 1.7	2.10 ± 0.32	2.47
[^{14}C]LBQ657, 40.0	-	13.8 ± 2.1	6.10 ± 1.6	2.26
[^{14}C]LBQ657, 90.0	-	25.3 ± 2.5	10.3 ± 1.8	2.46
[^{14}C]LBQ657, 240	-	66.6 ± 1.9	21.4 ± 2.6	3.11
[^{14}C]LBQ657, 490	-	129 ± 1.3	38.3 ± 7.4	3.37
[^{14}C]LBQ657, 40.0	Rifamycin SV, 25.0	7.74 ± 1.8	4.36 ± 1.4	1.78
[^{3}H]$E_2$17βG, 1.60	-	9.44 ± 0.23	1.43 ± 0.093	6.61
	Rifamycin SV, 25.0	1.78 ± 0.21	1.40 ± 0.069	1.28

[a] Accumulation ratio was calculated by dividing uptake into OATP1B1 cells by that into control cells.

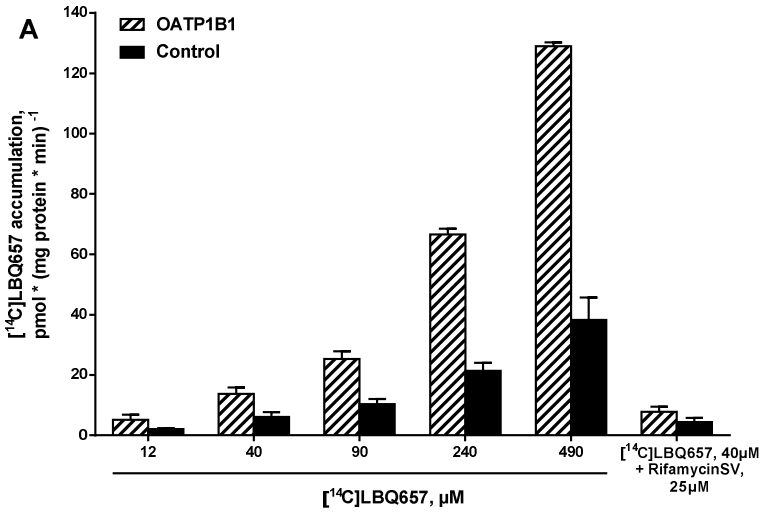
Study Titles:	Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)			
Uptake of [^{14}C]LBQ657 or the positive control substrate ([^{3}H]Estradiol 17-beta Glucuronide) into control or OATP1B3-expressing cells				
Compound, concentration, µM	Inhibitor, concentration, µM	Cellular accumulation, (pmol·mg protein-1·min-1)		Accumulation ratio OATP1B3-expressing versus Control [a]
		OATP1B3 cells	Control cells	
[^{14}C]LBQ657, 12.0	-	7.90 ± 0.61	2.10 ± 0.32	3.77
[^{14}C]LBQ657, 40.0	-	24.2 ± 1.2	6.10 ± 1.6	3.96
[^{14}C]LBQ657, 90.0	-	43.7 ± 3.0	10.3 ± 1.8	4.24
[^{14}C]LBQ657, 240	-	74.3 ± 3.6	21.4 ± 2.6	3.47
[^{14}C]LBQ657, 490	-	109 ± 5.2	38.3 ± 7.4	2.85
[^{14}C]LBQ657, 40.0	Rifamycin SV, 25.0	4.13 ± 0.57	4.36 ± 1.4	0.948
[^{3}H]$E_2$17βG, 1.60	-	3.72 ± 0.067	1.43 ± 0.093	2.6
	Rifamycin SV, 25.0	1.26 ± 0.039	1.40 ± 0.069	0.90

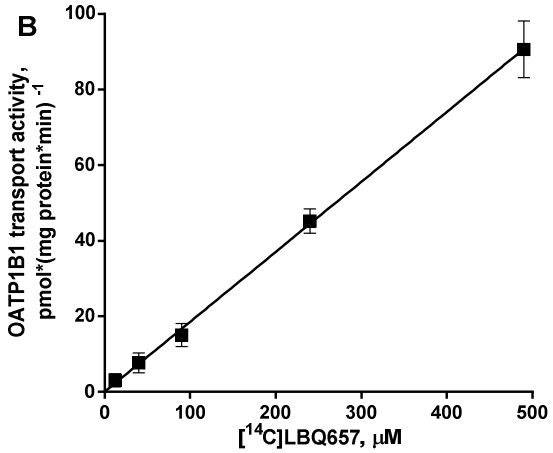
[a] Accumulation ratio was calculated by dividing uptake into OATP1B3 cells by that into control cells.

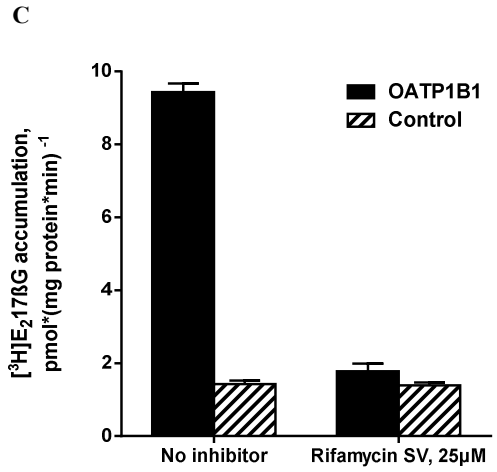
Study Titles:	Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)

Accumulation of [^{14}C]LBQ657 or the positive control substrate ([^{3}H]Estradiol 17-beta Glucuronide) into control, or OATP1B1- expressing cells

Uptake of [^{14}C]LBQ657 (12-490 µM) was conducted at 37°C for 4 minutes into control (hatched bars) or OATP1B1 expressing cells (solid bars). The bars below represent mean uptake activity normalized to protein concentration. Each of the data points on Panels A, B and C represent the mean (n=3) value ± SD. The effect of the OATP1B1 inhibitor, rifamycin SV (25 µM) on the active transport was assessed at the [^{14}C]LBQ657 concentration of 40 µM as indicated in Panel A. Non-linear regression analysis of OATP1B1-mediated transport versus [^{14}C]LBQ657 concentration is shown in Panel B. The effect of the model OATP inhibitor (rifamycin SV, 25 µM) on the accumulation of probe substrate [^{3}H]$E_2$17βG (1.6 µM) was also examined (Panel C).



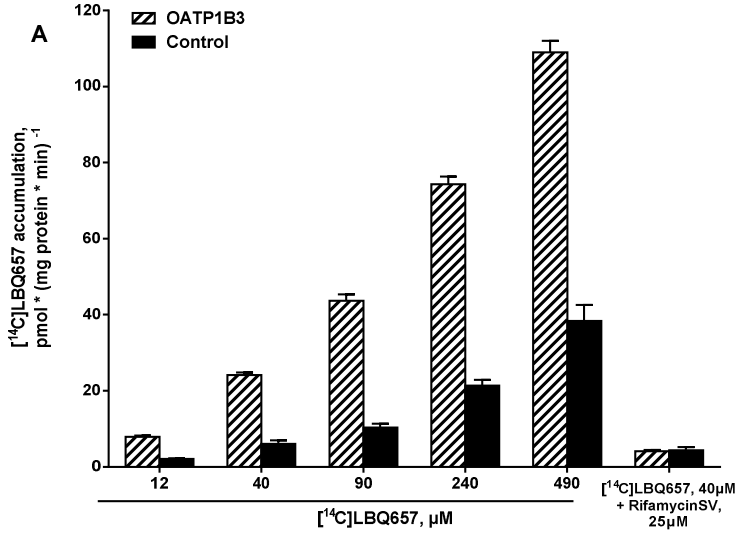


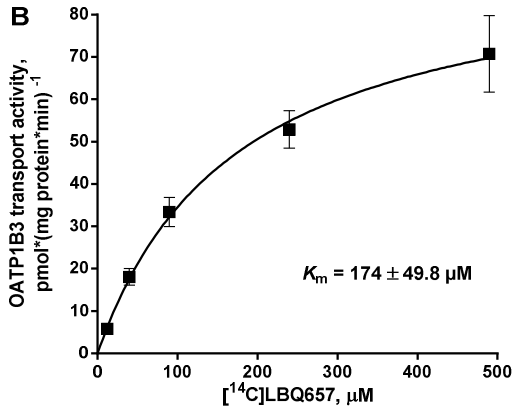


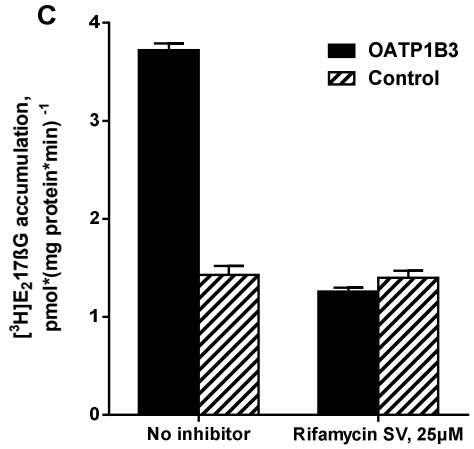
Study Titles:	Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)

Accumulation of [^{14}C]LBQ657 or the positive control substrate ([^{3}H]Estradiol 17-beta Glucuronide) into control, or OATP1B3-expressing cells

Uptake of [^{14}C]LBQ657 (12-490 µM) was conducted at 37°C for 4 minutes into control (hatched bars) or OATP1B3 expressing cells (solid bars). The bars below represent mean uptake activity normalized to protein concentration. Each of the data points on Panels A, B and C represent the mean (n=3) value ± SD. The effect of the OATP1B3 inhibitor, rifamycin SV (25 µM) on the active transport was assessed at the [^{14}C]LBQ657 concentration of 40 µM as indicated in Panel A. Non-linear regression analysis of OATP1B1-mediated transport versus [^{14}C]LBQ657 concentration is shown in Panel B. The K_m value for hOATP1B3-mediated [^{14}C]LBQ657 transport was estimated to be 174 ± 49.8 µM. The effect of the model OATP inhibitor (rifamycin SV, 25 µM) on the accumulation of probe substrate [^{3}H]$E_2$17βG (1.6 µM) was also examined (Panel C).







Conclusions: Active transport of LBQ657 into OATP1B1 and OATP1B3 expressing cells was observed. The Km value for [^{14}C]LBQ657 transport by OATP1B3 was estimated to be 174 ± 49.8 µM and the Km of OATP1B1 transport could not be estimated due to insufficient saturation of the activity. These *in vitro* results suggested that active transport by OATP1B1 and OATP1B3 contributes to the systemic clearance of LBQ657 which can be altered when LBQ657 is co-administered with drugs that inhibit such transport. The observation of an *in vivo* effect of such an inhibition would be dependent on the dose and the actual concentrations achieved therapeutically.

2.6.5.16G　薬物動態試験：その他

Test article: LBQ657

Study Titles:	Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)
Study nos.:	R1200044
GLP compliance:	not required
Location in CTD:	4.2.2.6-9
Study System:	Human embryonic kidney (HEK293) cells stably expressing OATP1B1 or OATP1B3.
Analyte/Assay:	LBQ657 and ([^{3}H]Estradiol 17-beta glucuronide / radioactivity

Effect of LBQ657 and the positive control inhibitor, rifamycin SV, on [^{3}H]$E_2$17βG accumulation in control or OATP1B1-expressing cells.

Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]$E_2$17βG accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**		**% Inhibition [b]**
		OATP1B1 cells	**Control cells**	
None	-	7.15 ± 0.11	0.41 ± 0.06	0
Rifamycin SV	25	0.37 ± 0.03	0.34 ± 0.06	99.7
LBQ657	5.0	7.19 ± 0.14	0.29 ± 0.03	NI [c]
	25	6.03 ± 0.08	0.34 ± 0.06	15.6
	50	4.93 ± 0.25	0.35 ± 0.02	32.1
	100	4.29 ± 0.17	0.32 ± 0.06	41.1
	250	2.43 ± 0.13	0.33 ± 0.13	68.9
	500	1.49 ± 0.08	0.33 ± 0.16	82.8

[a] [^{3}H]$E_2$17ßG was added to a final concentration of 1.3 µM

[b] % inhibition was calculated as follows: $\left(1-\left[\frac{(Accumulation)_{OATP,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OATP,0}-(Accumulation)_{Control,0}}\right]\right)\times 100$

[c] NI: No inhibition

Study Titles:	Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)		
Effect of LBQ657 and the positive control inhibitor, rifamycin SV, on [^{3}H]E$_2$17βG accumulation in control or OATP1B3-expressing cells			
Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]E$_2$17βG accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**	**% Inhibition [b]**
		OATP1B3 cells / **Control cells**	
None	-	4.61 ± 0.35 / 0.41 ± 0.06	-
Rifamycin SV	25	0.42 ± 0.03 / 0.34 ± 0.06	98.2
LBQ657	2.5	4.52 ± 0.09 / 0.29 ± 0.03	NI [c]
	10	6.27 ± 0.06 / 0.34 ± 0.06	NI
	25	5.71 ± 0.46 / 0.35 ± 0.02	NI
	50	6.06 ± 0.26 / 0.32 ± 0.06	NI
	100	6.94 ± 0.41 / 0.33 ± 0.13	NI
	250	7.98 ± 0.73 / 0.33 ± 0.16	NI

[a] [^{3}H]E$_2$17βG was added to a final concentration of 1.3 µM

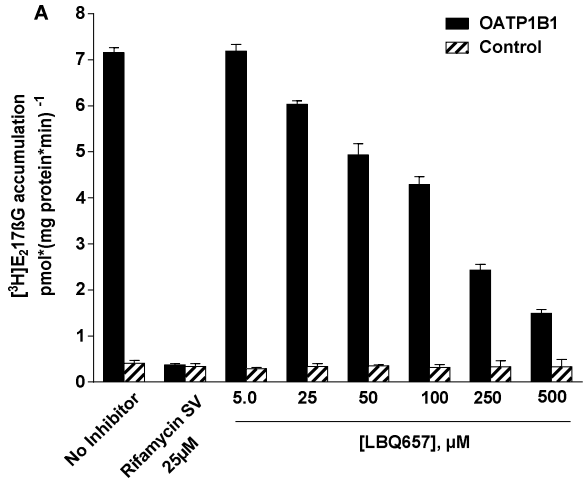
[b] % inhibition was calculated as follows: $\left(1-\left[\frac{(Accumulation)_{OATP,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OATP,0}-(Accumulation)_{Control,0}}\right]\right)\times 100$

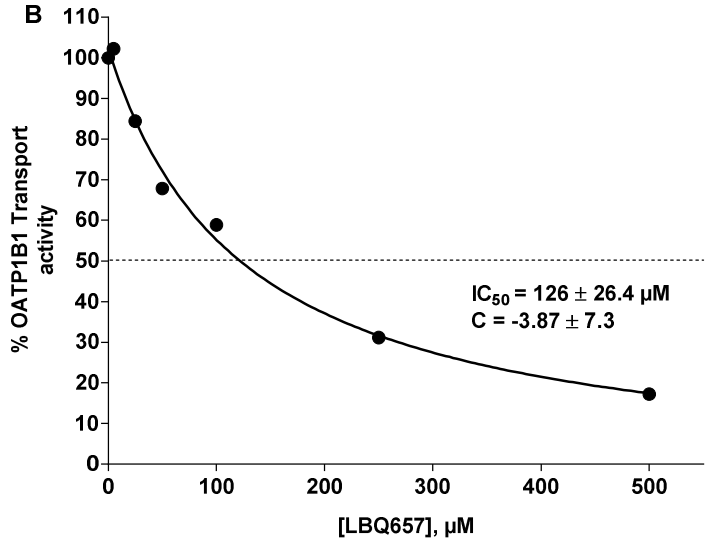
[c] NI: No inhibition

Study Titles: Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)

Assessment of LBQ657 as an inhibitor of OATP1B1-mediated uptake of [^{3}H]E$_2$17βG

HEK293 cells stably expressing OATP1B1 (black bars), or control cells (hatched bars) were incubated with [^{3}H]E$_2$17βG (1.3 µM) in the presence or absence of rifamycin SV (25 µM; positive control inhibition) or LBQ657 as indicated in the **Panel A**. Each bar represents the average pmol [^{3}H]E$_2$17βG accumulation per mg cellular protein per min ± standard deviation of three separate wells. The IC$_{50}$ was 126 ± 26.4 µM **(Panel B)**.

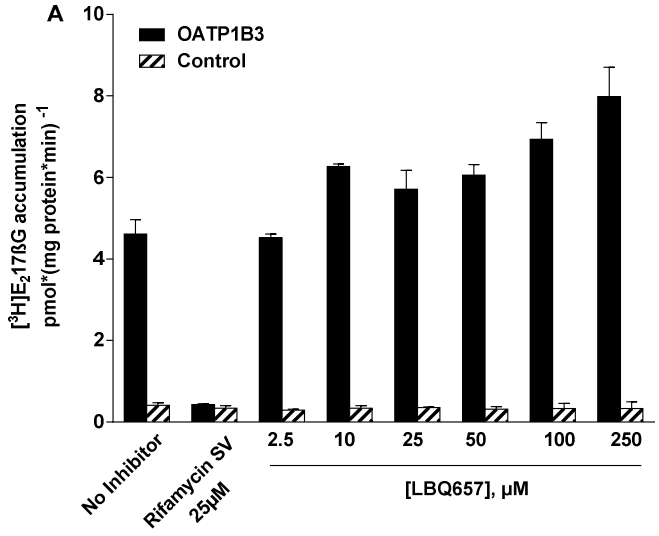


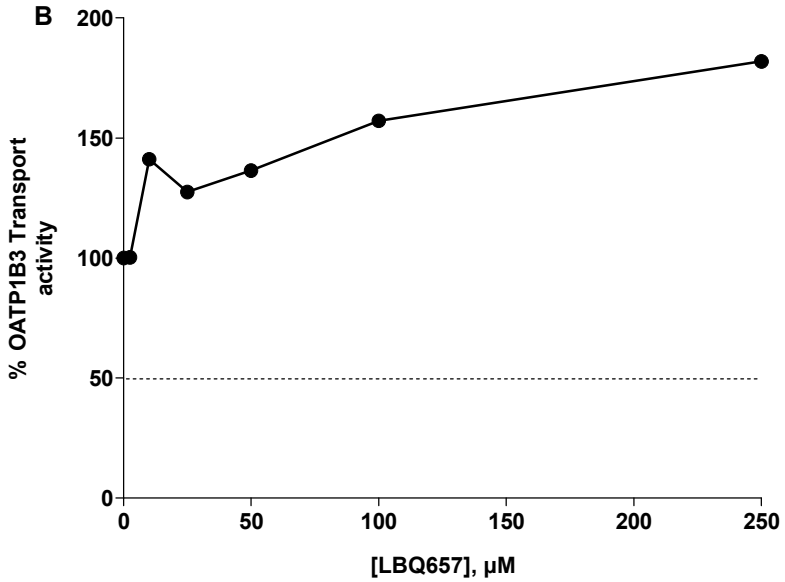


Study Titles:	Assessment of LBQ657 (active metabolite of AHU377) as a substrate of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)

Assessment of LBQ657 as an inhibitor of OATP1B3-mediated uptake of [^{3}H]E$_2$17βG

HEK293 cells stably expressing OATP1B3 (black bars), or control cells (hatched bars) were incubated with [^{3}H]E$_2$17βG (1.3 µM) in the presence or absence of rifamycin SV (25 µM; positive control inhibition) or LBQ657 as indicated as indicated in the **Panel A**. Each bar represents the average pmol [^{3}H]E$_2$17βG accumulation per mg cellular protein per min ± standard deviation of three separate wells. The IC$_{50}$ value was not estimated since LBQ657 did not appear to act as an inhibitor of OATP1B3 transport (**Panel B).**





Conclusion: LBQ657 was shown to be an in vitro inhibitor of OATP1B1 but not OATP1B3. The IC50 value for OATP1B1 inhibition was 126 ± 26 µM with a maximum inhibition of 82.8% at 500 µM. These in vitro findings suggested that LBQ657 may cause an increase in the systemic exposure of co-medications whose clearance is significantly mediated by OATP1B1 transport activity provided that sufficiently high LBQ657 concentrations are achieved in vivo.

2.6.5.16H　薬物動態試験：その他

Test article: AHU377

Study Titles:	Assessment of AHU377 as an inhibitor of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)
Study nos.:	R1200090
GLP compliance:	not required
Location in CTD:	4.2.2.6-10
Study System:	Human embryonic kidney (HEK293) cells stably expressing OATP1B1 or OATP1B3.
Analyte/Assay:	AHU377 and LBQ657 ([^{3}H]Estradiol 17-beta glucuronide / radioactivity

Effect of AHU377 and rifamycin SV on [^{3}H]$E_2$17βG accumulation in control or OATP1B1-expressing cells.

Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]$E_2$17βG accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**		**% Inhibition [b]**
		OATP1B1 cells	**Control cells**	
None	-	4.97 ± 0.46	0.51 ± 0.29	0.0
Rifamycin SV	20	0.55 ± 0.56	0.20 ± 0.06	92.2
AHU377	1.0	3.52 ± 0.60	0.27 ± 0.12	27.1
	2.5	1.83 ± 0.31	0.21 ± 0.07	63.5
	5.0	0.80 ± 0.14	0.66 ± 0.49	97.0
	10	0.36 ± 0.11	0.17 ± 0.03	95.7
	25	0.42 ± 0.27	0.23 ± 0.11	95.7
	50	0.22 ± 0.10	0.14 ± 0.01	98.3

[a] [^{3}H]$E_2$17βG was added to a final concentration of 1.1 µM

[b] % inhibition was calculated as follows: $\left(1-\left[\frac{(Accumulation)_{OATP,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OATP,0}-(Accumulation)_{Control,0}}\right]\right)\times 100$

Study Titles:	Assessment of AHU377 as an inhibitor of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)			
Effect of AHU377 and rifamycin SV on [^{3}H]$E_2$17βG accumulation in control or OATP1B3-expressing cells				
Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]$E_2$17βG accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**		**% Inhibition [b]**
		OATP1B3 cells	**Control cells**	
None	-	5.11 ± 0.20	0.51 ± 0.29	0.0
Rifamycin SV	20	0.77 ± 0.46	0.20 ± 0.06	87.7
AHU377	1.0	4.55 ± 0.79	0.27 ± 0.12	6.89
	2.5	3.87 ± 1.2	0.21 ± 0.07	20.2
	5.0	1.92 ± 0.01	0.66 ± 0.49	72.5
	10	1.08 ± 0.27	0.17 ± 0.03	80.2
	25	0.99 ± 0.12	0.23 ± 0.11	83.5
	50	0.61 ± 0.14	0.14 ± 0.01	89.9

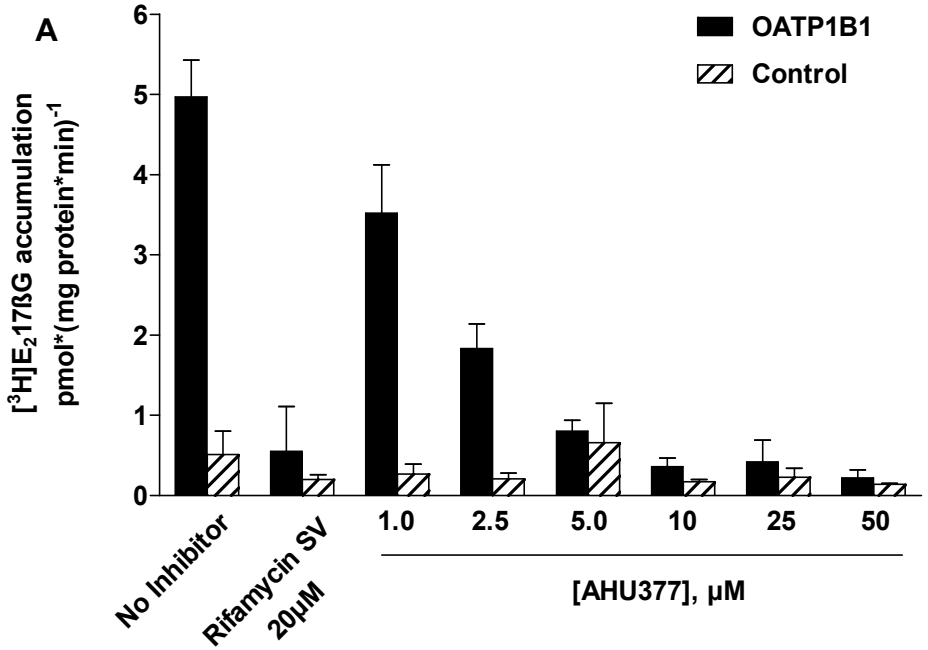
[a] [^{3}H]$E_2$17βG was added to a final concentration of 1.1 µM

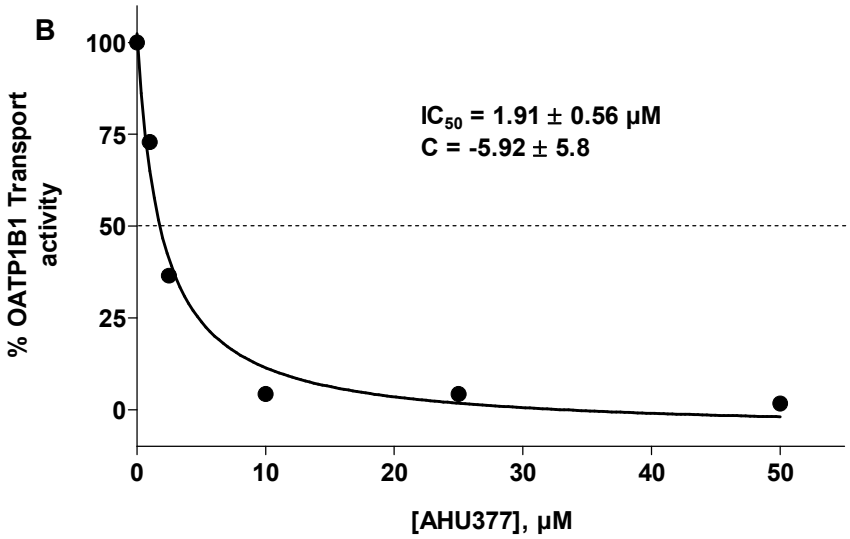
[b] % inhibition was calculated as follows: $\left(1-\left[\frac{(Accumulation)_{OATP,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OATP,0}-(Accumulation)_{Control,0}}\right]\right)\times 100$

Study Titles:	Assessment of AHU377 as an inhibitor of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)

Assessment of AHU377 as an inhibitor of OATP1B1-mediated uptake of [^{3}H]$E_2$17βG

HEK cells stably expressing OATP1B1 (black bars), or control cells (hatched bars) were incubated with [^{3}H]$E_2$17βG (1.1 µM) in the presence or absence of rifamycin SV (20 µM; positive control inhibition) or AHU377 as indicated in the **Panel A**. Each bar represents the average pmol [^{3}H]$E_2$17βG accumulation per mg cellular protein per min ± standard deviation of three separate wells. The IC_{50} was 1.91 ± 0.56 µM **(Panel B).**

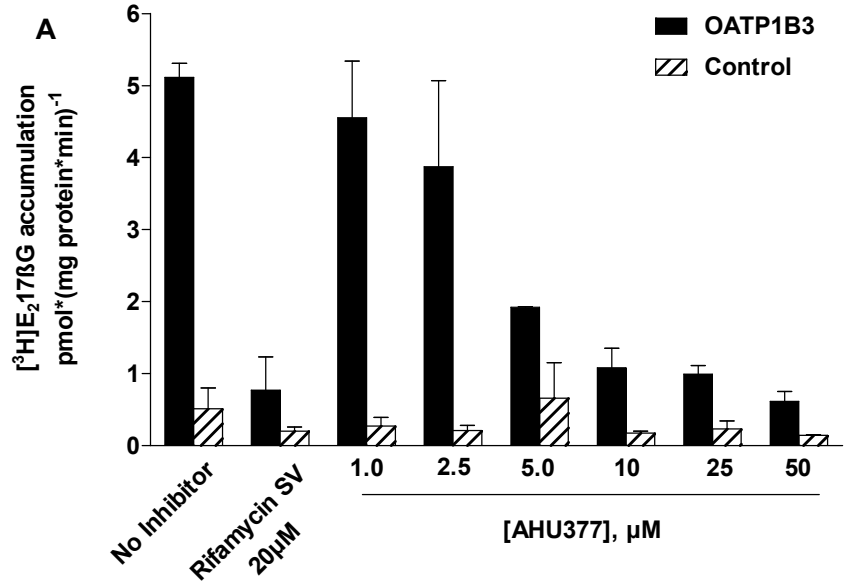


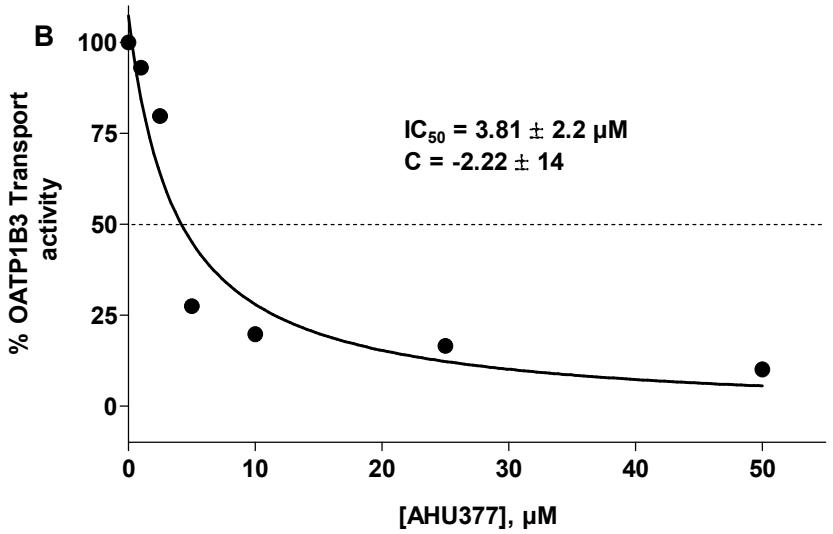


Study Titles:	Assessment of AHU377 as an inhibitor of human organic anion transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3)

Assessment of AHU377 as an inhibitor of OATP1B3-mediated uptake of [^{3}H]$E_2$17βG

HEK293 cells stably expressing OATP1B3 (black bars), or control cells (hatched bars) were incubated with [^{3}H]$E_2$17βG (1.1 µM) in the presence or absence of rifamycin SV (20 µM; positive control inhibition) or AHU377 as indicated as indicated in the **Panel A**. Each bar represents the average pmol [^{3}H]$E_2$17βG accumulation per mg cellular protein per min ± standard deviation of three separate wells. The IC_{50} value was estimated to be approximately 3.81 ± 2.2 µM (**Panel B**).





Conclusion: AHU377 was shown to inhibit the transport activities of OATP1B1 and OATP1B3 in vitro. The IC50 values of OATP1B1 and OATP1B3 inhibition of [^{3}H]$E_2$17βG transport were 1.91 ± 0.56 µM and 3.81 ± 2.2 µM, respectively. As a result, AHU377 may cause an increase in the systemic exposure of co-medications whose clearance is significantly mediated by OATP1B1 or OATP1B3 transport activity provided that sufficiently high AHU377 concentrations are achieved in vivo.

2.6.5.16I 薬物動態試験：その他

Test article: AHU377

Study Title:	Assessment of AHU377 as an inhibitor of human organic anion transporters 1 and 3 (OAT1 & OAT3)
Study nos.:	R1300221
GLP compliance:	not required
Location in CTD:	4.2.2.6-14
Study System:	Human embryonic kidney (HEK293) cells stably over-expressing OAT1 or OAT3.
Analyte/Assay:	AHU377 [^{3}H]cidofovir / radioactivity

Effect of AHU377 and the model inhibitor probenecid on [^{3}H]cidofovir accumulation in control or OAT1 expressing cells.

Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]cidofovir accumulation (pmol/mg protein/min) [a]**		**% inhibition [b]**
		Control cells	**OAT1 cells**	
None	0	0.286 ± 0.058	13.8 ± 0.65	0
Probenecid	100	0.321 ± 0.072	0.861 ± 0.032	96.0
AHU377	1.0	0.181 ± 0.053	13.4 ± 0.65	2.39
	2.5	0.154 ± 0.016	13.2 ± 0.60	3.51
	5.0	0.207 ± 0.053	12.0 ± 0.55	13.1
	10	0.196 ± 0.040	11.8 ± 0.68	13.8
	25	0.278 ± 0.076	10.6 ± 0.15	23.7
	50	0.201 ± 0.041	8.77 ± 0.21	36.6

[a] [^{3}H]cidofovir was added to a final concentration of 1.5 µM

[b] % inhibition was calculated as follows:

$$(1-[\frac{(Accumulation)_{transfected,I}-(Accumulation)_{untransfected,I}}{(Accumulation)_{transfected,0}-(Accumulation)_{untrasfected,0}}])\times 100$$

Study Title:	Assessment of AHU377 as an inhibitor of human organic anion transporters 1 and 3 (OAT1 & OAT3)			
Effect of AHU377 and the model inhibitor probenecid on [^{3}H]Estrone-3-sulfate accumulation in control or OAT3-expressing HEK293 cells				
Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]E$_3$S accumulation (fmol/mg protein/min) [a]**		**% Inhibition [b]**
		Control cells	**OAT3 cells**	
None	0	1.25 ± 0.085	14.1 ± 0.31	0
Probenecid	100	1.05 ± 0.018	1.39 ± 0.072	97.4
AHU377	0.10	1.13 ± 0.064	12.6 ± 0.20	10.9
	0.50	1.29 ± 0.11	9.66 ± 0.35	34.9
	1.0	1.09 ± 0.097	6.74 ± 0.23	56.1
	2.5	1.02 ± 0.061	4.20 ± 0.081	75.2
	5.0	0.965 ± 0.049	3.00 ± 0.044	84.2
	10	0.854 ± 0.052	2.00 ± 0.034	91.1

[a] [^{3}H]E$_3$S was added to a final concentration of 0.73 µM

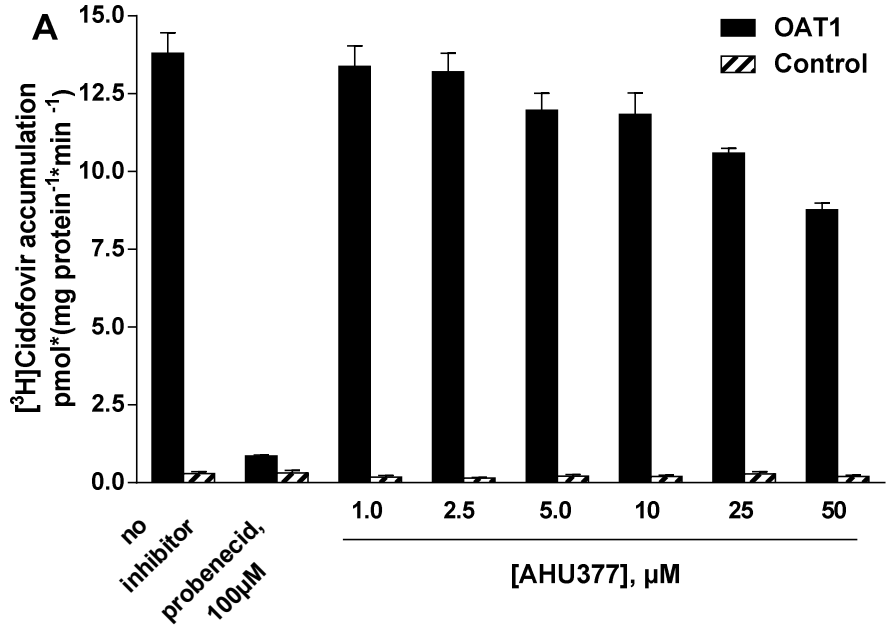
[b] % inhibition was calculated as follows:

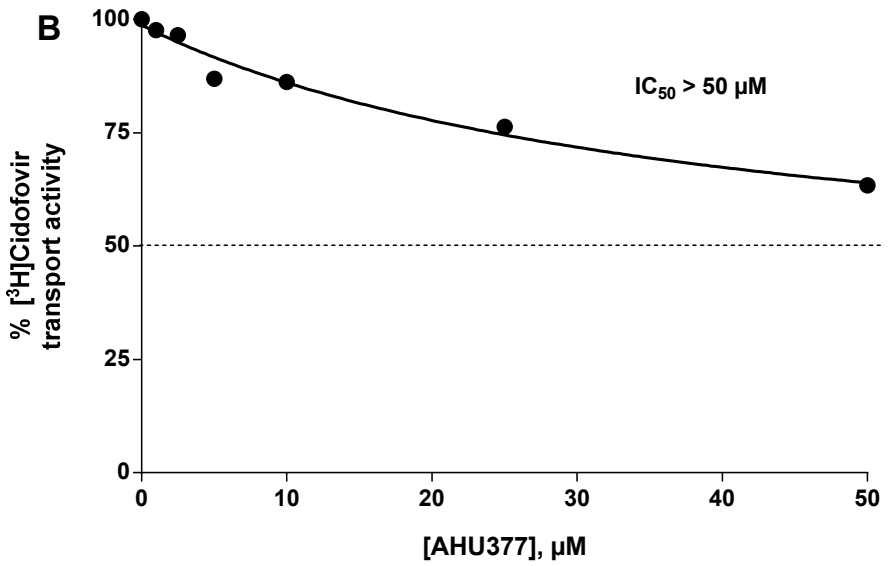
$$(1-[\frac{(Accumulation)_{transfected,I}-(Accumulation)_{untransfected,I}}{(Accumulation)_{transfected,0}-(Accumulation)_{untrasfected,0}}])\times 100$$

Study Title: Assessment of AHU377 as an inhibitor of human organic anion transporters 1 and 3 (OAT1 & OAT3)

Assessment of AHU377 as an inhibitor of OAT1

HEK293 cells stably expressing OAT1 (solid bars), or control untransfected HEK293 cells (hatched bars) were incubated with [^{3}H]cidofovir (1.5 µM) in the presence or absence of probenecid (100 µM; positive control inhibitior) or AHU377 as indicated in Panel A. Each bar represents the average pmol [^{3}H]cidofovir accumulation per mg cellular protein ± standard deviation of three separate wells. Non-linear regression analysis of the % transport activity relative to control (no inhibitor) is shown in Panel B. The IC_{50} value was estimated. The IC_{50} was >50 µM **(Panel B).**

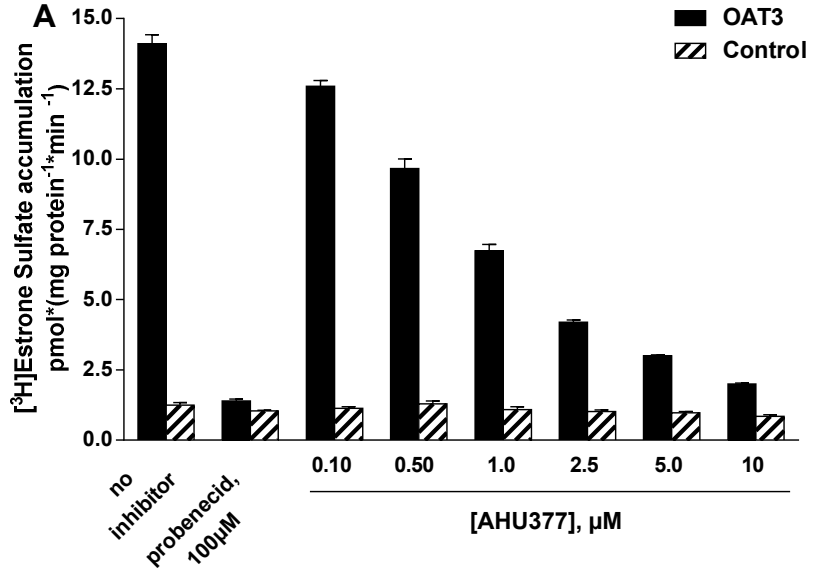


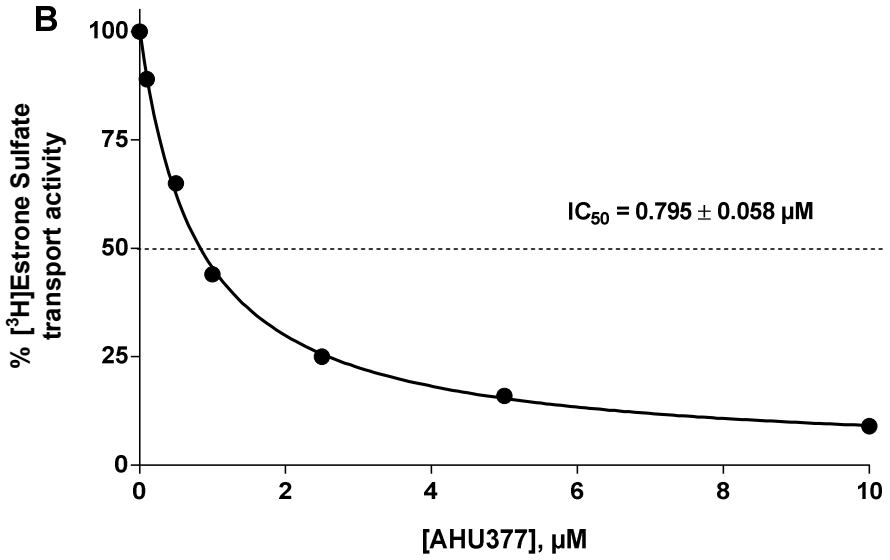


Study Title: Assessment of AHU377 as an inhibitor of human organic anion transporters 1 and 3 (OAT1 & OAT3)

Assessment of AHU377 as an inhibitor of OAT3

HEK293 cells stably expressing OAT3 (solid bars), or control untransfected HEK293 cells (hatched bars) were incubated with [^{3}H]E_3S (0.73 µM) in the presence or absence of probenecid (100 µM; positive control inhibitor) or AHU377 as indicated in Panel A. Each bar represents the average pmol [^{3}H]E_3S accumulation per mg cellular protein ± standard deviation of three separate wells. Non-linear regression analysis of the % transport activity relative to control (no inhibitor) is shown in Panel B. The IC_{50} value was estimated to be approximately 0.795 ± 0.058 µM (**Panel B**).





Conclusion: AHU377 was shown to inhibit the transport activities of OAT1 and OAT3 *in vitro*. At an AHU377 concentration of 50 µM or 10 µM, the transport activities of OAT1 and OAT3 were inhibited by 36.6% and 91.1%, respectively. The IC_{50} values of OAT1 and OAT3 inhibition were greater than 50 µM and 0.795 ± 0.058 µM, respectively. These *in vitro* findings indicated that AHU377 may cause an increase in the systemic exposure of co-medications whose clearance is significantly mediated by OAT1 and OAT3 transport activity provided that sufficiently high AHU377 concentrations are achieved *in vivo*.

2.6.5.16J　薬物動態試験：その他

Test article: AHU377 and LBQ657

Study Titles:	Assessment of AHU377 and its active metabolite, LBQ657, as inhibitors of organic cation transporters 1 and 2 (OCT1 & OCT2)
Study nos.:	R1200302
GLP compliance:	not required
Location in CTD:	4.2.2.6-11
Study System:	Human embryonic kidney (HEK293) cells stably expressing the human orthologs of OCT1 or OCT2.
Analyte/Assay:	AHU377 and LBQ657 / [^{3}H] 1-Methyl-4-phenylpyridinium acetate and [^{14}C]metformin radioactivity

Assessment of AHU377 to inhibit OCT1

Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]MPP^{+} accumulation, (pmol·mg protein^{-1}·min^{-1})**		
		Control cells	**OCT1 cells**	**% inhibition[a]**
None	0	0.020 ± 0.00035	0.265 ± 0.018	0
Decynium 22	20	0.015 ± 0.00045	0.074 ± 0.0047	75.9
AHU377	1.0	0.018 ± 0.00010	0.277 ± 0.018	NI[b]
	2.5	0.019 ± 0.0013	0.294 ± 0.018	NI
	5.0	0.018 ± 0.00052	0.278 ± 0.016	NI
	10	0.019 ± 0.00083	0.275 ± 0.0079	NI
	25	0.018 ± 0.00054	0.276 ± 0.0084	NI
	50	0.019 ± 0.00062	0.289 ± 0.0027	NI

[a] %inhibition was calculated as follows: $(1-[\frac{(Accumulation)_{OCT1,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OCT1,0}-(Accumulation)_{Control,0}}])\times 100$

[b] NI: No Inhibition

Study Titles:	Assessment of AHU377 and its active metabolite, LBQ657, as inhibitors of organic cation transporters 1 and 2 (OCT1 & OCT2)			
Assessment of AHU377 to inhibit OCT2				
Inhibitor	**Inhibitor concentration, µM**	**[^{14}C]metformin accumulation, (pmol·mg protein^{-1}·min^{-1})**		
		Control cells	**OCT2 cells**	**% inhibition[a]**
None	0	0.014 ± 0.00026	0.198 ± 0.014	0
Decynium 22	20	0.011 ± 0.00032	0.056 ± 0.0035	75.8
AHU377	1.0	0.013 ± 0.000075	0.207 ± 0.013	NI[b]
	2.5	0.014 ± 0.00094	0.220 ± 0.014	NI
	5.0	0.013 ± 0.00038	0.207 ± 0.012	NI
	10	0.014 ± 0.00060	0.206 ± 0.0059	NI
	25	0.013 ± 0.00039	0.206 ± 0.0063	NI
	50	0.014 ± 0.00045	0.216 ± 0.0020	NI

[a] %inhibition was calculated as follows:

$$(1-[\frac{(Accumulation)_{OCT,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OCT,0}-(Accumulation)_{Control,0}}])\times 100$$

[b] NI: No Inhibition

Assessment of LBQ657 to inhibit OCT1				
Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]MPP^{+} accumulation, (pmol·mg protein^{-1}·min^{-1})**		
		Control cells	**OCT1 cells**	**% inhibition[a]**
None	0	0.020 ± 0.000065	0.311 ± 0.0053	0
Decynium 22	20	0.016 ± 0.0014	0.074 ± 0.0049	80.09
LBQ657	5.0	0.020 ± 0.00096	0.298 ± 0.012	4.55
	25	0.019 ± 0.00077	0.314 ± 0.052	NI[b]
	50	0.020 ± 0.00053	0.296 ± 0.010	5.24
	100	0.020 ± 0.00027	0.288 ± 0.012	7.99
	250	0.019 ± 0.00018	0.289 ± 0.019	7.30
	500	0.019 ± 0.00049	0.292 ± 0.016	6.27

Study Titles:	Assessment of AHU377 and its active metabolite, LBQ657, as inhibitors of organic cation transporters 1 and 2 (OCT1 & OCT2)			
Assessment of LBQ657 to inhibit OCT2				
Inhibitor	**Inhibitor concentration, µM**	**[^{14}C]metformin accumulation, (pmol·mg protein^{-1}·min^{-1})**		
		Control cells	**OCT2 cells**	**% inhibition[a]**
None	0	0.0274 ± 0.00009	0.235 ± 0.0040	0
Decynium 22	20	0.0221 ± 0.0019	0.056 ± 0.0037	83.8
LBQ657	5.0	0.0275 ± 0.0013	0.225 ± 0.0094	4.85
	25	0.0267 ± 0.0011	0.237 ± 0.0040	NI[b]
	50	0.0275 ± 0.00073	0.223 ± 0.0075	5.70
	100	0.0271 ± 0.00038	0.217 ± 0.0089	8.43
	250	0.0264 ± 0.00025	0.218 ± 0.014	7.62
	500	0.0257 ± 0.00068	0.220 ± 0.012	6.24

[a] %inhibition was calculated as follows:

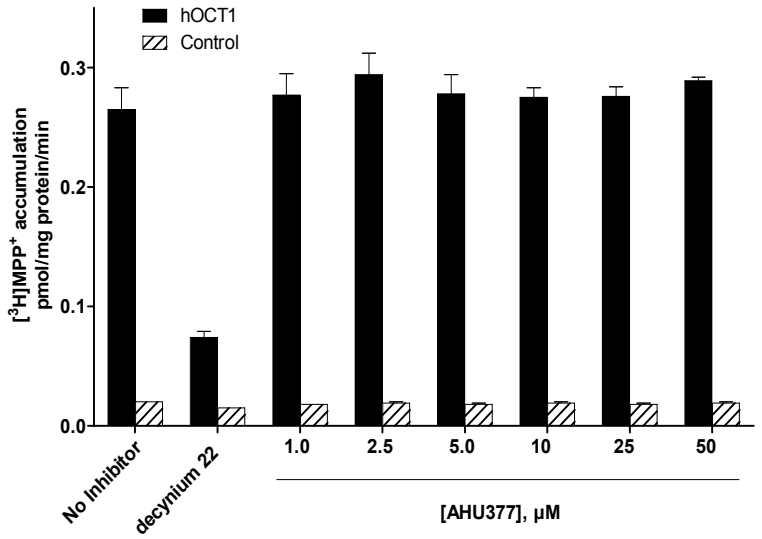
$$(1-[\frac{(Accumulation)_{OCT,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OCT,0}-(Accumulation)_{Control,0}}])\times 100$$

[b] NI: No Inhibition

Study Titles:	Assessment of AHU377 and its active metabolite, LBQ657, as inhibitors of organic cation transporters 1 and 2 (OCT1 & OCT2)

Assessment of AHU377 to inhibit OCT1

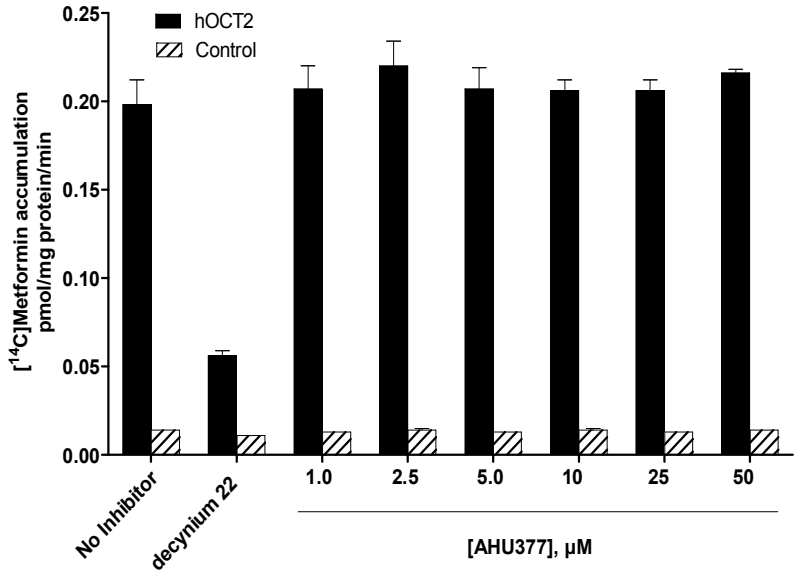
HEK Flp-In 293 cells expressing OCT1 (black bars), or control cells (hatched bars) were incubated with the model OCT1 substrate [^{3}H]MPP+ (17 nM), in the presence of the general OCT inhibition decynium 22 (20 µM) or AHU377 (0-50 µM). Each bar represents the average accumulation per mg cellular protein ± standard deviation of three separate wells.



Study Titles:	Assessment of AHU377 and its active metabolite, LBQ657, as inhibitors of organic cation transporters 1 and 2 (OCT1 & OCT2)

Assessment of AHU377 to inhibit OCT2

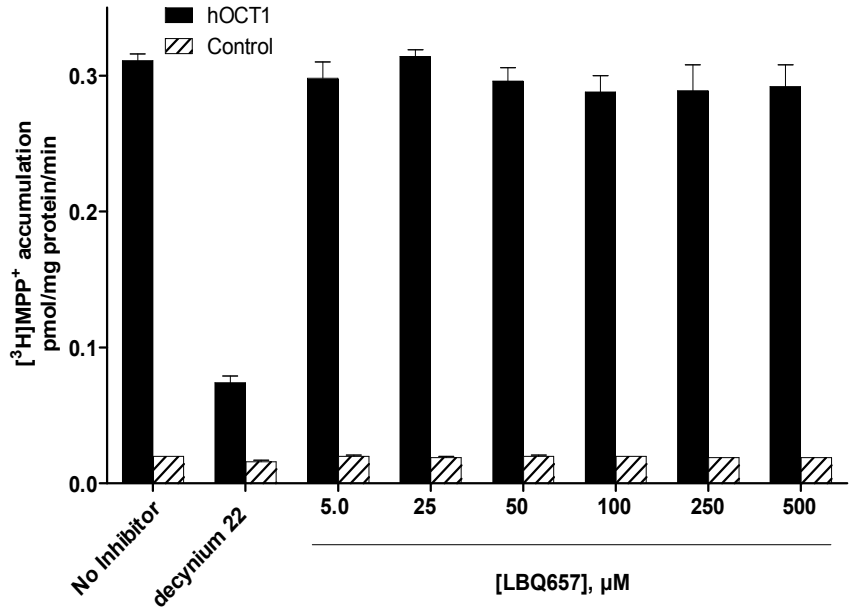
HEK Flp-In 293 cells expressing OCT2 (black bars), or control cells (hatched bars) were incubated with the model OCT2 substrate [^{14}C]metformin (12.7 µM), in the presence of the general OCT inhibition decynium 22 (20 µM) or AHU377 (0-50 µM). Each bar represents the average accumulation per mg cellular protein ± standard deviation of three separate wells



Study Titles:	Assessment of AHU377 and its active metabolite, LBQ657, as inhibitors of organic cation transporters 1 and 2 (OCT1 & OCT2)

Assessment of LBQ657 to inhibit OCT1

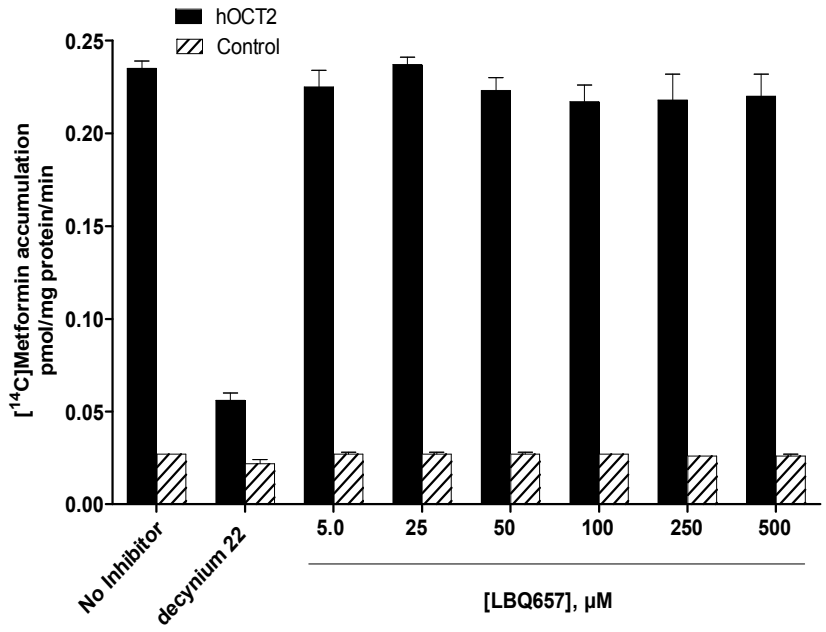
HEK Flp-In 293 cells expressing OCT1 (black bars), or control cells (hatched bars) were incubated with the model OCT1 substrate [^{3}H]MPP+ (17 nM), in the presence of the general OCT inhibition decynium 22 (20 µM) or LBQ657 (0-500 µM). Each bar represents the average accumulation per mg cellular protein ± standard deviation of three separate wells



Study Titles:	Assessment of AHU377 and its active metabolite, LBQ657, as inhibitors of organic cation transporters 1 and 2 (OCT1 & OCT2)

Assessment of LBQ657 to inhibit OCT2

HEK Flp-In 293 cells expressing OCT2 (black bars), or control cells (hatched bars) were incubated with the model OCT2 substrate [^{14}C]metformin (12.7 µM), in the presence of the general OCT inhibition decynium 22 (20 µM) or LBQ657 (0-500 µM). Each bar represents the average accumulation per mg cellular protein ± standard deviation of three separate wells.



Conclusion: The results of these studies demonstrated that neither AHU377 nor its active metabolite, LBQ657, is an in vitro inhibitor of human OCT1 or OCT2.

2.6.5.16K　薬物動態試験：その他

Test article: VAL489

Study Titles:	Assessment of VAL489 as an inhibitor of organic cation transporters 1 and 2 (OCT1 & OCT2)
Study nos.:	R1200303
GLP compliance:	not required
Location in CTD:	4.2.2.6-12
Study System:	Human embryonic kidney (HEK293) cells stably expressing the human orthologs of OCT1 or OCT2.
Analyte/Assay:	VAL489 and [^{3}H] 1-Methyl-4-phenylpyridinium acetate or [^{14}C] metformin / radioactivity

Effect of VAL489 and decynium 22 on [^{3}H]MPP^{+} accumulation in control or OCT1-expressing cells.

Inhibitor	**Inhibitor concentration, µM**	**[^{3}H]MPP^{+} accumulation [a] (pmol·mg protein^{-1}·min^{-1})**		
		Control cells	**OCT1 cells**	**% inhibition[b]**
None	-	118 ± 3.0	20.4 ± 0.65	0.0
Decynium 22	20	42.0 ± 4.1	15.0 ± 0.46	72.4
	1.0	122 ± 5.0	18.9 ± 1.0	NI [c]
	5.0	129 ± 3.5	18.9 ± 0.71	NI
VAL489	10	116 ± 6.8	19.2 ± 0.47	1.08
	25	111 ± 2.9	18.9 ± 0.90	6.10
	50	123 ± 6.2	18.2 ± 0.058	NI
	100	124 ± 2.9	17.1 ± 0.073	NI

[a] [^{3}H]MPP^{+} was added to a final concentration of 8.8 nM

[b] % inhibition was calculated as follows:

$$(1-[\frac{(Accumulation)_{OCT,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OCT,0}-(Accumulation)_{Control,0}}])\times 100$$

[c] NI: No Inhibition

Study Titles:	Assessment of VAL489 as an inhibitor of organic cation transporters 1 and 2 (OCT1 & OCT2)			
Effect of VAL489 and decynium 22 on [^{14}C]metformin accumulation in control or OCT2-expressing cells.				
Inhibitor	Inhibitor concentration, μM	[^{14}C]metformin accumulation [a] (pmol·mg protein^{-1}·min^{-1})		% Inhibition [b]
		OCT2 cells	Control cells	
None	-	135 ± 8.4	3.80 ± 0.36	0.0
Decynium 22	20	2.13 ± 0.30	2.38 ± 0.19	100
VAL489	1.0	139 ± 4.5	3.49 ± 0.40	NI [c]
	5.0	133 ± 0.85	3.38 ± 0.17	1.83
	10	133 ± 2.3	3.43 ± 0.23	1.27
	25	135 ± 6.7	3.39 ± 0.092	0.421
	50	137 ± 4.6	3.32 ± 0.15	NI
	100	123 ± 0.24	3.05 ± 0.21	8.87

[a] [^{14}C]metformin was added to a final concentration of 12.5 μM

[b] % inhibition was calculated as follows:

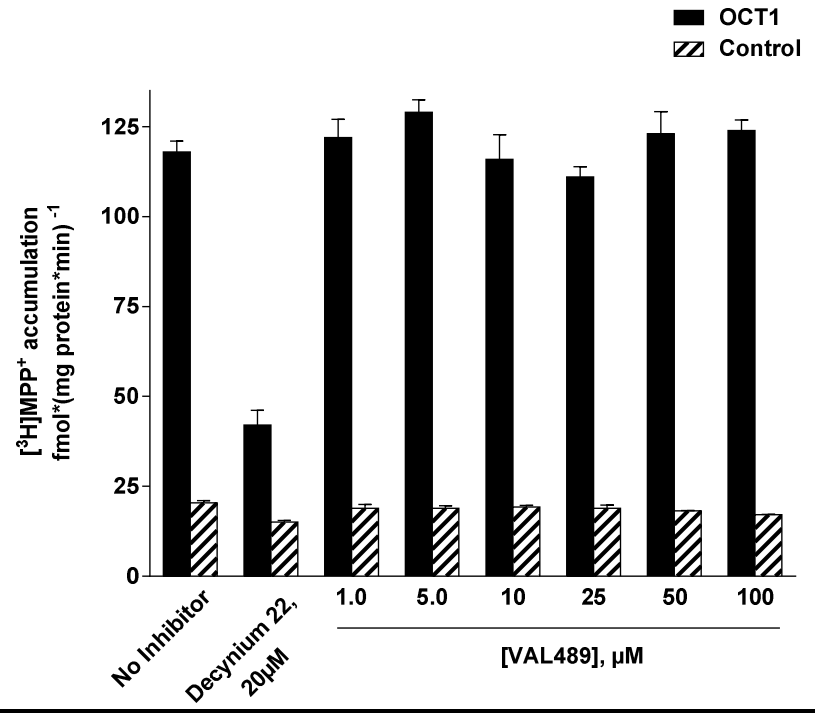
$$(1-[\frac{(Accumulation)_{OCT,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OCT,0}-(Accumulation)_{Control,0}}])\times 100$$

[c] NI: No Inhibition

Study Titles:	Assessment of VAL489 as an inhibitor of organic cation transporters 1 and 2 (OCT1 & OCT2)

Assessment of VAL489 as an inhibitor of OCT1-mediated uptake of $[^3H]MPP^+$

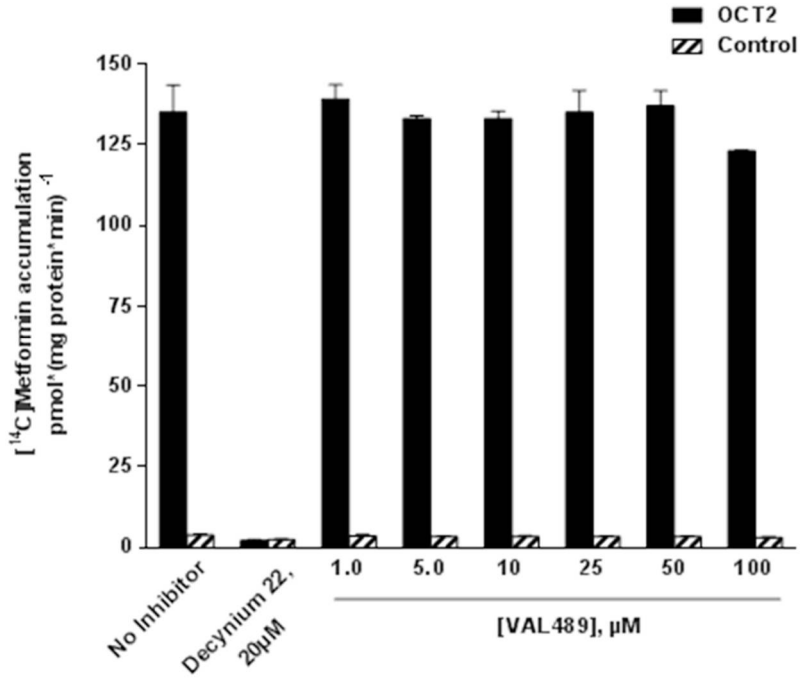
HEK cells stably expressing OCT1 (black bars), or control cells (hatched bars) were incubated with $[^3H]MPP+$ (8.8 nM) in the presence or absence of decynium 22 (20 µM; positive control inhibition) or VAL489 (1-100 µM). Each bar represents the average fmol $[^3H]MPP+$ accumulation per mg cellular protein per min ± standard deviation of three separate wells.



Study Titles:	Assessment of VAL489 as an inhibitor of organic cation transporters 1 and 2 (OCT1 & OCT2)

Assessment of VAL489 as an inhibitor of OCT2-mediated uptake of [^{14}C]metformin

HEK cells stably expressing OCT2 (black bars), or control cells (hatched bars) were incubated with [^{14}C]metformin (12.5 µM) in the presence or absence of decynium 22 (20 µM; positive control inhibition) or VAL489 (1-100 µM). Each bar represents the average pmol [^{14}C]metformin accumulation per mg cellular protein per min ± standard deviation of three separate wells.



Conclusion: The results of these studies demonstrated that VAL489 is not an in vitro inhibitor of human OCT1 or OCT2.

2.6.5.16L　薬物動態試験：その他

Test article: AHU377

Study Titles:	Assessment of AHU377 as an inhibitor of the multidrug and toxin extrusion (MATE) 1 and 2-K transporters
Study nos.:	[R1300681]
GLP compliance:	not required
Study System:	Human embryonic kidney (HEK293) cells stably expressing the human ortholog of MATE1 or MATE2-K.
Analyte/Assay:	AHU377 and [^{14}C] metformin / radioactivity

Effect of AHU377 and MATE inhibitor tetraethylammonium (TEA) on [^{14}C]metformin accumulation in control or MATE1-expressing cells

Inhibitor	**Inhibitor concentration, µM**	**[^{14}C]metformin accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**		**% Inhibition [b]**
		MATE1 cells	**Control cells**	
None	-	55.0 ± 2.3	2.94 ± 0.17	0.0
TEA	500	3.28 ± 0.44	1.06 ± 0.11	95.7
AHU377	5.0	50.7 ± 1.9	2.80 ± 0.13	8.07
	50	45.8 ± 1.3	2.77 ± 0.046	17.3

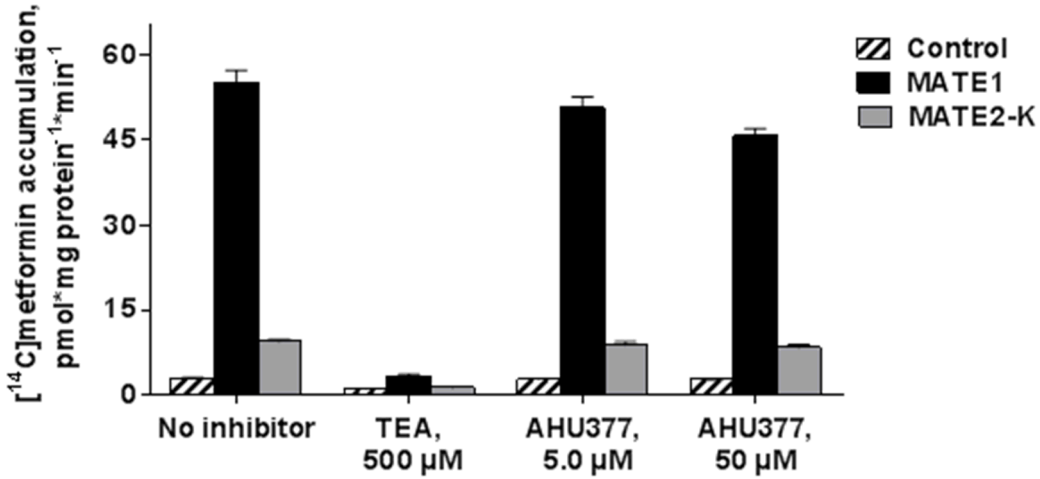
Effect of AHU377 and MATE inhibitor TEA on [^{14}C]metformin accumulation in control or MATE2-K-expressing cells

Inhibitor	**Inhibitor concentration, µM**	**[^{14}C]metformin accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**		**% Inhibition [b]**
		MATE2-K cells	**Control cells**	
None	-	9.48 ± 0.37	2.94 ± 0.17	0.0
TEA	500	1.36 ± 0.15	1.06 ± 0.11	95.5
AHU377	5.0	8.88 ± 0.56	2.80 ± 0.13	7.10
	50	8.41 ± 0.54	2.77 ± 0.046	13.8

[a] [^{14}C]metformin was added to a final concentration of 12.1 µM

[b] % inhibition was calculated as follows:

$$(1-[\frac{(Accumulation)_{OCT,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OCT,0}-(Accumulation)_{Control,0}}])\times 100$$

Study Titles:	Assessment of AHU377 as an inhibitor of the multidrug and toxin extrusion (MATE) 1 and 2-K transporters
HEK cells stably expressing MATE1 (black bars) or MATE2-K (grey bars), or control cells (hatched bars) were incubated with [^{14}C]metformin (12.1 µM) in the presence or absence of TEA (500 µM; positive control inhibition) or AHU377 (5.0 µM or 50 µM). Each bar represents the average pmol [^{14}C]metformin accumulation per mg cellular protein per min ± standard deviation of three separate wells.	

Conclusion: AHU377 (5.0 µM and 50 µM) was shown to be a weak inhibitor of MATE1 or MATE2-K in vitro. As a result, AHU377 is unlikely to increase the systemic exposure to co-medications whose clearance is significantly mediated by MATE1 or MATE2-K transport activities in vivo.

2.6.5.16M　薬物動態試験：その他

Test article: LBQ657

Study Titles:	Assessment of LBQ657 as an inhibitor of the multidrug and toxin extrusion (MATE) 1 and 2-K transporters
Study nos.:	[R1300680]
GLP compliance:	not required
Study System:	Human embryonic kidney (HEK293) cells stably expressing the human ortholog of MATE1 or MATE2-K.
Analyte/Assay:	LBQ657 and [^{14}C] metformin / radioactivity

Effect of LBQ657 and MATE inhibitor tetraethylammonium (TEA) on [^{14}C]metformin accumulation in control or MATE1-expressing cells

Inhibitor	**Inhibitor concentration, µM**	**[^{14}C]metformin accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**		**% Inhibition [b]**
		MATE1 cells	**Control cells**	
None	-	55.0 ± 2.3	2.94 ± 0.17	0.0
TEA	500	3.28 ± 0.44	1.06 ± 0.11	95.7
LBQ657	25.0	52.8 ± 3.4	3.05 ± 0.10	4.44
	250	49.5 ± 3.9	2.83 ± 0.062	10.4

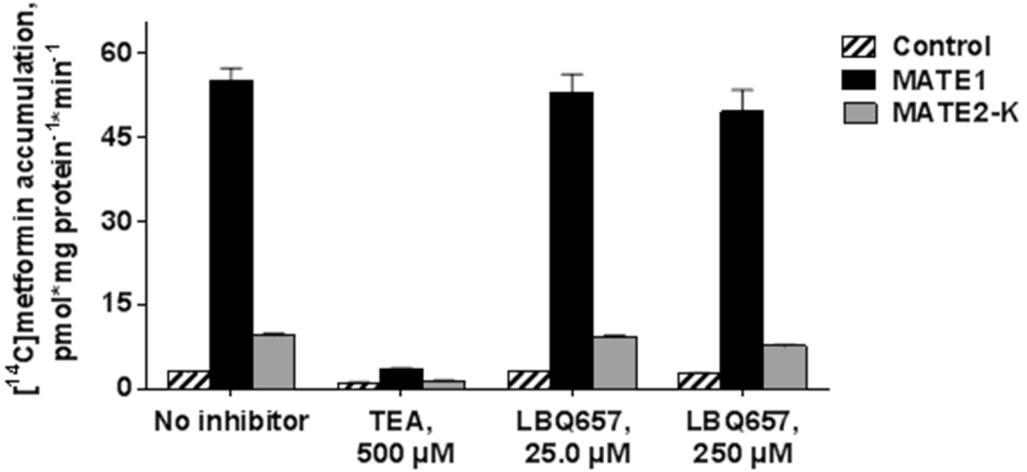
Effect of LBQ657 and MATE inhibitor TEA on [^{14}C]metformin accumulation in control or MATE2-K-expressing cells

Inhibitor	**Inhibitor concentration, µM**	**[^{14}C]metformin accumulation, [a] (pmol·mg protein^{-1}·min^{-1})**		**% Inhibition [b]**
		MATE2-K cells	**Control cells**	
None	-	9.48 ± 0.37	2.94 ± 0.17	0.0
TEA	500	1.36 ± 0.15	1.06 ± 0.11	95.5
LBQ657	25.0	9.17 ± 0.35	3.05 ± 0.10	6.40
	250	7.58 ± 0.32	2.83 ± 0.062	27.3

[a] [^{14}C]metformin was added to a final concentration of 12.1 µM

[b] % inhibition was calculated as follows:

$$(1-[\frac{(Accumulation)_{OCT,I}-(Accumulation)_{Control,I}}{(Accumulation)_{OCT,0}-(Accumulation)_{Control,0}}])\times 100$$

Study Titles:	Assessment of LBQ657 as an inhibitor of the multidrug and toxin extrusion (MATE) 1 and 2-K transporters
HEK cells stably expressing MATE1 (black bars) or MATE2-K (grey bars), or control cells (hatched bars) were incubated with [^{14}C]metformin (12.1 µM) in the presence or absence of TEA (500 µM; positive control inhibition) or LBQ657 (25.0 µM or 250 µM). Each bar represents the average pmol [^{14}C]metformin accumulation per mg cellular protein per min ± standard deviation of three separate wells.	

Conclusion: LBQ657 (25 µM and 250 µM) was shown to be a weak inhibitor of MATE1 or MATE2-K *in vitro*. As a result, LBQ657 is unlikely to increase the systemic exposure to co-medications whose clearance is significantly mediated by MATE1 or MATE2-K transport activities *in vivo*.

2.6.5.16N　薬物動態試験：その他

Test article: AHU377and LBQ657

Study Titles:	In vitro assessment of cytochrome P450 time-dependent inhibition by AHU377 and the metabolite, LBQ657, and reversible inhibition of CYP2B6
Study nos.:	[R1500389]
GLP compliance:	not required
Study System:	Pooled human liver microsomes (source: Corning)
Probe substrate/Assay::	Phenacetin, Bupropion, Amodiaquine, Diclofenac, S-mephenytoin, Bufuralol, Midazolam; The potential of AHU377 or LBQ657 to act as a time-dependent inhibitor of human CYP enzyme activity was investigated by measuring the CYP activity remaining after preincubation with AHU377 or LBQ657 for various time periods. Bupropion (whose metabolism is known to be selective for CYP2B6) concentrations used for these determinations were less than or equal to its reported Km value and bupropion metabolism was assessed by LC-MS/MS analysis of the hydroxybupropion formation.

Inhibitory effect of AHU377, LBQ657 on CYP enzyme-selective metabolic reactions

Time-dependent Inhibition

CYP enzyme	Probe reaction	Test compound	K_I (μM)	k_{inact}
			Total[a]	(1/min)
CYP1A2	phenacetin *O*-deethylation	Furafylline[b]	1.89 ± 0.759	0.0485 ± 0.00829
		AHU377	n.t.	n.t.
		LBQ657	n.t.	n.t.
CYP2B6	bupropion hydroxylation	Ticlopidine[b]	0.437 ± 0.287	0.0223 ± 0.00327
		AHU377	n.t.	n.t.
		LBQ657	n.t.	n.t.
CYP2C8	amodiaquine *N*-deethylation	Gemfibrozil glucuronide[b]	5.80 ± 1.87	0.0594 ± 0.00385
		AHU377	n.t.	n.t.
		LBQ657	n.t.	n.t.
CYP2C9	diclofenac 4′-hydroxylation	Tienilic acid[b]	0.626 ± 0.208	0.0410 ± 0.00421

Study Titles:	In vitro assessment of cytochrome P450 time-dependent inhibition by AHU377 and the metabolite, LBQ657, and reversible inhibition of CYP2B6			
		AHU377	n.t.	n.t.
		LBQ657	n.t.	n.t.
CYP2C19	*S*-mephenytoin 4′-hydroxylation	Ticlopidine[b]	4.12 ± 2.88	0.0434 ± 0.0132
		AHU377	n.t.	n.t.
		LBQ657	n.t.	n.t.
CYP2D6	bufuralol 1′-hydroxylation	Paroxetine[b]	0.0642 ± 0.0368	0.0365 ± 0.00332
		AHU377	n.t.	n.t.
		LBQ657	n.t.	n.t.
CYP3A4/5	midazolam 1′-hydroxylation	Troleandomycin[b]	1.80 ± 0.925	0.0651 ± 0.0139
		AHU377	n.t.	n.t.
		LBQ657	n.t.	n.t.

[a]total (bound + unbound) K_I value

[b]positive control

n.t.: no time-dependent inhibition observed

Inhibitory effect of AHU377 and LBQ657 on CYP2B6 metabolic reactions

Reversible Inhibition

CYP enzyme	Probe reaction	Total[a] IC_{50} value (μM)	
		AHU377	LBQ657
CYP2B6	bupropion hydroxylation	65	>100

a total (bound + unbound) IC50

Conclusion: AHU377 and LBQ657 are unlikely to inhibit the metabolic clearance of comedications metabolized by CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 in the time-dependent manner at concentrations of up to 100 μM. AHU377 may reversibly inhibit the metabolic clearance of CYP2B6 substrates if sufficient concentrations of AHU377 are achieved clinically.