ダーブロック錠1mg
ダーブロック錠2mg
ダーブロック錠4mg
ダーブロック錠6mg

製造販売承認申請書添付資料

第２部（モジュール２）CTDの概要（サマリー）

2.6. 非臨床試験の概要文及び概要表

2.6.4. 薬物動態試験の概要文
2.6.5. 薬物動態試験概要表

グラクソ・スミスクライン株式会社

非臨床概要 薬物動態試験の目次

2.6.4 及び 2.6.5 の略号等一覧

略語（略称）	内　容
AAG	α1-酸性糖蛋白質
ADME	吸収、分布、代謝、排泄
APCI/LC-MS	Atmospheric pressure chemical isolation liquid chromatography mass spectrometry
AUC	血漿中濃度－時間曲線下面積
Bcrp1	マウス breast cancer resistance protein
BCRP	ヒト breast cancer resistance protein
BDC	胆管カニュレーション処置
cDNA	相補的デオキシリボ核酸
CHO	チャイニーズハムスター卵巣細胞
CKD	慢性腎臓病
CL	クリアランス
CLi	固有クリアランス
CLr	腎クリアランス
Cmax	最高血漿中濃度
CYP	チトクローム P450
DRM	薬物関連物質
EG	エストラジオール 17β-D グルクロナイド
EPO	エリスロポエチン
F	バイオアベイラビリティ
FaSSIF	人工腸液
fu	血漿蛋白非結合率
GD	妊娠
GFR	糸球体ろ過率
GSK1278863	ダプロデュスタット
GSK2391220	ダプロデュスタットの代謝物 M2
GSK2391804	ダプロデュスタットの代謝物 M8
GSK2399022	ダプロデュスタットの代謝物 M9
GSK2487818	ダプロデュスタットの代謝物 M4（立体異性体）
GSK2499166	ダプロデュスタットの代謝物 M4（立体異性体）
GSK2506102	ダプロデュスタットの代謝物 M5（立体異性体）
GSK2506104	ダプロデュスタットの代謝物 M3（立体異性体）
GSK2531398	ダプロデュスタットの代謝物 M6（立体異性体）
GSK2531399	ダプロデュスタットの代謝物 M5（立体異性体）
GSK2531400	ダプロデュスタットの代謝物 M13（立体異性体）
GSK2531401	ダプロデュスタットの代謝物 M13（立体異性体）
GSK2531403	ダプロデュスタットの代謝物 M3（立体異性体）
GSK2531407	ダプロデュスタットの代謝物 M6（立体異性体）
GSK2531408	ダプロデュスタットの代謝物 M10
GSK2632557	ダプロデュスタットの代謝物 M22
HEK293 細胞	ヒト胎児由来腎臓 293 細胞
HPLC	高速液体クロマトグラフ
HSA	ヒト血清アルブミン
IC50	50%阻害濃度
LC-MS	液体クロマトグラフ／質量分析
LC-MS/MS	液体クロマトグラフ／タンデム質量分析

2.6.4 及び 2.6.5 の略号等一覧（続き）

略語（略称）	内　容
LD	授乳
LLQ	定量下限
M2	ダプロデュスタットの代謝物 GSK2391220
M3	ダプロデュスタットの代謝物（立体異性体） GSK2506104 及び／又は GSK2531403
M4	ダプロデュスタットの代謝物（立体異性体） GSK2487818 及び／又は GSK2499166
M5	ダプロデュスタットの代謝物（立体異性体） GSK2531399 及び／又は GSK2506102
M6	ダプロデュスタットの代謝物（立体異性体） GSK2531398 及び／又は GSK2531407
M8	ダプロデュスタットの代謝物
M9	ダプロデュスタットの代謝物
M10	ダプロデュスタットの代謝物
M13	ダプロデュスタットの代謝物（立体異性体） GSK2531401 及び／又は GSK2531400
M21	ダプロデュスタットの代謝物
M22	ダプロデュスタットの代謝物
M23	ダプロデュスタットの代謝物
M24	ダプロデュスタットの代謝物
MATE	Multidrug and toxin extrusion
MDCKII 細胞	Madin-Darby イヌ腎臓由来細胞 II
MDR1	Multidrug resistance protein 1
mRNA	メッセンジャーRNA（リボ核酸）
MS	質量分析
NADPH	ニコチンアミドアデニンジヌクレオチドリン酸
NMR	核磁気共鳴
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation
Papp	見かけの透過係数
P-gp	P-糖蛋白質
PK	薬物動態
PXR	プレグナン X 受容体
QWBA	定量的全身オートラジオグラフ
S2	昆虫由来培養細胞
t1/2	消失半減期
TK	トキシコキネティクス
tmax	最高血漿中濃度到達時間
UPLC	超高速高分離液体クロマトグラフ
Vss	定常状態における分布容積

2.6.4.　薬物動態試験の概要文

2.6.4.1.　まとめ

マウス、ラット、ウサギ、イヌ、サル及びヒト試料を用いてダプロデュスタット又は[14C]標識したダプロデュスタット（[14C]標識体）の吸収、分布、代謝及び排泄を検討した。臨床での投与経路である経口投与又は静脈内投与での薬物動態（PK）及びバイオアベイラビリティ（F）を評価した。一部の薬理試験及び安全性試験ではヒトでの代謝物を皮下投与した。また、開発中止となった他の適応症取得のために実施した経皮投与試験については、試験成績のみを 2.6.5 に示した。In vitro 試験では、ダプロデュスタット及び代謝物（一部の試験）の血漿蛋白結合率、血球移行性、膜透過性、トランスポーターを介した輸送及び阻害作用、CYP による代謝及び代謝酵素の誘導・阻害作用を検討した。動物は毒性試験と同様の系統を用い、毒性試験での曝露量（トキシコキネティクス、TK）を検討し、ヒトにおけるダプロデュスタットの安全性の検討が可能となるよう考慮した。ウサギ及びサルではヒトと類似した代謝物が生成されるため、これらの動物ではダプロデュスタットを投与したときの安全性を評価した。マウス及びラットではヒトにおける主な 3 種の代謝物［GSK2391220（M2）、GSK2506104（M3）及び GSK2531401（M13）］が十分量生成されないため、これらの代謝物を直接投与して安全性を評価した。これまでに得られたダプロデュスタットの PK 成績から、マウス、ラット、ウサギ、イヌ、ミニブタ及びサルを用いた評価は、ダプロデュスタットの臨床での安全性評価に有用であると考えられた。

健康成人に 25 mg の[14C]標識体を単回経口投与したとき、血漿中放射能の主な成分は未変化体であった。血漿中の主な代謝物は、M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）で、血漿中放射能の 7.6～8.3%であり、次いで M4（GSK2487818）、M5（GSK2506102、M14 と co-elute）、M6（GSK2531398）が 3.6～5.7%であった。その他に M15 及び M33 等もみられた。

慢性腎臓病（CKD）の腎性貧血患者にダプロデュスタットの 5 mg を反復経口投与したときの血漿中の主な 3 種の代謝物（M2、M3 及び M13：血漿中薬物関連物質の 10%超）及びその他の 3 つの代謝物（M4、M5 及び M6：10%未満）が認められた［2012N157420（PHI115573 試験）及び 2017N338206（200942 試験）］。これらの 6 種の代謝物がヒトで主に生成される代謝物であると考えられ、これらのうち M2 を除き、M3、M4、M5、M6 及び M13 はキラル中心を持ち、複数の立体異性体が存在すると考えられた。そのため、PHX111427 試験のヒト尿中の M3、M4、M5、M6 及び M13 をキラル分析した結果、M3、M4 及び M13 の立体異性体はそれぞれ M3（GSK2506104）、M4（GSK2487818）及び M13（GSK2531401）であることが示された。M5 及び M6 の立体異性体はそれぞれ 2 種あり、M5 は GSK2531399 及び GSK2506102、M6 は GSK2531398 及び GSK2531407 であった（表 2.6.4.1-1）。PPO116097 試験のヒト血漿をキラル分析した結果、M3 の立体異性体は GSK2506104 及び GSK2531403 で、GSK2531403 は GSK2506104 の 10%未満及び血漿中 M3 の 10%未満しか認められないことから、GSK2506104 が主な代謝物であると考えられた。また、M13 の立体異性体は GSK2531401 のみであった。更に、M3 の 2 つの立体異性体の比に

経時的な変化がみられなかったことから、ヒト生体内では立体異性体へ変換されないことが示された。

これらのことより、M3 及び M13 の主な立体異性体は GSK2506104 及び GSK2531401 であることが示された。

マウス及びラットではヒトでの主な代謝物が十分量生成されないため、これらの動物で実施した代謝物の毒性試験の多くでは、M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）を投与して評価した。

マウスにダプロデュスタット及び M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）を投与した 13 週間反復毒性試験で、血漿をキラル分析した結果、M3 及び M13 は投与した立体異性体のみが検出された。マウス血漿中に他の立体異性体（M3：GSK2531403、M13：GSK2531400）は検出されなかったことから、マウスでも立体異性体への変換は起こらないことが示された。

ヒトの主な代謝物（立体異性体を含む）を評価した臨床試験を表 2.6.4.1-1 に示す。また、各項の試験一覧には、該当する報告書の場所及び GLP の遵守状況を示した（表 2.6.4.2-2、表 2.6.4.3-1、表 2.6.4.4-1 及び表 2.6.4.5-2）。分析方法の詳細は別添 1 に、本項には含まなかった試験の一覧を別添 2 に示す。また、2.6.5 の table1 は本項では 2.6.5.1.というように記載した。

表 2.6.4.1-1　ヒトでの主な 6 種の代謝物（立体異性体を含む）を評価した試験一覧

Metabolite number	GSK compound number	Percent DRM (P+6M's) in human plasma of CKD patients at Steady State (Study 200942 and PHI115573) [Report 2017N338206 and 2012N157420][a]	Percent DRM (total radioactivity) in circulation in human plasma (Study 200232) [Report 2018N376203][a]	Relative Abundance of each enantiomer of metabolite M3 and M13 in circulation in human plasma (Study PPO116097) [Report 2014N221116][b]	Approximate relative abundance of each stereoisomeric form in human urine (Study PHX111427) [Report2012N137995][b]	Nonclinical species demonstrating metabolites in circulation via endogenous production[a]				
						Ms	Rt	Rb	D	Mo
Daprodustat	GSK1278863	16.0 to 39.4%	39.5 %	NA	NA	Y	Y	Y	Y	Y
M2	GSK2391220	14.8 to 19.3%	8.1 %	NA	100 % of M2 (Achiral)	N[c]	N[c]	Y	N	Y
M3	GSK2506104	18.4 to 25.1%	7.6 %	~94% of M3	100 % of M3	N[c]	N[c]	Y	N	Y
	GSK2531403			~6% of M3	0 % of M3 (not detectable)					
M4	GSK2487818	6.2 to 6.8%	5.7 %	NA	99 % of M4	N	N	Y	N	Y
	GSK2499166			NA	1 % of M4					
M5	GSK2506102	5.1 to 6.8%	<4.5 %	NA	79 % of M5	N	N	Y	N	Y
	GSK2531399			NA	20.5 % of M5					
M6	GSK2531398	5.6 to 7.1%	3.6 %	NA	89 % of M6	N	N	Y	N	Y
	GSK2531407			NA	10 % of M6					
M13	GSK2531400	12.9 to 20.7%	8.3 %	NQ	0 % of M13 (not detectable)	N[c]	N[c]	Y	N	Y
	GSK2531401			100% of M13	100 % of M13					

Key:
a = Methods used did not discriminate between chiral metabolites. b = Chiral methods where used for detection.
c = Mice and rats produced no or very limited metabolites M2, M3 and M13. Thus for several key studies, the relevant stereoisomeric forms for M2, M3 and M13 were administered SC with or without oral co-administration of daprodustat.
D = Dog; DRM = Drug-related material; M3, M4, M5, M6 and M13 = Stereoisomeric forms of chiral metabolites of daprodustat; M2 = Achiral metabolite of daprodustat;
Ms = Mouse; Mo = Monkey; N = Species did not sufficiently produce metabolite for metabolite qualification; NA = Not Appricable; NQ = Non-quantifiable; Rb = Rabbit; Rt =Rat; Y = Species produce sufficient metabolite for metabolite quantitation and qualification,
Note: The six predominant circulating human metabolites of daprodustat are M2, M3, M4, M5, M6 and M13.　Of these, M2, M3 and M13 are major (>10% DRM). The predominant stereoisomeric form of the human circulating metabolites that are chiral are separately shown.
Note: Systemic exposure of nonclinical species to the relevant stereoisomeric forms of the major metabolites, see Table 2.6.4.8.2.

本項では、マウス、ラット、ウサギ、イヌ及びサルで実施されたダプロデュスタットの吸収、分布、代謝及び排泄試験の要約及び考察を示す。In vitro 代謝試験はマウス、ラット、ウサギ（肝細胞のみ）、イヌ、サル、ハムスター（肝細胞のみ）、ミニブタ（肝細胞のみ）及びヒトの肝ミクロソーム及び肝細胞を用いて実施した。更に、ダプロデュスタットの代謝物について in vitro 及び in vivo 試験を実施した。

吸収

- ダプロデュスタット（溶液）を経口投与したときの F は、マウスで 88%及びラットで 100%と高く、イヌで 46%及びサルで 34%と中程度であった。また、イヌにダプロデュスタット（懸濁液）を経口投与したときの F は溶液よりも概ね低かった。
- マウス、ラット、イヌ及びサルに静脈内投与したときのダプロデュスタットのクリアランス（CL）は低く、分布容積（Vss）は総体液量よりも小さいか、同程度であった。
- マウス、ラット、イヌ及びサルに反復経口投与したときのダプロデュスタットの曝露量は、概して投与量増加の割合を下回って増加し、単回及び反復投与の曝露量に明らかな差はなく、また明らかな性差もみられなかった。

分布

- ラットに[14C]標識体を投与したとき放射能は速やかに広く組織に分布し、組織内放射能は血液中放射能よりも低く、脳内放射能は血液中放射能の約 2%であった。また、放射能は組織から速やかに消失した。
- In vitro において、動物（マウス、ラット、ウサギ、イヌ及びサル）及びヒトでのダプロデュスタットの血漿蛋白結合率は高く、それぞれ 93.6%以上及び 99%超であり、M2、M3、M6 及び M13 のヒト血漿蛋白結合率は 34%未満であった。
- 動物及びヒトでの血液／血漿比は、それぞれ 0.45～1.64 及び 0.75～1.23 であり、血球との結合は低いことが示された。

代謝

- M2、M3 及び M13 は、ウサギ（M13 除く）及びサルの血漿中で認められたが、マウス、ラット及びイヌの血漿中には認められなかった。
- ヒトに[14C]標識体を経口投与したとき、血漿中の主な成分は未変化体であった。また、ヒトにおいてダプロデュスタットは広範に代謝され、主にシクロヘキサン環が酸化された二酸化体及び三酸化体に代謝される。ヒト血漿中には、その他の酸化体がわずかに検出された。
- 慢性腎臓病（CKD）患者における臨床成績（200942 試験及び PHI115573 試験）から、定常状態での血漿中に主な 3 種の代謝物（M2、M3 及び M13）が認められ、それぞれ血中薬物関連物質（DRM）の 10%超であり、次いで 3 種の代謝物（M4、M5 及び M6）が 5.1～7.1%認められた。
- In vitro のヒト肝ミクロソームにおいて、ダプロデュスタットの反応性代謝物が生成される可能性は極めて低いことが示された。

排泄

- ラット、イヌ及びサルに[14C]標識体を経口投与したときの放射能は、主に糞中に排泄さ、尿中へは少量であった。BDC ラット、BDC イヌ及び BDC サルに[14C]標識体を経口投与したとき、放射能は、ラットでは多くが胆汁中に、サルでは主に胆汁中に排泄され、イヌでは一部が胆汁中に排泄された。ラット、イヌ及びサルの胆汁中には、それぞれ投与量の約 42、8 及び 53%が排泄され、胆汁及び尿中排泄率より、それぞれ投与量の少なくとも 49、8 及び 69%が吸収されたと考えられた。ラットにおける放射能の総回収率は 96%超、イヌで 86%超、サルで 93%超であった。

薬物動態学的薬物相互作用

- In vitro において、ダプロデュスタットの酸化的代謝には CYP2C8 が 95%寄与し、CYP3A4 の寄与は 5%とわずかであったことから、CYP2C8 阻害又は誘導剤との併用投与により薬物相互作用が生じる可能性がある。CYP2C8 阻害剤である gemfibrozil 又はトリメトプリムと併用投与した臨床薬物相互作用試験において、ダプロデュスタットの曝露量が増加することが示された。
- In vitro において、ダプロデュスタットは BCRP の基質であったことから、ダプロデュスタットを強力な BCRP 阻害剤と併用投与する場合、曝露量が上昇する可能性がある。
- In vitro において、ダプロデュスタットは CYP1A2、CYP2C9、CYP2C19 及び CYP2D6 の基質ではなかった。また、P-gp、OATP1B1、OATP1B3、OATP2B1、OCT1、OAT1、OAT3、OCT2、MATE1 及び MATE2-K の基質でもなかった。
- M3 及び M13 は OAT3 の基質であり、M2 は OAT1 及び OAT3 の弱い基質であったことから、OAT1 又は OAT3 阻害薬との併用は、M2、M3 又は M13 の曝露量を増加させる可能性がある。
- In vitro において、ダプロデュスタットは CYP2C8、OATP1B1 及び OATP1B3 を阻害したが、いずれの IC50 も臨床推定用量での血漿中ダプロデュスタット濃度よりも高かった。CYP2C8 の基質であるピオグリタゾン又は OATP1B1 及び OATP1B3 の基質であるロスバスタチンとの臨床薬物相互作用試験の結果から、ダプロデュスタットはピオグリタゾン又はロスバスタチンの曝露量に影響を及ぼさなかった。
- In vitro において、ダプロデュスタットは CYP1A2、CYP2A6、CYP2B6、CYP2C9、CYP2C19、CYP2D6 及び CYP3A4、P-gp、BCRP、OAT1、OAT3、OCT2、MATE1 及び MATE2-K を阻害しなかった。また、ダプロデュスタットは CYP1A2、CYP2B6 及び CYP3A4 を誘導しなかった。
- In vitro において、M2（GSK2391220）、M3（GSK2531403）、M4（GSK2487818）、M5（GSK2506102）、M6（GSK2531398）及び M13（GSK2531401）は、CYP1A2、CYP2C8、CYP2C9、CYP2C19、CYP2D6 及び CYP3A4 を阻害せず、M3（GSK2506104）は CYP2B6 を阻害しなかった。また、M2（GSK2391220）、M3（GSK2506104）、M4（GSK2487818）、M5（GSK2506102）、M6（GSK2531398）及び M13（GSK2531401）は、OAT1、OAT3、OCT2、MATE1 及び MATE2-K を阻害せず、M3（GSK2531403）は OATP1B1 を阻害しなかった。

2.6.4.1.1.　　被験物質

試験には、特記しない限り親化合物を用い、投与量及び濃度は親化合物で表した。試験には非標識体及び複素環の炭素を安定的に標識した[14C]ダプロデュスタット（[14C]標識体）を用いた（図 2.6.4.1-1）。また、ヒトでの主な代謝物の構造を図 2.6.4.1-2 に示す。

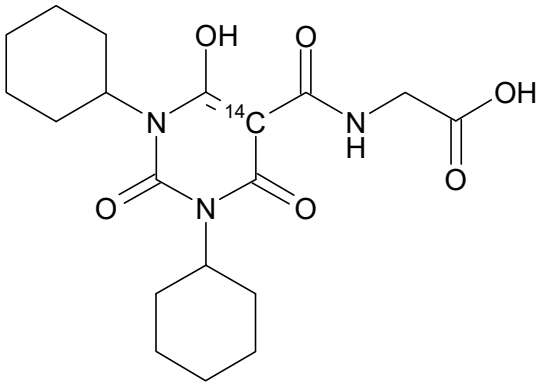
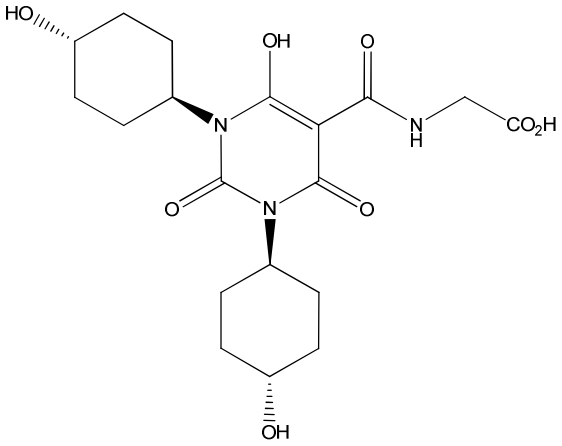
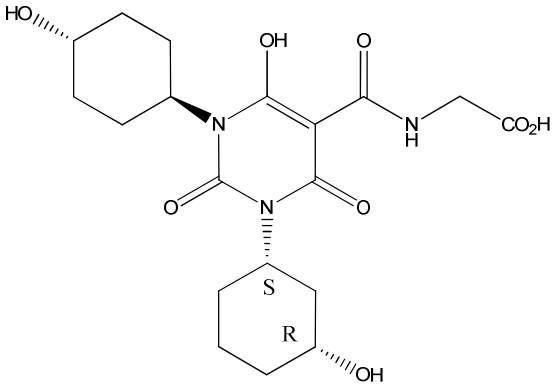


図 2.6.4.1-1　　[14C]標識体の標識位置

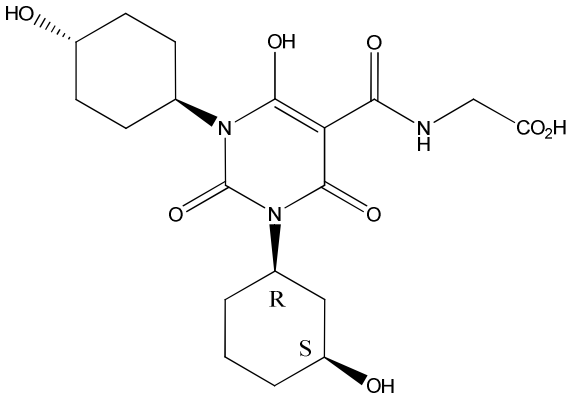


M2 (GSK2391220)

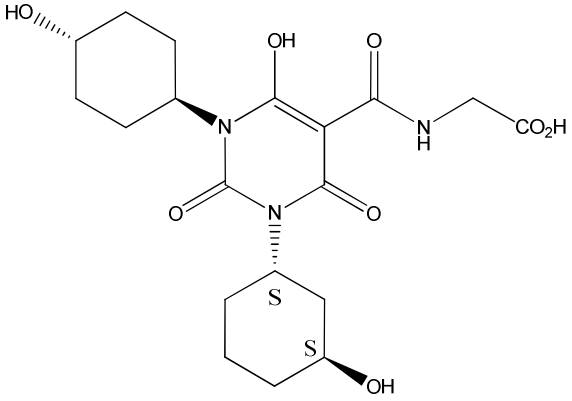
図 2.6.4.1-2　　ヒトでの主な代謝物の構造



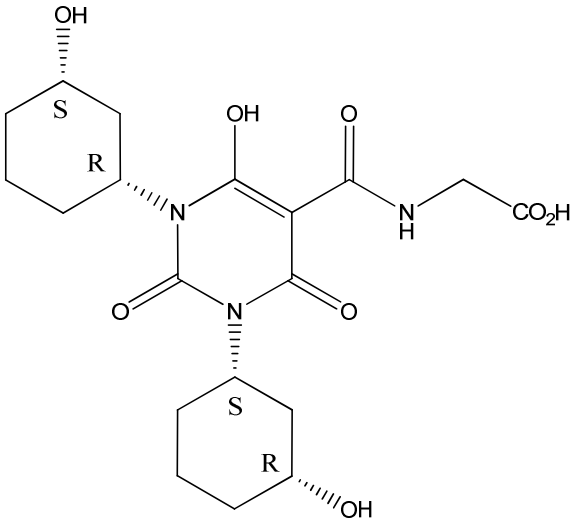
M3 (GSK2531403 stereoisomer)



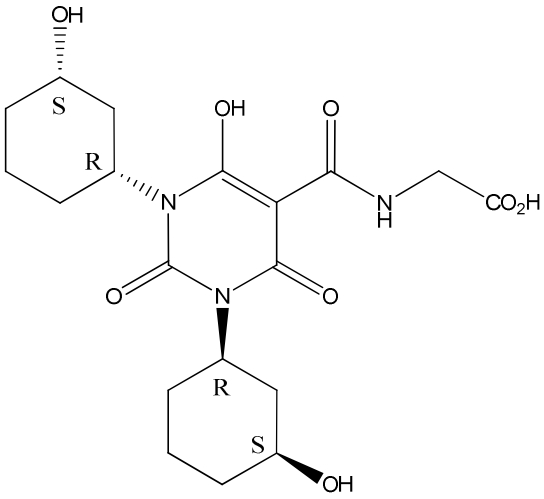
M3 (GSK2506104 stereoisomer)



M4 (GSK2487818 stereoisomer)

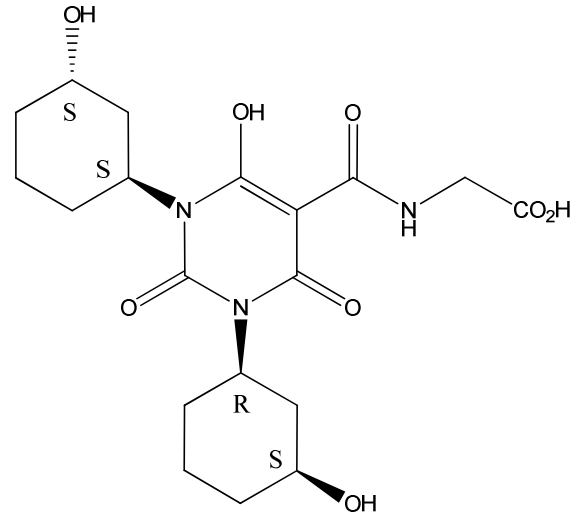


M5 (GSK2531399 stereoisomer)

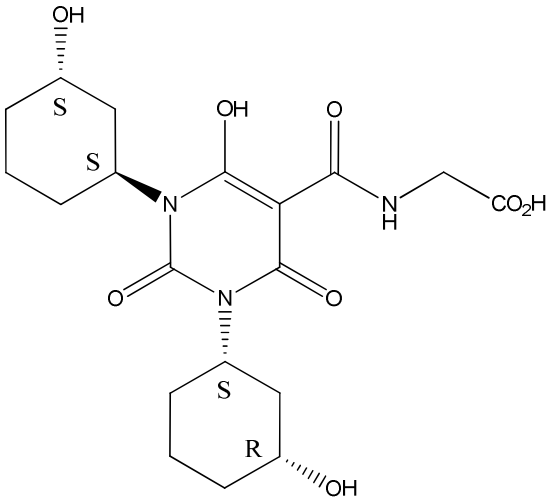


M5 (GSK2506102 stereoisomer)

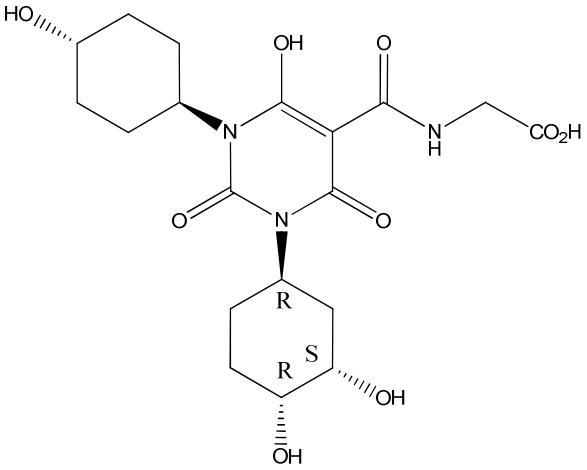
図 2.6.4.1-2　　ヒトでの主な代謝物の構造（続き）



M6 (GSK2531398 stereoisomer)



M6 (GSK2531407 stereoisomer)



M13 (GSK2531401 stereoisomer)

図 2.6.4.1-2　　ヒトでの主な代謝物の構造（続き）

2.6.4.1.2.　　分析法

マウス、ラット、ウサギ、イヌ及びサルの血漿中未変化体は、蛋白質沈殿法（必要に応じて固相抽出法）で試料を前処理した後、LC-MS/MS を用いて定量した。生体試料中の未変化体の定量には、既知濃度のダプロデュスタットを添加した試料より得られた検量線（回帰直線）を用いた。各分析法の妥当性は、真度、精度、直線性、範囲及び定量限界の評価により確認した。更に、QC 試料を同時に分析し、分析法の経時的な安定性を保証した。

ダプロデュスタットの安全性試験では、マウス、ラット、ウサギ、イヌ及びサルの血漿中（20、25 又は 50 μL）の未変化体濃度の定量に、十分な真度及び精度を有するバリデートされた分析法を用いた。測定法は 50～50000 ng/mL の濃度範囲でバリデートされた。

同様の真度及び精度で、ヒト血漿及び尿中（50 μL）の未変化体を 1～1000 ng/mL の濃度範囲で、更にヒト血漿中（100 μL）の未変化体を 0.100～100 ng/mL の濃度範囲で測定可能な分析法を開発した。

また、マウス及びサル血漿中（50 μL）の M2、M3、M4、M5、M6 及び M13（ヒトでの主な代謝物）の定量のため、1～1000 ng/mL の濃度範囲でバリデートされた LC-MS/MS 測定法を開発した。これらの方法はアキラルな測定法であるため、マウス及びサル血漿中に存在する代謝物の立体異性体については検討できない。また、ヒト血漿中（50 μL）での主な 6 種の代謝物を定量するため、1～1000 ng/mL の濃度範囲でバリデートされた LC-MS/MS 測定法も開発した。

ヒトでの 6 種の主な代謝物のうち、M2（GSK2391220）を除き、M3、M4、M5、M6 及び M13 はキラル中心を持つため、複数の立体異性体が存在すると考えられた。そのため、PHX111427 試験（2012N137995）のヒト尿試料及び PPO116097 試験（2012N221116）のヒト血漿試料で、立体異性体の純物質を用いてキラル分析を行い、M3、M4、M5、M6 及び M13 の主な立体異性体を特定した。その結果、主な立体異性体は、M3 では GSK2506104、M4 では GSK2487818、M5 では GSK2506102、M6 では GSK2531398 及び M13 では GSK2531401 であることが示された。これらの主な立体異性体は、その後の生体試料分析法（アキラル HPLC）の開発及びバリデーションで使用されたが、キラル分析は立体異性体の純物質が使用可能になった後に導入されたため、マウス、サル及びヒトのアキラル分析の開発では標準物質として使用した立体異性体とは一致していない。しかしながら、アキラルの生体試料分析法の開発及びバリデートにおいては、十分なブリッジング試験が実施され、1 つの代謝物の標準物質に対して複数の立体異性体の標準物質を用いた場合に同等の真度、感度及び特異性が得られている。測定法の手法及びバリデーションの概要を Appendix 1 に示す。

[14C]標識体投与後の生体試料中の放射能の測定は、液体シンチレーションカウンター又は定量的全身オートラジオグラフィーで行った。代謝物のプロファイリングと同定には、放射能及び UV 検出器付き HPLC、放射能検出器付き LC-MS、APCI/LC-MS、LC-MS/MS 及び NMR を用いた。

PK 及びトキシコキネティクス（TK）はノンコンパートメント法で解析した。

2.6.4.2.　吸収

マウス、ラット、ウサギ、イヌ及びサルにダプロデュスタットを単回及び反復投与したときの血漿中の PK 及び TK を検討した。投与は主に臨床投与経路である経口投与、及び静脈内投与で行った。また、一部の試験では、ダプロデュスタット又は代謝物を単独投与したときの代謝物濃度を測定した。更に、げっ歯類においてヒト血漿中の主な代謝物（M2、M3 及び M13）が十分量生成されないことから、一部の安全性試験では、ダプロデュスタットとこれらの代謝物を併用投与したときの代謝物濃度についても測定した。なお、測定した代謝物のうち、GSK コード番号を持つ代謝物は次の通りであった。すなわち、M2（GSK2391220）、M3（GSK2506104 及び GSK2531403）、M4（GSK2487818 及び GSK2499166）、M5（GSK2506102 及び GSK2531399）、M6（GSK2531398 及び GSK2531407）、M8（GSK2391804）、M9（GSK2399022）、M10（GSK2531408）、M13（GSK2531400 及び GSK2531401）、及び M22（GSK2632557）であった。

2.6.4.2.1.　単回投与

2.6.4.2.1.1.　マウス

####### 2.6.4.2.1.1.1.　経口投与

######## 2.6.4.2.1.1.1.1.　ダプロデュスタット

絶食下で雄マウスにダプロデュスタットの 1.9 及び 3.9 mg/kg（溶液）で単回経口投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した静脈内投与したときの成績は 2.6.4.2.1.1.2.に記載した。

試験成績を 2.6.5.3.1.に示す。1.9 mg/kg を投与したときの経口バイオアベイラビリティ（F）は、88%と高かった。

######## 2.6.4.2.1.1.1.2.　代謝物

単回投与

非絶食下で雄マウスに M13（GSK2531401）の 50 mg/kg（懸濁液）を単回経口投与したときの PK を検討した（2012N137345）。

試験成績を 2.6.5.3.2.に示す。M13 の血漿中濃度は投与後 6 時間まで定量可能であった。M13 の Cmax は 0.171 μg/mL、AUC0-t は 0.564 μg·h/mL、tmax は投与後 0.5 時間であった。

単回投与

非絶食下で雄マウスに M13（GSK2531401）の 150 mg/kg（懸濁液）を単回経口投与したときの PK を検討した（2011N130731）。

試験成績を 2.6.5.3.2.に示す。M13 の血漿中濃度は投与後 24 時間のすべての採血ポイントまで定量可能であった。M13 の Cmax は 0.476 μg/mL、AUC0-t は 2.13 μg·h/mL、tmax は投与後 0.5 時間であった。

2.6.4.2.1.1.2.　静脈内投与

2.6.4.2.1.1.2.1.　ダプロデュスタット

単回投与

雄マウスにダプロデュスタットの 1.3 mg/kg を単回静脈内投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した経口投与したときの成績は 2.6.4.2.1.1.1.に記載した。

試験成績を表 2.6.4.2-1 及び 2.6.5.3.1.に示す。全身クリアランス（CL）は 0.7 mL/min/kg（42 mL/h/kg）と低かった。定常状態の分布容積（Vss）は 0.3 L/kg であり、総体液量[Davies, 1993]よりも小さかった。

表 2.6.4.2-1　各動物に単回静脈内投与したときの薬物動態パラメータ

Species	Dose (mg/kg)	CL (mL/min/kg)	Vss (L/kg)	MRT (h)	$t_{1/2}$ (h)
Mouse – Non-fasted	1.3	0.7	0.3	7.8	-
Rat - Non-fasted	0.6	0.2	0.4	36.7	33.5
Dog - Fasted	1.2	0.9	0.5	8.8	6.6
Monkey - Fasted	1.1	8.4	0.8	1.6	1.9

Data source: UH2008/00002

2.6.4.2.1.1.2.2.　代謝物

単回投与

雄マウスに M13（GSK2531401）の 40 mg/kg を単回静脈内投与したときの PK を検討した（2012N132320）。なお、本試験においては、所定の採血時間に肝臓及び腎臓内 M13 濃度も測定し、試験成績は 2.6.4.3.2.1.に記載した。

試験成績を 2.6.5.3.2.に示す。マウスでの M13 の CL は速やかで、血漿中 M13 濃度は概して投与後 8 時間までしか定量できなかったため、PK パラメータを算出できなかった。

上記の試験で M13 の PK データが得られなかったことから、追加試験を実施した。

単回投与

雄マウスに M13（GSK2531401）の 40 及び 100 mg/kg、又はダプロデュスタットの 20 mg/kg を単回静脈内投与したときの PK を検討した（2012N156583）。なお、所定の採血時間に腎組織内の M13 及びダプロデュスタット濃度も測定し、成績を 2.6.4.3.2.1.に記載した。

試験成績を 2.6.5.3.1.及び 2.6.5.3.2.に示す。40 及び 100 mg/kg の用量において、血漿中 M13 濃度はそれぞれ投与後 24 及び 8 時間まで定量可能であった。一方、ダプロデュスタットの血漿中濃度は投与後 24 時間のすべての採血ポイントまで定量可能であった。

M13 の曝露量（Cmax 及び AUC0-t）は、投与量増加に伴って増加した。CL は 1.090～1.210 L/h/kg と低かったが、ダプロデュスタットと比較すると約 40 倍高かった。M13 の Vss は約 0.4 L/kg であり、ダプロデュスタットと同程度で、総体液量[Davies, 1993]よりも小さかった。

2.6.4.2.1.1.3.　皮下投与

代謝物

雄マウスにヒト血漿中の主な代謝物である M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）を含むカクテルをそれぞれ 2.0、2.5 及び 1.3 mg/kg（溶液）の投与量で単回皮下投与したときの各代謝物の PK を検討した（2013N174038）。なお、M2、M3 及び M13 の実投与量は目標の投与量の 60%より低かったものの、試験の検討には影響はないと考えられた。

試験成績を 2.6.5.3.3.に示す。M2、M3 及び M13 の曝露量（Cmax 及び AUC0-2）はほぼ類似していることが示された。

2.6.4.2.1.2.　ラット

2.6.4.2.1.2.1.　経口投与

2.6.4.2.1.2.1.1.　ダプロデュスタット

単回投与

雌ラットにダプロデュスタットの 0.02、0.1、0.8 及び 7.0 mg/kg（溶液）を単回経口投与したときの曝露量を検討した（2013N184197）。

試験成績を 2.6.5.3.1.に示す。曝露量（Cmax 及び AUC0-t）は、投与量増加の割合に伴って増加した。

単回投与

絶食下の雄ラットにダプロデュスタットの 1.6 mg/kg（溶液）又は 31.1 mg/kg（懸濁液）を単回経口投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した静脈内投与したときの成績は 2.6.4.2.1.2.2.に記載した。

試験成績を 2.6.5.3.1.に示す。1.6 mg/kg を投与したときの F は、100%と高かった。

2.6.4.2.1.2.1.2.　代謝物

M8 は、ヒト肝ミクロームを用いた in vitro 代謝試験で、ダプロデュスタットの酸化的代謝物として同定され、CYP2C8 阻害剤によりもっとも生成が阻害された代謝物である（2.6.4.4.1.1.9.）。M13 は、ヒトでの 6 種の主な代謝物のうちの 1 つである。

単回投与

雄ラットに代謝物である M8（GSK2391804）の 100 mg/kg を単回経口投与したときの M8（GSK2391804）、M2（GSK2391220）、M3（GSK2531403）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）及び M13（GSK2531401）の PK を検討した（2012N153861）。

試験成績を 2.6.5.3.4.に示した。M8（GSK2391804）の Cmax、AUC0-t 及び tmax は、それぞれ 0.434 µg/mL、2.33 µg·h/mL 及び投与後 0.5 時間であり、F は 2%未満であった。M2 は少なくとも M8 の投与後 3 時間まで定量可能であったが、M3、M4、M5、M6 及び M13 は定量できなかった。また、M2 の M8 の曝露量に対する比は 0.008 未満であった。

単回投与

雄ラットに M13（GSK2531401）の 150 mg/kg を単回経口投与したときの M13（GSK2531401）の PK を検討した（2012N145868）。

試験成績を 2.6.5.3.2.に示した。Cmax、AUC0-t 及び tmax は、それぞれ 0.35 µg/mL、1.72 µg/h/mL 及び投与後 0.5 時間であり、F は 0.85%であった。

2.6.4.2.1.2.2.　静脈内投与

2.6.4.2.1.2.2.1.　ダプロデュスタット

単回投与

雄ラットにダプロデュスタットの 0.6 mg/kg を 1 時間持続静脈内投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した経口投与したときの成績は 2.6.4.2.1.2.1.に記載した。

試験成績を表 2.6.4.2-1 及び 2.6.5.3.1.に示す。CL は 0.2 mg/min/kg（12 mL/h/kg）と低く、Vss は 0.4 L/kg であり、総体液量よりも小さかった[Davies, 1993]。また、$t_{1/2}$ は 33.5 時間であった。

単回投与

雄ラットにダプロデュスタットの 17.5 mg/kg（溶液）を 22 分間持続静脈内投与、又は 11.1 mg/kg（溶液）を 2 時間持続静脈内投与したときの PK を検討した（CD2008/01285）。

試験成績を 2.6.5.3.1.に示す。22 分間持続投与したときの Cmax 及び AUC0-t はそれぞれ 39.2 µg/mL 及び 371 µg.h/mL で、2 時間持続投与したときの Cmax 及び AUC0-t は、それぞれ 54.6 µg/mL 及び 423 µg·h/mL であった。

2.6.4.2.1.2.2.2.　代謝物

単回投与

雄ラットに M8（GSK2391804）の 40 mg/kg を単回静脈内投与したときの M8（GSK2391804）、M2（GSK2391220）、M3（GSK2531403）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）及び M13（GSK2531401）の PK を検討した（2012N153861）。

試験成績を 2.6.5.3.4.に示す。M8 の CL は 12.2 mL/min/kg と低く、Vdss は 0.485 L/kg であり、総体液量よりも小さく[Davies, 1993]、$t_{1/2}$ は 5.37 時間であった。M8 の Cmax 及び AUC0-inf はそれぞれ 134 µg/mL 及び 52.9 µg.h/mL であった。

M2 は M8 の投与後 3 時間まで定量可能であった。M3 及び M4 は概して投与後 1 時間まで定量可能であったが、M5、M6 及び M13 は定量できなかった。また、M2、M3 又は M4 の M8 の曝露量に対する比は 0.008 未満であった。

単回投与

雄ラットに M13（GSK2531401）の 40 mg/kg を単回静脈内投与したときの M13（GSK2531401）の PK を検討した（2012N145868）。なお、実質投与量は 19.2 mg/kg であった。

試験成績を 2.6.5.3.2.に示した。CL は 14.8 mL/min/kg と低く、Vdss は 0.83 L/kg であり、総体液量よりも大きく[Davies, 1993]、$t_{1/2}$ は 0.93 時間で、MRT は 0.96 時間であった。

2.6.4.2.1.3.　イヌ

2.6.4.2.1.3.1.　経口投与

単回投与

雄イヌにダプロデュスタットを絶食下で 3.2 mg/kg（親化合物、溶液）又は非絶食下で 5 mg/kg（親化合物、カリウム塩又はトリス一水和物／カプセル剤)、並びに非絶食下で 1.0、3.1 又は 30.6 mg/kg（親化合物、懸濁液）の用量で単回経口投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した静脈内投与したときの成績は 2.6.4.2.1.3.2.に記載した。

試験成績を 2.6.5.3.1.に示す。絶食下でダプロデュスタットの 3.2 mg/kg を溶液として経口投与したときの F は 46%であったが、非絶食下で 1.0～30.6 mg/kg を懸濁液として経口投与したときの F は、16～40%であった。また、ダプロデュスタット（親化合物、カリウム塩及びトリス一水和物／カプセル剤）の 5 mg/kg を経口投与したときの曝露量（Cmax 及び AUC0-t）は同程度であった。

単回投与

雌雄イヌにダプロデュスタットの 120 mg/kg を単回経口投与したときの TK を検討した（CD2006/01837）。なお、本試験は雌雄それぞれ 1 匹ずつの成績である。

試験成績を 2.6.5.3.5.に示す。ダプロデュスタットの血漿中濃度は投与後 24 時間のすべての採血ポイントまで定量可能であった。雄の曝露量（Cmax 及び AUC0-24）は 11.8 μg/mL 及び 99.0 μg·h/mL であり、雌では 25.2 μg/mL 及び 313 μg·h/mL で、雄に比べて雌の方がそれぞれ 2.1 倍及び 3.2 倍高かったが、雄では投与後約 0.47～4 時間の間に 3 回嘔吐がみられたことから、嘔吐が雄の曝露量に影響したと考えられる。

2.6.4.2.1.3.2.　静脈内投与

雄イヌにダプロデュスタットの 1.2 mg/kg（溶液）を 1 時間持続静脈内投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した経口投与したときの成績は 2.6.4.2.1.3.1.に記載した。

試験成績を表 2.6.4.2-1 及び 2.6.5.3.1.に示す。CL は 0.9 mL/min/kg（54 mL/h/kg）と低く、Vss は 0.5 L/kg であり、総体液量[Davies, 1993]よりもやや小さかった。また、$t_{1/2}$ は 6.6 時間であった。

2.6.4.2.1.4.　ミニブタ

2.6.4.2.1.4.1.　経皮投与

本申請は経口剤のため、ミニブタの皮膚にダプロデュスタットを単回塗布したときの試験成績のみを 2.6.5.3.6.に示す（2013N180775）。

2.6.4.2.1.5.　サル

2.6.4.2.1.5.1.　経口投与

単回投与

絶食下の雄サルにダプロデュスタットの 3.0 mg/kg（溶液）を単回経口投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した静脈内投与したときの成績は 2.6.4.2.1.5.2.に記載した。

試験成績を表 2.6.4.2-1 及び 2.6.5.3.1.に示す。F は約 34%と中程度であった。

単回投与

雌雄サルにダプロデュスタットの 10～600 mg/kg（懸濁液）を単回経口投与したときの TK を検討した（CD2009/00639）。なお、300 mg/kg 以上の投与量で嘔吐がみられ、600 mg/kg では忍容性が認められなかった。全般的に、300 mg/kg から 600 mg/kg への増量に伴って AUC0-t が減少したが、このことは恐らく嘔吐によるものと考えられる。

試験成績を 2.6.5.3.7.に示す。曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。また、概して Cmax 及び AUC0-t は投与量増加の割合を下回って増加したが、その一因として、消化管内における被験物質の溶解性が律速となり吸収が制限された可能性又は嘔吐（300 mg/kg 以上）が考えられた。

2.6.4.2.1.5.2.　静脈内投与

雄サルにダプロデュスタットの 1.1 mg/kg（溶液）を 1 時間持続静脈内投与したときの PK を検討した（UH2008/00002）。なお、本試験で同時に実施した経口投与したときの成績は 2.6.4.2.1.5.1.に記載した。

試験成績を表 2.6.4.2-1 及び 2.6.5.3.1.に示す。CL は 8.4 mL/min/kg（504 mL/h/kg）と低く、Vss は 0.8 L/kg であり、総体液量[Davies, 1993]と同程度であった。また、$t_{1/2}$ は 1.9 時間であった。

2.6.4.2.1.6.　ヒト

2.6.4.2.1.6.1.　経皮投与

本申請は経口剤のため、ヒト皮膚にダプロデュスタットを塗布したときの成績は、試験成績のみを 2.6.5.に示した。

経皮吸収

ヒト皮膚にダプロデュスタットを塗布したとき吸収についての成績（2012N152036）を 2.6.5.5.1.に示す。

皮膚透過性

ヒト皮膚にダプロデュスタットを塗布したときの皮膚組織（表皮及び真皮）への透過性の成績を 2.6.5.5.2.に示す（2012N152038）。

ダプロデュスタットの異なる粒子径を用いた皮膚透過性

ヒト皮膚にダプロデュスタット（マイクロナイズ製剤又は非マイクロナイズ製剤）を塗布したときの皮膚透過性の成績を 2.6.5.5.3.に示す（2015N230888）。

2.6.4.2.2.　反復投与

2.6.4.2.2.1.　マウス

2.6.4.2.2.1.1.　経口投与

2.6.4.2.2.1.1.1.　ダプロデュスタット

14 日間反復投与

雌雄マウスにダプロデュスタットの 30 mg/kg/日（懸濁液）を 14 日間反復経口投与したときの投与 14 日目の TK を検討した（CD2009/00900）。

試験成績を 2.6.5.4.2.に示す。ダプロデュスタットの血漿中濃度は投与 14 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。雌雄の tmax は、それぞれ約 4 及び 1 時間であった。また、雌雄の曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。

13 週間反復投与

雌雄マウスにダプロデュスタットの 3、30 又は 60 mg/kg/日（懸濁液）を 13 週間反復経口投与したときの投与 1 日目及び 4 週目に TK を検討した（2011N112096）。

試験成績を 2.6.5.4.3.に示す。ダプロデュスタットの血漿中濃度は概して投与後 24 時間のすべての採血ポイントまで定量可能であった。雌雄の tmax は投与後 0.5～8 時間であった。各投与量での曝露量（Cmax 及び AUC0-t）に明らかな性差はなく、投与 1 日目及び 4 週目の間でも明らかな差はみられなかった。また、曝露量は投与量増加の割合を下回って増加したが、その一因として、消化管内における被験物質の溶解性が律速となり吸収が制限された可能性が考えられた。

2.6.4.2.2.1.1.2.　代謝物

13 週間反復投与

雌雄マウスにダプロデュスタットの 30 mg/kg/日（懸濁液）を 13 週間反復経口投与したときの投与 7 日目のダプロデュスタット及び代謝物［M2（GSK2391220）、M3（GSK2531403）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）及び M13（GSK2531400）］の TK を検討した（2011N117145）。

試験成績を 2.6.5.4.1.に示す。ダプロデュスタットの血漿中濃度は投与後 24 時間のすべての採血ポイントまで定量可能であった。ダプロデュスタットの tmax は雄で投与後 1 時間、雌で投与後 4 時間であった。曝露量（Cmax 及び AUC0-t）に、明らかな性差はみられなかった。また、6 種の代謝物のうち、M2 が 1 例のマウスにおいて投与後 1 時間に定量されたのみであった。

2.6.4.2.2.1.1.3.　　代謝物との併用投与

13 週間反復投与

雌雄マウスにダプロデュスタット及びヒト血漿中の主な代謝物を併用で 13 週間反復投与したときの投与 1 日目及び 4 週目の TK を検討した（2014N199031）。ダプロデュスタットは 6 又は 20 mg/kg/日（懸濁液）を経口で、M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）はカクテル（それぞれ 2、2.5 及び 1.3 mg/kg/日）で皮下投与した。

試験成績を 2.6.5.4.4.に示す。ダプロデュスタットの血漿中濃度は、投与 1 日目及び 4 週目の投与後 24 時間のすべての採血ポイントまで定量可能であった。一方、代謝物の血漿中濃度は投与 1 日目及び 4 週目において少なくとも 8 時間まで定量可能であった。ダプロデュスタット及び代謝物の tmax はそれぞれ投与後 1～8 及び 0.083～0.25 時間であった。投与 1 日目の M13 を除き、概して、ダプロデュスタット及び代謝物の曝露量（Cmax 及び AUC0-t）に、明らかな性差はなく、投与 1 日目及び 4 週目の間でも明らかな差はみられなかった。ダプロデュスタットの曝露量は、投与量増加に伴って増加した。

ダプロデュスタットと代謝物を併用投与したとき、代謝物の投与量はダプロデュスタットの投与量（6 又は 20 mg/kg/日）に関わらず同じであり、代謝物の曝露量も同程度であったことが確認された。

2 年間反復投与

雌雄マウスにダプロデュスタット及び代謝物を併用で 2 年間反復投与したときの投与 4 及び 26 週目の TK を検討した（2014N221714）。ダプロデュスタットの 0.2、0.8 又は 3 mg/kg/日を経口で、M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）のカクテル（それぞれ 2、2.5 及び 1.3 mg/kg/日）で皮下投与した。

試験成績を 2.6.5.4.5.に示す。ダプロデュスタットの血漿中濃度は、投与 4 週目及び 26 週目の投与後 24 時間のすべての採血ポイントまで定量可能であった。一方、代謝物の血漿中濃度は、M13 を除き、投与 4 週目及び 26 週目において 8 時間まで定量可能であった。

ダプロデュスタットの tmax は投与後 0.25～8 時間であり、雄よりも雌の曝露量（AUC0-t）が 2.1～2.4 倍となった投与 26 週目を除き、概してダプロデュスタットの曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。また、投与 4 及び 26 週目の曝露量は概して投与量増加の割合に伴って増加した。更に、投与 4 及び 26 週目のダプロデュスタットの曝露量（Cmax 及び AUC0-t）に明らかな差はみられなかった。

代謝物の tmax は投与 4 及び 26 週目で投与後 0.083～0.25 時間であり、代謝物の曝露量（Cmax 及び AUC0-t）に性差はなかった。また、投与 4 及び 26 週間目の代謝物の曝露量（Cmax 及び AUC0-t）に明らかな差はみられなかった。

ダプロデュスタットと代謝物を併用したとき、代謝物の投与量はダプロデュスタットの投与量（0.2、0.8 又は 3 mg/kg/日）に関わらず同じであり、代謝物の曝露量も同程度であったことが確認された。

2.6.4.2.2.1.2.　皮下投与

2.6.4.2.2.1.2.1.　代謝物

14 日間反復投与（4 週間反復投与による薬理試験）

雌マウスに M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）のカクテル（それぞれ 2.0、2.5 及び 1.3 mg/kg/日）を 14 日間反復皮下投与したときの TK を検討した（2014N208198）。

試験成績を 2.6.5.4.6.に示す。代謝物の血漿中濃度は、投与 14 日目において投与後 8 時間まで定量可能であった。M2、M3 及び M13 の tmax はそれぞれ投与後 0.25、0.08 及び 0.25 時間であった。M2、M3 及び M13 の Cmax はそれぞれ 2.67、3.03 及び 2.04 μg/mL であり、AUC0-t はそれぞれ 1.61、1.68 及び 1.26 μg·h/mL であった。

2.6.4.2.2.2.　ラット

2.6.4.2.2.2.1.　経口投与

2.6.4.2.2.2.1.1.　ダプロデュスタット

5 日間反復投与

雄ラットにダプロデュスタットの 30 mg/kg/日（懸濁液）を 5 日間反復経口投与したときの投与 5 日目のダプロデュスタット及び代謝物［M2（GSK2391220）M3（GSK2531403）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）、M8（GSK2391804）、M10（GSK2531408）、M13（GSK2531401）及び M9（GSK2399022）／M22（GSK2632557）］の PK を検討した（2012N153858）。

試験成績を 2.6.5.4.8.に示す。ダプロデュスタット及び M2、M8、M9/M22 及び M10 の血漿中濃度は、投与 5 日目において投与後 24 時間のすべての採血ポイントまで定量可能であった。一方、M3、M4、M5、M6 及び M13 は、投与 5 日目のいずれの時点においても定量されなかった。投与 5 日目において、定量された代謝物の曝露量（Cmax 及び AUC）のダプロデュスタットに対する比は低かった（0.0003 未満）。

14 日間反復投与

雄ラットにダプロデュスタットの 10、30 又は 60 mg/kg/日（懸濁液）を 14 日間反復経口投与したときの投与 1 及び 14 日目おける TK を検討した（RD2006/01192）。

試験成績を 2.6.5.4.10.に示す。ダプロデュスタットの血漿中濃度は、投与 1 及び 14 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。tmax は概して投与後 1～4 時間であった。曝露量（Cmax 及び AUC0-24）は投与量増加の割合を下回って増加した。また、いずれの用量においても、投与 1 及び 14 日目の曝露量に明らかな差はみられなかった。

14 日間反復投与

雌ラットにダプロデュスタットの 100、250 又は 500 mg/kg/日（懸濁液）を 14 日間反復経口投与したときの投与 1 及び 14 日目の TK を検討した（CD2008/01306）。なお、250 mg/kg/日以上の用量では忍容性が認められず、500 mg/kg/日群では全例を投与 14 日目より前に死亡又は安楽死させたため、500 mg/kg/日投与群については投与 14 日目の TK パラメータが得られなかった。

試験成績を 2.6.5.4.11.に示す。ダプロデュスタットの血漿中濃度は、投与 1 及び 14 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。投与 1 日目において、tmax は投与後 2～24 時間であり、投与 14 日目では tmax は投与後 1～1.5 時間であった。いずれの投与量の曝露量も、同程度であった。また、いずれの投与量でも投与 1 と 14 日目の曝露量（Cmax 及び AUC0-t）に明らかな差はなかった。

4 週間反復投与

雌雄ラットにダプロデュスタットの 2、7 又は 20 mg/kg/日（懸濁液）を 4 週間反復経口投与したときの投与 1 日目及び 4 週目の TK を検討した（RD2007/01333）。

試験成績を 2.6.5.4.13.に示す。ダプロデュスタットの血漿中濃度は、投与 1 日目及び 4 週目の投与後 24 時間のすべての採血ポイントまで定量可能であった。tmax は概して投与後 1～2 時間であった。いずれの用量においても、投与 1 日目及び 4 週目の曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。曝露量は投与量増加の割合を下回って増加した。

5 週間反復投与（代謝物との併用）

出生前及び出生後の発生並びに母体の機能に関する試験において、妊娠ラットにダプロデュスタット及び代謝物を併用して 5 週間反復投与したときの妊娠 9 日目及び授乳 21 日目のダプロデュスタット及び代謝物の TK を検討した（2018N365151）。ダプロデュスタットの 0.8、7 及び 40 mg/kg/日を経口で、妊娠 6 日目から授乳 21 日目まで M2（GSK2391220）M3（GSK2506104）及び M13（GSK2531401）を 2.5、3.2 及び 1.8 mg/kg/日（1 日 2 回）で皮下投与した。

試験成績を 2.6.5.4.12.に示す。妊娠 9 日目では、ダプロデュスタット及び M2 の血漿中濃度は投与後 24 時間のすべての採血ポイントまで、M3 及び M13 は投与後 12 時間まで定量可能であった。授乳 21 日目では、ダプロデュスタット、M2、M3 及び M13 は投与後 24 時間まで定量可能であった。妊娠 9 日目及び授乳 21 日目でダプロデュスタットの曝露量（Cmax 及び AUC0-t）は、投与量増加の割合を下回って増加した。ダプロデュスタット、M2、M3 及び M13 の曝露量（Cmax 及び AUC0-t）は妊娠 9 日目及び授乳 21 日目で同程度であった。ダプロデュスタットと代謝物を併用投与したとき、代謝物の投与量はダプロデュスタットの投与量（0.8、7 及び 40 mg/kg/日）に関わらず同じであり、代謝物の曝露量も同程度であったことが確認された。

13 週間反復投与

雌雄ラットにダプロデュスタットの 0.8、4、20 又は 100 mg/kg/日（懸濁液）を 13 週間反復経口投与したときの投与 1 日目、4 及び 13 週目の TK を検討した（CD2009/00951）。なお、100 mg/kg/日群では死亡や状態悪化により忍容性が認められず、100 mg/kg/日投与群については投与 13 週目の TK パラメータが得られなかった。

試験成績を 2.6.5.4.14.に示す。ダプロデュスタットの血漿中濃度は、投与 1 日目、4 及び 13 週目のすべての採血ポイントまで定量可能であった。投与 1 日目、4 及び 13 週目の tmax はそれぞれ投与後 1～8、0.5～8 及び 0.5～2 時間であった。曝露量（Cmax 及び AUC0-t）は、4 mg/kg/日までは概して投与量増加に伴って増加したが、それ以降の投与量では投与量増加の割合を下回って増加した。また、0.8 及び 4 mg/kg/日群の投与 4 及び 13 週目（20 mg/kg/日群では雌の 13 週目の Cmax のみ）での曝露量（Cmax 及び AUC0-t）は投与 1 日目と比較して概して 1.5～2 倍高かったが、20 mg/kg/日及び 100 mg/kg/日群では明らかな差はみられなかった。ラットではダプロデュスタットの消失半減期が長く（33.5～37.2 時間、2.6.5.3.1.）、1 日 1 回の反復投与によって蓄積性がみられる可能性が考えられたが、投与 4 又は 13 週目の Cmax 及び AUC0-t が投与 1 日目の 2 倍を超えたのは計 28 の評価群及び時点のうち 2 点のみで、その程度は 2 倍をわずかに超えた程度（2.02 及び 2.13）であった。試験全体を評価した結果、1 日 1 回反復経口投与後のダプロデュスタットの曝露量に顕著な蓄積性は認められないと判断した。更に、明らかな性差もみられなかった。

26 週間反復投与

雌雄ラットにダプロデュスタットの 0.8、4 又は 10 mg/kg/日（懸濁液）を 26 週間反復経口投与したときの投与 4、13 及び 26 週目の TK を検討した（2011N126130）。なお、10 mg/kg/日を投与した雄（全例）は投与 26 週目以前に死亡又は安楽死させたため、投与 26 週目の雄の TK パラメータが得られなかった。

試験成績を 2.6.5.4.15.に示す。tmax は概して投与後 0.5～2 時間であった。投与 4、13 及び 26 週目の曝露量（Cmax 及び AUC0-t）に、明らかな差はなく、明らかな性差もみられなかった。曝露量は概して投与量増加に伴って増加した。

2 年間反復投与

雄ラットにダプロデュスタットの 0.02、0.1、0.8 又は 4 mg/kg/日、雌には 0.02、0.1、0.8 又は 7 mg/kg/日を 2 年間反復経口投与したときの投与 4 及び 26 週目の TK を検討した（2014N196080）。

試験成績を 2.6.5.4.16.に示す。ダプロデュスタットの血漿中濃度は、投与後 24 時間のすべての採血ポイントまで定量可能であった。投与 4 週目及び 26 週目の tmax は投与後 0.5～2 時間であった。いずれの投与量でも投与 4 及び 26 週目の曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。また、曝露量（Cmax 及び AUC0-t）は、投与量増加に伴って増加した。いずれの投与量においても、投与 4 及び 26 週目の曝露量に明らかな差はみられなかった。

2.6.4.2.2.2.1.2.　代謝物

5 日間反復投与

妊娠ラットにダプロデュスタット及び代謝物を併用で 5 日間反復投与したときの妊娠 10 日目のダプロデュスタット及び代謝物の TK を検討した（2016N305715）。

第 1 フェーズでは、妊娠ラットにダプロデュスタットの 60 mg/kg を経口で、M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）を 2.5、3.2 及び 1.8 mg/kg/日又は 7.5、9.6 及び 5.4 mg/kg/日をカクテルとして皮下に妊娠 6～10 日目まで投与した。

第 2 フェーズでは、妊娠ラットにダプロデュスタットの 10 及び 40 mg/kg を経口で、M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）を 2.5、3.2 及び 1.8 mg/kg/日をカクテルとして皮下に妊娠 6～10 日目まで投与した。また、40 mg/kg 単独投与も行った。

試験成績を 2.6.5.4.9.に示す。投与 1 日目においてダプロデュスタット、M2、M3 及び M13 の血漿中濃度は、投与後 24 時間のすべての採血ポイントまで定量可能であった。ダプロデュスタットの tmax は投与後 2 時間であり、代謝物では投与後 0.25 時間であった。代謝物の曝露量（Cmax 及び AUC0-t）は投与量増加に伴って増加した。

2.6.4.2.2.2.2.　静脈内投与

14 日間反復投与

雄ラットにダプロデュスタットの 20 又は 40 mg/kg/日を 14 日間反復静脈内投与したときの投与 1 及び 14 日目の TK を検討した（CD2008/01740）。

試験成績を 2.6.5.4.7.に示す。ダプロデュスタットの血漿中濃度は、投与 1 及び 14 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。tmax は、概して投与後 2 及び 4 時間であった。曝露量（Cmax 及び AUC0-t）は投与量増加の割合を下回って増加した。また、投与 1 及び 14 日目の Cmax 及び AUC0-t に明らかな差はみられなかった。

2.6.4.2.2.3.　ウサギ

2.6.4.2.2.3.1.　経口投与

5 日間反復投与

妊娠 7～11 日目のウサギにダプロデュスタットの 60 mg/kg/日（懸濁液）を 5 日間反復経口投与したときのダプロデュスタット及び代謝物［M2（GSK2391220）、M3（GSK2531403）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）及び M13（GSK2531400）］の投与 5 日目の TK を検討した（2012N140611）。

試験成績を 2.6.5.4.17.に示す。ダプロデュスタット、M2、M3、M4、M5 及び M13 の血漿中濃度は、投与 5 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。一方、M6 は投与後 8 時間まで定量可能であった。概して、ダプロデュスタット及び代謝物の tmax は投与後 2～4 時間であった。ダプロデュスタットの曝露量（Cmax 及び AUC0-t）は、いずれの代謝物よりも 16 倍以上高かった。

13 日間反復投与

妊娠 7～19 日目のウサギにダプロデュスタットの 4、30、60、125 又は 250 mg/kg/日（懸濁液）を 13 日間反復経口投与したときの投与 5 及び 13 日目（Ctrough のみ）の TK を検討した（CD2008/01041）。なお、250 mg/kg/日群の動物では、状態悪化のため、投与 13 日目の TK パラメータが得られなかった。

試験成績を 2.6.5.4.18.に示す。ダプロデュスタットの血漿中濃度は、投与 5 及び 13 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。tmax は投与後 4～8 時間でみられた。また、いずれの用量においても、投与 5 日目と 13 日目の投与 24 時間後における血漿中濃度に明らかな差はみられなかった。

13 日間反復投与

妊娠 7～11 日目のウサギにダプロデュスタットの 4、30 又は 60 mg/kg/日（懸濁液）を 5 日間反復経口投与したときの投与 5 日目の TK を検討した（CD2009/00390）。

試験成績を 2.6.5.4.19.に示す。投与 5 日目の tmax は投与後 4～8 時間であった。曝露量（Cmax 及び AUC0-t）は投与量増加の割合を下回って増加した。

2.6.4.2.2.3.2.　　経皮投与

本申請は経口剤のため、ウサギの皮膚にダプロデュスタットを反復塗布したときの試験成績のみを 2.6.5.4.20.に示す（2011N125481）。

2.6.4.2.2.4.　　イヌ

2.6.4.2.2.4.1.　　経口投与

3 日間反復投与

雌雄イヌにダプロデュスタットの 90 mg/kg をカプセル剤（絶食下）又は懸濁液（非絶食下）として 3 日間反復経口投与したときの投与 1 日目の曝露量を比較した（RD2007/01367）。

試験成績を 2.6.5.4.21.に示す。ダプロデュスタットの血漿中濃度は、投与後 24 時間のすべての採血ポイントまで定量可能であった。tmax は投与後 4～8 時間であった。曝露量（Cmax 及び AUC0-t）には、それぞれの剤形で明らかな性差はなかった。カプセル剤（絶食下で投与）と懸濁液（非絶食下で投与）との間で、曝露量に明らかな差はみられなかった。

7 日間反復投与

雌雄イヌにダプロデュスタットの 20、60 又は 120 mg/kg/日（懸濁液）を 7 日間反復経口投与したときの投与 1 及び 7 日目の TK を検討した（CD2007/00353）。なお、120 mg/kg 群の投与 7 日目の投与後 0.5 時間までに嘔吐があったため、120 mg/kg 群で投与 1 及び 7 日目の曝露量（Cmax 及び AUC0-t）を比較することは適切でないと考えられた。

試験成績を 2.6.5.4.22.に示す。ダプロデュスタットの血漿中濃度は、投与 1 及び 7 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。曝露量（Cmax 及び AUC0-

t）に明らかな性差はみられなかった。投与 1 日目の曝露量（Cmax 及び AUC0-t）は投与量増加の割合を下回って増加した。

4 週間反復投与

雌雄イヌにダプロデュスタットの 3、30 又は 90 mg/kg/日（カプセル剤）を 4 週間反復経口投与したときの投与 1 日目及び 4 週目の TK を検討した（RD2007/01336）。なお、90 mg/kg/日投与群の動物では、状態悪化のため、投与 4 週目の TK パラメータが得られなかった。

試験成績を 2.6.5.4.23.に示す。ダプロデュスタットの血漿中濃度は投与後 24 時間のすべての採血ポイントまで定量可能であった。tmax は概して投与後 1～4 時間であった。投与 1 日目及び 4 週目（90 mg/kg 群は除く）の曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。曝露量は投与 1 日目及び 4 週目のいずれにおいても、概して投与量増加の割合を下回って増加した。いずれの用量でも投与 1 日目と 4 週目の曝露量（Cmax 及び AUC0-t）に明らかな差はみられなかった。

13 週間反復投与

雌雄イヌにダプロデュスタットの 1、3 又は 30 mg/kg/日（カプセル剤）を 13 週間反復経口投与したときの投与 1 日目、17 日目、7 週目及び 13 週目の TK を検討した（CD2009/00952）。なお、雌の 30 mg/kg/日投与群では投与 17 日目以降 15 mg/kg/日に減量した。

試験成績を 2.6.5.4.24.に示す。ダプロデュスタットの血漿中濃度は投与後 24 時間のすべての採血ポイントまで定量可能であった。tmax は概して投与後 1～24 時間であった。曝露量（Cmax 及び AUC0-t）は、概して投与量増加の割合を下回って増加した。3 mg/kg 群の投与 7 週を除いて、概して投与 7 及び 13 週目の曝露量に明らかな差はみられなかった。また、曝露量に概して性差はみられなかった。

2.6.4.2.2.5.　ミニブタ

2.6.4.2.2.5.1.　経皮投与

本申請は経口剤のため、ミニブタの皮膚にダプロデュスタットを反復塗布したときの試験成績のみを 2.6.5.に示した。

14 日間反復投与

ミニブタの皮膚にダプロデュスタットを 14 日間反復塗布したときの PK 成績（2012N148386）を 2.6.5.4.25.に示す。

14 日間反復投与

皮膚刺激性試験において、ミニブタの皮膚にダプロデュスタットを 14 日間反復塗布したときの PK 成績（2013N185591）を 2.6.5.4.26.に示す。

28 日間反復投与

ミニブタの皮膚にダプロデュスタットを 28 日間反復塗布したときの PK 成績（2015N231048）を 2.6.5.4.27.に示す。

13 週間反復投与

ミニブタの皮膚にダプロデュスタットを 13 週間反復塗布したときの PK 成績（2016N273683）を 2.6.5.4.28.に示す。

2.6.4.2.2.6.　サル

2.6.4.2.2.6.1.　経口投与

14 日間

雌雄サルにダプロデュスタットの 10、30 又は 100 mg/kg/日（懸濁液）を 14 日間反復経口投与したときの投与 1 及び 14 日目の TK を検討した（CD2009/00780）。

試験成績を 2.6.5.4.29.に示す。ダプロデュスタットの血漿中濃度は、10 mg/kg/日群では投与 1 日目の投与後 8 時間及び投与 14 日目の投与後 24 時間、30 又は 100 mg/kg/日群では投与 1 及び 14 日目の投与後 24 時間のすべての採血ポイントまで定量可能であった。100 mg/kg/日群での投与 1 日目において雄の曝露量（Cmax 及び AUC0-t）が雌に比べてそれぞれ 2.4 及び 2.1 倍高かったのを除き、明らかな性差はみられなかった。AUC0-t は概して投与量増加の割合に伴って増加したが、Cmax は概して投与量増加の割合を下回って増加した。また、10 mg/kg/日群の雌を除き、概して投与 1 及び 14 日目の曝露量（Cmax 及び AUC0-t）に明らかな差はみられなかった。

13 週間反復投与

雌雄サルにダプロデュスタットの 5、20 又は 100 mg/kg/日（懸濁液）を 13 週間反復経口投与したときの投与 1 日目、4 週目及び 13 週目のダプロデュスタットの TK を検討した（2010N108482）。

試験成績を 2.6.5.4.30.に示す。ダプロデュスタットの血漿中濃度は、5 及び 20 mg/kg/日群では概して投与 1 日目、4 週目及び 13 週目の投与後 8 時間まで、100 mg/kg/日群では投与 1 日目、4 週目及び 13 週目の投与後 24 時間のすべての採血ポイントまで定量可能であった。ダプロデュスタットの tmax は、5 及び 20 mg/kg/日群では投与後 0.5～3 時間、100 mg/kg/日群では投与後 2～6 時間であった。いずれの用量群及び評価時点においても、曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。また、概して曝露量は投与量増加の割合を下回って増加した。いずれの用量群においても投与 1 日目、4 週目及び 13 週目の曝露量明らかな差はみられなかった。

13 週間反復投与

雌雄サルにダプロデュスタットの 5、20 又は 100 mg/kg/日（懸濁液）を 13 週間反復経口投与したときの投与 1 日目、4 週目及び 13 週目のダプロデュスタット及び代謝物［M2

（GSK2391220）、M3（GSK2531403）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）及び M13（GSK2531400）］の TK を検討した（2011N117143）。

試験成績を 2.6.5.4.31.に示す。6 種の代謝物の血漿中濃度は、投与後 24 時間のすべて採血ポイントまで定量可能であった。各代謝物の tmax は概して投与後 1～8 時間であった。各代謝物の曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。概して、投与 1 日目、4 週目及び 13 週目での 6 種の代謝物の曝露量（Cmax 及び AUC0-t）は投与量増加の割合を下回って増加した。また、いずれの投与量群においても、投与 1 日目、4 週目及び 13 週目の代謝物の曝露量に明らかな差はみられなかった。

39 週間反復投与

雌雄サルにダプロデュスタットの 3、10 又は 50 mg/kg/日（懸濁液）を 39 週間反復経口投与したときのダプロデュスタット及び代謝物［M2（GSK2391220）、M3（GSK2531403）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）及び M13（GSK2531400）］の投与 1 日目、4、26 及び 39 週目の TK を検討した（2011N126131）。

試験成績を 2.6.5.4.32.に示す。ダプロデュスタットの tmax は概して投与後 0.5～8 時間であった。いずれの投与量においても、6 種の代謝物が定量され、代謝物の tmax は投与後 1～8 時間であった。いずれの投与量群、及び投与 1 日目、4 週目、26 週目及び 39 週目の評価時点においても、概してダプロデュスタットの曝露量（Cmax 及び AUC0-t）に明らかな性差はみられなかった。ダプロデュスタットの AUC0-t は概ね投与量増加の割合に伴って増加したが、Cmax は投与量増加の割合を下回って増加した。また、いずれの投与量群においても、投与 1 日目、4 週目、26 週目及び 39 週目のダプロデュスタットの曝露量に明らかな差はみられなかった。投与 39 週目において、ダプロデュスタットに対する各代謝物の AUC0-t 比率（代謝物：ダプロデュスタット比）はいずれも 0.09 未満であった。

表 2.6.4.2-2　　吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Percutaneous absorption	Human skin	3M	Ex vivo	A	5 mg/cm^2 (0.2% cream, gel, ointment)	Single	No	[redacted]	2012N152036 (R10-1824)	m4.2.2.2
Percutaneous absorption	Human skin	3M	Ex vivo	A	10 mg/cm^2 (0.2% cream)	Single	Yes	[redacted]	2012N152038 (GK1202-01)	m4.2.2.2
Percutaneous absorption	Human skin	3F	Ex vivo	A	1, 5, 15, 30% (w/w)	Up to 24hrs	No	[redacted]	2015N230888	m4.2.2.2
Pharmacokinetics	Mouse (CD-1)	27M[a,b] 24M[a,c]	IV (bolus) Oral (gavage)	A	1.3 1.9, 3.9	Single	No	GSK	UH2008/00002	m4.2.2.2
Pharmacokinetics	Rat (SD)	3M[b] 3M[c] 3M[c]	IV (1 hour infusion) Oral (gavage) Oral (gavage)	A	0.6 1.6 31.1	Single	No	GSK	UH2008/00002	m4.2.2.2
Pharmacokinetics	Rat (SD)	3M	IV (22 min. infusion)[d] (2 hour infusion)[d]	A	17.5 11.1	Single	No	GSK	CD2008/01285 (08DMM039)	m4.2.2.2
Toxicokinetics	Rat (SD)	3F	Oral (gavage)	A	0.02, 0.1, 0.8, 7	Single	No	GSK	2013N184197 (R70458N)	m4.2.2.2
Pharmacokinetics	Dog (beagle)	3M[c] 3M[c] 3M[b]	IV (1 hour infusion) Oral (gavage) Oral (gavage)	A	1.2 3.2 1, 3.1, 30.6	Single	No	GSK	UH2008/00002	m4.2.2.2
Pharmacokinetics	Dog (beagle)	3M[c]	Oral (capsule)	A B C	5 5[e] 5[f]	Single	No	GSK	UH2008/00002	m4.2.2.2

表 2.6.4.2-2　　吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Dog (beagle)	1M/1F	Oral (gavage)	A	120	Single	No	GSK	CD2006/01837 (D06386)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	2M	Topical	A	10%, 35%	Single	No	GSK	2013N180775 (F43142N)	m4.2.2.2
Pharmacokinetics	Monkey (cynomolgus)	3M[c] 3M[c]	IV (1 hour infusion) Oral (gavage)	A	1.1 3.0	Single	No	GSK	UH2008/00002	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	18M/18F	Oral (gavage)	A	30	14 days (Day 14)	No	GSK	CD2009/00900 (D09169)	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	42M/42F	Oral (gavage)	A	3, 30, 60	13 weeks (Day 1, Week 4)	Yes	[redacted]	2011N112096 (G10121)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites	Mouseg (CD-1)	21M/21F	Oral (gavage)	A	30	13 weeks (Day 7)	Yes	GSK	2011N117145 (10DMM034)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites (co-administered)	Mouse (CD-1)	48M/48F	Oral (gavage) SC (Injection)	A	6, 20 (daprodustat) 2.0 (M2), 2.5 (M3), 1.3 (M13)	13 weeks (Day 1, Week 4)	Yes	[redacted]	2014N199031 (M70456G)	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	51M/51F	Oral (gavage)	A	0.2, 0.8, 3[t]	104 weeks (Weeks 4 and 26)	Yes	[redacted]	2014N221714 (M70585G)	m4.2.2.2
Toxicokinetics	Rat (SD)	6M	IV (4 hour infusion)	A	20, 40	14 days (Days 1 & 14)	No	GSK	CD2008/01740 (I08185)	m4.2.2.2
Pharmacokinetics	Rat (SD)	3M	Oral (gavage)	A	30	5 days (Days 5)	No	GSK	2012N153858 (12DMM037)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites	Rat (SD)	6F (pregnant)	Oraln (gavage)	A	10[o], 40[p], 40[q], 60[r], 60[s]	5 days (GD 6 to GD10)	No	[redacted]	2016N305715 (R71028N)	m4.2.2.2

表 2.6.4.2-2 吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rat (SD)	3M	Oral (gavage)	A	10, 30, 60	14 days (Days 1 & 14)	No	GSK	RD2006/01192 (R41921)	m4.2.2.2
Toxicokinetics	Rat (SD)	3F	Oral (gavage)	A	100, 250, 500	14 days (Days 1 & 14)	No	[redacted]	CD2008/01306 (D08114)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites w	Rat (SD)	6F (pregnant/ lactating)	Oral (gavage) SC	A	0.8, 7, 40 2.5 (M2) 3.2 (M3) 1.8 (M13)	38 days (GD6, GD9 and LD21)	Yes	[redacted]	2018N365151 (R71242G)	m4.2.2.2
Toxicokinetics	Rat (SD)	3M/3F	Oral (gavage)	A	2, 7, 20	4 weeks (Days 1 & 28)	Yes	GSK	RD2007/01333 (R42197)	m4.2.2.2
Toxicokinetics	Rat (SD)	3M/3F	Oral (gavage)	A	0.8, 4, 20, 100	13 weeks (Day 1, Weeks 4 & 13)	Yes	[redacted]	CD2009/00951 (G09057)	m4.2.2.2
Toxicokinetics	Rat (SD)	9M/9F	Oral (gavage)	A	0.8, 4, 10	26 weeks (Weeks 4, 13 & 26)	Yes	GSK	2011N126130 (G10276)	m4.2.2.2
Toxicokinetics	Rat (SD)	3-9M 3-9F	Oral (gavage)	A	0.02, 0.1, 0.8, or 4 0.02, 0.1, 0.8, or 7	Up to 99 weeks (Weeks 4 and 26) 99 weeks (Weeks 4 and 26)	Yes	[redacted]	2014N196080 (R70375G)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites	Rabbits (DB) (pregnant)	4F	Oral (gavage)	A	60	5 days (Day 5 of dosing/ Day 11 GD)	Yes	GSK	2012N140611 (G12109)	m4.2.2.2
Toxicokinetics	Rabbits (DB) (pregnant)	3 or 4F	Oral (gavage)	A	4, 30, 60, 125, 250	13 days (Day 5/Day 11 GD) & Day 13/Day 19 GD)	No	GSK	CD2008/01041 (D08163)	m4.2.2.2

表 2.6.4.2-2　　吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rabbits (DB) (pregnant)	9F	Oral (gavage)	A	4, 30, 60	13 days (Gestational Day 5 of dosing/ Day 11)	Yes	[redacted]	CD2009/00390 (G09061)	m4.2.2.2
Toxicokinetics	Rabbit (NZW)	3 or 6M	Topical	A	2%	14 days (Days 1 & 14)	Yes	GSK	2011N125481 (L42845)	m4.2.2.2
Toxicokinetics	Dog (beagle)	2M/2F	Oral (gavage & capsule)	A	90[i]	3 days (Day 1)	No	GSK	RD2007/01367 (D42209)	m4.2.2.2
Toxicokinetics	Dog (beagle)	1M/1F	Oral (gavage)	A	20, 60, 120	7 days (Days 1 & 7)	No	GSK	CD2007/00353 (D07078)	m4.2.2.2
Toxicokinetics	Dog (beagle)	3M/3F	Oral (gavage)	A	3, 30, 90	4 weeks (Days 1 & 28)	Yes	GSK	RD2007/01336 (D42196)	m4.2.2.2
Toxicokinetics	Dog (beagle)	4 or 6M/ 4 or 6F	Oral (gavage)	A	1, 3, 30/15[h]	13 weeks (Day 1, Weeks 7 & 13)	Yes	[redacted]	CD2009/00952 (G69058)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	1M/1F	Topical	A	0.5%, 1%, 2% w/w applied daily to 10% BSA	14 days (Days 1 & 14)	No	[redacted]	2012N148386 (D12086)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	1M/1F	Topical	A	10, 20, 35% w/w applied daily to 10% BSA	14 Days (Days 1 & 14) Days 2, 3, 4, and 7: one sample 21 hours post-dose	No	[redacted]	2013N185591 (F70438N)	m4.2.2.2

表 2.6.4.2-2　吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Minipig (Göttingen)	3M	Topical	A	2, 10, 35% w/w applied daily to 10% BSA	28 days (Days 1 & 28)	No	[redacted]	2015N231048 (F70727N)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	4M/4F	Topical	A	5, 10, 20% (w/w) (42, 84, 168 mg/kg/day)	13 weeks (Weeks 4 and 13)	Yes	[redacted]	2016N273683 (F70916G)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	1M/1F	Oral (gavage)	A	10, 30, 100	14 days (Days 1 & 14)	No	GSK	CD2009/00780 (D09121)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	4-6M/ 4-6F	Oral (gavage)	A	5, 20, 100	13 weeks (Day 1, Weeks 1 & 14)	Yes	[redacted]	2010N108482 (G10005)	m4.2.2.2
Toxicokinetics of circulating metabolites	Monkeyj (cynomolgus)	4-6M/ 4-6F	Oral (gavage)	A	5, 20, 100	13 weeks (Day 1, Weeks 1 & 14)	Yes	GSK	2011N117143 (10DMM033)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites	Monkey (cynomolgus)	4M/4F	Oral (gavage)	A	3, 10, 50	39 weeks (Day 1, Weeks 4, 26 & 39)	Yes	[redacted]	2011N126131 (G10277)	m4.2.2.2

表 2.6.4.2-2　吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Studies with Metabolite GSK2531401 (M13)										
Pharmacokinetics of daprodustat and GSK2531401 (M13)	Mouse (CD-1)	Daprodustat 15M GSK2531401 18M/16M[u] 15M[u]	IV (bolus)	A E	20[m] 40/100 (M13)	Single	No	GSK	2012N156583 (12DMM041)	m4.2.2.2
Pharmacokinetics	Mouse (CD-1)	18M[b,k,v]	Oral (gavage)	E	150	Single	No	GSK	2011N130731 (11DMM060)	m4.2.2.2
Pharmacokinetics	Mouse (CD-1)	18M[b,l,v]	Oral (gavage)	E	50	Single	No	GSK	2012N137345 (12DMM004)	m4.2.2.2
Pharmacokinetics and EPO response	Mouse (CD-1)	15M[v]	IV (bolus)	E	40	Single	No	GSK	2012N132320 (11DMM049)	m4.2.2.2
Pharmacokinetics	Rat (SD)	4M[b] 4M[b]	IV (bolus) Oral (gavage)	E	19.2 218	Single	No	GSK	2012N145868 (12DMM019)	m4.2.2.2
Studies with Metabolite GSK2391804 (M8)										
Pharmacokinetics	Rat (SD)	4M[b]	IV (bolus) Oral (gavage)	M	40 100	Single	No	GSK	2012N153861 (12DMM038)	m4.2.2.2

表 2.6.4.2-2 吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Studies with Metabolites GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13)										
Pharmacokinetics	Mouse (CD-1)	24M	SC (Injection)	D, E, N	1.2 (M2), 1.5 (M3) 0.77 (M13)	Single	No	GSK	2013N174038 (13DMM016)	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	15F	SC (Injection)	D, E, N	2.0 (M2), 2.5 (M3) 1.3 (M13)	14 days	No	GSK	2014N208198 (M70590N)	m4.2.2.2

Key: BSA = Body surface area, DB = Dutch Belted, EPO = Erythropoietin, IV = Intravenous, pc = post coitum, w/w = weight/weight ratio, M2 = GSK2391220, M3 = GSK2506104, M13 = GSK2531401, GD = Gestation day, LD = Lactation day, NZW = New Zealand White, SC = Subcutaneous, SD = Sprague Dawley.
A = Daprodustat (Parent form).
B = Daprodustat (Potassium salt).
C = Daprodustat (Tris monohydrate salt).
D = M2 metabolite of daprodustat, GSK2391220 (Parent form).
E = M3 metabolite of daprodustat, GSK2506104 (Parent form).
M = M8 metabolite of daprodustat, GSK2391804 (Parent form).
N = M13 metabolite of daprodustat, GSK2531401 (Parent form).

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]

表 2.6.4.2-2　吸収試験一覧

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD

a = Composite sampling design; n=3/time point.
b = Non-fasted animals.
c = Fasted animals.
d = Animals received either a 22 or 120 minute IV infusion.
e = GSK1278863B, the potassium salt of daprodustat was used.
f = GSK1278863D, the tris monohydrate salt of daprodustat was used.
g = Samples obtained from the 13 week toxicity study in the mouse, [Report 2011N112096].
h = On Day 17, the dose was reduced from 30 mg/kg/day to 15 mg/kg/day for females only.
i = 2M/2F fasted animals were administered the 90 mg/kg/day dose in a capsule, and 2M/2F fed animals were administered an oval gavage dose of 90 mg/kg/day.
j = Samples obtained from the 13 week toxicity study in the monkey, [Report 2010N108482].
k = Suspension in 1% aqueous methylcellulose.
l = Amorphous spray dried dispersion suspension in hydroxypropylmethylcellulose.
m = GSK2531401 (M13) dosed at either 40 mg/kg/day or 100 mg/kg/day.　Daprodustat dosed at 20 mg/kg/day.
n = Daprodustat was administered orally, but some groups were also administered a cocktail of metabolites (M2,3 and 13,) of daprodustat or vehicle subcutaneously.
o = Given daprodustat at 10 mg/kg/day and metabolite cocktail - 2.5, 3.2, and 1.8 mg/kg/day (for M2, 3 or 13, respectively).
p = Given daprodustat at 40 mg/kg/day and vehicle (25 mM Phosphate Buffer, pH 7.4).
q = Given daprodustat at 40 mg/kg/day and metabolite cocktail - 2.5, 3.2, and 1.8 mg/kg/day (for M2, 3 and 13, respectively).
r = Given daprodustat at 60 mg/kg/day and metabolite cocktail - 2.5, 3.2, and 1.8 mg/kg/day (for M2, 3 and 13, respectively).
s = Given daprodustat at 60 mg/kg/day and metabolite cocktail - 7.5, 9.6, and 5.4 mg/kg/day (for M2, 3 and 13, respectively).
t = 0.2, 0.8 and 3 mg/kg/day daprodustat groups were also administered a cocktail of metabolites (M2, 3 and 13)at 2, 2.5, and 1.3 mg/kg/day, respectively.
u = 2 to 3 mice/timepoint.
v = 3 mice/timepoint.
w = Daily Subcutaneous Dose metabolite cocktail [GSK2391220 (M2); GSK2506104 (M3): GSK2531401 (M13)] mg/kg/day [dosed as 1.25, 1.8 and　0.9 mg/kg/dose BID every 6 hours].

2.6.4.3.　分布

ダプロデュスタットの血漿蛋白結合率、血球移行、トランスポーター（P-gp、OATP1B1及びOATP1B3、OAT1、OAT3、OCT2、MATE1、MATE-K及びBCRP）を介した輸送及びトランスポーター阻害、並びに組織内分布をin vitro及びin vivoで評価した。また、ヒトでの主な6種の代謝物［M2（GSK2391220）、M3（GSK2506104及びGSK2531403）、M4（GSK2487818及びGSK2499166）、M5（GSK2506102及びGSK2531399）、M6（GSK2531398）及びM13（GSK2531401及びGSK2531400）］についても一部のin vitro試験を実施した。

試験一覧を表 2.6.4.3-1に示す。

2.6.4.3.1.　In vitro

2.6.4.3.1.1.　血漿蛋白結合率及び結合蛋白

2.6.4.3.1.1.1.　ダプロデュスタットの血漿蛋白結合率

マウス、ラット、ウサギ、イヌ、サル及びヒトの血漿とダプロデュスタット（0.2、1、10及び50 µg/mL）をインキュベートしたときの血漿蛋白結合率を平衡透析法及びLC-MS-MSを用いて検討した（CD2010/00340）。

試験成績を2.6.5.7.1.に示す。動物血漿でのダプロデュスタットの血漿蛋白結合率は93.6%以上、ヒト血漿では99%超といずれも高かった。なお、予備的な試験（UH2008/00002）として、マウス、ラット、イヌ、サル及びヒト血漿でのダプロデュスタット（1及び10 µg/mL）の血漿蛋白結合率を検討しており、試験成績のみを本試験と同様の2.6.5.7.1.に示した。

2.6.4.3.1.1.2.　ダプロデュスタットの蛋白結合

ダプロデュスタット（0.2、1、10及び30 µg/mL）のヒト血清アルブミン（HSA、0.600 mM）及びα1-酸性糖蛋白質（AAG、0.015及び0.150 mM）への結合を平衡透析法及びLC-MS-MSを用いて検討した（CD2010/00340）。

試験成績を 2.6.5.7.3.に示す。ダプロデュスタットの HSA への結合率は 99.3%以上と高く、AAG への結合率は AAG の濃度にかかわらず 20%未満と低かった。また、0.2～30 µg/mL の濃度範囲で HSA 及び AAG への蛋白結合に濃度依存性はみられなかった。

2.6.4.3.1.1.3.　代謝物の血漿蛋白結合率

ヒトの血漿とヒトでの主な6種の代謝物［M2（GSK2391220）、M3（GSK2531403及びGSK2506104）、M4（GSK2499166）、M5（GSK2531399）、M6（GSK2531398）及びM13（GSK2531400及びGSK2531401）］を18時間インキュベートしたときの血漿蛋白結合率を平衡透析法及びUPLC-MS-MSを用いて検討した。各代謝物の濃度は10、100、250及び500 ng/mLとした（2012N131897）。

試験成績を2.6.5.7.2.に示す。M2、M3（GSK2531403及びGSK2506104）、M6及びM13（GSK2531400及びGSK2531401）の血漿蛋白結合率はいずれの濃度でも34%未満と低く、

濃度依存性はみられなかった。一方、M4（M4 の主ではない立体異性体）及び M5（M5 の主ではない立体異性体）の低濃度（10 ng/mL）での血漿蛋白結合率は低く（それぞれ 68 及び 74%）、濃度の増加に伴い 98%まで上昇し、濃度に依存すると考えられた。なお、各代謝物は 18 時間のインキュベート後も濃度の低下はみられず、安定していた。

2.6.4.3.1.2.　血球移行性

マウス、ラット、イヌ、サル及びヒトの血液とダプロデュスタット（1 及び 10 μg/mL）を 30 分間インキュベートしたときの血球移行について経時的に検討した（UH2008/00002）。

試験成績を 2.6.5.7.4.に示す。動物及びヒトでのダプロデュスタットの血液／血漿比はそれぞれ 0.45～1.64 及び 0.75～1.23 と、ダプロデュスタットの血球移行は概してわずかであった。

2.6.4.3.1.3.　マイクロダイアリシス

皮膚にダプロデュスタットを塗布したときの検討法としてマイクロダイアリシスを開発した（2015N228833）が、本申請は経口剤のため、試験成績のみを 2.6.5.9.2.に示す。

2.6.4.3.1.4.　膜透過性及び P-gp を介した輸送

2.6.4.3.1.4.1.　受動的膜透過性

MDCKII-MDR1 細胞を用いて、P-gp 阻害薬である GF120918（2 μM）の存在下で、[14C]標識体（3 及び 5 μM）の受動的膜透過性について検討した。apical 側の媒体として pH7.4 の DMEM を、又は胃腸管の状態を再現するために pH5.5 及び pH7.4 の人工腸液（FaSSIF）を用いた（UH2008/00018 及び CD2008/00421）。

試験成績を 2.6.5.9.1.及び 2.6.5.9.3.に示す。[14C]標識体（3 及び 5 μM）の受動的膜透過性は pH7.4 の DMEM 下で中程度（それぞれ 21 及び 20 nm/s）であった。また、pH5.5 及び pH7.4 の FaSSIF 下での[14C]標識体（3 μM）の膜透過係数は 325 及び 191 nm/s と、いずれも高い受動的膜透過性を示し、特に pH5.5 で高かった。

2.6.4.3.1.4.2.　P-gp を介した輸送

MDCKII-MDR1 細胞を用いて、P-gp 阻害薬である GF120918（2 μM）の非存在及び存在下でのダプロデュスタット（5 μM）の P-gp を介した輸送について検討した。GF120918 の非存在下での efflux ratio が 2 より大きい場合に P-gp の基質と判断した（UH2008/00018）。

試験成績を 2.6.5.9.3.に示す。ダプロデュスタットの efflux ratio は、GF120918 の非存在下で 1 未満であったことから、ダプロデュスタットは P-gp の基質ではないと判断された。

2.6.4.3.1.5.　P-gp 阻害

MDCKII-MDR1 細胞を用いて、[3H]ジゴキシン（0.030 μM）の P-gp を介した basolateral 側から apical 側への輸送に対するダプロデュスタット（0.1～100 μM）の阻害作用について検討した（CD2008/00669）。

試験成績を 2.6.5.9.11.に示す。ダプロデュスタットは P-gp を阻害しなかった。

2.6.4.3.1.6.　BCRP を介した輸送

2.6.4.3.1.6.1.　マウス Bcrp1

マウス BCRP1 を発現させた MDCKII-Bcrp1 細胞を用いて、BCRP 阻害薬である GF120918（5 μM）の非存在及び存在下でのダプロデュスタット（3 μM）の Bcrp を介した輸送について検討した。efflux ratio が 2 以上の場合に Bcrp1 の基質と判断した（UH2008/00018）。

試験成績を 2.6.5.9.4.に示す。ダプロデュスタットの efflux ratio はそれぞれ 60 及び 5.5 であった。見かけの透過係数（Papp）は 79 nm/sec と高かったが、GF120918 の存在下（Bcrp1 の飽和状態）においても透過がみられたことから、実際の透過係数は更に高いと考えられる。ダプロデュスタットはマウス Bcrp1 の基質であることが示された。ダプロデュスタットは in vitro においてマウス Bcrp1 の基質であり、MDCKII に対して中程度から高度の透過性を示した。

2.6.4.3.1.6.2.　ヒト BCRP

ヒト BCRP を発現させた MDCKII-BCRP 細胞を用いて、BCRP 阻害薬である GF120918 の非存在下及び存在下でのダプロデュスタット（3 μM）のヒト BCRP を介した輸送について検討した（2015N237800）。efflux ratio が GF120918 非存在下では 2 以上であり、GF120918 存在下では約 1 に低下する場合に BCRP の基質と判断した。

試験成績を 2.6.5.9.5.に示す。GF120918 の非存在下及び存在下におけるダプロデュスタットの efflux ratio はそれぞれ 4.4 及び 0.65 であったことから、ダプロデュスタットは 3 μM の濃度でヒト BCRP の基質であることが示された。

2.6.4.3.1.7.　BCRP 阻害

MDCKII-BCRP 細胞を用いて、[14C]シメチジン（0.5 μM）の BCRP を介した basolateral 側から apical 側への輸送に対するダプロデュスタット（0.01～100 μM）の阻害作用について検討した（2012N151642）。

試験成績を 2.6.5.9.12.に示す。[14C]シメチジンの basolateral 側から apical 側への輸送速度は 10±0.75 pmole/cm^2/hr であり、ダプロデュスタットはいずれの濃度でも[14C]シメチジン輸送を阻害しなかった。

2.6.4.3.1.8.　OATP1B1 及び OATP1B3 を介した輸送

HEK293-OATP1B1 及び HEK293-OATP1B3 細胞を用いてダプロデュスタット及び 6 種の代謝物（M2；GSK2391220、M3；GSK2506104、M4；GSK2487818、M5；GSK2506102、M6；GSK2531398 及び M13；GSK2531401）の OATP1B1 及び OATP1B3 を介した輸送について検討した。ダプロデュスタットの濃度は 0.1～100 μM を、M2 及び M3 の濃度はいずれも 0.01～10 μM を、M4、M5、M6 及び M13 の濃度はいずれも 1～30 μM を用いた（2015N255319）。

試験成績を 2.6.5.9.7.に示す。ダプロデュスタットは、OATP1B1 の基質であったが、OATP1B3 の基質ではなかった。M13 は OATP1B1 の基質ではなかったが、OATP1B3 の基質

であった。M2、M4、M5 及び M6 は OATP1B1 及び OATP1B3 の基質であったが、M3 はいずれの基質でもなかった。

2.6.4.3.1.9.　OATP1B1 及び OATP1B3 阻害

2.6.4.3.1.9.1.　ダプロデュスタット

CHO-OATP1B1 及び HEK-MSRII-OATP1B3 細胞を用いて、OATP1B1 及び OATP1B3 を介した[3H]Estradiol 17β-D-glucuronide（[3H]EG）の輸送に対するダプロデュスタット（0.1～100 μM）の阻害作用について検討した（CD2008/00231）。

試験成績を 2.6.5.9.13.に示す。ダプロデュスタットは OATP1B1 及び OATP1B3 を阻害し、IC50 はそれぞれ 6 及び 11 μM であった。

2.6.4.3.1.9.2.　代謝物

HEK293-MSRII-OATP1B1 細胞を用いて、メトトレキセートの OATP1B1 を介した輸送に対するダプロデュスタットの 6 種の代謝物（M2；GSK2391220、M3；GSK2531403、M4；GSK2487818、M5；GSK2506102、M6；GSK2531398 及び M13；GSK2531401）の阻害作用について検討した（2013N166580）。各代謝物の濃度はいずれも 0.085～50 μM を用いた。

試験成績を 2.6.5.9.14.に示す。M2、M3、M4、M5、M6 及び M13 は概して 50 μM の濃度まで OATP1B1 を阻害しなかった。

2.6.4.3.1.10.　OAT1、OAT3、OCT2、MATE1 及び MATE2-K

2.6.4.3.1.10.1.　OAT1、OAT3、OCT2、MATE1 及び MATE2-K を介した輸送

S2-OAT1、S2-OAT3、HEK293-OCT2、HEK293-MATE1-及び HEK293-MATE2-K 細胞を用いて、ダプロデュスタット及び 6 種の代謝物（M2；GSK2391220、M3；GSK2506104、M4；GSK2487818、M5；GSK2506102、M6；GSK2531398 及び M13；GSK2531401）の OAT1、OAT3、OCT2、MATE1 及び MATE2-K を介した輸送について検討した。ダプロデュスタットの濃度は 0.1～100 μM を、M2 の濃度は 0.01～10 μM（OAT1、OAT3 及び OCT2）又は 1～30 μM（MATE1 及び MATE2-K）を、M3、M4、M5、M6 及び M13 の濃度は 1～30 μM を用いた（2015N249257）。

試験成績を 2.6.5.9.9.及び 2.6.5.9.10.に示す。M3 及び M13 は OAT3 の基質であったが、OAT1、OCT2、MATE1 及び MATE2-K の基質ではなかった。M2 は OAT1 及び OAT3 の弱い基質であったが、OCT2、MATE1 及び MATE2-K の基質ではなかった。ダプロデュスタット、M4、M5 及び M6 は OAT1、OAT3、OCT2、MATE1 及び MATE2-K の基質ではなかった。

2.6.4.3.1.10.2.　OAT1、OAT3、OCT2、MATE1 及び MATE2-K 阻害

S2-OAT1、S2-OAT3、HEK293-OCT2、HEK293-MATE1 及び HEK293-MATE2-K 細胞を用いて、ダプロデュスタット及び 6 種の代謝物（M2；GSK2391220、M3；GSK2506104、M4；GSK2487818、M5；GSK2506102、M6；GSK2531398 及び M13；GSK2531401）の

OAT1、OAT3、OCT2、MATE1 及び MATE2-K に対する阻害作用について検討した（2015N242070）。試験には放射能標識した各プローブ基質及び被験物質カクテル（ダプロデュスタット；1.4 μM、M2；20 μM、M3；20 μM、M4；10 μM、M5；5 μM、M6；10 μM 及び M13；10 μM）を用いた。

試験成績を 2.6.5.9.15.に示す。ダプロデュスタット及び 6 種の代謝物はいずれも OAT1、OAT3、OCT2、MATE1 及び MATE2-K を阻害しなかった。

2.6.4.3.1.11.　その他の in vitro での分布試験

2.6.4.3.1.11.1.　ヒト肝細胞への取込み

2.6.4.3.1.11.1.1.　ダプロデュスタット

ヒト肝細胞を用いて、阻害薬カクテルの存在下で、ダプロデュスタット（0.3～0.5 μM）の肝細胞への受動的取込みについて検討した。試験には OATP1B1、OATP1B3、OATP2B1 及び OCT1 の阻害薬カクテル［OATP1B1 及び OATP1B3；リファマイシン（10 μM）及びシクロスポリン A（10 μM）、OATP2B1；モンテルカスト（10 μM）及び OCT1；イミプラミン（100 μM））］を用いた（2015N232657）。

試験成績を 2.6.5.9.6.に示す。ダプロデュスタットのヒト肝細胞への取込みに、阻害薬カクテルによる明らかな阻害はみられなかったことから、ダプロデュスタットは肝細胞に主に受動的に取り込まれると考えられ、OATP1B1、OATP1B3、OATP2B1 及び OCT1 の基質ではないと考えられた。本成績は、各種トランスポーター発現系での成績（2.6.4.3.1.8.）とは完全には一致しなかったが、発現系よりも肝細胞での本試験の方がより完全な試験系であることから、ダプロデュスタットは OATP1B1、OATP1B3、OATP2B1 及び OCT1 の基質ではないと考えられた。

2.6.4.3.1.11.1.2.　M4（GSK2487818）

ヒト肝細胞を用いて、阻害薬カクテルの存在下で、M4（GSK2487818）（0.2～15 μM）の肝細胞への受動的取込みについて検討した（2015N259558）。試験には OATP1B1、OATP1B3、OATP2B1 及び OCT1 阻害薬カクテルを用いた。

試験成績を 2.6.5.9.8.に示す。M4（GSK2487818）のヒト肝細胞への取込みに、阻害薬カクテルによる明らかな阻害はみられなかったことから、M4 は肝細胞に主に受動的に取り込まれると考えられ、OATP1B1、OATP1B3、OATP2B1 及び OCT1 の基質ではないと考えられた。本成績は、各種トランスポーター発現系での成績（2.6.4.3.1.8.）とは完全には一致しなかったが、発現系よりも肝細胞での本試験の方がより完全な試験系であることから、M4 は OATP1B1、OATP1B3、OATP2B1 及び OCT1 の基質ではないと考えられた。

2.6.4.3.2.　In vivo

2.6.4.3.2.1.　マウス

2.6.4.3.2.1.1.　腎臓内濃度

雄マウスにダプロデュスタットの 20 mg/kg 又は M13（GSK2531401）の 40 及び 100 mg/kg を単回静脈内投与したときの血漿、腎臓及び肝臓内濃度を検討した（2012N132320 及び 2012N156583）。

試験成績を 2.6.5.6.2.及び 2.6.5.6.3.に示す。血漿中濃度と同様に、M13 の腎臓及び肝臓内濃度は、それぞれ投与後 12 及び 8 時間まで定量された。ダプロデュスタットの投与量は M13 に比べ低かったにも関わらず、腎臓内ダプロデュスタット濃度は M13 の 15～500 倍であった。

2.6.4.3.2.2.　ラット

2.6.4.3.2.2.1.　血液／血漿比及び肝臓／血液比

雌雄ラットに[14C]標識体の 10 mg/kg を単回経口投与したときのダプロデュスタットの血液／血漿中濃度比及び肝臓／血液中濃度比を検討した（CD2008/00100）。血液及び肝臓サンプルは、投与 1、4、8、24 及び 168 時間（血液のみ）後に採取し、血液、血漿及び肝臓内の放射能を測定した。

試験成績を 2.6.5.7.5.に示す。雄ラットの血液及び血漿中放射能は投与 1 時間後で最大となり、それぞれ 37.4 及び 72.1 μg eq./g であった。雌ラットでも投与 1 時間後で最大となり、それぞれ 41.4 及び 76.1 μg eq./g であった。その後、経時的に低下し、168 時間後では 0.15 μg eq./g 未満となった。また、投与後 168 時間までの放射能の血液／血漿中濃度比は 0.505～0.560 であり、血球移行は低いと考えられた。

また、雌雄ラットの肝臓内放射能は投与 1 時間後で最大となり、それぞれ 22.2 及び 30.7 μg eq./g であった。放射能の肝臓／血液中濃度比は 0.319～0.825 であった。

2.6.4.3.2.2.2.　QWBA

雄の有色ラットに[14C]標識体の 10 mg/kg を単回経口投与し、投与 1、4、8 及び 24 時間後、並びに 3、7 及び 35 日後の組織内分布について QWBA を用いて検討した（CD2008/00271）。

試験成績を 2.6.5.6.1.に示す。放射能は速やかに広く組織に分布し、ほとんどの組織内放射能は血液中放射能（62.510 μg eq./g）よりも低く、ダプロデュスタットの分布容積が小さいことと一致した。脳内放射能は血液中放射能の約 2%であった。放射能は、ほとんどの組織で投与 1 時間後に最も高く、投与 7 日目までに定量下限（0.038 μg eq./g）未満となった。眼球及び皮膚のメラニン含有組織への放射能の結合はみられなかった。

2.6.4.3.2.3.　イヌ

2.6.4.3.2.3.1.　血液／血漿比

雌雄の無処置イヌ及び雄の胆管カニューレ処置（BDC）イヌにダプロデュスタットの 20 mg/kg を単回経口投与したときの血液／血漿中濃度比を検討した（CD2008/00025）。

試験成績を 2.6.5.7.5.に示す。ダプロデュスタットの血液／血漿濃度比は、無処置イヌでは投与 24 時間後まで 0.72～0.87、BDC イヌでは投与 72 時間後で 0.84 と、血球移行は低かった。

2.6.4.3.2.4.　サル

2.6.4.3.2.4.1.　血液／血漿比

雄の無処置及び BDC サルにダプロデュスタットの 10 mg/kg を単回経口投与したときの血液／血漿中濃度比を検討した（2018N355713）。

試験成績を 2.6.5.7.5.に示す。ダプロデュスタットの血液／血漿濃度比は、無処置サルで 0.615～0.839、BDC サルで 0.606～0.668 であり、明らかな血球移行はないと考えられた。

2.6.4.3.2.5.　胎盤通過及び胎児への移行

ラットの胚・胎児発生に関する試験では、60 mg/kg/日群において、胎児に着床後胚死亡の増加及び骨格変異が認められており（2.6.6.6.4.1.1.）、この理由として同群でみられた母動物毒性の可能性、及びダプロデュスタットが胎盤を通過する可能性が考えられた。また、ダプロデュスタットは膜透過性が高いこと、一般に分子量が 300～600 程度の薬剤は、胎盤を通過しやすく[杉本充弘, 2018]、ダプロデュスタットの分子量は 393.43 であることから、胎盤を通過する可能性が考えられた。

これらのことから、ダプロデュスタットは胎盤通過する可能性があると考えられた。

表 2.6.4.3-1　　分布試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Plasma protein binding	Mouse (CD-1), Rat (SD), Dog (beagle), Monkey (Cynomolgus), Human	NA	In vitro	A	1 and 10 µg/mL	NA	No	GSK	UH2008/00002	m4.2.2.3
Plasma protein, HSA and AAG binding	Mouse, Rat, Rabbit, Dog, Monkey, Human	NA	In vitro	A	0.2, 1, 10, 30[a], 50 µg/mL	NA	No	GSK	CD2010/00340 (09DMM060)	m4.2.2.3
Blood cell association	Mouse (CD-1), Rat (Sprague Dawley), Dog (beagle), Monkey (Cynomolgus), Human	NA	In vitro	A	1 and 10 µg/mL	NA	No	GSK	UH2008/00002	m4.2.2.3
Kidney distribution	Mouse (CD-1)	15M[i]	IV (bolus)	N	40	Single	No	GSK	2012N132320 (11DMM049)	m4.2.2.3
Kidney distribution	Mouse (CD-1)	15M 18M/ 16M[h] 15M[h]	IV (bolus)	A N	20[g] 40/100 (M13)	Single	No	GSK	2012N156583 (12DMM041)	m4.2.2.3
Blood to plasma and blood to liver ratio	Rat	3M/F; 3M BDC	Oral (gavage)	[14C]	10[b]	Single (intact: up to 7 days) (BDC: up to 4 days)	Yes	[redacted]	CD2008/00100 (7717-705)	m4.2.2.3
Blood to plasma ratio	Dog (beagle)	3M/3F; 3M BDC	Oral (gavage)	[14C]	20[b]	Single (intact: up to 7 days) (BDC: up to 3 days)	Yes	[redacted]	CD2008/00025 (AFA00607)	m4.2.2.3
Blood to plasma ratio	Monkey (cynomolgus)	3M; 2M BDC	Oral (gavage)	[14C]	10[b]	Single (Up to 168 hours)	No	[redacted]	2018N355713 (8361764)	m4.2.2.3

表 2.6.4.3-1　　分布試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Hepatic uptake	Human	NA	In vitro	A	0.5 & 0.3 μM	NA	No	GSK	2015N232657 (14DMM039)	m4.2.2.3
Inhibition of OAT1, OAT3, OCT2, MATE1 and MATE2-K transporters (daprodustat and 6 metabolites)	S2 cells HEK293 cells	NA	In vitro	A	Varying[c]	NA	No	[redacted]	2015N242070 (XS-0661)	m4.2.2.3
Substrate for OATP1B1 and OATP1B3 transporters (daprodustat and 6 metabolites)	HEK293 cells	NA	In vitro	A	Varying[d]	NA	No	[redacted]	2015N255319 (XS-0674)	m4.2.2.3
Substrate for OAT1, OAT3, OCT2, MATE1 and MATE2-K transporters (daprodustat and 6 metabolites)	S2 cells HEK293 cells	NA	In vitro	A	Varying[f] (see key)	NA	No	[redacted]	2015N249257 (XS-0662)	m4.2.2.3
Interaction with P-gp and membrane permeability	Human	NA	In vitro	A	5 μM	NA	No	GSK	UH2008/00018	m4.2.2.3
P-gp-mediated transport inhibition	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/00669 (08DMM040)	m4.2.2.3

表 2.6.4.3-1 分布試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Passive and absorptive membrane permeability	NA	NA	In vitro	[14C]	3 μM[b]	NA	No	GSK	CD2008/00421 (08DMM021)	m4.2.2.3
Microdialysis	NA	NA	In vitro	A	2.5 and 25 ng/mL	NA	No	[redacted]	2015N228833	m4.2.2.3
OATP1B1 and OATP1B3 transport	HEK-MSRII	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/00231 (07DMM163)	m4.2.2.3
Interaction with Bcrp1 and membrane permeability	MDCKII-murine Bcrp1 cells	NA	In vitro	A	3 μM	NA	No	GSK	UH2008/00018	m4.2.2.3
Evaluation as substrate	MDCKII-BCRP cells	NA	In vitro	A	3 μM[a]	NA	No	GSK	2015N237800 (15DMM007)	m4.2.2.3
BCRP mediated transport inhibition	Human	NA	In vitro	A	0.01 to 100 μM	NA	No	GSK	2012N151642 (12DMM014)	m4.2.2.3
Whole body autoradiography	Rat (Long Evans)	7M	Oral (gavage)	[14C]	10[b]	Singl[e] (Up to 35 days)	Yes	[redacted]	CD2008/00271 (85-0708)	m4.2.2.3
Studies with Metabolites										
Plasma protein binding	Human	NA	In vitro	D, E, G, J, K, N[c]	10, 100, 250 and 500 ng/mL	NA	No	GSK	2012N131897 (11DMM027)	m4.2.2.3
Hepatic uptake of GSK2487818	Human (cryopreserved hepatocytes)	NA	In vitro	Gd	0.2, 2, 15 μM	NA	No	GSK	2015N259558 (15DMM035)	m4.2.2.3
OATP1B1 transport	Human	NA	In vitro	D, E, G, J, K, N[e]	85 nM to 50 μM	NA	No	GSK	2013N166580	m4.2.2.3

表 2.6.4.3-1　分布試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD

A = Daprodustat (Parent form). D = M2 metabolite of daprodustat, GSK2391220 (Parent form). E = M3 metabolite of daprodustat, GSK2506104 (Parent form). G = M4 metabolite of daprodustat, GSK2487818 (Parent form). J = M5 metabolite of daprodustat, GSK2506102 (Parent form). K = M6 metabolite of daprodustat, GSK2531398 (Parent form). N = M13 metabolite of daprodustat, GSK2531401 (Parent form). [14C] = 14C labelled daprodusat.
AAG = α1-acid glycoprotein.　Bcrp1 = Murine breast cancer resistant protein 1.
BCRP = Human breast cancer resistant protein.　BDC = Bile duct cannulated.
HEK-MSRII =Human embryonic kidney expressing macrophage scavenger receptor II.
hMDR1 = Human multi-drug resistant 1 gene.　HSA = Human serum albumin.
MATE1 = Multidrug and toxin extrusion transporter 1.　MATE2-K = Multidrug and toxin extrusion transporter 2-K.
MCDK = Madin Darby canine kidney.　NA = Not applicable.　OAT1 = Organic anion transporter 1.
OAT3 = Organic anion transporter 3.　OATP1B1 = Organic anion transporting polypeptide 1B1.
OATP1B3 = Organic anion transporting polypeptide 1B3.　OCT2 = Organic cation transporter 2.
P-gp = P-glycoprotein. w/w = weight/weight ratio.
a = 30 μg/mL was employed in the binding of HSA and AAG only.
b = [14C]GSK1278863.
c = 1.4 μM GSK1278863, 20 μM M2 (GSK2391220), 20 μM M3 (GSK2506104), 10 μM M4 (GSK2487818), 5 μM M5 (GSK2506102), 10 μM M6 (GSK2531398), and 10 μM M13 (GSK2531401).
d = 0.1, 1, 10, 100 μM　- GSK1278863,　0.01, 0.1, 1, 10 μM – M2 (GSK2391220) & M3 (GSK2506104), 1, 3, 10, 30 μM　for M13 (GSK2531401), M4 (GSK2487818), M5 (GSK2506102) & M6 (GSK2531398).
e = 0.1, 1, 10, 100 μM　- GSK1278863,　0.01, 0.1, 1, 10 μM and 1, 3, 10, 30 μM (for MATE1 assay only) – M2 (GSK2391220), 1, 3, 10, 30 μM　for M3 (GSK2506104), M13 (GSK2531401), M4 (GSK2487818), M5 (GSK2506102) & M6 (GSK2531398).
f = GSK1278863D, the tris monohydrate salt of daprodustat was used.
g = GSK2531401 (M13) dosed at either 40 mg/kg/day or 100 mg/kg/day.　Daprodustat dosed at 20 mg/kg/day.
h = 2 to 3 mice/timepoint.
i = 3 mice/timepoint.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.

2.6.4.4.　代謝

In vitro 及び in vivo でのダプロデュスタットの代謝を検討した。代謝試験の一覧を表 2.6.4.4-1 に、推定代謝経路を 2.6.5.12.に示す。

2.6.4.4.1.　In Vitro 試験

2.6.4.4.1.1.　ダプロデュスタットの代謝試験

2.6.4.4.1.1.1.　血中での安定性

ヒト血液及びダプロデュスタット（1 及び 10 µg/mL）を 37°C で最長 2 時間までインキュベートしたときの血中での安定性を検討した（UH2008/00002）。

試験成績を 2.6.5.11.1 に示す。ダプロデュスタットはヒト血中で少なくとも 2 時間安定であった。

2.6.4.4.1.1.2.　肝ミクロソーム及び肝細胞での固有クリアランス

マウス、ラット、イヌ、サル及びヒト肝ミクロソーム及び肝細胞及びダプロデュスタット（0.5 µM）を 37°C で 30 分間インキュベートし、固有クリアランスを検討した（UH2008/00018）。固有クリアランスがそれぞれ 0.5～5 mL/min/g 肝、5～8 mL/min/g 肝及び 8 mL/min/g 肝超のとき、それぞれ低い、中程度及び高いとした。

試験成績を 2.6.5.11.2.に示す。マウス、ラット、イヌ、サル及びヒトの肝ミクロソーム及び肝細胞でのダプロデュスタットの固有クリアランスはいずれも低かった（1.1 mL/min/g 肝以下）。

2.6.4.4.1.1.3.　ラット肝 S9 画分での代謝

アロクロール 1254 で誘導させたラットの肝臓から調製した S9 及び[14C]標識体を、細菌を用いる復帰突然変異試験又はマウスリンフォーマ試験（2.6.6.4.2.1.1.及び 2.6.6.4.2.2.1.）で用いたのと同じ条件下でインキュベートしたときの代謝物を検討した（2011N114076）。

試験成績を 2.6.5.11.5.に示す。いずれの試験条件でも未変化体のみがみられ、代謝物は検出されなかった。

2.6.4.4.1.1.4.　ラット摘出肝灌流標本での代謝

雄 BDC ラットから作製した摘出肝灌流標本（IPRL）を用い、[14C]標識体の 30 mg/kg を投与したときの胆汁及び血漿中代謝物を検討した。また、雄ラットに[14C]標記体の 10 mg/kg を単回経口投与したときの投与 4、8、24 及び 48 時間後の血漿中代謝物を検討した（CD2008/00941）。

試験成績を 2.6.5.10.2.及び 2.6.5.10.10.に示す。IPRL では、胆汁中放射能の回収率は、投与量の 14～34%であり、胆汁中の主な成分は未変化体であったが、その他に 3 種の一酸化体（M8、M9 及び M10）及び二酸化体（M2）が検出された。

無処置ラットの血漿中放射能は、投与 4、8、24 及び 48 時間後でそれぞれ 53918、49961、23683 及び 14578 ng equivalents/g であった。各時点での血漿中放射能の主な成分は未変化体で、それぞれ血漿中総放射能の 87、94、89 及び 87%であり、経時的に減少した。

2.6.4.4.1.1.5.　肝細胞での代謝物の同定

マウス、ラット、ウサギ、イヌ、サル及びヒトの肝細胞及び[14C]標識体を 37°C で最長 24 時間インキュベートし、代謝物を検討した（CD2008/01286）。

試験成績を 2.6.5.11.4.に示す。ヒト肝細胞で同定された一酸化体である M8、M9 及び M10 は、2 種以上の動物において検出された（M8：マウス、ラット、ウサギ及びサル、M9：ラット、ウサギ及びサル、M10：ウサギ及びサル）。サル肝細胞での代謝プロファイルがヒト肝細胞と最も類似していたが、イヌ肝細胞はダプロデュスタットを代謝しなかった。

未変化体は、すべての動物の肝細胞で検出された。M8 は、ピリミジンジオン環の窒素原子に対してシクロヘキシル環の 4 位の炭素に酸化反応が生じたものであり（トランス異性体）、マウス、ラット、ウサギ及びサルの肝細胞で認められた。対応するシス異性体である M9 はラット、ウサギ及びサルの肝細胞で認められた。M10 は、ピリミジンジオン環の窒素原子に対してシクロヘキシル環の 3 位の炭素に酸化が生じたものであり、ウサギ及びサルの肝細胞で認められた。二酸化体である M1、M2、M3 及び M4 は、ウサギ及びサルの肝細胞で認められ、M5、M6 及び M7 は、サルの肝細胞で認められた。特性が明らかにされていないグルクロン酸抱合体（M12）はサルの肝細胞で認められた。

これらのことから、動物及びヒト肝細胞でのダプロデュスタットの代謝経路は、一酸化及び二酸化であり、ヒト肝細胞で生成された代謝物は 1 種以上の動物で生成が確認された。

2.6.4.4.1.1.6.　ミニブタ肝細胞での代謝

ミニブタ肝細胞及びダプロデュスタット（19 μM）を 37°C で 24 時間インキュベートし、代謝物を検討した（2011N125815）。

試験成績を 2.6.5.11.4.に示す。ダプロデュスタットは酸化され、4 種の一酸化体（M8、M9、M10 及び M22）及び 6 種の二酸化体（M2、M3、M4、M21、M23 及び M24）が同定された。2 種の代謝物（M23 及び M24）は、以前の試験では認められなかったが、2 つのシクロヘキシル基がそれぞれ 1 個所ずつ酸化されたものであり、M2、M3、及び M4 の立体異性体又は位置異性体である。また、未変化体も検出された。

ミニブタ肝細胞で生成された代謝物は、ヒト、サル、及びウサギ肝細胞と同様であった（2.6.4.4.3.6.1.及び 2.6.4.4.1.1.5.）。

2.6.4.4.1.1.7.　ハムスター肝細胞での代謝

ハムスター肝細胞及びダプロデュスタット（20 μM）を 37°C で最大 24 時間インキュベートし、代謝物を検討した（2013N160562）。

試験成績を 2.6.5.11.4.に示す。ハムスター肝細胞で、ダプロデュスタットは酸化的に代謝され、4 種の一酸化体（M8、M9、M10 及び M22）が同定され、未変化体も検出された。この他の代謝物も存在していたと考えられるが、本試験条件下では検出できなかった。また、

ハムスター肝細胞でみられた一酸化体は、これ以前の試験でヒト、サル、及びウサギ肝細胞で検出された代謝物と同様であった（2.6.4.4.3.6.1.及び 2.6.4.4.1.1.5.）。

2.6.4.4.1.1.8.　肝細胞及び CYP 発現系での代謝物の同定

マウス、ラット、ウサギ、イヌ、サル及びヒトの肝細胞及び CYP（CYP1A2、CYP2C9、CYP2C19、CYP2D6 及び CYP3A4）発現系でダプロデュスタットの代謝を予備的に検討した（UH2008/00018）。

試験成績を 2.6.5.11.3.に示す。予備的な検討では、肝細胞で 19 種の代謝物がみられ、ケトン体、一酸化体、一酸化体のケトン体、二酸化体、三酸化体、グルクロン酸抱合体、一酸化体のグルクロン酸抱合体であった。このうち 14 種の代謝物はヒト肝細胞でもみられた。

CYP2C9、CYP2D6 及び CYP3A4 発現系では 3 種の一酸化体がみられたが、CYP1A2 及び CYP2C19 では代謝物はみられなかった。

2.6.4.4.1.1.9.　代謝酵素の同定

ヒト肝ミクロソーム及び CYP（CYP1A2、CYP2C8、CYP2C9、CYP2C19、CYP2D6 及び CYP3A4）発現系を用いて[14C]標識体（5 μM）の酸化的代謝に関与する酵素を検討した。ヒト肝ミクロソームを用いた試験では、選択的 CYP 阻害剤であるアザムリン、スルファフェナゾール、キニジン、モンテルカスト、ベンジルニルバノール及びフラフィリン（それぞれ CYP3A4、CYP2C9、CYP2D6、CYP2C8、CYP2C19 及び CYP1A2 の阻害剤）を用いた（CD2010/00300）。

試験成績を 2.6.5.11.6.及び 2.6.5.11.7.に示す。ヒト肝ミクロソームにおいて未変化体及び 3 種の一酸化体（M8、M9／M22 及び M10）がみられた。その他に、いくつかのピークがみられたが、構造は特定できなかった。これらの代謝物の生成は CYP2C8 の選択的阻害剤であるモンテルカストにより最大 45%阻害されたが、その他の CYP の選択的阻害剤では明らかな阻害は見られなかった。また、CYP2C8 発現系では M2、M3、M4、M5、M6、M7、M8、M9/M22、M10 及び M17 がみられたが、M2～M7（二酸化体）はヒト肝ミクロソームでは検出されなかった。CYP3A4 発現系では M8 及び M9/M22 のみがみられ、5%程度が代謝されたにすぎなかった。CYP1A2、CYP2C9、CYP2C19 及び CYP2D6 発現系では代謝物は検出されなかった。

更に、ダプロデュスタットの酸化的代謝への CYP2C8 及び CYP3A4 の寄与率は、それぞれ 95%及び 5%であった。

これらのことから、in vitro においてダプロデュスタットの酸化的代謝には主に CYP2C8 が関与し、CYP3A4 の寄与はわずかあることが示唆された。

2.6.4.4.1.1.10.　反応性代謝物の生成

ヒト肝ミクロソームを NADPH の非存在及び存在下で[14C]標識体（10 μM）と 37°C で 60 分インキュベート後に試料をろ過し、ろ過フィルター上に保持された放射能（共有結合した分子）を測定することにより、反応性代謝物が生成される可能性を検討した（CD2007/01464）。なお、陽性対照にはアセトアミノフェンを用いた。

試験成績を 2.6.5.11.8.に示す。ろ過フィルター上に保持された放射能は、NADPH 非存在及び存在下でそれぞれ 1.74±1.05 及び 0 pmol/mg protein であり、NADPH 依存性の結合がみられなかった。なお、アセトアミノフェンでは NADPH 非存在及び存在下でそれぞれ 1.50±5.65 及び 212±14.7 pmol/mg protein であった。

このことから、この試験条件下ではダプロデュスタットの反応性代謝物が生成される可能性は極めて低いことが示された。

2.6.4.4.1.2.　ダプロデュスタットによる酵素誘導・阻害

2.6.4.4.1.2.1.　CYP 誘導

培養ヒト肝細胞及びダプロデュスタット（0.1～100 μM）を 48 時間インキュベートしたときの CYP1A2、2B6 及び 3A4 の mRNA レベルに対する影響を評価した（CD2008/01088）。

試験成績を 2.6.5.13.1.に示す。ダプロデュスタットにより、CYP1A2、2B6 及び 3A4 の mRNA レベルに明らかな増加は認められなかった。

このことから、検討した濃度範囲内においてダプロデュスタットは CYP1A2、2B6 及び 3A4 を誘導しないと考えられた。

2.6.4.4.1.2.2.　ヒト肝ミクロソームでの CYP 阻害（予備検討）

ダプロデュスタット（0.1～100 μM）が CYP1A2、2C9、2C19、2D6 及び 3A4 を直接的あるいは時間依存的に阻害する可能性を、ヒト肝ミクロソームを用いて検討した（UH2008/00018）。直接的阻害の評価では、ダプロデュスタット及びヒト肝ミクロソームを各 CYP の選択的プローブ基質と 37°C で 5 分プレインキュベートし、NADPH とインキュベートした。時間依存的阻害の評価では、NADPH 存在下で肝ミクロソーム及びダプロデュスタットを 30 分プレインキュベートし、各プローブ基質とインキュベートした。

試験成績を 2.6.5.13.2.に示す。ダプロデュスタットは CYP1A2、2C9、2C19、2D6 及び 3A4 を直接的に阻害せず（IC50：すべて 100 μM 超）、時間依存的にも阻害しなかった。

2.6.4.4.1.2.3.　ヒト肝ミクロソームでの CYP 阻害

ダプロデュスタット（0.1～100 μM）による CYP1A2、CYP2A6、CYP2B6、CYP2C8、CYP2C9、CYP2C19、CYP2D6 及び CYP3A4 の直接的及び時間依存的に阻害する可能性を、ヒト肝ミクロソームを用いて検討した（CD2008/01013）。直接的阻害の評価では、ダプロデュスタット及びヒト肝ミクロソームを各 CYP の選択的プローブ基質と約 37°C で約 5 分プレインキュベートし、NADPH とインキュベートした。時間依存的阻害の評価では、NADPH の存在下で肝ミクロソーム及びダプロデュスタットを 20 分プレインキュベートし、各プローブ基質とインキュベートした。

試験成績を 2.6.5.13.3.に示す。ダプロデュスタットは CYP2C8 を阻害し、その IC50 は 21 μM であったが、CYP2C8 を時間依存的には阻害しなかった。また、CYP1A2、CYP2A6、CYP2B6、CYP2C9、CYP2C19、CYP2D6 及び CYP3A4 に対しては、100 μM までの濃度で直接的あるいは時間依存的な阻害のいずれも示さなかった。

これらのことから、ダプロデュスタットはCYP2C8を阻害することが示された。

2.6.4.4.1.3.　代謝物による酵素阻害

2.6.4.4.1.3.1.　各種代謝物による阻害

M2（GSK2391220）、M3（GSK2531403）、M4（GSK2487818）、M5（GSK2506102）、M6（GSK2531398）及びM13（GSK2531401）によるCYPの直接的及び時間依存的な阻害をCYP1A2、CYP2C8、CYP2C9、CYP2C19、CYP2D6及びCYP3A4発現系を用いて検討した（2013N167801）。直接的及び時間依存的阻害の評価において、各代謝物（それぞれ0.1～100 μM）及びCYP発現系を各CYPの選択的プローブ基質（CYP3A4ではジエトキシフルオレセイン又は7-ベンジルオキシキノリン）と37°Cで5分プレインキュベートした。

試験成績を2.6.5.13.4.に示す。本試験条件下において、M2（GSK2391220）、M3（GSK2531403）、M5（GSK2506102）、M6（GSK2531398）及びM13（GSK2531401）はCYPを直接的あるいは時間依存的に阻害しなかった。一方、M4（GSK2487818）はCYPを直接的に阻害しなかったが、CYP2C8、CYP2C19及びCYP3A4（基質：ジエトキシフルオレセイン）を時間依存的に阻害した。

2.6.4.4.1.3.2.　M4による阻害

M4（GSK2487818、0.1～100 μM）によるCYP2C8、CYP2C19及びCYP3A4の直接的及び時間依存的な阻害を、ヒト肝ミクロソームを用いて検討した（2014N223000）。直接的阻害の評価では、M4（GSK2487818）及びヒト肝ミクロソームを各CYPの選択的プローブ基質と約37°Cで20分プレインキュベートしたのち、NADPHを添加した。時間依存的阻害の評価では、NADPHの存在下でM4（GSK2487818）及び肝ミクロソームを20分プレインキュベートしたのち、各CYPの選択的プローブ基質を添加した。

試験成績を2.6.5.13.6.に示す。M4（GSK2487818）は、CYP2C8、CYP2C19及びCYP3A4のいずれも直接的あるいは時間依存的に阻害しなかった。

2.6.4.4.1.3.3.　M3による阻害

M3（GSK2506104、0.07～100 μM）によるCYP1A2、CYP2B6、CYP2C8、CYP2C9、CYP2C19、CYP2D6及びCYP3A4の直接的及び時間依存的な阻害を、ヒト肝ミクロソームを用いて検討した（2018N382188）。直接的阻害の評価では、M3（GSK2506104）及びヒト肝ミクロソームを各CYPの選択的プローブ基質と約37°Cで30分プレインキュベートしたのち、NADPHを添加した。時間依存的阻害の評価では、NADPHの存在下でM3（GSK2506104）及び肝ミクロソームを30分プレインキュベートしたのち、各CYPの選択的プローブ基質を添加した。

試験成績を2.6.5.13.5.に示す。M3（GSK2506104）は、CYP1A2、CYP2B6、CYP2C8、CYP2C9、CYP2C19、CYP2D6及びCYP3A4のいずれも直接的あるいは時間依存的に阻害しなかった。

2.6.4.4.2.　Ex Vivo 試験

2.6.4.4.2.1.　代謝物の立体異性体の検討

M3 及び M13 はキラル中心を持つため、立体異性体として、M3 には GSK2506104 及び GSK2531403 が、M13 には GSK2531401 及び GSK2531400 が存在する可能性がある。このことから、ヒト及びマウス血漿中でのこれらの立体異性体を確認し、更に立体異性体の生体内での変換（すなわち M3 では GSK2506104 から GSK2531403 への変換、及び M13 では GSK2531401 から GSK2531400 への変換）の可能性を評価した（2014N221116）。ヒト血漿は PPO116097 試験（2014N194711）から、マウス血漿はダプロデュスタットと M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）を同時投与した 13 週間毒性試験（2014N199031）から得た検体を用いた。

試験成績を 2.6.5.10.11.に示す。ヒト血漿中では、M3 は GSK2506104 及び GSK2531403 の両方が存在したが、GSK2531403 は GSK2506104 の 10%未満であり、割合に経時的な変化はなかった。このことから、ヒト生体内で M3 の立体異性体への変換はないことが示された。また、ヒト血漿中には M13 は GSK2531401 のみが存在した。

マウスにダプロデュスタットと代謝物 M2、M3 及び M13 を投与したときの血漿中には、投与した M3 及び M13 の立体異性体のみが存在した。M3 及び M13 のそれぞれに対応する立体異性体がみられなかったことから、マウス生体内で M3 及び M13 の立体異性体への変換はないことが示された。

これらのことから、ヒト及びマウスともに、血漿中 M3 及び M13 の主な立体異性体はそれぞれ GSK2506104 及び GSK2531401 であることが確認された。また、ヒト及びマウスのいずれにおいても生体内での立体異性体への変換はないことが示された。

2.6.4.4.3.　In Vivo 試験

2.6.4.4.3.1.　マウス

2.6.4.4.3.1.1.　反復投与したときの血漿中代謝物

マウスにダプロデュスタットの 30 mg/kg/日を反復経口投与したときの血漿中代謝物を検討した（2010N109722）。なお、検討には別試験（CD2009/00900）で得た血漿を用いた。

試験成績を 2.6.5.10.1 に示す。血漿中には未変化体及び一酸化体（M8、M9 及び M22）がみられた。

2.6.4.4.3.2.　ラット

2.6.4.4.3.2.1.　単回経口投与したときの血漿、胆汁及び尿糞中代謝物

雌雄の未処置及び雄の BDC ラットに[14C]標識体の 10 mg/kg を単回経口投与したときの血漿（未処置ラットのみ）、胆汁（BDC ラットのみ）及び尿糞中の代謝物を検討した（CD2008/01663）。なお、ラットの排泄に関する成績は、2.6.4.5.1.に示す。

試験成績を 2.6.5.10.3.及び 2.6.5.10.8.に示す。雌雄の未処置ラットの血漿中には未変化体のみが認められた。雄の未処置ラットの尿糞中の主な成分は代謝物であり、糞中では一酸化体及び二酸化体が投与量の 46%、尿中では 6%であった。代謝物は一酸化体（M8 及び M9）が

二酸化体よりも多かった。糞及び尿中の未変化体はそれぞれ投与量の 33 及び 1%であった。雌の未処置ラットの代謝プロファイルは雄と同様であった。

雄の BDC ラットの糞中未変化体は投与量の 33%であり、未吸収の成分であると考えられた。また、胆汁及び尿中未変化体はそれぞれ 18 及び 0.3%であった。胆汁中の代謝物（一酸化体及び二酸化体）は 20%、尿中には 2%がみられた。未処置ラットと同様に、代謝物は一酸化体（M8 及び M9）が二酸化体よりも多かった。

これらのことから、ラットの血漿中には未変化体のみがみられ、尿糞及び胆汁中には酸化体及び未変化体がみられた。

2.6.4.4.3.2.2.　反復経口投与したときの血漿中代謝物

雌雄ラットにダプロデュスタットの 20 mg/kg/日を反復経口投与したときの血漿中代謝物を検討した（2010N109722）。なお、検討には別試験（CD2009/00951）で得た血漿を用いた。

試験成績を 2.6.5.10.1 に示す。血漿中には未変化体及び一酸化体（M9 及び M22）がみられた。血漿中の代謝物プロファイルに性差はみられなかった。

2.6.4.4.3.3.　ウサギ

2.6.4.4.3.3.1.　反復経口投与したときの血漿中代謝物

雌雄ラットにダプロデュスタットの 60 mg/kg/日を反復経口投与したときの血漿中代謝物を検討した（2010N109722）。なお、検討には別試験（CD2009/00390）で得た血漿を用いた。

試験成績を 2.6.5.10.1 に示す。血漿中には未変化体、一酸化体及び二酸化体（M2、M3、M4、M5、M8、M9、M10 及び M22）がみられた。ウサギの血漿中の代謝物プロファイルはサルと類似していたが、ウサギではサルでみられた M6、M7、M13 及び M21 はみられなかった。

2.6.4.4.3.4.　イヌ

2.6.4.4.3.4.1.　単回経口投与したときの血漿、胆汁及び尿糞中代謝物

雌雄の未処置イヌ及び雄の BDC イヌに[14C]標識体の 20 mg/kg を単回経口投与したときの血漿（未処置イヌのみ）、胆汁（BDC イヌのみ）及び尿糞中代謝物を検討した（CD2008/01662）。なお、イヌの排泄に関する成績は、2.6.4.5.2.に示す。

試験成績を 2.6.5.10.4.及び 2.6.5.10.9.に示す。雌雄の未処置イヌの血漿中には未変化体のみがみられた。雌雄の未処置イヌ及び雄の BDC イヌの糞中にも未変化体のみがそれぞれ投与量の 80%超（雌雄）及び 74%認められ、BDC イヌの成績から多くが未吸収の成分であると考えられた。また、雌雄の未処置イヌの尿中排泄率は低く、主に未変化体として排泄された。雄の BDC イヌの胆汁中でも主に未変化体（6%）がみられた。雌の未処置イヌの代謝プロファイルは雄と同様であった。

2.6.4.4.3.5.　サル

2.6.4.4.3.5.1.　反復経口投与したときの血漿中代謝物

サルにダプロデュスタットの 100 mg/kg/日を反復経口投与したときの血漿中代謝物を検討した（2010N109722）。なお、検討には別試験（CD2009/00639 及び CD2009/00780）より得た血漿を用いた。

試験成績を 2.6.5.10.1 に示す。血漿中には未変化体、一酸化体（M8、M9、M10 及び M22）、二酸化体（M2、M3、M4、M5、M6、M7 及び M21）及び三酸化体（M13）がみられた。M9 及び M22 を除き、血漿中に同定された代謝物濃度に反復投与で明らかな増加はみられなかったが、M9 及び M22 は投与 1 日目と 14 日目でわずかに増加した。

2.6.4.4.3.5.2.　単回経口投与したときの血漿、胆汁及び尿糞中代謝物

雄の未処置サル及び BDC サルに[14C]標識体の 10 mg/kg を単回経口投与したときの血漿（未処置サルのみ）、胆汁（BDC サルのみ）及び尿糞中代謝物を検討した（2018N386289）。血漿は投与後 1、4、8 及び 24 時間で採血した。なお、サルの排泄に関する成績は、2.6.4.5.1.3.に示す。

試験成績を 2.6.5.10.5.に示す。未処置サルの投与後 1～24 時間の血漿中には主に未変化体がみられ（血漿中放射能の 47%超）、M18（グルクロン酸抱合体）が 20%超でみられた。その他に、一酸化体（M8、M9、M10 及び M22）及び二酸化体（M2）がわずかにみられた。糞中には主に未変化体が投与量の 39%みられ、その他に複数の酸化体が合計で 30%みられた。

BDC サルの糞中には未変化体が投与量の 17%認められ、未吸収の成分であると考えられた。尿中には主に酸化体として排泄され、グルクロン酸抱合体は少なく、未変化体はわずかであった。胆汁中には未変化体が胆汁中放射能の 33%、酸化体が 46%、複数のグルクロン酸抱合体（M18～M20、M25～M29）が約 20%排泄された。しかしながら、糞中にはグルクロン酸抱合体は認められないことから、胆汁に分泌されたグルクロン酸抱合体は腸内細菌叢により加水分解されたと考えられた。

2.6.4.4.3.6.　ヒト

2.6.4.4.3.6.1.　単回経口投与したときの血漿及び尿中代謝物（予備検討）

健康成人にダプロデュスタットの最大 300 mg を単回経口投与したときの血漿及び尿中代謝物を予備的に検討した（2010N109720）。検討に使用した血漿及び尿は第 1 相試験（PHX111427 試験、ZM2009/00008）より得た。

試験成績を 2.6.5.10.6.に示す。投与後 0～8 時間のプール血漿中には未変化体に加え、一酸化体（M8、M9、M10 及び M22）、二酸化体（M2、M3、M4、M5、M6、M7 及び M21）並びに三酸化体（M13）がみられた。その他に一酸化反応及び脱水素反応による生成物（M17）並びに一酸化反応と更なる酸化反応／脱水素反応の組み合わせによる 3 種の代謝物（M14、M15 及び M16）が含まれていた。投与後 8～24 時間のプール血漿中には、未変化

体に加え、M2、M3 及び M13 がみられた。血漿中代謝物はすべて、尿中においても検出された。

以上、ヒトにダプロデュスタットを経口投与したときの血漿中には未変化体及び複数の酸化体（一酸化体、二酸化体及び三酸化体）がみられた。その他に、尿中にはグルクロン酸抱合体（M18、M19 及び M20）がみられた。

2.6.4.4.3.6.2.　単回経口投与したときの血漿、胆汁及び尿糞中代謝物

健康成人に[14C]標識体の 50 μg を単回静脈内に、及びダプロデュスタットの 6 mg を単回経口で併用投与後に、[14C]標識体の 25 mg を単回経口投与したときの血漿、尿糞及び胆汁中代謝物を検討した（2018N376203）。

試験成績を 2.6.5.10.7.に示す。[14C]標識体の 25 mg を単回経口投与したときの投与後 0～8 時間のプール血漿中には主に未変化体がみられ、血漿中放射能の 40%であった。ほとんどの代謝物はシクロヘキシル環の水酸化体であり、M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）が血漿中放射能の 7.6～8.3%と多く、次いで M4（GSK2487818）、M5（GSK2506102、M14 と co-elute）及び M6（GSK2531398）が 3.6～5.7%であり、その他に M15 及び M33 等も認められた。更に、尿中には投与量の 21%が排泄され、一酸化体、二酸化体及び、三酸化体がみられ、未変化体及びグルクロン酸抱合体は検出されなかった。また、糞中には 74%が排泄され、このうち未変化体は 0.5%であった。血漿及び尿糞中の主な代謝物は同様であった。

微量の[14C]標識体を単回静脈内投与したときの胆汁中の代謝物は、経口投与後の糞中の代謝物と同様であった。このことから、ダプロデュスタットを経口投与したとき、大部分が吸収され、主に酸化的代謝により体内から消失し、肝胆道系及び尿中排泄経路を介して排泄されると考えられた。

表 2.6.4.4-1 代謝試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Blood stability	Human	NA	In vitro	A	1, 10 µg/mL	NA	No	GSK	UH2008/00002	m4.2.2.4
Clearance in liver microsomes	Mouse, Rat, Dog, Monkey, Human	NA	In vitro	A	0.5 µM	NA	No	GSK	UH2008/00018	m4.2.2.4
Clearance in hepatocytes	Mouse, Rat, Dog, Monkey, Human	NA	In vitro	A	0.5 µM	NA	No	GSK	UH2008/00018	m4.2.2.4
Bioactivation in liver microsomes	Human	NA	In vitro	[14C]	10 µM	NA	No	GSK	CD2007/01464 (07DMM137)	m4.2.2.4
Qualitative identification of metabolites following incubation with human CYP enzymes	Human	NA	In vitro	A	10 µM	NA	No	GSK	UH2008/00018	m4.2.2.4
Preliminary identification of metabolites following incubation with hepatocytes	Mouse, Rat, Dog, Monkey, Human	NA	In vitro	A	10 µM	NA	No	GSK	UH2008/00018	m4.2.2.4
Metabolism in hepatocytes	Mouse, Rat, Rabbit, Dog, Monkey, Human	NA	In vitro	[14C]	12.5 µM	NA	No	GSK	CD2008/01286 (07DMM139)	m4.2.2.4
Metabolism in hepatocytes	Minipig (Göttingen)	NA	In vitro	A	19 µM	NA	No	GSK	2011N125815 (11DMM021)	m4.2.2.4
Metabolism in hepatocytes	Hamster (Syrian)	NA	In vitro	A	20 µM	NA	No	GSK	2013N160562 (12DMM025)	m4.2.2.4

表 2.6.4.4-1 代謝試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administ-ration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolism in rat liver S9	Rat liver S9	NA	In vitro	[14C]	4, 40, 400 μM	NA	No	GSK	2011N114076 (10DMM007)	m4.2.2.4
Oxidative enzymology in liver microsomes	Human	NA	In vitro	[14C]	5 μM	NA	No	GSK	CD2010/00300 (09DMM051)	m4.2.2.4
CYP induction in hepatocytes	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/01088 (08DMM042)	m4.2.2.4
Inhibition of human CYP enzymes in liver microsomes	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	UH2008/00018	m4.2.2.4
Inhibition of human CYP enzymes in liver microsomes	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/01013 (07DMM149)	m4.2.2.4
Metabolism in isolated perfused rat liver	Rat (Sprague Dawley)	3	Ex vivo	[14C]	30	NA	No	GSK	CD2008/00941 (07DMM151	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	3M/3F; 3M (BDC)	Oral (gavage)	[14C]	10	Single	No	GSK	CD2008/01663 (07DMM161)	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	14M	Oral (gavage)	[14C]	10	Single	No	GSK	CD2008/00941 (07DMM151	m4.2.2.4
In vivo metabolism	Dog (beagle)	3M/3F; 3M (BDC)	Oral (gavage)	[14C]	20	Single	No	GSK	CD2008/01662 (07DMM160)	m4.2.2.4
Metabolite characterization	Mouse, Rat, Rabbit, Monkey[a], Human[b]	NS	Oral	A	NS	NS	NS	GSK	2010N109722 (09DMM062)	m4.2.2.4

表 2.6.4.4-1　代謝試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolite characterization	Monkey (cynomolgus)	3M; 2M (BDC)	Oral (gavage)	[14C]	10	Single (Up to 168 hours)	No	[redacted]	2018N386289 (8361764)	m4.2.2.4
Metabolite characterization	Human[c]	4 (NS)	Oral	A	300 mg	Single	No	GSK	2010N109720 (08DMM117)	m4.2.2.4
Metabolite characterization	Human	4M	IV Oral Oral	[14C] A [14C]	50 µg 6 mg 25 mg	Single	No	GSK	2018N376203 (17DMM043)	m4.2.2.4
Studies with Metabolites										
Inhibition of human CYP enzymes	Human	NA	In vitro	D, E, G, J, K, N[c]	0.0 to 100 µM	NA	No	GSK	2013N167801 (12DMM032)	m4.2.2.4
Inhibition of CYP Enzymes by GSK2506104 (M13)	Human	NA	In vitro	E[d]	0.0729 to 100 µM	NA	No	[redacted]	2018N382188 (180538)	m4.2.2.4
Inhibition of CYP Enzymes by GSK2487818 (M4)	Human (Liver Microsomes)	NA	In vitro	G[e]	0.1 to 100 µM	NA	No	GSK	2014N223000 (13DMM003)	m4.2.2.4
Determination of stereoisomeric forms & stereoisomeric conversion	Mouse (plasma) Human (plasma)	NA	Ex vivo	A[f] A[g]	NA	NA	No	GSK	2014N221116 (14DMM007)	m4.2.2.4

表 2.6.4.4-1　代謝試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD

Key:
A = Daprodustat (Parent form).　D = M2 metabolite of daprodustat, GSK2391220 (Parent form).
E = M3 metabolite of daprodustat, GSK2506104 (Parent form).　G = M4 metabolite of daprodustat, GSK2487818 (Parent form).
J = M5 metabolite of daprodustat, GSK2506102 (Parent form).　K = M6 metabolite of daprodustat, GSK2531398 (Parent form).
N = M13 metabolite of daprodustat, GSK2531401 (Parent form).　[14C] = 14C labelled daprodusat.　BDC = Bile duct cannulated.
CYP = Cytochrome P450.　NA = Not applicable.　NS = Not specified.
a = Samples obtained during toxicity studies CD2009/00639/00, CD2009/00780/00, CD2009/00900/00, CD2009/00390/00 and CD2009/00951/00.
b = Samples obtained from clinical study PHX111427 (GSK Document Number ZM2009/00008/00).
c = Study assessed metabolites M2 (GSK2391220), M3 (GSK2531403), M4 (GSK2487818), M5 (GSK2506102), M6 (GSK2531398), M13 (GSK2531401).
d = Study assessed metabolite M3 (GSK2506104).
e = Study assessed metabolite M4 (GSK2487818).
f = Daprodustat was administered in vivo, but metabolites M2 (GSK2391220), M3 (GSK2506104) and M13 (GSK2531401) were monitored in the study.
g = Daprodustat was administered in vivo, but stereoconversion between metabolite form for M3 (GSK2506104 to GSK2531403) and M13 (GSK2531401 to GSK2531400) were monitored in the study.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]

2.6.4.5.　排泄

ラット、イヌ及びサルにダプロデュスタットを経口投与したときの排泄を検討した。試験の一覧を表 2.6.4.5-2 に示す。

2.6.4.5.1.　ラット

雌雄の未処置ラット及び雄 BDC ラットに[14C]標識体の 10 mg/kg を単回経口投与したときの、放射能の尿糞中及び胆汁中への排泄を検討した（CD2008/00100）。

試験成績を表 2.6.4.5-1 及び 2.6.5.14.1.並びに 2.6.5.15.1.に示す。雌雄の未処置ラットでは、放射能の大部分が糞中に排泄され、それぞれ投与量の 87.5 及び 86.3%であり、尿中には、それぞれ 8.74 及び 10.5%が排泄された。雌雄ラットの投与 168 時間後までの放射能の総回収率は、それぞれ投与量の 96.9 及び 97.6%であり、排泄に明らかな性差はみられなかった。

雄の BDC ラットの胆汁には投与量の 42.0%が排泄され、糞及び尿中へは、それぞれ 45.7 及び 6.61%が排泄された。胆汁及び尿中排泄率から、経口投与した投与量の少なくとも 48.6%が吸収されたと考えられた。投与 96 時間後までの放射能の総回収率は 96.4%であった。

2.6.4.5.2.　イヌ

雌雄の未処置イヌ及び雄 BDC イヌに[14C]標識体の 20 mg/kg を単回経口投与したときの放射能の尿糞中及び胆汁中への排泄を検討した（CD2008/00025）。

試験成績を表 2.6.4.5-1 及び 2.6.5.14.1.並びに 2.6.5.15.1.に示す。雌雄の未処置イヌでは、放射能の大部分が糞中に排泄され、それぞれ投与量の 81.4 及び 86.4%であり、尿中への排泄は約 2%とわずかであった。雌雄イヌの投与 168 時間までの放射能の総回収率は、それぞれ 88.0 及び 90.2%であり、排泄に明らかな性差はみられなかった。

雄の BDC イヌの胆汁には投与量の 7.77%が排泄され、糞及び尿中へは、それぞれ 76.3 及び 0.52%が排泄された。胆汁及び尿中への排泄率から、経口投与した投与量の少なくとも 8%が吸収されたと考えられる。投与 72 時間後までの放射能の総回収率は 86.8%であった。

2.6.4.5.3.　サル

雄の未処置サル及び雄 BDC サルに[14C]標識体の 10 mg/kg を単回経口投与したときの放射能の尿糞中及び胆汁中への排泄を検討した（2018N355713）。

試験成績を表 2.6.4.5-1 及び 2.6.5.14.1.並びに 2.6.5.15.1.に示す。未処置サル及び BCD サルのいずれにおいても排泄は速やかで、投与 48 時間後でそれぞれ投与量の 85 及び 89%であった。

未処置サルでは、放射能の大部分が糞中に排泄され、尿中への排泄は比較的少なく、それぞれ 78 及び 13%であり、投与 168 時間後までの放射能の総回収率は 93%であった。

BDC サルでは、放射能の大部分が胆汁中へ排泄され、投与量の 53%であった。尿及び糞中へは、それぞれ 16 及び 24%が排泄された。胆汁及び尿中への排泄率から、経口投与した投与量の少なくとも 69%が吸収されたと考えられる。投与 168 時間までの放射能の総回収率は 94%であった。

表 2.6.4.5-1　　ラット、イヌ及びサルに[14C]標識体を経口投与したときの尿糞及び胆汁中排泄率

Report No.	Species/ Status	Sex/ Number	Route	Dose (mg/kg)	Percent of Dose Recovered in:			
					Feces	Urine	Bile	Total[a]
CD2008/00100	Rat / Intact	3M	Oral	10	86.3	10.5	-	97.6
		3F	Oral	10	87.5	8.74	-	96.9
	Rat / BDC	3M	Oral	10	45.7	6.61	42.0	96.4
CD2008/00025	Dog / Intact	3M	Oral	20	86.38	1.79	-	90.16
		3F	Oral	20	81.41	2.03	-	87.95
	Dog / BDC	2M	Oral	20	76.29	0.52	7.77	86.80
2018N355713	Monkey / Intact	3M	Oral	10	77.5	13.4	-	93.1
	Monkey / BDC	2M	Oral	10	24.1	15.8	53.0	93.9

Key:
Data shown are means
a = Includes, as appropriate, radioactivity recovered in cage rinses/washes/wipes/debris GI tract and residual carcass.
- = Not determined.
BDC = Bile duct cannulated.

2.6.4.5.4.　　ヒト

健康成人男性に[14C]標識体を単回投与したときのマスバランス試験の結果は 2.7.2.2.1.2.1.4.に示し、各試料中の代謝物については、2.6.4.4.3.6.2.及び 2.6.5.10.7.に示す（2018N376203）。

2.6.4.5.5.　　乳中への移行

ラット母動物にダプロデュスタット及び代謝物カクテル［M2（GSK2391220）、M3（GSK2506104）及び M13（GSK2531401）］を投与した出生前及び出生後の発生並びに母体の機能に関する試験（2018N365151）において、出生児（生後 10 日）の血漿中にダプロデュスタット及び 3 種のヒト代謝物が検出されたことから、本剤の乳汁移行性が示唆された（2.6.5.8.2.及び 2.6.6.6.5.1.1.）。

表 2.6.4.5-2　排泄試験一覧

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Elimination	Rat (Sprague Dawley)	15M/15F; 3M (BDC)	Oral (gavage)	[14C]	10	Single (Up to 168 hours)	Yes	[redacted]	CD2008/00100 (7717-705)	m4.2.2.5
Elimination	Dog (beagle)	3M/3F 3M (BDC)	Oral (gavage)	[14C]	20	Single (intact: up to 168 hours) (BDC: up to 72 hours)	Yes	[redacted]	CD2008/00025 (AFA00607)	m4.2.2.5
Elimination	Monkey (cynomolgus)	3M; 2M (BDC)	Oral (gavage)	[14C]	10	Single (up to 168 hours)	No	[redacted]	2018N355713 (8361764)	m4.2.2.5
Milk transfer	Rat (SD)	6F (pregnant/ lactating)	Oral (gavage) SC	A	0.8, 7, 40 2.5 (M2) 3.2 (M3) 1.8 (M13)	38 days (GD6, GD9 and LD21)	Yes	[redacted]	2018N365151 (R71242G)	m4.2.2.5

Key:
BDC = Bile duct cannulated.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]

2.6.4.6.　薬物動態学的薬物相互作用

ダプロデュスタット及びヒトでの主な代謝物である M2（GSK2391220）、M3（GSK2506104）、M4（GSK2487818）、M5（GSK2506102）、M6（GSK2531398）及び M13（GSK2531401）が CYP 又はトランスポーターの基質となる、又は阻害する可能性について in vitro 試験で評価した。また、ダプロデュスタットによる CYP 誘導についても in vitro で評価した。これらの結果から、臨床で薬物動態学的薬物相互作用が起こる可能性を検討した。

2.6.4.6.1.　ダプロデュスタットの曝露量に及ぼす影響（被相互作用薬の可能性）

In vitro において、ダプロデュスタットの酸化的代謝には主に CYP2C8 が関与し、CYP3A4 の関与はわずかであった。ダプロデュスタットと CYP2C8 阻害剤である gemfibrozil 又はトリメトプリムとの臨床薬物相互作用試験で、ダプロデュスタットの曝露量が増加することが示された（2.7.2.3.4.1.）。

In vitro において、ダプロデュスタットは BCRP の基質であることが示された。このため、ダプロデュスタットを強力な BCRP 阻害剤と併用した場合、ダプロデュスタットの曝露量が増加する可能性が考えられたが、母集団薬物動態解析により、BCRP 阻害剤はダプロデュスタットの薬物動態に影響を与えないと考えられた（2.7.2.3.1.2.1.）。

ヒトでの 6 種の主な代謝物の OATP1B1 及び OATP1B3 による輸送を OATP1B1 及び OATP1B3 発現細胞を用いて検討した結果、M4 が最も OATP1B1 及び OATP1B3 で輸送された。しかしながら、M4 はヒト肝細胞において主に受動的に肝に取り込まれると考えられたことから、代謝物が OATP1B1 及び OATP1B3 を介した薬物相互作用を起こす可能性は低いと考えられた。

In vitro において、M3 及び M13 は OAT3 の基質で、M2 は OAT1 及び OAT3 の弱い基質であった。ヒトでの M2、M3 及び M13 の CLr（PHI115573 試験）は、血漿蛋白非結合率で補正した糸球体ろ過率（fu*GFR）よりも低かったことから、これらトランスポーターを介した尿細管分泌はわずかであると考えられ、腎トランスポーターを介した薬物相互作用の可能性は低いと考えられた。

2.6.4.6.2.　併用薬の曝露量に及ぼす影響（相互作用薬の可能性）

In vitro において、ダプロデュスタットは CYP2C8 を阻害し、IC50 は 21 μM（8.3 μg/mL）であった。臨床推定用量での血漿中ダプロデュスタット濃度（0.485 μg/mL、表 2.6.4.8-1）を考慮すると、ダプロデュスタットの CYP2C8 阻害作用により臨床で薬物相互作用が起こる可能性は低い[MHLW, 2018]と考えられた。CYP2C8 の基質であるピオグリタゾンと併用投与した臨床薬物相互作用試験において、ダプロデュスタットはピオグリタゾンの曝露量に影響を及ぼさなかった（2.7.2.2.1.3.2.）。In vitro において、ダプロデュスタットは CYP1A2、CYP2B6 及び CYP3A4 を誘導しなかった。

ヒトでの 6 種の主な代謝物による P-gp 及び BCRP の阻害作用は検討していないものの、代謝物は主に肝の CYP2C8 によって生成されることから、主に腸管での吸収に関わる P-gp 及び BCRP の阻害による代謝物の薬物動態への影響は小さいと考えられる。In vitro におい

て、ダプロデュスタットは OATP1B1 及び OATP1B3 を阻害したが、臨床薬物相互試験でダプロデュスタットはロスバスタチンの曝露量に影響を及ぼさなかったことが確認されている（2.7.2.2.1.3.2.）。

2.6.4.7.　その他の薬物動態試験

該当なし。

2.6.4.8.　考察及び結論

ウサギ及びサルでのダプロデュスタットの代謝物プロファイルは、ヒトと類似していた。その他の動物ではヒトで生成されるダプロデュスタットの主な代謝物の一部しか生成されないため、マウス及びラットを用いた安全性及び薬物動態試験ではヒトでの主な 3 種の代謝物［GSK2391220（M2）、GSK2506104（M3）及び GSK2531401（M13）］を投与し、安全性プロファイルを検討した。ダプロデュスタットを経口投与したときのダプロデュスタットの動物／ヒト曝露量比を表 2.6.4.8-1 に、ヒトでの主な代謝物の動物／ヒト曝露量比を表 2.6.4.8-2 に示す。

ダプロデュスタット（溶液）を経口投与したときの F は、マウスで 88%及びラットで 100%と高く、イヌで 46%及びサルで 34%と中程度であった。また、イヌにダプロデュスタット（懸濁液）を経口投与したときの F は溶液よりも概ね低く、ダプロデュスタットの溶解性が低いことによると考えられた。マウス、ラット、イヌ及びサルに静脈内投与したときのダプロデュスタットの CL は低く、Vss は総体液量[Davies, 1993]よりも小さいか、同程度であった。

マウス、ラット、イヌ及びサルに反復経口投与したときのダプロデュスタットの曝露量は、概して投与量増加の割合を下回って増加し、単回及び反復投与の曝露量に明らかな差はなく、また明らかな性差もみられなかった。

ダプロデュスタットは速やかに広く組織に分布し、組織内放射能は血液中放射能よりも低く、脳内放射能は血漿中放射能の約 2%であった。また、放射能は組織から速やかに消失した。

In vitro において、動物及びヒトでのダプロデュスタットの血漿蛋白結合率は高く、それぞれ 93.6%以上及び 99%超であった。動物及びヒトでの血液／血漿比は、それぞれ 0.45～1.64 及び 0.75～1.23 であり、血球との結合は低いことが示された。

ヒトに[14C]標識体を経口投与したとき、血漿中の主な成分は未変化体であった。CKD 患者にダプロデュスタットを投与した臨床成績（200942 試験及び PHI115573 試験）から、定常状態での血漿中に主な 3 種の代謝物（M2、M3 及び M13）が認められ、それぞれ DRM の 10%超であり、次いで 3 種の代謝物（M4、M5 及び M6）が 10%未満であった。

ヒト肝ミクロソームを用いた in vitro 試験において、ダプロデュスタットが反応性代謝物を生成する可能性は極めて低いことが示された。

ラット、イヌ及びサルに[14C]標識体を経口投与したときの主な排泄経路は、糞中であった。BDC ラット、BDC イヌ及び BDC サルに[14C]標識体を経口投与したとき、ラット及びサルでは放射能は主に胆汁中に排泄され、イヌでは一部が胆汁中に排泄された。ラット、イ

ヌ及びサルの胆汁中には、それぞれ投与量の約 42、8 及び 53%が排泄され、胆汁及び尿中排泄率より、それぞれ投与量の少なくとも 49、8 及び 69%が吸収されたと考えられた。

In vitro において、ダプロデュスタットの酸化的代謝には CYP2C8 が 95%寄与し、CYP3A4 の寄与は 5%とわずかであったことから、CYP2C8 阻害又は誘導剤との併用投与により薬物相互作用が生じる可能性が考えられた。CYP2C8 阻害剤である gemfibrozil 又はトリメトプリムと併用投与した臨床薬物相互作用試験において、ダプロデュスタットの曝露量が増加することが示された（2.7.2.3.4.1.）。

In vitro で、ダプロデュスタットは CYP2C8 を阻害し、その IC50 は 21 μM であった。しかしながら、CYP2C8 の基質であるピオグリタゾンと併用投与した臨床薬物相互作用試験において、ダプロデュスタットはピオグリタゾンの曝露量に影響を及ぼさなかった（2.7.2.2.1.2.4.2.）。

ヒト肝細胞において、ダプロデュスタットによる CYP1A2、CYP2B6 又は CYP3A4 の誘導は認められなかった。

また、ダプロデュスタットは BCRP の基質であったが、母集団薬物動態解析により、BCRP 阻害剤はダプロデュスタットの薬物動態に影響を与えないと考えられた（2.7.2.3.1.2.1）。

In vitro でダプロデュスタットは OATP1B1 及び OATP1B3 を阻害し、その IC50 はそれぞれ 6 及び 11 μM であった。しかしながら、OATP1B1 及び OATP1B3 の基質であるロスバスタチンと併用投与した臨床薬物相互作用試験で、ダプロデュスタットはロスバスタチンの曝露量に影響を及ぼさなかった（2.7.2.2.1.3.2.）。

表 2.6.4.8-1　ダプロデュスタットの経口投与時の動物／ヒト曝露量比

動物種 (投与期間) [報告書番号]	投与量[b] (mg/kg/日)	性	Cmax (μg/mL)	AUC(0-24h) (μg·hr/mL)	動物/ヒト 曝露量比[a]	
			投与期間終了時[c]		Cmax	AUC
マウス (13 週間) [2011N112096]	**3 (無毒性量)**	♂♀	**14.5**	**181**	**29.9**	**153**
	30	♂♀	50.7	669	105	565
	60	♂♀	60.5	833	125	704
マウス (2 年間)[h] M2,M3,M13 併用 [2014N221714]	0.2	♂♀	1.35	20.6	2.78	17.4
	0.8[g] (無毒性量)	♂♀	**4.93**	**68.0**	**10.2**	**57.4**
	3	♂♀	14.9	238	30.7	201
ラット (26 週間)[d] [2011N126130]	0.8	♂♀	14.5	233	29.9	197
	4 (無毒性量)	♂♀	**46.8**	**785**	**96.5**	**663**
	10[d]	♂♀	75.0	1377	155	1163
ラット (2 年間) [2014N196080]	0.02	♂♀	0.484	8.47	0.998	7.15
	0.1	♂♀	2.64	40.9	5.44	34.5
	0.8[g] (無毒性量)	♂♀	**18.0**	**274**	**37.1**	**231**
	4	♂	58.0	889	120	751
	7	♀	83.5	1430	172	1208
ラット (胚・胎児発生) [CD2009/00388]	**7[f] (無毒性量)**	♀	**69.7**	**1086**	**144**	**917**
ラット (出生前後発生)[h] M2,M3,M13 併用 [2018N365151]	0.8	♀	8.51	146	17.5	123
	7 (無毒性量)	♀	**52.0**	**855**	**107**	**722**
	40	♀	128	2240	264	1892
ウサギ (胚・胎児発生)[h] [CD2009/00390]	4	♀	1.49	22.8	3.07	19.3
	30	♀	9.24	133	19.1	112
	60 (無毒性量)	♀	**11.8**	**197**	**24.3**	**166**
イヌ (13 週間) [CD2009/00952]	1	♂♀	1.31	7.73	2.70	6.53
	3 (無毒性量)	♂♀	**2.34**	**19.1**	**4.82**	**16.1**
	30/15[e]	♂♀	NA	NA	NA	NA
サル (13 週間) [2010N108482]	5	♂♀	0.758	4.55	1.56	3.84
	20 (無毒性量)	♂♀	**1.40**	**14.8**	**2.89**	**12.5**
	100	♂♀	3.34	43.3	6.89	36.6
サル (39 週間) [2011N126131]	**3 (無毒性量)**	♂♀	**0.655**	**3.49**	**1.35**	**2.95**
	10	♂♀	1.30	9.46	2.68	7.99
	50	♂♀	2.58	29.8	5.32	25.2
ヒト(推定)[a]	24 mg/日	♂♀	0.485	1.184	NA	NA

注：太字は無毒性量を示す。表中の曝露量は、雌雄それぞれにつきマウス及びウサギ（コンポジットサンプリング）では n=2～3、ラット、イヌ及びサルでは n=3～6 の平均値。NA = 該当せず

a. 動物の曝露量（投与期間終了時又は最終測定日）と、定常状態のヒト推定曝露量（Cmax 及び AUC）を用いて算出した。血液透析患者（日本人、体重 54 kg）に 24 mg 投与したときの定常状態（非透析日）における曝露量を推定した（2019N405983）

b. イヌの 13 週間投与試験（カプセル投与）を除き、強制経口投与によりダプロデュスタットを投与した

c. 試験終了時の TK 測定値を示した。ラットの 2 年間投与試験、マウスの 13 週間投与試験及び 2 年間投与試験、並びにウサギの胚・胎児発生試験については、それぞれ試験 26 週、4 週及び 26 週、並びに妊娠 11 日（投与 5 日目）の測定値を示した

d. 雌のみの値。雄は試験 20 週に生存率低下のため安楽死させた

e. 試験終了前に死亡又は一般状態悪化のために安楽死させた

f. ラットの 4 週間経口投与試験の試験 4 週の曝露量

g. ラット 2 年間投与試験（ダプロデュスタット単独経口投与）及びマウス 2 年間投与試験（ダプロデュスタットを経口投与、更に 3 種のヒト代謝物を皮下投与）における非腫瘍性所見についての無毒性量

h. 3 種のヒト代謝物（M2、M3 及び M13）の曝露量については表 2.6.4.8-2 参照

表 2.6.4.8-2　サル及び妊娠ウサギへのダプロデュスタットの経口投与又はマウス及び妊娠ラットへの直接皮下投与によるヒト代謝物の動物／ヒト曝露量比

代謝物	動物種	ダプロデュスタット経口投与量（マウス及びラットは代謝物の皮下投与量）(mg/kg/日)	平均全身曝露量 サル (試験 39 週) ウサギ (妊娠 11 日) ラット (妊娠 9 日) マウス (試験 26 週 [b])		動物/ヒト曝露量比 [a]	
			Cmax (µg/mL)	AUC(0-24h) (µg·hr/mL)	Cmax	AUC
GSK2391220 (M2)	サル	3 (39 週間、無毒性量)	0.0312	0.266	0.32	0.21
		50 (39 週間、最大耐量)	0.0899	1.27	0.91	0.99
	ウサギ	60 (胚・胎児発生、無毒性量)	1.02	11.7	10.4	9.11
	ラット	2.5 M2 (出生前後発生) [c]	1.55	2.50	15.7	1.95
	マウス	2 M2 (2 年間) [b]	3.87	1.66	39.3	1.29
	ヒト [a] (推定)	24 mg/日	0.0985	1.285	NA	
GSK2506104 (M3)	サル	3 (39 週間、無毒性量)	0.0110	0.0789	0.10	0.04
		50 (39 週間、最大耐量)	0.0329	0.468	0.29	0.27
	ウサギ	60 (胚・胎児発生、無毒性量)	0.222	2.69	1.98	1.53
	ラット	3.2 M3 (出生前後発生) [c]	1.72	2.47	15.4	1.40
	マウス	2.5 M3 (2 年間) [b]	4.55	1.84	40.6	1.04
	ヒト [a] (推定)	24 mg/日	0.1120	1.761	NA	
GSK2531401 (M13)	サル	3 (39 週間、無毒性量)	0.00642	0.0362	0.14	0.04
		50 (39 週間、最大耐量)	0.0171	0.247	0.38	0.28
	ウサギ	60 (胚・胎児発生、無毒性量)	0.0158	0.194	0.35	0.22
	ラット	1.8 M13 (出生前後発生) [c]	1.19	1.94	26.4	2.24
	マウス	1.3 M13 (2 年間) [b]	2.70	1.34	59.9	1.54
	ヒト [a] (推定)	24 mg/日	0.0451	0.868	NA	

注：　NA = 該当せず

a. 動物の曝露量（投与期間終了時又は最終測定日）と、定常状態のヒト推定曝露量（Cmax 及び AUC）を用いて算出した。血液透析患者（日本人、体重 54 kg）に 24 mg 投与したときの定常状態（非透析日）における曝露量を推定した（2019N405983）

b. マウス 2 年間がん原性試験の試験 26 週における代謝物の平均曝露量。投薬群には 3 種のヒト代謝物（M2: 2.0 mg/kg/日、M3: 2.5 mg/kg/日、M13: 1.3 mg/kg/日）の混合液を 1 日 1 回皮下投与し、更に、0.2、0.8 又は 3 mg/kg/日のダプロデュスタットを経口投与した。表中の値はダプロデュスタットが投与された 3 群の平均値（3 群ともに代謝物の投与量は同じ)

c. ラットの出生前後発生に関する試験の 7 mg/kg/日（＋代謝物）（無毒性量）群の妊娠 9 日における代謝物の平均曝露量

Data source: 2014N221714, 2018N365151, 2012N140611, 2011N126131

2.6.4.9.　図表

本文中に記載した。

2.6.4.10.　参考文献

Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharm Res. 1993;10:1093-5.

GlaxoSmithKline Document Number 2012N137995 Study ID NA. Chiral method development and analysis of GSK1278863A metabolites in human urine isolates. Report Date 30-Nov-2018.

GlaxoSmithKline Document Number 2014N194711 Study ID NA. Quantitation of GSK1278863, GSK2391220, GSK2531403, GSK2487818, GSK2506102, GSK2531398, and GSK2531401 in Human Plasma from Study PPO116097 via UPLC® with MS/MS Detection. Report Date 1-May-2015.

GlaxoSmithKline Document Number 2015N246875 Study ID NA. Population pharmacokinetic and pharmacodynamic analysis of GSK1278863 and its metabolites in non-dialysis dependent and hemodialysis dependent patients with chronic kidney disease. Report Date 7-Dec-2015.

GlaxoSmithKline Document Number 2015N247095 Study ID PPO116097. A Phase II, Randomized, Placebo Controlled, Double-Blind (Sponsor Open) Study of GSK1278863, a HIF-Prolyl Hydroxylase Inhibitor, to Reduce Ischemic Events in Patients Undergoing Thoracic Aortic Aneurysm Repair. Report Date 14-Feb-2017.

GlaxoSmithKline Document Number 2017N338206 Study ID 200942. A repeat-dose, open-label, parallel-group study to assess the pharmacokinetics of GSK1278863 and metabolites in subjects with End Stage Renal Disease undergoing peritoneal dialysis. Report Date 6-Jul-2018.

GlaxoSmithKline Document Number 2019N405983 Study ID NA. Population Pharmacokinetic Modeling, including Covariate Analysis, of Daprodustat (GSK1278863) and its Metabolites in Healthy Volunteers and Chronic Kidney Disease patients from Phase 1 and Phase 2 data. Report Date 4-Jul-2019.

MHLW. 医薬品開発と適正な情報提供のための薬物相互作用ガイドライン. Available at URL (2019.7.10): https://www.mhlw.go.jp/hourei/doc/tsuchi/T180724I0150.pdf. 2018 Jul 23:.

杉本充弘. I. 妊婦・授乳婦への薬物療法の基本的考え方. In: 杉本充弘, editor. 妊婦・授乳婦の薬 改訂 2 版. 2018:1-5.

APPENDIX 1 ANALYTICAL METHODS USED FOR THE DETERMINATION OF DAPRODUSTAT IN BIOLOGICAL FLUIDS

Mouse Assays

Five methods for the determination of daprodustat (and/or metabolites of daprodustat) in mouse plasma have been validated using HPLC-MS/MS. Daprodustat was extracted from mouse plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard. Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective, and summaries of the validation data are tabulated below.

	Report CD2009/00955 (GSK1278863MOPLVALA)	**Report 2010N109787 (GSK1278863MOPLVALB)**
Calibration model	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS interface	Turbolon Spray	Turbolon Spray
Validated range	50 to 50000 ng/mL	1 to 1000 ng/mL for all metabolites
Precision (%CV) within-assay	≤6.8%	GSK2391220: ≤4.6% GSK2531403: ≤4.0% GSK2499166: ≤4.4% GSK2531399: ≤3.2% GSK2531398: ≤3.7% GSK2531400: ≤6.2%
Precision (%CV) between-assay	NA	NA
Accuracy (% bias)	-4.0%≤%Bias≤2.2%	GSK2391220: -3.8%≤ Bias ≤13.7% GSK2531403: -2.6%≤ Bias ≤8.0% GSK2499166: -2.5%≤ Bias ≤11.8% GSK2531399: -2.0%≤ Bias ≤11.6% GSK2531398: -1.9%≤ Bias ≤12.7% GSK2531400: -1.6%≤ Bias ≤6.4%
Stability in mouse plasma	At least 24 hours at room temperature	At least 24 hours at ambient temperature At least 160 days at -20°C and -80°C (In mouse whole blood: At least 4 hours at ambient temperature)
Freeze-thaw stability	At least 3 cycles from -20°C to room temperature	At least 3 cycles from -20°C to ambient temperature
Processed extract stability	NA	At least 8 days at ambient temperature

Mouse Assays (continued)

	Report 2013N182744 (P1248)	Report 2014N198503[a] (P1248)	Report 2014N219756[a] (P1248)
Calibration model	Linear, 1/concentration^2	NA	NA
MS/MS interface	Turbo Ion Spray	NA	NA
Validated range	10 to 10000 ng/mL (GSK1278863) 0.200 to 200 ng/mL (GSK2391220, GSK2506104, and GSK2531401)	NA	NA
Precision (%CV) within-assay	≤6.33% (GSK1278863) ≤8.02% (GSK2391220) ≤12.1% (GSK2506104) ≤7.69% (GSK2531401)	NA	NA
Precision (%CV) between-assay	≤4.55% (GSK1278863) ≤7.32% (GSK2391220) ≤9.07% (GSK2506104) ≤5.26% (GSK2531401)	NA	NA
Accuracy (% bias)	-6.29 to 6.45% (GK1278863) -4.32 to 9.68% (GK2391220) -4.02 to 6.24% (GSK2506104) -4.06 to 6.16% (GSK2531401)	NA	NA
Stability in mouse plasma	16 days at -20 °C and -70 °C (all analytes)	NA	165 days at -20 °C and -70 °C
Freeze-thaw stability	Five cycles thawed at room temperature and frozen at -20 °C and -70 °C (all analytes)	NA	NA
Processed extract stability	321.75 hours at 2 to 8 °C (GSK1278863) 386 hours at 2 to 8 °C (GSK2391220, GSK2506104 and GSK2531401)	NA	NA

Key:
a = Supplemental validation for study 2013N182744.

Rat Assays

Eight methods for the determination of daprodustat (and/or metabolites of daprodustat) in rat plasma have been validated using HPLC-MS/MS. Daprodustat was extracted from rat plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard. Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective and summaries of the validation data are tabulated below.

	Report CD2007/01258 (GSK1278863RTPLVALB)	**Report 2011N122686[a] (GSK1278863RTPLVALB)**	**Report 2010N108487 ([redacted]8203526)**
Calibration model	Linear weighted $1/x^2$	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS interface	Turbolon Spray	Turbolon Spray	Turbolon Spray
Validated range	50 to 50000 ng/mL	50 to 50000 ng/mL	50 to 50000 ng/mL
Precision (%CV) within-assay	≤3.2%	≤5.8%	≤5.2%
Precision (%CV) between-assay	≤5.0%	NA	≤7.2%
Accuracy (% bias)	-6.2%≤%Bias≤12.0%	-9.5% to 6.8%	-9.0% to 4.4%
Stability in rat plasma	At least 24 hours at room temperature	NA	81 days at -20 °C
Freeze-thaw stability	At least 3 cycles at -20°C	NA	3 additional cycles
Processed extract stability	At least 96 hours at room temperature	NA	3 days at 4 °C

Rat Assays (continued)

	Report 2013N180658 (P1242)	Report 2014N219980[b] (P1242)	Report 2016N308027 (P1484)
Calibration model	Linear 1/concentration^2	Linear 1/concentration^2	Linear, 1/concentration^2
MS/MS interface	Turbo Ion Spray	Turbo Ion Spray	Turbo Ion Spray
Validated range	50 to 50000 ng/mL	50 to 50000 ng/mL	150 to 150000 ng/mL (GSK1278863) 0.200 to 200 ng/mL (GSK2391220, GSK2506104 and GSK2531401)
Precision (%CV) within-assay	≤7.05%	NA	≤8.35% (GSK1278863) ≤9.49% (GSK2391220) ≤5.99% (GSK2506104) ≤6.70% (GSK2531401)
Precision (%CV) between-assay	≤7.98%	NA	≤6.44% GSK1278863) ≤7.66% (GSK2391220) ≤4.56%(GSK2506104)| ≤5.18% (GSK2531401)
Accuracy (% bias)	-6.20%≤%Bias≤16.2%	NA	-5.56 to 2.66% (GSK1278863) -3.34 to 5.16% (GSK2391220) -3.31 to 4.49% (GSK2506104) -4.16 to 6.73% (GSK2531401)
Stability in rat plasma	6 days at -20 °C and -70 °C	367 days at -20 °C and -70 °C	10 days at -20 °C and 9 days at -70 °C for all analytes
Freeze-thaw stability	Five cycles thawed at room temperature and frozen at -20 °C and -70 °C	NA	Five cycles frozen at -20 °C or -70 °C and thawed at room temperature
Processed extract stability	52.75 hours at 2 to 8 °C	145.5 hours at 2 to 8 °C	91.27 hours at 2 to 8 °C (GSK1278863); 91.62 hours at 2 to 8 °C for all metabolites

Rat Assays (continued)

	Report 2018N362125[c] (P1484)
Calibration model	Linear, 1/concentration^2
MS/MS interface	Turbo Ion Spray
Validated range	150 to 150000 ng/mL
Precision (%CV) within-assay	NA
Precision (%CV) between-assay	NA
Accuracy (% bias)	NA
Stability in rat plasma	68 Days at -20 and -70°C
Freeze-thaw stability	NA
Processed extract stability	NA

Key:
a = Supplemental validation for study CD2007/01258.
b = Supplemental validation for study 2013N180658.
c = Supplemental validation for study 2016N308027.

Rabbit Assays

Five methods for the determination of daprodustat (and/or metabolites of daprodustat) in rabbit plasma have been validated using HPLC-MS/MS. Daprodustat was extracted from rabbit plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard. Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective and summaries of the validation data are tabulated below.

	Report CD2008/01209 (GSK1278863RBPLVALA)	**Report 2010N108826 ([redacted]8204240)**	**Report 2011N119256 (GSK1278863RBPLVALB)**
Calibration model	Linear weighted $1/x^2$	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS interface	Turbolon Spray	Turbolon Spray	Turbolon Spray
Validated range	50 to 50000 ng/mL	50 to 50000 ng/mL	1 to 1000 ng/mL
Precision (%CV) within-assay	≤3.2%	≤7.7%	≤5.7%
Precision (%CV) between-assay	NA	≤7.4%	≤3.9%
Accuracy (% bias)	-8.3%≤%Bias≤1.2%	-5.4% to 6.6%	-6.3%≤%Bias≤6.2%
Stability in rabbit plasma	At least 24 hours at room temperature	73 days at -20 °C	At least 24 hours at ambient temperature
Freeze-thaw stability	At least 3 cycles from -20°C to room temperature	3 cycles from -20 °C to room temperature	At least 3 cycles from -20°C to ambient temperature
Processed extract stability	At least 96 hours at room temperature	3 days after initial injection stored at 4 °C	At least 96 hours at ambient temperature

Rabbit Assays (continued)

	Report 2012N132981[a] (GSK1278863RBPLVALB)	Report 2012N139919 (GSK1278863RBPLVALC)
Calibration model	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS interface	Turbolon Spray	Turbolon Spray
Validated range	1 to 1000 ng/mL	1 to 1000 ng/mL
Precision (%CV) within-assay	NA	GSK2391220: ≤6.2% GSK2531403: ≤5.4% GSK2499166: ≤5.8% GSK2531399: ≤5.9% GSK2531398: ≤4.9% GSK2531400: ≤7.5%
Precision (%CV) between-assay	NA	GSK2391220: ≤3.4% GSK2531403: ≤4.7% GSK2499166: ≤4.2% GSK2531399: ≤2.8% GSK2531398: ≤2.0% GSK2531400: ≤1.6%
Accuracy (% bias)	NA	GSK2391220: -12.7%≤ Bias ≤7.9% GSK2531403: -12.1%≤ Bias ≤10.0% GSK2499166: -10.9%≤ Bias ≤12.9% GSK2531399: -10.8%≤ Bias ≤5.3% GSK2531398: -8.5%≤ Bias ≤6.1% GSK2531400: -13.6%≤ Bias ≤4.3%
Stability in rabbit plasma	NA	At least 24 hours at ambient temperature
Freeze-thaw stability	NA	At least 3 cycles from -20 °C to ambient temperature
Processed extract stability	NA	At least 3 days at ambient temperature

Key:
a = Supplemental validation for study 2011N119256.

Dog Assays

Two methods for the determination of daprodustat (and/or metabolites of daprodustat) in dog plasma have been validated using HPLC-MS/MS. Daprodustat was extracted from dog plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard. Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective and summaries of the validation data are tabulated below.

	Report CD2007/01259 (GSK1278863DOPLVALC)	**Report 2010N108824 ([redacted]8203527)**
Calibration model	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS interface	Turbolon Spray	Turbolon Spray
Validated range	50 to 50000 ng/mL	50 to 50000 ng/mL
Precision (%CV) within-assay	≤4.8%	≤6.4%
Precision (%CV) between-assay	NA	≤7.0%
Accuracy (% bias)	-5.2%≤%Bias≤2.2%	-4.7% to 5.4%
Stability in dog plasma	At least 24 hours at room temperature	At least 24 hours at room temperature 63 days at -20 °C
Freeze-thaw stability	At least 3 cycles at -20°C	At least 3 cycles at -20 °C
Processed extract stability	At least 96 hours at room temperature	At least 70 hours stored at 4 °C

Minipig Assays

Five methods for the determination of daprodustat in minipig plasma have been validated using HPLC-MS/MS. Daprodustat was extracted from minipig plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard. Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective and summaries of the validation data are tabulated below.

	Report 2014N213579 (P1298)	**Report 2015N232986 (P1352)**	**Report 2015N232987 (P1351)**
Calibration model	Linear, 1/concentration^2	Linear, 1/concentration^2	Linear, 1/concentration^2
MS/MS interface	Turbo Ion Spray	Turbo Ion Spray	Turbo Ion Spray
Validated range	0.1 to 100 ng/mL	0.5 to 500 ng/mL	0.5 to 500 ng/mL
Precision (%CV) within-assay	≤11.4%	≤13.5%	≤2.99%
Precision (%CV) between-assay	≤8.10%	NA	NA
Accuracy (% bias)	-13.0 to 8.13%	-2.69%≤%Bias≤7.40%	-3.26%≤%Bias≤6.20%
Stability in minipig plasma	8 days at -20 °C and -70 °C Whole blood: Two hours on ice or at room temperature	NA	NA
Freeze-thaw stability	Five cycles thawed at room temperature	NA	NA
Processed extract stability	105.28 hours at 2 to 8 °C	NA	NA

Minipig Assays (continued)

	Report 2015N237487 (P1370)	**Report 2016N272071[a] (P1370)**
Calibration model	Linear, 1/concentration^2	Linear, 1/concentration^2
MS/MS interface	Turbo Ion Spray	Turbo Ion Spray
Validated range	0.100 to 100 ng/mL	0.100 to 100 ng/mL
Precision (%CV) within-assay	≤8.68%	NA
Precision (%CV) between-assay	≤9.19%	NA
Accuracy (% bias)	-14.1%≤%Bias≤2.75%	NA
Stability in minipig plasma	42 days stored at -20 °C and -70 °C Whole blood: Two hours at room temperature, in an ice bath and at 37 °C	280 days stored at -20 °C and -70 °C
Freeze-thaw stability	Five cycles thawed at room temperature and frozen at -20 °C and -70 °C	NA
Processed extract stability	207.63 hours at 2 to 8 °C	NA

Key:
a = Supplemental validation for study 2015N237487.

Monkey Assays

Three methods for the determination of daprodustat (and/or metabolites of daprodustat) in monkey plasma have been validated using HPLC-MS/MS. Daprodustat was extracted from monkey plasma by protein precipitation using acetonitrile containing an isotopically labeled internal standard. Extracts were analyzed by HPLC-MS/MS using multiple reaction monitoring. The methods are selective, and summaries of the validation data are tabulated below.

	Report CD2009/00712 (GSK1278863CYPLVALA)	Report CD2010/00387 (GSK1278863CYPLVALB)	Report 2011N126360 (GSK1278863CYPLVALB)
Calibration model	Linear weighted $1/x^2$	Linear weighted $1/x^2$	Linear weighted $1/x^2$
MS/MS interface	Turbolon Spray	Turbolon Spray	Turbolon Spray
Validated range	50 to 50000 ng/mL	1 to 1000 ng/mL	1 to 1000 ng/mL
Precision (%CV) within-assay	≤11.1%	≤10.0% for GSK2391220 ≤10.0% for GSK2531403 ≤8.0% for GSK2499166 ≤8.4% for GSK2531399 ≤12.4% for GSK2531398 ≤14.5% for GSK2531400	NA
Precision (%CV) between-assay	NA	≤3.5% for GSK2391220 ≤8.6% for GSK2531403 ≤6.2% for GSK2499166 ≤3.4% for GSK2531399 ≤2.5% for GSK2531398 ≤2.9% for GSK2531400	NA
Accuracy (% bias)	-1.1%≤%Bias≤1.4%	-10.3% ≤ Bias ≤11.0% for GSK2391220 -9.9% ≤ Bias ≤19.0% for GSK2531403 -7.9% ≤ Bias ≤18.2% for GSK2499166 -9.8% ≤ Bias ≤10.1% for GSK2531399 -7.1% ≤ Bias ≤13.9% for GSK2531398 -8.5% ≤ Bias ≤6.6% for GSK2531400	NA
Stability in monkey plasma	At least 24 hours at room temperature	At least 24 hours at room temperature (Whole blood: At least 4 hours at room temperature)	The metabolites stored at -20 °C for at least 301 days and -80 °C for at least 296 days
Freeze-thaw stability	At least 3 cycles from -20°C to room temperature	At least 3 cycles from -20°C to room temperature	NA
Processed extract stability	NA	At least 72 hours at room temperature	NA

Key:
a = Supplemental validation for study CD2010/00387.

Human Assays

Method validation study for human samples can be found in m2.7.1, Summary of Biopharmaceutic Studies and Associated Analytical Methods for Daprodustat.

APPENDIX 2 ADDITIONAL INFORMATION

The following reports have been reviewed within GlaxoSmithKline (GSK) and the information is considered to have no bearing on safety. These reports are not included in the study listing tables for the following reasons:

Report No. (Study No.)	Title	Reason for Exclusion from Study Listing Tables
FD2007/00411 (Z27930)	Assessment of stability of GSK1278863 in dimethyl sulphoxide	Stability reports for formulations used in nonclinical studies.
FD2007/00417 (Z27944)	Assessment of stability of GSK1278863 in dimethyl formamide	
2011N122988 (S42847)	GSK1278863A: Assessment of Stability and Homogeneity of a Formulation of GSK1278863A in Petrolatum (S42847)	
2011N124494	Assessment of Stability of GSK2531401 in 0.9% (w/v) Aqueous Sodium Chloride, Final Formulation Adjusted to pH 7 (±0.2)	
2012N131899 (S29987)	GSK2531401A. Further Assessment of Stability of GSK2531401 in 0.9% (w/v) Aqueous Sodium Chloride, Final Formulation Adjusted to pH 7 (±0.2). (S29987).	
2012N145434 (S30226)	Assessment of Stability of GSK2487818 in Dimethyl Sulfoxide	
2012N150097 (S30350G)	Assessment of Stability of GSK2506102 in Dimethyl Sulfoxide	
2011N126848	Vehicle Assessment of Intravenous formulations	
2016N270208 (S70894G)	GSK1278863: Analytical Method Validation and Assessment of Homogeneity and Stability of Topical Formulations in Petrolatum	
2011N120068 (S29755)	Assessment of Homogeneity and Stability of GSK1278863 in Butylated Hydroxy Toluene (BHT) (0.2% w/w) , Eumulgin B2 (2.5% w/w), Sophiderm (5% w/w), Phenoxyethanol (1% w/w), Carbopol 980NF (0.2% w/w), Pemulen TR-1 (0.2% w/w), Propylene Glycol (1% w/w) in Water, final formulation pH adjusted to pH 5 (+/-0.2), using Trolamine	
2012N150298 (S30351G)	Assessment of Stability of GSK2531398 in Dimethyl Sulfoxide	
2017N318288 (S71192G)	GSK2391220A (M2), GSK2506104A (M3) and GSK2531401A (M13): Analytical method partial validation followed by formulation stability assessment in 0.5% sodium chloride with aqueous 25mM phosphate buffer, pH 7.4 (. 0.2)	
CD2007/01207 (G07246)	GSK1278863A: Formulation Stability Assessment in 1% Methylcellulose (400 cps. @ 2%)	

CD2008/01498 (G08108)	GSK1278863A: Formulation Stability Assessment in 1% Methylcellulose (400 cps. @ 2%) at Concentrations of 0.02, 0.05 & 0.1 mg/mL	
2014N192456	GSK1278863A: Formulation stability assessment in 1% Methylcellulose (400 cps@2%), 2% Tween 80, 5mM NaOH in water, pH 6(+/-).2)	
2014N188533	(S70459G)" GSK2391220A (M2), GSK2506104A (M3) and GSK2531401A (M13): Formulation Stability Assessment in Aqueous 25mM Phosphate Buffer, pH 7.4 (± 0.2)	
2013N187566	Validation of a high performance liquid chromatographic method for the determination of GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) in dose formulations	Method validation studies for dose formulations
2013N187566	Validation of a High Performance Liquid Chromatographic Method for the Determination of GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) in Dose Formulations	

Studies listed in Appendix 2 comprise those conducted during the formal research and development of the compound. Early screening studies completed on multiple compounds during the candidate selection phase have not been included.

MODULE 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

1. PHARMACOKINETICS: OVERVIEW

Table 1.1 Listing of Absorption Studies with Daprodustat and Metabolites

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Percutaneous absorption	Human skin	3M	Ex vivo	A	5 mg/cm^2 (0.2% cream, gel, ointment)	Single	No	[redacted]	2012N152036 (R10-1824)	m4.2.2.2
Percutaneous absorption	Human skin	3M	Ex vivo	A	10 mg/cm^2 (0.2% cream)	Single	Yes	[redacted]	2012N152038 (GK1202-01)	m4.2.2.2
Percutaneous absorption	Human skin	3F	Ex vivo	A	1, 5, 15, 30% (w/w)	Up to 24hrs	No	[redacted]	2015N230888	m4.2.2.2
Pharmacokinetics	Mouse (CD-1)	27M[a,b] 24M[a,c]	IV (bolus) Oral (gavage)	A	1.3 1.9, 3.9	Single	No	GSK	UH2008/00002	m4.2.2.2
Pharmacokinetics	Rat (SD)	3M[b] 3M[c] 3M[c]	IV (1 hour infusion) Oral (gavage) Oral (gavage)	A	0.6 1.6 31.1	Single	No	GSK	UH2008/00002	m4.2.2.2
Pharmacokinetics	Rat (SD)	3M	IV (22 min. infusion)[d] (2 hour infusion)[d]	A	17.5 11.1	Single	No	GSK	CD2008/01285 (08DMM039)	m4.2.2.2
Toxicokinetics	Rat (SD)	3F	Oral (gavage)	A	0.02, 0.1, 0.8, 7	Single	No	GSK	2013N184197 (R70458N)	m4.2.2.2

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Pharmacokinetics	Dog (beagle)	3M[c]	IV (1 hour infusion)	A	1.2	Single	No	GSK	UH2008/00002	m4.2.2.2
		3M[c]	Oral (gavage)		3.2					
		3M[b]	Oral (gavage)		1, 3.1, 30.6					
Pharmacokinetics	Dog (beagle)	3M[c]	Oral (capsule)	A	5	Single	No	GSK	UH2008/00002	m4.2.2.2
				B	5[e]					
				C	5[f]					
Toxicokinetics	Dog (beagle)	1M/1F	Oral (gavage)	A	120	Single	No	GSK	CD2006/01837 (D06386)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	2M	Topical	A	10%, 35%	Single	No	GSK	2013N180775 (F43142N)	m4.2.2.2
Pharmacokinetics	Monkey (cynomolgus)	3M[c]	IV (1 hour infusion)	A	1.1	Single	No	GSK	UH2008/00002	m4.2.2.2
		3M[c]	Oral (gavage)		3.0					
Toxicokinetics	Monkey (cynomolgus)	1M/1F	Oral (gavage)	A	10, 30, 100, 300, 600	Single	No	GSK	CD2009/00639 (D09120)	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	18M/18F	Oral (gavage)	A	30	14 days (Day 14)	No	GSK	CD2009/00900 (D09169)	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	42M/42F	Oral (gavage)	A	3, 30, 60	13 weeks (Day 1, Week 4)	Yes	[redacted]	2011N112096 (G10121)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites	Mouse[g] (CD-1)	21M/21F	Oral (gavage)	A	30	13 weeks (Day 7)	Yes	GSK	2011N117145 (10DMM034)	m4.2.2.2

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics of daprodustat and metabolites (co-administered)	Mouse (CD-1)	48M/48F	Oral (gavage) SC (Injection)	A	6, 20 (daprodustat) 2.0 (M2), 2.5 (M3), 1.3 (M13)	13 weeks (Day 1, Week 4)	Yes	[redacted]	2014N199031 (M70456G)	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	51M/51F	Oral (gavage)	A	0.2, 0.8, 3[t]	104 weeks (Weeks 4 and 26)	Yes	[redacted]	2014N221714 (M70585G)	m4.2.2.2
Toxicokinetics	Rat (SD)	6M	IV (4 hour infusion)	A	20, 40	14 days (Days 1 & 14)	No	GSK	CD2008/01740 (I08185)	m4.2.2.2
Pharmacokinetics	Rat (SD)	3M	Oral (gavage)	A	30	5 days (Days 5)	No	GSK	2012N153858 (12DMM037)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites	Rat (SD)	6F (pregnant)	Oral[n] (gavage)	A	10[o], 40[p], 40[q], 60[r], 60[s]	5 days (GD 6 to GD10)	No	[redacted]	2016N305715 (R71028N)	m4.2.2.2
Toxicokinetics	Rat (SD)	3M	Oral (gavage)	A	10, 30, 60	14 days (Days 1 & 14)	No	GSK	RD2006/01192 (R41921)	m4.2.2.2
Toxicokinetics	Rat (SD)	3F	Oral (gavage)	A	100, 250, 500	14 days (Days 1 & 14)	No	[redacted]	CD2008/01306 (D08114)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites[w]	Rat (SD)	6F (pregnant/ lactating)	Oral (gavage) SC	A	0.8, 7, 40 2.5 (M2) 3.2 (M3) 1.8 (M13)	38 days (GD6, GD9 and LD21)	Yes	[redacted]	2018N365151 (R71242G)	m4.2.2.2
Toxicokinetics	Rat (SD)	3M/3F	Oral (gavage)	A	2, 7, 20	4 weeks (Days 1 & 28)	Yes	GSK	RD2007/01333 (R42197)	m4.2.2.2

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rat (SD)	3M/3F	Oral (gavage)	A	0.8, 4, 20, 100	13 weeks (Day 1, Weeks 4 & 13)	Yes	[redacted]	CD2009/00951 (G09057)	m4.2.2.2
Toxicokinetics	Rat (SD)	9M/9F	Oral (gavage)	A	0.8, 4, 10	26 weeks (Weeks 4, 13 & 26)	Yes	GSK	2011N126130 (G10276)	m4.2.2.2
Toxicokinetics	Rat (SD)	3-9M	Oral (gavage)	A	0.02, 0.1, 0.8, or 4	Up to 99 weeks (Weeks 4 and 26)	Yes	[redacted]	2014N196080 (R70375G)	m4.2.2.2
		3-9F			0.02, 0.1, 0.8, or 7	99 weeks (Weeks 4 and 26)				
Toxicokinetics of daprodustat and metabolites	Rabbits (DB) (pregnant)	4F	Oral (gavage)	A	60	5 days (Day 5 of dosing/ Day 11 GD)	Yes	GSK	2012N140611 (G12109)	m4.2.2.2
Toxicokinetics	Rabbits (DB) (pregnant)	3 or 4F	Oral (gavage)	A	4, 30, 60, 125, 250	13 days (Day 5/Day 11 GD) & Day 13/Day 19 GD)	No	GSK	CD2008/01041 (D08163)	m4.2.2.2
Toxicokinetics	Rabbits (DB) (pregnant)	9F	Oral (gavage)	A	4, 30, 60	13 days (Gestational Day 5 of dosing/ Day 11)	Yes	[redacted]	CD2009/00390 (G09061)	m4.2.2.2

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rabbit (NZW)	3 or 6M	Topical	A	2%	14 days (Days 1 & 14)	Yes	GSK	2011N125481 (L42845)	m4.2.2.2
Toxicokinetics	Dog (beagle)	2M/2F	Oral (gavage & capsule)	A	90[i]	3 days (Day 1)	No	GSK	RD2007/01367 (D42209)	m4.2.2.2
Toxicokinetics	Dog (beagle)	1M/1F	Oral (gavage)	A	20, 60, 120	7 days (Days 1 & 7)	No	GSK	CD2007/00353 (D07078)	m4.2.2.2
Toxicokinetics	Dog (beagle)	3M/3F	Oral (gavage)	A	3, 30, 90	4 weeks (Days 1 & 28)	Yes	GSK	RD2007/01336 (D42196)	m4.2.2.2
Toxicokinetics	Dog (beagle)	4 or 6M/ 4 or 6F	Oral (gavage)	A	1, 3, 30/15[h]	13 weeks (Day 1, Weeks 7 & 13)	Yes	[redacted]	CD2009/00952 (G69058)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	1M/1F	Topical	A	0.5%, 1%, 2% w/w applied daily to 10% BSA	14 days (Days 1 & 14)	No	[redacted]	2012N148386 (D12086)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	1M/1F	Topical	A	10, 20, 35% w/w applied daily to 10% BSA	14 Days (Days 1 & 14) Days 2, 3, 4, and 7: one sample 21 hours post-dose	No	[redacted]	2013N185591 (F70438N)	m4.2.2.2

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Minipig (Göttingen)	3M	Topical	A	2, 10, 35% w/w applied daily to 10% BSA	28 days (Days 1 & 28)	No	[redacted]	2015N231048 (F70727N)	m4.2.2.2
Toxicokinetics	Minipig (Göttingen)	4M/4F	Topical	A	5, 10, 20% (w/w) (42, 84, 168 mg/kg/day)	13 weeks (Weeks 4 and 13)	Yes	[redacted]	2016N273683 (F70916G)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	1M/1F	Oral (gavage)	A	10, 30, 100	14 days (Days 1 & 14)	No	GSK	CD2009/00780 (D09121)	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	4-6M/ 4-6F	Oral (gavage)	A	5, 20, 100	13 weeks (Day 1, Weeks 1 & 14)	Yes	[redacted]	2010N108482 (G10005)	m4.2.2.2
Toxicokinetics of circulating metabolites	Monkey[j] (cynomolgus)	4-6M/ 4-6F	Oral (gavage)	A	5, 20, 100	13 weeks (Day 1, Weeks 1 & 14)	Yes	GSK	2011N117143 (10DMM033)	m4.2.2.2
Toxicokinetics of daprodustat and metabolites	Monkey (cynomolgus)	4M/4F	Oral (gavage)	A	3, 10, 50	39 weeks (Day 1, Weeks 4, 26 & 39)	Yes	[redacted]	2011N126131 (G10277)	m4.2.2.2

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Studies with Metabolite GSK2531401 (M13)										
Pharmacokinetics of daprodustat and GSK2531401 (M13)	Mouse (CD-1)	Daprodustat 15M GSK2531401 18M/16M[u] 15M[u]	IV (bolus)	A E	20[m] 40/100 (M13)	Single	No	GSK	2012N156583 (12DMM041)	m4.2.2.2
Pharmacokinetics	Mouse (CD-1)	18M[b,k,v]	Oral (gavage)	E	150	Single	No	GSK	2011N130731 (11DMM060)	m4.2.2.2
Pharmacokinetics	Mouse (CD-1)	18M[b,l,v]	Oral (gavage)	E	50	Single	No	GSK	2012N137345 (12DMM004)	m4.2.2.2
Pharmacokinetics and EPO response	Mouse (CD-1)	15M[v]	IV (bolus)	E	40	Single	No	GSK	2012N132320 (11DMM049)	m4.2.2.2
Pharmacokinetics	Rat (SD)	4M[b] 4M[b]	IV (bolus) Oral (gavage)	E	19.2 218	Single	No	GSK	2012N145868 (12DMM019)	m4.2.2.2
Studies with Metabolite GSK2391804 (M8)										
Pharmacokinetics	Rat (SD)	4M[b]	IV (bolus) Oral (gavage)	M	40 100	Single	No	GSK	2012N153861 (12DMM038)	m4.2.2.2

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Studies with Metabolites GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13)										
Pharmacokinetics	Mouse (CD-1)	24M	SC (Injection)	D, E, N	1.2 (M2), 1.5 (M3) 0.77 (M13)	Single	No	GSK	2013N174038 (13DMM016)	m4.2.2.2
Toxicokinetics	Mouse (CD-1)	15F	SC (Injection)	D, E, N	2.0 (M2) 2.5 (M3) 1.3 (M13)	14 days	No	GSK	2014N208198 (M70590N)	m4.2.2.2

Key: BSA = Body surface area, DB = Dutch Belted, EPO = Erythropoietin, IV = Intravenous, pc = post coitum, w/w = weight/weight ratio, M2 = GSK2391220, M3 = GSK2506104, M13 = GSK2531401, GD = Gestation day, LD = Lactation day, NZW = New Zealand White, SC = Subcutaneous, SD = Sprague Dawley.
A = Daprodustat (Parent form).
B = Daprodustat (Potassium salt).
C = Daprodustat (Tris monohydrate salt).
D = M2 metabolite of daprodustat, GSK2391220 (Parent form).
E = M3 metabolite of daprodustat, GSK2506104 (Parent form).
M = M8 metabolite of daprodustat, GSK2391804 (Parent form).
N = M13 metabolite of daprodustat, GSK2531401 (Parent form).

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]

Listing of Absorption Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD

a = Composite sampling design; n=3/time point.
b = Non-fasted animals.
c = Fasted animals.
d = Animals received either a 22 or 120 minute IV infusion.
e = GSK1278863B, the potassium salt of daprodustat was used.
f = GSK1278863D, the tris monohydrate salt of daprodustat was used.
g = Samples obtained from the 13 week toxicity study in the mouse, [Report 2011N112096].
h = On Day 17, the dose was reduced from 30 mg/kg/day to 15 mg/kg/day for females only.
i = 2M/2F fasted animals were administered the 90 mg/kg/day dose in a capsule, and 2M/2F fed animals were administered an oval gavage dose of 90 mg/kg/day.
j = Samples obtained from the 13 week toxicity study in the monkey, [Report 2010N108482].
k = Suspension in 1% aqueous methylcellulose.
l = Amorphous spray dried dispersion suspension in hydroxypropylmethylcellulose.
m = GSK2531401 (M13) dosed at either 40 mg/kg/day or 100 mg/kg/day. Daprodustat dosed at 20 mg/kg/day.
n = Daprodustat was administered orally, but some groups were also administered a cocktail of metabolites (M2,3 and 13,) of daprodustat or vehicle subcutaneously.
o = Given daprodustat at 10 mg/kg/day and metabolite cocktail - 2.5, 3.2, and 1.8 mg/kg/day (for M2, 3 or 13, respectively).
p = Given daprodustat at 40 mg/kg/day and vehicle (25 mM Phosphate Buffer, pH 7.4).
q = Given daprodustat at 40 mg/kg/day and metabolite cocktail - 2.5, 3.2, and 1.8 mg/kg/day (for M2, 3 and 13, respectively).
r = Given daprodustat at 60 mg/kg/day and metabolite cocktail - 2.5, 3.2, and 1.8 mg/kg/day (for M2, 3 and 13, respectively).
s = Given daprodustat at 60 mg/kg/day and metabolite cocktail - 7.5, 9.6, and 5.4 mg/kg/day (for M2, 3 and 13, respectively).
t = 0.2, 0.8 and 3 mg/kg/day daprodustat groups were also administered a cocktail of metabolites (M2, 3 and 13)at 2, 2.5, and 1.3 mg/kg/day, respectively.
u = 2 to 3 mice/timepoint.
v = 3 mice/timepoint.
w = Daily Subcutaneous Dose metabolite cocktail [GSK2391220 (M2); GSK2506104 (M3): GSK2531401 (M13)] mg/kg/day [dosed as 1.25, 1.8 and 0.9 mg/kg/dose BID every 6 hours].

Table 1.2 Listing of Distribution Studies with Daprodustat and Metabolites

Type of Study	Species (Strain)/ Test System	No./Sex / Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Plasma protein binding	Mouse (CD-1), Rat (SD), Dog (beagle), Monkey (Cynomolgus), Human	NA	In vitro	A	1 and 10 μg/mL	NA	No	GSK	UH2008/00002	m4.2.2.3
Plasma protein, HSA and AAG binding	Mouse, Rat, Rabbit, Dog, Monkey, Human	NA	In vitro	A	0.2, 1, 10, 30[a], 50 μg/mL	NA	No	GSK	CD2010/00340 (09DMM060)	m4.2.2.3
Blood cell association	Mouse (CD-1), Rat (Sprague Dawley), Dog (beagle), Monkey (Cynomolgus), Human	NA	In vitro	A	1 and 10 μg/mL	NA	No	GSK	UH2008/00002	m4.2.2.3
Kidney distribution	Mouse (CD-1)	15M[i]	IV (bolus)	N	40	Single	No	GSK	2012N132320 (11DMM049)	m4.2.2.3
Kidney distribution	Mouse (CD-1)	15M	IV (bolus)	A	20[g]	Single	No	GSK	2012N156583 (12DMM041)	m4.2.2.3
		18M/ 16M[h] 15M[h]		N	40/100 (M13)					

Listing of Distribution Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Blood to plasma and blood to liver ratio	Rat	3M/F; 3M BDC	Oral (gavage)	[^{14}C]	10[b]	Single (intact: up to 7 days) (BDC: up to 4 days)	Yes	[redacted]	CD2008/00100 (7717-705)	m4.2.2.3
Blood to plasma ratio	Dog (beagle)	3M/3F; 3M BDC	Oral (gavage)	[^{14}C]	20[b]	Single (intact: up to 7 days) (BDC: up to 3 days)	Yes	[redacted]	CD2008/00025 (AFA00607)	m4.2.2.3
Blood to plasma ratio	Monkey (cynomolgus)	3M; 2M BDC	Oral (gavage)	[^{14}C]	10[b]	Single (Up to 168 hours)	No	[redacted]	2018N355713 (8361764)	m4.2.2.3
Hepatic uptake	Human	NA	In vitro	A	0.5 & 0.3 μM	NA	No	GSK	2015N232657 (14DMM039)	m4.2.2.3
Inhibition of OAT1, OAT3, OCT2, MATE1 and MATE2-K transporters (daprodustat and 6 metabolites)	S_2 cells HEK293 cells	NA	In vitro	A	Varying[c]	NA	No	[redacted]	2015N242070 (XS-0661)	m4.2.2.3

Listing of Distribution Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex / Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Substrate for OATP1B1 and OATP1B3 transporters (daprodustat and 6 metabolites)	HEK293 cells	NA	In vitro	A	Varying[d]	NA	No	[redacted]	2015N255319 (XS-0674)	m4.2.2.3
Substrate for OAT1, OAT3, OCT2, MATE1 and MATE2-K transporters (daprodustat and 6 metabolites)	S_2 cells HEK293 cells	NA	In vitro	A	Varying[f] (see key)	NA	No	[redacted]	2015N249257 (XS-0662)	m4.2.2.3
Interaction with P-gp and membrane permeability	Human	NA	In vitro	A	5 μM	NA	No	GSK	UH2008/00018	m4.2.2.3
P-gp-mediated transport inhibition	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/00669 (08DMM040)	m4.2.2.3
Passive and absorptive membrane permeability	NA	NA	In vitro	[^{14}C]	3 μM[b]	NA	No	GSK	CD2008/00421 (08DMM021)	m4.2.2.3

Listing of Distribution Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex / Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Microdialysis	NA	NA	In vitro	A	2.5 and 25 ng/mL	NA	No	[redacted]	2015N228833	m4.2.2.3
OATP1B1 and OATP1B3 transport	HEK-MSRII	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/00231 (07DMM163)	m4.2.2.3
Interaction with Bcrp1 and membrane permeability	MDCKII-murine Bcrp1 cells	NA	In vitro	A	3 μM	NA	No	GSK	UH2008/00018	m4.2.2.3
Evaluation as substrate	MDCKII-BCRP cells	NA	In vitro	A	3 μM[a]	NA	No	GSK	2015N237800 (15DMM007)	m4.2.2.3
BCRP mediated transport inhibition	Human	NA	In vitro	A	0.01 to 100 μM	NA	No	GSK	2012N151642 (12DMM014)	m4.2.2.3
Whole body autoradiography	Rat (Long Evans)	7M	Oral (gavage)	[^{14}C]	10[b]	Single (Up to 35 days)	Yes	[redacted]	CD2008/00271 (85-0708)	m4.2.2.3
Studies with Metabolites										
Plasma protein binding	Human	NA	In vitro	D, E, G, J, K, N[c]	10, 100, 250 and 500 ng/mL	NA	No	GSK	2012N131897 (11DMM027)	m4.2.2.3

Listing of Distribution Studies with Daprodustat and Metabolites (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex / Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Studies with Metabolites (Continued)										
Hepatic uptake of GSK2487818	Human (cryopreserved hepatocytes)	NA	In vitro	G[d]	0.2, 2, 15 μM	NA	No	GSK	2015N259558 (15DMM035)	m4.2.2.3
OATP1B1 transport	Human	NA	In vitro	D, E, G, J, K, N[e]	85 nM to 50 μM	NA	No	GSK	2013N166580	m4.2.2.3

Type of Study	Species (Strain)/ Test System	No./Sex / Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD

A = Daprodustat (Parent form). D = M2 metabolite of daprodustat, GSK2391220 (Parent form). E = M3 metabolite of daprodustat, GSK2506104 (Parent form). G = M4 metabolite of daprodustat, GSK2487818 (Parent form). J = M5 metabolite of daprodustat, GSK2506102 (Parent form). K = M6 metabolite of daprodustat, GSK2531398 (Parent form). N = M13 metabolite of daprodustat, GSK2531401 (Parent form). [^{14}C] = ^{14}C labelled daprodusat.
AAG = α1-acid glycoprotein. Bcrp1 = Murine breast cancer resistant protein 1.
BCRP = Human breast cancer resistant protein. BDC = Bile duct cannulated.
HEK-MSRII =Human embryonic kidney expressing macrophage scavenger receptor II.
hMDR1 = Human multi-drug resistant 1 gene. HSA = Human serum albumin.
MATE1 = Multidrug and toxin extrusion transporter 1. MATE2-K = Multidrug and toxin extrusion transporter 2-K.
MCDK = Madin Darby canine kidney. NA = Not applicable. OAT1 = Organic anion transporter 1.
OAT3 = Organic anion transporter 3. OATP1B1 = Organic anion transporting polypeptide 1B1.
OATP1B3 = Organic anion transporting polypeptide 1B3. OCT2 = Organic cation transporter 2.
P-gp = P-glycoprotein. w/w = weight/weight ratio.
a = 30 μg/mL was employed in the binding of HSA and AAG only.
b = [^{14}C]GSK1278863.
c = 1.4 μM GSK1278863, 20 μM M2 (GSK2391220), 20 μM M3 (GSK2506104), 10 μM M4 (GSK2487818), 5 μM M5 (GSK2506102), 10 μM M6 (GSK2531398), and 10 μM M13 (GSK2531401).
d = 0.1, 1, 10, 100 μM - GSK1278863, 0.01, 0.1, 1, 10 μM – M2 (GSK2391220) & M3 (GSK2506104), 1, 3, 10, 30 μM for M13 (GSK2531401), M4 (GSK2487818), M5 (GSK2506102) & M6 (GSK2531398).
e = 0.1, 1, 10, 100 μM - GSK1278863, 0.01, 0.1, 1, 10 μM and 1, 3, 10, 30 μM (for MATE1 assay only) – M2 (GSK2391220), 1, 3, 10, 30 μM for M3 (GSK2506104), M13 (GSK2531401), M4 (GSK2487818), M5 (GSK2506102) & M6 (GSK2531398).
f = GSK1278863D, the tris monohydrate salt of daprodustat was used.
g = GSK2531401 (M13) dosed at either 40 mg/kg/day or 100 mg/kg/day. Daprodustat dosed at 20 mg/kg/day.
h = 2 to 3 mice/timepoint.
i = 3 mice/timepoint.

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
GSK = GlaxoSmithKline.

Table 1.3 Listing of Metabolism Studies with Daprodustat

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Blood stability	Human	NA	In vitro	A	1, 10 μg/mL	NA	No	GSK	UH2008/00002	m4.2.2.4
Clearance in liver microsomes	Mouse, Rat, Dog, Monkey, Human	NA	In vitro	A	0.5 μM	NA	No	GSK	UH2008/00018	m4.2.2.4
Clearance in hepatocytes	Mouse, Rat, Dog, Monkey, Human	NA	In vitro	A	0.5 μM	NA	No	GSK	UH2008/00018	m4.2.2.4
Bioactivation in liver microsomes	Human	NA	In vitro	[^{14}C]	10 μM	NA	No	GSK	CD2007/01464 (07DMM137)	m4.2.2.4
Qualitative identification of metabolites following incubation with human CYP enzymes	Human	NA	In vitro	A	10 μM	NA	No	GSK	UH2008/00018	m4.2.2.4

Listing of Metabolism Studies with Daprodustat (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administ-ration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Preliminary identification of metabolites following incubation with hepatocytes	Mouse, Rat, Dog, Monkey, Human	NA	In vitro	A	10 μM	NA	No	GSK	UH2008/00018	m4.2.2.4
Metabolism in hepatocytes	Mouse, Rat, Rabbit, Dog, Monkey, Human	NA	In vitro	[^{14}C]	12.5 μM	NA	No	GSK	CD2008/01286 (07DMM139)	m4.2.2.4
Metabolism in hepatocytes	Minipig (Göttingen)	NA	In vitro	A	19 μM	NA	No	GSK	2011N125815 (11DMM021)	m4.2.2.4
Metabolism in hepatocytes	Hamster (Syrian)	NA	In vitro	A	20 μM	NA	No	GSK	2013N160562 (12DMM025)	m4.2.2.4
Metabolism in rat liver S9	Rat liver S9	NA	In vitro	[^{14}C]	4, 40, 400 μM	NA	No	GSK	2011N114076 (10DMM007)	m4.2.2.4
Oxidative enzymology in liver microsomes	Human	NA	In vitro	[^{14}C]	5 μM	NA	No	GSK	CD2010/00300 (09DMM051)	m4.2.2.4

Listing of Metabolism Studies with Daprodustat (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administ-ration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
CYP induction in hepatocytes	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/01088 (08DMM042)	m4.2.2.4
Inhibition of human CYP enzymes in liver microsomes	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	UH2008/00018	m4.2.2.4
Inhibition of human CYP enzymes in liver microsomes	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	CD2008/01013 (07DMM149)	m4.2.2.4
Metabolism in isolated perfused rat liver	Rat (Sprague Dawley)	3	Ex vivo	[^{14}C]	30	NA	No	GSK	CD2008/00941 (07DMM151	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	3M/3F; 3M (BDC)	Oral (gavage)	[^{14}C]	10	Single	No	GSK	CD2008/01663 (07DMM161)	m4.2.2.4
In vivo metabolism	Rat (Sprague Dawley)	14M	Oral (gavage)	[^{14}C]	10	Single	No	GSK	CD2008/00941 (07DMM151	m4.2.2.4

Listing of Metabolism Studies with Daprodustat (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
In vivo metabolism	Dog (beagle)	3M/3F; 3M (BDC)	Oral (gavage)	[^{14}C]	20	Single	No	GSK	CD2008/01662 (07DMM160)	m4.2.2.4
Metabolite characterization	Mouse, Rat, Rabbit, Monkey[a], Human[b]	NS	Oral	A	NS	NS	NS	GSK	2010N109722 (09DMM062)	m4.2.2.4
Metabolite characterization	Monkey (cynomolgus)	3M; 2M (BDC)	Oral (gavage)	[^{14}C]	10	Single (Up to 168 hours)	No	[redacted]	2018N386289 (8361764)	m4.2.2.4
Metabolite characterization	Human[c]	4 (NS)	Oral	A	300 mg	Single	No	GSK	2010N109720 (08DMM117)	m4.2.2.4
Metabolite characterization	Human	4M	IV Oral Oral	[^{14}C] A [^{14}C]	50 μg 6 mg 25 mg	Single	No	GSK	2018N376203 (17DMM043)	m4.2.2.4

Listing of Metabolism Studies with Daprodustat (Continued)

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Studies with Metabolites										
Inhibition of human CYP enzymes	Human	NA	In vitro	D, E, G, J, K, N[c]	0.0 to 100 μM	NA	No	GSK	2013N167801 (12DMM032)	m4.2.2.4
Inhibition of CYP Enzymes by GSK2506104 (M13)	Human	NA	In vitro	E[d]	0.0729 to 100 μM	NA	No	[redacted]	2018N382188 (180538)	m4.2.2.4
Inhibition of CYP Enzymes by GSK2487818 (M4)	Human (Liver Microsomes)	NA	In vitro	G[e]	0.1 to 100 μM	NA	No	GSK	2014N223000 (13DMM003)	m4.2.2.4
Determination of stereoisomeric forms & stereoisomeric conversion	Mouse (plasma)	NA	Ex vivo	A[f]	NA	NA	No	GSK	2014N221116 (14DMM007)	m4.2.2.4
	Human (plasma)			A[g]						

Type of Study	Species (Strain)/ Test System	No./Sex /Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD

Key:

A = Daprodustat (Parent form). D = M2 metabolite of daprodustat, GSK2391220 (Parent form).
E = M3 metabolite of daprodustat, GSK2506104 (Parent form). G = M4 metabolite of daprodustat, GSK2487818 (Parent form).
J = M5 metabolite of daprodustat, GSK2506102 (Parent form). K = M6 metabolite of daprodustat, GSK2531398 (Parent form).
N = M13 metabolite of daprodustat, GSK2531401 (Parent form). [^{14}C] = ^{14}C labelled daprodusat. BDC = Bile duct cannulated.
CYP = Cytochrome P450. NA = Not applicable. NS = Not specified.
a = Samples obtained during toxicity studies CD2009/00639/00, CD2009/00780/00, CD2009/00900/00, CD2009/00390/00 and CD2009/00951/00.
b = Samples obtained from clinical study PHX111427 (GSK Document Number ZM2009/00008/00).
c = Study assessed metabolites M2 (GSK2391220), M3 (GSK2531403), M4 (GSK2487818), M5 (GSK2506102), M6 (GSK2531398), M13 (GSK2531401).
d = Study assessed metabolite M3 (GSK2506104).
e = Study assessed metabolite M4 (GSK2487818).
f = Daprodustat was administered in vivo, but metabolites M2 (GSK2391220), M3 (GSK2506104) and M13 (GSK2531401) were monitored in the study.
g = Daprodustat was administered in vivo, but stereoconversion between metabolite form for M3 (GSK2506104 to GSK2531403) and M13 (GSK2531401 to GSK2531400) were monitored in the study.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]

Table 1.4 Listing of Excretion Studies with Daprodustat

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Elimination	Rat (Sprague Dawley)	15M/15F; 3M (BDC)	Oral (gavage)	[^{14}C]	10	Single (Up to 168 hours)	Yes	[redacted]	CD2008/00100 (7717-705)	m4.2.2.5
Elimination	Dog (beagle)	3M/3F 3M (BDC)	Oral (gavage)	[^{14}C]	20	Single (intact: up to 168 hours) (BDC: up to 72 hours)	Yes	[redacted]	CD2008/00025 (AFA00607)	m4.2.2.5
Elimination	Monkey (cynomolgus)	3M; 2M (BDC)	Oral (gavage)	[^{14}C]	10	Single (up to 168 hours)	No	[redacted]	2018N355713 (8361764)	m4.2.2.5
Milk transfer	Rat (SD)	6F (pregnant / lactating)	Oral (gavage) SC	A	0.8, 7, 40 2.5 (M2) 3.2 (M3) 1.8 (M13)	38 days (GD6, GD9 and LD21)	Yes	[redacted]	2018N365151 (R71242G)	m4.2.2.5

Key:
BDC = Bile duct cannulated.
A = Daprodustat (Parent form).

Testing Facility:
[redacted] = [redacted]
[redacted] = [redacted]

2. ANALYTICAL METHODS AND VALIDATION REPORTS

Table 2.1 Pharmacokinetics: Analytical Methods and Validation Reports

Type of Study	Species	Quantification Limits	Report No. (Method Reference)	Location in CTD
Abbreviated method for the determination of GSK1278863 in acidified mouse plasma using HPLC-MS/MS	Mouse	50 to 50000 ng/mL	CD2009/00955 (GSK1278863MOPLVALA)	m4.2.2.1
Abbreviated method for the determination of metabolites of GSK1278863 (GSK2391220, GSK2531403, GSK2499166, GSK2531399, GSK2531398 and GSK2531400) in acidified mouse plasma using HPLC-MS/M	Mouse	1 to 1000 ng/mL	2010N109787 (GSK1278863MOPLVALB)	m4.2.2.1
Quantitation of GSK1278863, GSK2391220, GSK2506104, and GSK2531401 in Acidified Mouse Plasma via UPLC with MS/MS Detection	Mouse	10 to 10000 ng/mL (GSK1278863) 0.200 to 200 ng/mL (metabolites)	2013N182744 (AHMJ2)	m4.2.2.1
Quantitation of GSK1278863, GSK2391220, GSK2506104, and GSK2531401 in Acidified Mouse Plasma via UPLC with MS/MS Detection - Supplemental Validation Data 2	Mouse	10 to 10000 ng/mL	2014N219756 (AHMJ2)	m4.2.2.1
Quantitation of GSK1278863, GSK2391220, GSK2506104, and GSK2531401 in Acidified Mouse Plasma via UPLC with MS/MS Detection - Supplemental Validation Data 2	Mouse	10 to 10000 ng/mL	2014N198503 (AHMJ2)	m4.2.2.1
Validation of a method for the determination of GSK1278863 in acidified rat plasma using HPLC-MS/MS	Rat	50 to 50000 ng/mL	CD2007/01258 (GSK1278863RTPLVALB)	m4.2.2.1
Supplemental validation data to “The validation of a method for the determination of GSK1278863 in acidified rat plasma using HPLC-MS/MS”	Rat	50 to 50000 ng/mL	2011N122686 (GSK1278863RTPLVALB)	m4.2.2.1
Validation of an analytical procedure for the determination of GSK1278863 in rat plasma using protein precipitation and LC-MS/MS	Rat	50 to 50000 ng/mL	2010N108487 ([redacted] 8203526)	m4.2.2.1

Pharmacokinetics: Analytical Methods and Validation Reports (Continued)

Type of Study	Species	Quantification Limits	Report No. (Method Reference)	Location in CTD
Validation of a method for the determination of GSK26442654 in rat plasma using UHPLC-MS/MS	Rat	10 to 10000 ng/mL	2011N124217 (GSK2642654RTPLVALA)	m4.2.2.1
Supplemental validation data to "The validation of a method for the determination of GSK2642654 using UHPLC-MS/MS"	Rat	10 to 10000 ng/mL	2012N131536 (GSK2642654RTPLVALA)	m4.2.2.1
Validation of an analytical procedure for the determination of GSK1278863 in acidified rat plasma via HPLC-MS/MS	Rat	50 to 50000 ng/mL	2013N180658 (P1242)	m4.2.2.1
Quantitation of GSK1278863 in Acidified Rat Plasma via HPLC with MS/MS Detection	Rat	50 to 50000 ng/mL	2014N219980 (P1242)	m4.2.2.1
Quantitation of GSK1278863, GSK2391220, GSK2506104, and GSK2531401 in acidified rat plasma via UHPLC with MS/MS Detection	Rat	150 to 150000 ng/mL (GSK1278863) 0.200 to 200 ng/mL (Metabolites)	2016N308027 (P1484)	m4.2.2.1
Supplemental validation data to "Quantitation of GSK1278863, GSK2391220, GSK2506104, and GSK2531401 in Acidified Rat Plasma via UHPLC with MS/MS Detection"	Rat	150 to 150000 ng/mL	2018N36125 (P1484)	m4.2.2.1
Abbreviated validation of a method for the determination of GSK1278863 in acidified rabbit plasma using HPLC-MS/MS	Rabbit	50 to 50000 ng/mL	CD2008/01209 (GSK1278863RBPLVALA)	m4.2.2.1
Validation of an analytical procedure for the determination of GSK1278863 in rabbit plasma using protein precipitation and LC-MS/MS	Rabbit	50 to 50000 ng/mL	2010N108826 ([redacted] 8204240)	m4.2.2.1
Validation of a method for the determination of GSK1278863 in acidified rabbit plasma using UHPLC-MS/MS	Rabbit	1 to 1000 ng/mL	2011N119256 (GSK1278863RBPLVALB)	m4.2.2.1
Supplemental validation data to "The validation of a method for the determination of GSK1278863 in acidified rabbit plasma using UHPLC-MS/MS	Rabbit	1 to 1000 ng/mL	2012N132981 (GSK1278863RBPLVALB)	m4.2.2.1
The validation of the method for the determination of the metabolites of GSK1278863 (GSK2391220, GSK2531403, GSK2499166, GSK2531399, GSK2531398 and GSK2531400) range 1 to 1000 ng/mL in acidified rabbit plasma using UHPLC/MS/MS	Rabbit	1 to 1000 ng/mL	2012N139919 (GSK1278863RBPLVALC)	m4.2.2.1

Pharmacokinetics: Analytical Methods and Validation Reports (Continued)

Type of Study	Species	Quantification Limits	Report No. (Method Reference)	Location in CTD
Abbreviated validation of a method for the determination of GSK1278863 in acidified dog plasma using HPLC-MS/MS	Dog	50 to 50000 ng/mL	CD2007/01259 (GSK1278863DOPLVALC)	m4.2.2.1
Validation of an analytical procedure for the determination of GSK1278863 in dog plasma using protein precipitation and LC-MS/MS	Dog	50 to 50000 ng/mL	2010N108824 ([redacted] 8203527)	m4.2.2.1
Quantitation of GSK1278863 in Gottingen minipig plasma via UPLC with MS/MS detection	Minipig	0.100 to 100 ng/mL	2014N213579 (P1298)	m4.2.2.1
Quantitation of GSK1278863 in Acidified Gottingen Mini Pig Plasma via UPLC with MS/MS Detection	Minipig	0.100 to 100 ng/mL	2015N232986 (P1352)	m4.2.2.1
Quantitation of GSK1278863 in Minipig Epidermis via UPLC with MS/MS Detection - method performance evaluation	Minipig	0.500 to 500 ng/mL	2015N232987 (P1351)	m4.2.2.1
Quantitation of GSK1278863 in Minipig Dermis via UPLC with MS/MS Detection - Method Perfomance Evalution	Minipig	0.500 to 500 ng/mL	2015N237487 (P1370)	m4.2.2.1
Quantitation of GSK1278863 in acidified Gottingen mini pig plasma via UPLC with MS/MS detection	Minipig	0.100 to 100 ng/mL	2016N272071 (P1370)	m4.2.2.1
Abbreviated validation of a method for the determination of GSK1278863 in acidified monkey plasma using HPLC-MS/MS	Monkey	50 to 50000 ng/mL	CD2009/00712 (GSK1278863CYPLVALA)	m4.2.2.1
Method for the determination of metabolites of GSK1278863 (GSK2391220, GSK2531403, GSK2499166, GSK2531399, GSK2531398 and GSK2531400) in acidified monkey plasma using UHPLC-MS/MS	Monkey	1 to 1000 ng/mL	CD2010/00387 (GSK1278863CYPLVALB)	m4.2.2.1
Supplemental validation data to “The validation of a method for the determination of the metabolites of GSK1278863 (GSK2391220, GSK2531403, GSK2499166, GSK2531399, GSK2531398, and GSK2531400) in acidified monkey plasma using UHPLC-MS/MS	Monkey	1 to 1000 ng/mL	2011N126360 (GSK1278863CYPLVALB)	m4.2.2.1

Key:
HPLC = High performance liquid chromatography, LC = liquid chromatography, MS/MS = Tandem mass-spectrometry, NA = Not applicable, UHPLC = Ultra high performance liquid chromatography.

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

Table 3.1 Absorption of Daprodustat after a Single Dose

Test Article: GSK1278863

Location in CTD: m4.2.2.2

Report No.: UH2008/00002 **Study No.:** -

Species (Strain):	**Mouse (CD-1)**			**Rat (SD)**		
Gender (M/F)/Number of Animals:	3M/time point	3M/time point	3M/time point	3M	3M	3M
Feeding Condition:	Non-fasted	Fasted	Fasted	Non-fasted	Fasted	Fasted
Vehicle/Formulation:	2% DMSO, 49% of 40%(w/v) Captisol™1 in water, 49% isotonic saline, pH~7.0-7.5	2% DMSO, 20% (w/v) Captisol™1 in water, pH~7/Solution	50% PEG-400, 10% Ethanol, and 40% of 40% (w/v) Encapsin in water/Solution	2%DMSO, 49% of 40% (w/v) Captisol™1 in water, 49% isotonic saline, pH~7.0	2% DMSO, 20% (w/v) Captisol™1 in water, pH~6.5-7.0/Solution	1% (w/v) Methylcellulose in water/Suspension
Method of Administration:	IV (bolus)	Oral (gavage)	Oral (gavage)	IV (1 hour infusion)	Oral (gavage)	Oral (gavage)
Dose (mg/kg):	1.3	1.9	3.9	0.6	1.6	31.1
Sample:	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	GSK1278863	GSK1278863	GSK1278863	GSK1278863	GSK1278863	GSK1278863
Assay:	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS
PK Parameters:						
Cmax (µg/mL)	4.227	4.863	5.603	3.033 ± 0.312	2.863 ± 0.506	62.4 ± 3.78
AUC0-t (µg.h/mL)	31.923	41.817	71.430	53.5569 ± 6.3543	99.4573 ±14.9089	941.020 ± 68.912
AUC0-inf (µg.h/mL)	-	-	-	54.8169 ± 7.0091	106.626 ± 19.9696	-
tmax[a] (h)	-	1.0	2.0	-	4.0 [1.0 to 6.0]	2.0 [1.0 to 4.0]
t1/2 (h)	-	-	-	33.5 ± 6.2	37.2 ± 12.5	-
CL (mL/h/kg)	42	-	-	12 ± 1.2	-	-
Vss (L/kg)	0.3	-	-	0.4 ± 0.1	-	-
MRT (h)	7.8	-	-	36.7 ± 4.7	-	-
F (%)	-	88	-	-	100	-

Additional Information: Mean or Mean ± SD

- = not applicable; IV = intravenous, SD = Sprague-Dawley; HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry

a: Median [range].

Absorption of Daprodustat after a Single Dose (Continued)

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: UH2008/00002 **Study No.:** -

Species (Strain):	**Dog (beagle)**				
Gender (M/F)/Number of Animals:	3M	3M	3M	3M	3M
Feeding Condition:	Fasted	Fasted	Non-fasted	Non-fasted	Non-fasted
Vehicle/Formulation:	2% DMSO, 49% of 40% (w/v) Captisol™1 in water, 49% isotonic saline, pH~8.0	2% DMSO, 20% (w/v) Captisol™1 in water, pH~8.5/Solution	-/Capsule	-/Capsule	-/Capsule
Method of Administration:	IV (1 hour infusion)	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)
Dose (mg/kg):	1.2	3.2	5	5 (potassium salt)	5 (tris monohydrate)
Sample:	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	GSK1278863	GSK1278863	GSK1278863	GSK1278863	GSK1278863
Assay:	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS
PK Parameters:					
Cmax (µg/mL)	3.257 ± 0.232	2.537 ± 0.09	2.720 ± 0.249	1.413 ± 0.337	2.667 ± 0.901
AUC0-t (µg.h/mL)	21.4529 ± 4.5992	25.1892 ± 4.823	24.1541 ± 4.7588	13.3425 ± 1.8105	24.9266 ±9.9981
AUC0-inf (µg.h/mL)	23.6067 ±6.6162	28.3774 ± 6.8046	24.5485 ± 4.8559	13.6248 ± 1.8947	25.2100 ± 10.0479
tmax[a] (h)	-	1.5 [0.8 to 4.0]	4.0 [3.0 to 4.0]	3.0 [2.0to 4.0]	4.0 [2.0 to 4.0]
t1/2 (h)	6.6 ± 2.1	7.1 ± 1.7	7.5 ± 1.9	5.5 ± 0.6	6.7 ± 1.5
CL (mL/h/kg)	54 ± 12	-	-	-	-
Vss (L/kg)	0.5 ± 0.1	-	-	-	-
MRT (h)	8.8 ± 2.7	-	-	-	-
F (%)	-	46 ± 6	25	15	25

Additional Information: Mean ± SD
- = not applicable; IV = intravenous, SD = Sprague-Dawley; HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: Median [range]

Absorption of Daprodustat after a Single Dose (Continued)

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: UH2008/00002 **Study No.:** -

Species (Strain):	**Dog (beagle)**			**Monkey (cynomolgus)**	
Gender (M/F)/Number of Animals:	3M	3M	3M	3M	3M
Feeding Condition:	Non-fasted	Non-fasted	Non-fasted	Fasted	Fasted
Vehicle/Formulation:	1% (w/v) Methylcellulose in water, pH~5.7/Suspension	1% (w/v) Methylcellulose in water, pH~6.1/Suspension	1% (w/v) Methylcellulose in water, pH~5.3/Suspension	2% DMSO, 49% of 40% (w/v) Captisol™1 in water, 49% isotonic saline, pH~8.0-8.5	2% DMSO, 20% (w/v) Captisol™1 in water/Solution
Method of Administration:	Oral (gavage)	Oral (gavage)	Oral (gavage)	IV (1 hour infusion)	Oral (gavage)
Dose (mg/kg):	1	3.1	30.6	1.1	3
Sample:	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte:	GSK1278863	GSK1278863	GSK1278863	GSK1278863	GSK1278863
Assay:	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS
PK Parameters:					
Cmax (µg/mL)	0.439 ± 0.047	2.543 ± 0.582	7.887 ± 1.755	1.437 ± 0.169	0.374 ± 0.096
AUC0-t (µg.h/mL)	5.6859 ± 0.8618	22.4373 ± 6.5537	90.2177 ± 22.2743	2.0924 ± 0.43	1.7152 ± 0.3148
AUC0-inf (µg.h/mL)	6.8203 ± 1.4394	32.551, 15.6544	110.4849 ± 33.0166	2.1919 ± 0.557	2.0406 ± 0.4475
tmax[a] (h)	2.0 [1.0 to 4.0]	3.0 [1.5 to 4.0]	4.0 [4.0 to 6.0]	-	-
t1/2 (h)	8.5 ± 2.4	9.1, 5.2	9.0 ± 1.2	1.9 ± 0.1	6.0 ± 3.8
CL (mL/h/kg)	-	-	-	504 ± 12	-
Vss (L/kg)	-	-	-	0.8 ± 0.1	-
MRT (h)	-	-	-	1.6 ± 0.1	-
F (%)	32	40	16	-	34 ± 8

Additional Information: Mean ± SD (n=2 - 3)
- = not applicable; IV = intravenous, HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: Median [range]

Absorption of Daprodustat after a Single Dose (Continued)

Test Article: GSK1278863

	Location in CTD: m4.2.2.2		
	Report No.: 2012N156583	**Report No.:** CD2008/01285	
	Study No.: 12DMM041	**Study No.:** 08DMM039	
Species (Strain):	**Mouse (CD-1)**	**Rat (SD)**	**Rat (SD)**
Gender (M/F)/Number of Animals:	3M/time point	3M	3M
Feeding Condition:	Non-fasted	-	-
Vehicle/Formulation:	10% Captisol[a]/Solution	6% Cavitron/Solution	6% Cavitron/Solution
Method of Administration:	IV (bolus)	IV infusion, 22-minute	IV infusion, 2-hour
Dose (mg/kg):	20	17.5	11.1
Sample:	Plasma	Plasma	Plasma
Analyte:	GSK1278863	GSK1278863	GSK1278863
Assay:	UHPLC-MS/MS	LC/MS/MS	LC/MS/MS
PK Parameters:			
Cmax (µg/mL)	88.0	39.2 ± 3.1	54.6 ± 0.26
AUC0-t (µg.h/mL)	614	371 ± 57	423 ± 49
AUC0-inf (µg.h/mL)	685	NA	NA
tmax (h)	0.083	1.42 [0.40, 2.43][b]	2.00 [2.00, 2.50][b]
t1/2 (h)	7.28	NA	NA
CL (mL/h/kg)	26.6	NA	NA
Vss (L/kg)	0.270	NA	NA
MRT (h)	10.2	NA	NA

Additional Information: Mean ± SD

- = unknown; IV = intravenous, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MC = methylcellulose; NA = not calculated/not applicable; SD = Sprague-Dawley; UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

The systemic exposure (AUC0-t and Cmax) increased proportionally with increasing dose from 0.02 to 7.0 mg/kg.

a: 10% Captisol™ in 25 mM Tris buffer (final formulation pH 9)

b: Median [range]

Absorption of Daprodustat after a Single Dose (Continued)

Test Article: GSK1278863

	Location in CTD: m4.2.2.2 **Report No.:** 2013N184197	**Study No.:** R70458N		
Species (Strain):	**Rat (SD)**	**Rat (SD)**	**Rat (SD)**	**Rat (SD)**
Gender (M/F)/Number of Animals:	3F	3F	3F	3F
Feeding Condition:	-	-	-	-
Vehicle/Formulation:	1% MC[a]/Solution	1% MC[a]/Solution	1% MC[a]/Solution	1% MC[a]/Solution
Method of Administration:	Oral (gavage)	Oral (gavage)	Oral (gavage)	Oral (gavage)
Dose (mg/kg):	0.02	0.1	0.8	7.0
Sample:	Plasma	Plasma	Plasma	Plasma
Analyte:	GSK1278863	GSK1278863	GSK1278863	GSK1278863
Assay:	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS
PK Parameters:				
Cmax (µg/mL)	0.137 [0.135, 0.139]	0.850 [0.723, 0.995]	6.29 [6.07, 6.68]	46.5 [43.3, 50.4]
AUC0-t (µg.h/mL)	2.35 [2.12, 2.63]	11.5 [9.57, 12.7]	94.3 [92.2, 96.0]	704 [648, 790]
tmax (h)	4.0 [2.0, 8.0]	2.0 [2.0, 2.0]	2.0 [2.0, 2.0]	2.0 [2.0, 2.0]
t1/2 (h)	NA	NA	NA	NA

Additional Information: Mean [range]

- = unknown; IV = intravenous, LC-MS/MS = liquid chromatography-tandem mass spectrometry; MC = methylcellulose; NA = not calculated/not applicable; SD = Sprague-Dawley; UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

The systemic exposure (AUC0-t and Cmax) increased proportionally with increasing dose from 0.02 to 7.0 mg/kg.

a: 1% methylcellulose (400cps @ 2%) (w/v)/ 0.2% Tween 80 (v/v)/ 5 mM NaOH in water (final pH 6.0)

Table 3.2 Absorption of GSK2531401 (M13) after a Single Dose

Test Article: GSK2531401 (M13)

	Location in CTD: m4.2.2.2 **Report No.:** 2012N156583 **Study No.:** 12DMM041		**Report No.:** 2012N132320 **Study No.:** 11DMM049	**Report No.:** 2011N130731 **Study No.:** 11DMM060
Species (Strain):	**Mouse (CD-1)**	**Mouse (CD-1)**	**Mouse (CD-1)**	**Mouse (CD-1)**
Gender (M/F)/Number of Animals:	2 or 3M/time point	2M/time point	3M/time point	3M/time point
Feeding Condition:	Non-fasted	Non-fasted	Non-fasted	Non-fasted
Test Article:	GSK2531401	GSK2531401	GSK2531401	GSK2531401
Vehicle/Formulation:	Saline/Solution	Saline/Solution	Saline/Solution	1% MC/Suspension
Method of Administration:	IV (bolus)	IV (bolus)	IV (bolus)	Oral (gavage)
Dose (mg/kg):	40	100	40	150
Sample:	Plasma	Plasma	Plasma	Plasma
Analyte:	GSK2531401	GSK2531401	GSK2531401	GSK2531401
Assay:	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS
PK Parameters:				
Cmax (µg/mL)	81.5	210	NA[b]	0.476
AUC0-t (µg.h/mL)	27.8	92.8	NA[b]	2.13
AUC0-inf (µg.h/mL)	27.9	92.9	NA[b]	NA
tmax (h)	0.083	0.083	NA[b]	0.5
t1/2 (h)	4.61	1.04	NA[b]	NA
CL (mL/h/kg)	1210	1090	NA[b]	NA
Vss (L/kg)	0.433	0.418	NA[b]	NA
MRT (h)	0.357	0.384	NA[b]	NA

Additional Information: Mean**,** IV = intravenous; MC = methylcellulose; NA = not calculated/not applicable; UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

Both GSK2531401 and GSK1278863 (see Table 3.1 for data after GSK1278863 administration) were characterized by low plasma clearance and a low steady state volume of distribution in male CD-1 mice. GSK2531401 (metabolite of GSK1278863) exhibited approximately 40-fold higher clearance than GSK1278863.

a: 10% Captisol™ in 25 mM Tris buffer (final formulation pH 9)

b: Plasma concentrations were quantifiable only up to the 8 hour sampling period, therefore pharmacokinetic analysis of GSK2531401 was not performed due to insufficient data points for accurate analysis.

Absorption of GSK2531401 (M13) after a Single Dose (Continued)

Test Article: GSK2531401 (M13)

	Location in CTD: m4.2.2.2		
	Report No.: 2012N137345	**Report No.:** 2012N145868	
	Study No.: 12DMM004	**Study No.:** 12DMM019	
Species (Strain):	**Mouse (CD-1)**	**Rat (SD)**	**Rat (SD)**
Gender (M/F)/Number of Animals:	3M/time point	4M	4M
Feeding Condition:	Non-fasted	Non-fasted	Non-fasted
Test Article:	GSK2531401[a]	GSK2531401	GSK2531401
Vehicle/Formulation:	1% MC/Suspension	Saline/Solution	1% MC/Suspension
Method of Administration:	Oral (gavage)	IV[b]	Oral (gavage)
Dose (mg/kg):	50	40	150
Sample:	Plasma	Plasma	Plasma
Analyte:	GSK2531401	GSK2531401	GSK2531401
Assay:	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS
PK Parameters:			
Cmax (µg/mL)	0.171	28.1 ± 2.07	0.346 ± 0.0913
AUC0-t (µg.h/mL)	0.564	22.2 ± 4.85	1.72 ± 0.426
AUC0-inf (µg.h/mL)	NA	22.2 ± 4.85	2.17 ± 0.506
tmax (h)	0.5	NA	0.53 [0.50 - 0.55][c]
t1/2 (h)	NA	0.93 ± 0.29	NA
CL (mL/min/kg)	NA	14.8 ± 2.45	NA
Vss (L/kg)	NA	0.828 ± 0.0562	NA
MRT (h)	NA	0.96 ± 0.19	NA
F (%)	NA	NA	0.85[d]

Additional Information: Mean ± SD

IV = intravenous; MC = methylcellulose; NA = not calculated/not applicable; SD = Sprague-Dawley; UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

The low volume of distribution in male rats suggests limited distribution of GSK2531401 beyond the central compartment.

a: amorphous spray-dried

b: IV pharmacokinetic parameters were analyzed after a 2 minute infusion.

c: Median [range]

d: F (%) calculated using mean AUC0-inf for IV and oral doses.

Table 3.3 Absorption of GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) after a Single Co-administration Dose

Test Article: GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13)
Location in CTD: m4.2.2.2
Report No.: 2013N174038 **Study No.:** 13DMM016

Species (Strain):	**Mouse (CD-1)**	**Mouse (CD-1)**	**Mouse (CD-1)**
Gender (M/F)/Number of Animals:	3M/time point	3M/time point	3M/time point
Feeding Condition:	Non-fasted	Non-fasted	Non-fasted
Test Article:	M2	M3	M13
Vehicle/Formulation:	Phosphate buffer[a]/Solution	Phosphate buffer[a]/Solution	Phosphate buffer[a]/Solution
Method of Administration:	SC (as cocktail of M2, M3 and M13)	SC (as cocktail of M2, M3 and M13)	SC (as cocktail of M2, M3 and M13)
Dose (mg/kg):	2.0	2.5	1.3
Sample:	Plasma	Plasma	Plasma
Analyte:	M2	M3	M13
Assay:	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS
PK Parameters:			
C_{max} (µg/mL)	5.670	7.160	5.490
AUC_{0-2} (µg.h/mL)	1.510	1.960	1.510
AUC_{0-t} (µg.h/mL)	1.520	1.970	1.510
t_{max} (h)	0.083	0.083	0.083
t_{last} (h)	4.00	8.00	2.00

Additional Information: Mean
SC = Subcutaneous Injection, UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
The AUC_{0-2} and C_{max} of all three metabolites were comparable.
a: 25 mM phosphate buffer containing 4.75 mg/mL NaCl (pH = 7.35 - 7.40).

Table 3.4 Absorption of GSK2391804 (M8) after a Single Dose

Test Article: GSK2391804 (M8)

Location in CTD: m4.2.2.2
Report No.: 2012N153861
Study No.: 12DMM038

Species (Strain):	**Rat (SD)**	**Rat (SD)**
Gender (M/F)/Number of Animals:	4M	4M
Feeding Condition:	Non-fasted	Non-fasted
Test Article:	GSK2391804	GSK2391804
Vehicle/Formulation:	Saline/Solution	1% MC/Suspension
Method of Administration:	IV[a] (bolus)	Oral (gavage)
Dose (mg/kg):	40	100
Sample:	Plasma	Plasma
Analyte:	GSK2391804	GSK2391804
Assay:	UHPLC-MS/MS	UHPLC-MS/MS
PK Parameters:		
C_{max} (µg/mL)	134 ± 9.81	0.434 ± 0.0885
AUC_{0-t} (µg.h/mL)	NA	2.33 ± 0.726
AUC_{0-inf} (µg.h/mL)	52.9 ± 10.1	NA
t_{max} (h)	NA	0.54 [0.52 - 0.57][b]
$t_{1/2}$ (h)	5.37 ± 0.23	NA
CL (mL/min/kg)	12.2 ± 2.00	NA
V_{ss} (L/kg)	0.485 ± 0.0804	NA
MRT (h)	0.66 ± 0.02	NA
F (%)	NA	1.53[c]

Additional Information: Mean ± SD
IV = intravenous; MC = methylcellulose; NA = not calculated/not applicable; SD = Sprague-Dawley;
UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
As the bioanalytical assay was achiral, the exposure values correspond to the sum of the circulating stereoisomeric forms of GSK2391804 (metabolite of GSK1278863).
In male rats, GSK2391804 was characterized by a low plasma clearance, a low steady state volume of distribution and a long terminal half-life.
a: IV pharmacokinetic parameters were analyzed after a 2 minute infusion.
b: Median [range]
c: F (%) calculated using mean AUC0-inf for IV and mean AUC0-t oral due to the high % extrapolation of AUC_{t-inf} oral.

Absorption of GSK2391804 (M8) after a Single Dose (Continued)

Test Article: GSK2391804

Location in CTD: m4.2.2.2

Report No.: 2012N153861 **Study No.:** 12DMM038

Species (Strain):	**Rat (SD)**	**Rat (SD)**	**Rat (SD)**	**Rat (SD)**
Gender (M/F)/Number of Animals:	4M	4M	4M	4M
Feeding Condition:	Non-fasted	Non-fasted	Non-fasted	Non-fasted
Test Article:	GSK2391804	GSK2391804	GSK2391804	GSK2391804
Vehicle/Formulation:	Saline/Solution	Saline/Solution	Saline/Solution	1% MC/Suspension
Method of Administration:	IV[a]	IV[a]	IV[a]	Oral (gavage)
Dose (mg/kg):	40	40	40	100
Sample:	Plasma	Plasma	Plasma	Plasma
Analyte:	GSK2391220 (M2)	GSK2531403 (M3)	GSK2499166 (M4)	GSK2391220 (M2)
Assay:	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS
PK Parameters:				
C_{max} (µg/mL)	0.0702 ± 0.0147	0.00329 ± 0.000272	0.00386 ± 0.000286	0.00352 ± 0.000606
AUC_{0-t} (µg.h/mL)	0.0884 ± 0.0212	NA	NA	0.0197 ± 0.0121
t_{max} (h)[b]	0.52 [0.50 - 0.60)	0.11 [0.10 - 0.13)	0.11 [0.10 - 0.13)	0.54 [0.52 - 0.57)

Additional Information: Mean ± SD

IV = intravenous; MC = methylcellulose; NA = not available (not enough data points to calculate); SD = Sprague-Dawley; UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

As the bioanalytical assay was achiral, the exposure values correspond to the sum of the circulating stereoisomeric forms of each chiral metabolite.

Metabolites GSK2531403 (M3) and GSK2499166 (M4) were generally quantifiable to 1 hour post-intravenous dosing while they were non-quantifiable after oral administration. Metabolites GSK2531399 (M5), GSK2531398 (M6) and GSK2531401 (M13) were not quantifiable at any time point regardless of dose route.

Following either a single oral or intravenous administration of GSK2391804 (M8) to rats, plasma exposure (AUC_{0-t} and C_{max}) of selected, known hydroxylated metabolites of GSK1278863 was low (mean systemic exposure metabolite : M8 ratios <0.008).

a: IV pharmacokinetic parameters were analyzed as a 2 minute infusion.

b: Median [range]

Table 3.5 Dog single dose study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: CD2006/01837 **Study No.:** D06386

Species (Strain):	**Dog (beagle)**	
Test Article:	GSK1278863	
Vehicle/Formulation:	1% (w/v) methylcellulose/ suspension	
Method of Administration:	Oral (gavage)	
Sample:	Plasma	
Analyte:	GSK1278863	
Assay:	HPLC-MS/MS	
Gender (M/F)/Number of Animals:	1M/1F	
Dose (mg/kg/day):	120	
Duration of dosing (weeks):	Single	
PK Parameters:		
	Male	Female
C_{max} (µg/mL)	11.8[a]	25.2
AUC_{0-24} (µg.h/mL)	99.0[a]	313
t_{max} (h)	2.00[a]	8.00

Additional Information: Individual (n=1), - =unknown
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: Three episodes of emesis were noted for the male (D07M-219) between approximately 0.47 and 4 hours post dose.

Table 3.6 Absorption of Daprodustat after a Single Dermal Dose to Minipigs

Test Article:	GSK1278863	**Location in CTD:**	m4.2.2.2
Study Number:	F43142N	**Report No.:**	2013N180775
Study Title:	Single Application Dermal Irritation and Toxicokinetic Study in Minipigs		

PK Parameters:

Parameter		Dose Concentration of GSK1278863 (% w/w)[b]	
		10	**35**
AUC $_{0\text{-}t}$[a] (ng.h/mL)	Mean (n=2)	84.9	132
	Min	48.7	4.99
	Max	121	260
C_{max} (ng/mL)	Mean (n=2)	9.49	15.6
	Min	2.77	1.55
	Max	16.2	29.6
T_{max} (h)	Median (n=2)	12.5	14.0
	Min	1.0	4.0
	Max	24.0	24.0

Noteworthy Findings: No dermal irritation was observed in the study

Key:

a = $AUC_{0\text{-}t}$ was referred to the area under the concentration curve from time zero to the last quantifiable concentration up to 24 hours post dose.

b = The dose was administered as 1 g formulation/kg for at least 23 ± 1 hour duration of exposure.

Table 3.7 Absorption of Daprodustat after a Single Oral Dose to Monkeys

Test Article: GSK1278863

Location in CTD: m4.2.2.2

Species (Strain): Monkey (Cynomolgus) **Report No.:** CD2009/00639 **Study No.:** D09120

Test Article: GSK1278863

Vehicle/Formulation: 1% (w/v) aqueous methylcellulose/Suspension

Method of Administration: Oral (gavage)

Sample: Plasma

Analyte: GSK1278863

Assay: HPLC-MS/MS

Gender (M/F)/Number of Animals: 2M/2F

Dose (mg/kg/day): 10, 30, 100, 300, 600

Duration of dosing (weeks): Single

PK Parameters:

	Male					Female				
Dose (mg/kg/day)	10	30	100	300	600	10	30	100	300	600
C_{max} (µg/mL)	1.30 [1.05, 1.55]	2.60 [2.09, 3.10]	6.04 [5.40, 6.69]	7.24 [5.24, 9.23]	16.2[a] [11.3, 21.1]	1.75 [1.31, 2.19]	2.94 [2.77, 3.10]	6.92 [5.41, 8.44]	10.1[b] [8.05, 12.2]	10.6[c] [8.03, 13.1]
AUC_{0-t} (µg.h/mL)	5.67 [4.28, 7.05]	19.9 [14.5, 25.3]	53.6 [53.4, 53.8]	103 [77.5, 128]	87.5[a] [86.5, 88.5]	8.36 [8.01, 8.70]	21.1 [20.8, 21.3]	72.5 [52.1, 92.9]	92.8[b] [90.4, 95.3]	70.6[c] [51.9, 89.3]
t_{max} (h)[d]	2.00 [2.00, 2.00]	1.50 [1.00, 2.00]	5.00 [2.00, 8.00]	6.00 [4.00, 8.00]	4.00[a] [4.00, 4.00]	2.00 [2.00, 2.00]	3.00 [2.00, 4.00]	5.00 [2.00, 8.00]	4.00[b] [4.00, 4.00]	3.00[c] [2.00, 4.00]

Additional Information: Mean [range] (n=2)

HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry

a: Both male animals given 600 mg/kg, had 2 episodes each of emesis.

b: One female animal given 300 mg/kg, had 3 episodes of emesis.

c: One female animal had a single episode of emesis and the other had multiple episodes of emesis given 600 mg/kg.

d: Median [range]

4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES

Table 4.1 Mouse 13 week study with Daprodustat (sampled after a duration of dosing for 7 days)

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2011N117145 **Study No.:** 10DMM034

Species (Strain):	**Mouse (CD-1)**			
Test Article:	GSK1278863			
Vehicle/Formulation:	1% methylcellulose (400cps) in water/Suspension			
Method of Administration:	Oral (gavage)			
Sample:	Plasma			
Analyte:	GSK1278863		M2, M3, M4, M5, M6, M13	
Assay:	LC-MS/MS		UHPLC-MS/MS	
Gender (M/F)/Number of Animals:	21M, 3/time point	21F, 3/time point	21M, 3/time point	21F, 3/time point
Dose (mg/kg/day):	30	30	30	30
Duration of dosing (days):	7	7	7	7
PK Parameters:				
C_{max} (µg/mL)	66.0 ± 5.3	75.8 ± 3.1	NA	NA
AUC_{0-t} (µg.h/mL)	819	1118	NA	NA
t_{max} (h)	1.00	4.00	NA	NA

Additional Information: Mean or Mean ± SD

LC-MS/MS = liquid chromatography-tandem mass spectrometry; NA = not available; UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

M2 (GSK2391220), M3 (GSK2531403), M4 (GSK2499166), M5 (GSK2531399), M6 (GSK2531398) and M13 (GSK2531400) are metabolites of GSK1278863.

Of the six metabolites analyzed, only M2 at the 1 hour time point, in one animal only, was quantifiable.

There was no marked (>2-fold) difference in systemic exposure (AUC_{0-t} and C_{max} values) between male and female mice.

Table 4.2 Mouse 14 day study with Daprodustat

Test Article: GSK1278863 **Location in CTD:** m4.2.2.2
Study Number: D09169 **Report No.:** CD2009/00900
Study Title: GSK1278863A: 14-Day Oral Toxicity Study in Mice
Plasma Toxicokinetic Values

Parameter	Period	Sex	Dose (mg/kg/day)
n=2 or 3 mice per timepoint			**30**
AUC_{0-t} (μg.h/mL)	Day 14	Male	818
		Female	992
C_{max} (μg/mL)	Day 14	Male	64.6
		Female	73.2
T_{max} (h)	Day 14	Male	4.0
		Female	1.0

Table 4.3 Mouse 13 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2011N112096 **Study No.:** G10121

Species (Strain):		**Mouse (CD-1)**					
Test Article:		GSK1278863					
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension					
Method of Administration:		Oral (gavage)					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		42M/42F					
Dose (mg/kg/day):		3, 30, 60					
Duration of dosing (weeks):		13					
PK Parameters:							
		Male			Female		
Dose (mg/kg/day)		3	30	60	3	30	60
C_{max} (µg/mL)	Day1	11.5 ± 1.6	62.9 ± 12.8	78.2 ± 6.3	11.1 ± 1.4	63.9 ± 8.4	94.8 ± 14.4
	Week4	12.3	46.0 ± 8.8	52.0 ± 13.3	16.7 ± 2.7	55.3 ± 7.8	68.9
AUC_{0-t} (µg.h/mL)	Day1	140	629	1002	176	829	1229
	Week4	151	520	722	211	817	943
t_{max} (h)	Day1	1.00	2.00	2.00	0.50	2.00	2.00
	Week4	2.00	2.00	4.00	2.00	4.00	8.00

Additional Information: Mean or Mean ± SD (n= 2 - 4/point)
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry

Table 4.4 Mouse 13 week study with Daprodustat and metabolites

Test Article: GSK1278863, GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13)

Location in CTD: m4.2.2.2

Report No.: 2014N199031 **Study No.:** M70456G

Species (Strain):	**Mouse (CD-1)**
Test Article:	GSK1278863
Vehicle/Formulation:	1% (w/v) aqueous methylcellulose/Suspension
Method of Administration:	GSK1278863: Oral (gavage), Metabolite cocktail (M2, M3, M13): Subcutaneous
Sample:	Plasma
Analyte:	GSK1278863, M2, M3, M13
Assay:	UHPLC-MS/MS
Gender (M/F)/Number of Animals:	48M/48F
Dose (mg/kg/day):	GSK1278863: 6, 20, Metabolite cocktail (M2, M3, M13): 2/2.5/1.3
Duration of dosing (weeks):	13
PK Parameters:	

Analyte: GSK1278863		Male		Female	
	Dose (mg/kg/day)	6	20	6	20
C_{max} (µg/mL)	Day1	15.8 ± 1.8	31.8 ± 13.2	17.2 ± 1.6	37.4 ± 2.5
	Week4	19.3 ± 3.4	38.4 ± 8.0	30.4 ± 6.0	59.5 ± 13.0
AUC_{0-t} (µg.h/mL)	Day1	260	422	291	628
	Week4	274	525	464	897
t_{max}	Day1	8.0	2.0	1.0	8.0
	Week4	2.0	8.0	2.0	1.0

Mouse 13 week study with Daprodustat (Continued)

Analyte: Metabolite

			Male			Female		
	Analyte:		M2	M3	M13	M2	M3	M13
	Dose of GSK1278863	Dose (mg/kg/day)	2.0	2.5	1.3	2.0	2.5	1.3
C_{max} (µg/mL)	6 mg/kg/day	Day1	2.71 ± 0.29	3.30 ± 0.31	2.08 ± 0.20	2.58 ± 1.06	2.96 ± 1.28	2.09 ± 0.85
		Week4	2.22 ± 0.28	2.76 ± 0.32	1.72 ± 0.54	3.02 ± 0.26	3.48 ± 0.34	2.32 ± 0.21
	20 mg/kg/day	Day1	2.48 ± 0.27	3.01 ± 0.29	1.99 ± 0.12	3.82 ± 0.66	4.50 ± 0.92	2.77 ± 0.39
		Week4	1.79 ± 0.64	2.34 ± 0.85	1.48 ± 0.50	2.95 ± 0.22	3.51 ± 0.28	2.23 ± 0.18
AUC_{0-t} (µg.h/mL)	6 mg/kg/day	Day1	1.21	1.49	1.07	1.11	1.22	0.371
		Week4	1.27	1.60	1.16	1.50	1.69	1.19
	20 mg/kg/day	Day1	1.15	1.42	1.05	1.37	1.50	0.539
		Week4	1.23	1.58	1.18	1.65	1.90	1.33
t_{max} (h)	6 mg/kg/day	Day1	0.083	0.083	0.083	0.250	0.250	0.250
		Week4	0.083	0.083	0.250	0.250	0.250	0.250
	20 mg/kg/day	Day1	0.083	0.083	0.250	0.083	0.083	0.083
		Week4	0.250	0.250	0.250	0.250	0.250	0.250

Additional Information: Mean or Mean ± SD (n= 3)
UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

Table 4.5 Mouse 2 year study with Daprodustat

Test Article: GSK1278863, GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13)

Location in CTD: m4.2.2.2

Report No.: 2014N221714 **Study No.:** M70585G

Species (Strain):		**Mouse (CD-1)**					
Test Article:		GSK1278863					
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension (daprodustat). 25 mM sodium phosphate, pH7.4 (metabolite cocktail)					
Method of Administration:		Oral (gavage), SC injection for metabolite cocktail					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		UHPLC-MS/MS					
Gender (M/F)/Number of Animals:		51M/51F					
Dose (mg/kg/day):		GSK1278863: 0.2, 0.8, 3, Metabolite cocktail (M2, M3, M13): 2/2.5/1.3					
Duration of dosing (years):		2					
PK Parameters:							
	Analyte	GSK1278863					
		Male			Female		
	Dose (mg/kg/day)	0.2	0.8	3	0.2	0.8	3
C_{max} (µg/mL)	Week4	0.987 ± 0.195	3.92 ± 0.14	10.6 ± 2.1	1.42 ± 0.23	5.31 ± 0.72	17.0 ± 5.4
	Week26	0.935 ± 0.134	3.83 ± 0.88	10.2 ± 4.4	1.76 ± 0.28	6.02 ± 0.42	19.5 ± 4.1
AUC_{0-t} (µg.h/mL)	Week4	13.8	49.1	145	22.3	82.7	278
	Week26	13.1	43.2	141	28.1	92.8	335
t_{max} (h)	Week4	1.00	2.00	0.50	1.00	4.00	4.00
	Week26	2.00	0.25	1.00	8.00	4.00	0.50

Mouse 2 year study with Daprodustat (Continued)

Analyte: Metabolite

			Male			Female		
		Analyte:	M2	M3	M13	M2	M3	M13
	Dose of GSK1278863	Dose (mg/kg/day)	2.0	2.5	1.3	2.0	2.5	1.3
C_{max} (µg/mL)	0.2 mg/kg/day	Week4	2.37 ± 0.30	2.82 ± 0.43	2.01 ± 0.28	2.73 ± 1.23	3.20 ± 1.50	1.88 ± 0.84
		Week26	3.39 ± 1.25	4.06 ± 1.51	2.37 ± 0.95	3.60 ± 0.19	3.89 ± 1.11	2.58 ± 0.25
	0.8 mg/kg/day	Week4	2.11 ± 0.88	2.45 ± 0.99	1.53 ± 0.05	2.51 ± 0.69	2.86 ± 0.87	1.98 ± 0.61
		Week26	3.79 ± 0.55	4.56 ± 0.63	2.71 ± 0.45	4.61 ± 0.67	5.50 ± 0.97	3.12 ± 0.46
	3 mg/kg/day	Week4	2.69 ± 0.64	3.14 ± 0.75	1.96 ± 0.44	2.41 ± 1.00	2.87 ± 1.17	1.82 ± 0.15
		Week26	4.00 ± 0.89	4.70 ± 1.04	2.83 ± 0.63	3.83 ± 0.53	4.57 ± 0.60	2.57 ± 0.25
AUC_{0-t} (µg.h/mL)	0.2 mg/kg/day	Week4	1.05	1.27	0.979	1.25	1.34	0.983
		Week26	1.24	1.46	1.08	1.95	2.02	1.48
	0.8 mg/kg/day	Week4	1.02	1.23	0.913	1.08	1.14	0.916
		Week26	1.53	1.86	1.37	1.87	1.95	1.42
	3 mg/kg/day	Week4	1.27	1.52	1.18	1.26	1.37	1.01
		Week26	1.50	1.79	1.29	1.86	1.98	1.40
t_{max} (h)	0.2 mg/kg/day	Week4	0.250	0.250	0.250	0.083	0.083	0.083
		Week26	0.083	0.083	0.083	0.250	0.083	0.250
	0.8 mg/kg/day	Week4	0.083	0.083	0.250	0.250	0.250	0.250
		Week26	0.083	0.083	0.083	0.083	0.083	0.083
	3 mg/kg/day	Week4	0.083	0.083	0.083	0.083	0.083	0.250
		Week26	0.083	0.083	0.083	0.083	0.083	0.083

Additional Information: Mean or Mean ± SD (n= 3)
UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

Table 4.6 Mouse 14 day study with metabolites GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13)

Test Article: GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13)
Location in CTD: m4.2.2.2
Report No.: 2014N208198 **Study No.:** M70590N

Species (Strain):	**Mouse (CD-1)**	**Mouse (CD-1)**	**Mouse (CD-1)**
Gender (M/F)/Number of Animals:	3F/time point	3F/time point	3F/time point
Feeding Condition:	Non-fasted	Non-fasted	Non-fasted
Test Article:	M2, M3, M13	M2, M3, M13	M2, M3, M13
Vehicle/Formulation:	Phosphate buffer[a]/Solution	Phosphate buffer[a]/Solution	Phosphate buffer[a]/Solution
Method of Administration:	SC	SC	SC
Dose (mg/kg/day):	M2/M3/M13 = 2.0/2.5/1.3	M2/M3/M13 = 2.0/2.5/1.3	M2/M3/M13 = 2.0/2.5/1.3
Sample:	Plasma	Plasma	Plasma
Analyte:	M2	M3	M13
Assay:	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS
PK Parameters:			
C_{max} (µg/mL)	2.67 ± 0.49	3.03 ± 0.28	2.04 ± 0.38
AUC_{0-t} (µg.h/mL)[b]	1.61	1.68	1.26
t_{max} (h)	0.25	0.08	0.25

Additional Information: Mean or Mean ± SD
SC = Subcutaneous Injection, HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: The 3 metabolites were administered as a cocktail via 1 injection per day of dosing.
b: Plasma concentrations of GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) were not quantifiable at 24 hours in all three animals

Table 4.7 Rat 14 day IV study with Daprodustat

Test Article: GSK1278863 **Location in CTD:** m4.2.2.2
Study Number: I08185 **Report No.:** CD2008/01740
Study Title: GSK1278863A: An Intravenous (Infusion) Once Daily Investigative Toxicology Study in the Male Rat

Plasma Toxicokinetic Values

Parameter[a]	Period	Dose (mg/kg/day)	
Male (n=3)		**20**	**40**
AUC_{0-t} (μg.h/mL)	Day 1	1224 [1189-1284]	1643 [1587-1738]
	Day 14[b]	1689 [1252-1937]	2022 [1968-2060]
C_{max} (μg/mL)	Day 1	93.1 [82.8-101]	106 [101-115]
	Day 14	110 [94.5-135]	109 [99.5-118]
Median T_{max} (h)	Day 1	4.0 [4.0-4.0]	2.0 [2.0-4.0]
	Day 14	4.0 [4.0-4.5]	2.0 [2.0-4.0]

Key:
a – Results are reported as mean and [range]
b – For the purpose of Day 14 AUC calculation, the concentration at 24 hours was assigned to the predose concentration value

Table 4.8 Rat 5 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2012N153858 **Study No.:** 12DMM037

Species (Strain):	**Rat (Sprague Dawley)**				
Test Article:	GSK1278863				
Vehicle/Formulation:	1% (w/v) aqueous methylcellulose/Suspension				
Method of Administration:	Oral (gavage)				
Sample:	Plasma				
Analyte:	GSK1278863, M2, M3, M4, M5, M6, M13, M8, M10 and M9/M22				
Assay:	UHPLC-MS/MS				
Gender (M/F)/Number of Animals:	3M				
Dose (mg/kg/day):	30				
Duration of dosing (days):	5				
PK Parameters:					
Analyte	GSK1278863	M2 (GSK2391220)	M8 (GSK2391804)	M9 (GSK2399022) / M22 (GSK2632557)	M10 (GSK2531408)
C_{max} (µg/mL)	112 ± 9.87	0.00682 ± 0.00273	0.0302 ± 0.0093	0.00931 ± 0.00274	0.0168 ± 0.0023
AUC_{0-t} (µg.h/mL)	1980 ± 69.3	0.0946 ± 0.0255	0.458 ± 0.088	0.159 ± 0.027	0.333 ± 0.042
t_{max} (h)	1.03	1.07	1.07	1.07	1.07

Additional Information: Mean or Mean ± SD (n=3)
UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry

Table 4.9 Rat 5 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2016N305715 **Study No.:** R71028N

Initial Phase

Species (Strain):	**Pregnant Rat (SD)**
Test Article:	GSK1278863
Vehicle/Formulation:	1% (w/v) aqueous methylcellulose/Suspension
Method of Administration:	Daprodustat: Oral (gavage) Metabolite Cocktail: Subcutaneous
Sample:	Plasma
Analyte:	GSK1278863, M2, M3, M13
Assay:	HPLC-MS/MS
Gender (M/F)/Number of Animals:	6F
Dose (mg/kg/day):	60 + metabolite cocktail[a], 60 + metabolite cocktail[b]
Duration of dosing (days):	5 (GD6 to GD10)
PK Parameters:	

	GSK1278863 (60 mg/kg/day) plus GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) (Cocktail at 2.5, 3.2, and 1.8 mg/kg/day, respectively)[a]				GSK1278863 (60 mg/kg/day) plus GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) (Cocktail at 7.5, 9.6, and 5.4 mg/kg/day, respectively)[b]			
	GSK1278863	M2	M3	M13	GSK1278863	M2	M3	M13
C_{max} (µg/mL)	126 ± 14	1.68 ± 0.18	1.87 ± 0.16	1.34 ± 0.13	141 ± 3	6.63 ± 1.02	7.84 ± 1.44	4.99 ± 0.69
AUC_{0-t} (µg.h/mL)	2300	3.33	3.27	2.79	2510	9.61	9.92	8.00
t_{max} (h)	2.00	0.250	0.250	0.250	2.00	0.250	0.250	0.250

Additional Information: Mean or Mean ± SD (n=3)
HPLC-MS/MS = High performance liquid chromatography-tandem mass spectrometry
a = GSK1278863A at 60 mg/kg/day and total daily Dose of Metabolite Cocktail at 2.5, 3.2, and 1.8 mg/kg/day (for M2, 3, or 13, respectively).
b = GSK1278863A at 60 mg/kg/day and total daily Dose of Metabolite Cocktail at 7.5, 9.6, and 5.4 mg/kg/day(for M2, 3, or 13, respectively).

Rat 5 day study with Daprodustat (Continued)

Test Article: GSK1278863

Location in CTD: m4.2.2.2

Report No.: 2016N305715 **Study No.:** R71028N

Extension Phase

Species (Strain):	**Pregnant Rat (SD)**		
Test Article:	GSK1278863		
Vehicle/Formulation:	1% (w/v) aqueous methylcellulose/Suspension		
Method of Administration:	Oral (gavage)		
Sample:	Plasma		
Analyte:	GSK1278863		
Assay:	HPLC-MS/MS		
Gender (M/F)/Number of Animals:	6F		
Dose (mg/kg/day):	10, 40		
Duration of dosing (days):	5 (GD6 to GD10)		
PK Parameters:		GSK1278863[e]	
	10[a]	40[b]	40[c]
C_{max} (µg/mL)	72.4 ± 5.6	118 ± 4.7	108 ± 10.0
	[65.9, 75.6]	[113, 122]	[100, 119]
AUC_{0-t} (µg.h/mL)	1030 ± 56	2100 ± 310	2100 ± 166
	[959, 1060]	[1750, 2340]	[1910, 2220]
t_{max} (h)[d]	2.00	2.00	2.00
	[2.00, 2.00]	[2.00, 4.00]	[0.500, 4.00]

Additional Information: Mean or Mean ± SD [range] (n=3)

HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry

a: Animals were dosed once daily with GSK1278863 by oral gavage at 10 mg/kg/day, and twice daily with GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) by subcutaneous injection at 2.5, 3.2, and 1.8 mg/kg/day (1.25, 1.6, or 0.9 mg/kg/dose), respectively.

b: Animals were dosed once daily with GSK1278863 by oral gavage at 40 mg/kg/day, and twice daily by subcutaneous injection with vehicle.

c: Animals were dosed once daily with GSK1278863 by oral gavage at 40 mg/kg/day, and twice daily with GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) by subcutaneous injection at 2.5, 3.2, and 1.8 mg/kg/day (1.25, 1.6, or 0.9 mg/kg/dose), respectively.

d: Median [range]

e: Toxicokinetics were assessed for only GSK1278863 in the extension phase of this study.

Table 4.10 Rat 14 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: RD2006/01192 **Study No.:** R41921

Species (Strain):		**Rat (SD)**		
Test Article:		GSK1278863		
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension		
Method of Administration:		Oral (gavage)		
Sample:		Plasma		
Analyte:		GSK1278863		
Assay:		HPLC-MS/MS		
Gender (M/F)/Number of Animals:		3M		
Dose (mg/kg/day):		10, 30, 60		
Duration of dosing (days):		14		
PK Parameters:				
		10	30	60
C_{max} (µg/mL)[a]	Day1	40.8 [35.7 to 45.6]	65.0 [58.2 to 73.1]	88.6 [76.2 to 100]
	Day14	54.8 [43.2 to 62.7]	64.2 [56.3 to 72.7]	93.8 [85.6 to 104]
AUC_{0-24} (µg.h/mL)	Day1	725 [691 to 777]	1135 [963 to 1352]	1590 [1468 to 1700]
	Day14	946 [696 to 1124]	1241 [1036 to 1432]	1745 [1516 to 1924]
t_{max} (h)[a]	Day1	2 [2 to 8]	1 [1 to 8]	4 [2 to 4]
	Day14	2 [0.5 to 4]	2 [2]	2 [1 to 2]

Additional Information: Mean [range] (n=3)
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: Median and [range].

Table 4.11 Rat 14 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: CD2008/01306 **Study No.:** D08114

Species (Strain):		**Rat (SD)**		
Test Article:		GSK1278863		
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension		
Method of Administration:		Oral (gavage)		
Sample:		Plasma		
Analyte:		GSK1278863		
Assay:		HPLC-MS/MS		
Gender (M/F)/Number of Animals:		3F		
Dose (mg/kg/day):		100, 250, 500		
Duration of dosing (days):		14		
PK Parameters:				
		100	250	500
C_{max} (µg/mL)	Day1	173 [160 – 179]	136 [131 – 139]	136 [129 – 141]
	Day14	187 [172 – 208]	147[b] [139, 155]	NA[c]
AUC0-t (µg.h/mL)	Day1	3083 [2707 – 3452]	2939 [2788 – 3019]	2708 [2451 – 2954]
	Day14	2994 [2808 – 3321]	3184[b] [3044, 3324]	NA[c]
t_{max} (h)[a]	Day1	4.00 [1.00 – 4.00]	2.00 [1.00 – 24.00]	24.00 [1.00 – 24.00]
	Day14	1.00 [1.00 – 2.00]	1.50[b] [1.00, 2.00]	NA[c]

Additional Information: Mean [range] (n=3)
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: Median [range].
b: n = 2. Rat 3505 was found dead prior to Day 14.
c: NA: Not applicable. All rats given 500 mg/kg/day were either found dead or euthanized prior to Day 14.

Table 4.12 Rat 38 day study with Daprodustat (sampled after a duration of dosing for 14 days)

Test Article: GSK1278863A plus M2 (GSK2391220), M3 (GSK2506104), and M13 (GSK2531401)

Location in CTD: m4.2.2.2

Report No.: 2018N365151 **Study No.:** R71242G

Species (Strain):	**Rat (SD)**
Test Article:	GSK1278863 + metabolites
Vehicle/Formulation:	Daprodustat: 1% (w/v) aqueous methylcellulose/Suspension Metabolite Cocktail (subcutaneous): Aqueous 25 mM Phosphate Buffer, pH 7.4/ Solution
Method of Administration:	Daprodustat: Oral (gavage) Metabolite Cocktail: Subcutaneous
Sample:	Plasma
Analyte:	GSK1278863, GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13)
Assay:	HPLC-MS/MS
Number of Animals:	24F
Dose (mg/kg/day):	Daprodustat: 0.8, 7, 40 Metabolites cocktail: 2.5, 3.2, 1.8 (GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13) mg/kg/day [dosed as 1.25, 1.8 and 0.9 mg/kg/dose BID every 6 hrs])
Duration of dosing (days):	Gestation Day(GD) 6 to Lactation Day 21 or GD6 to GD9

PK Parameters:		LD21 0.8/ (2.5, 3.2, 1.8)	LD21 7/ (2.5, 3.2, 1.8)	LD21 40/ (2.5, 3.2, 1.8)	GD9 0.8/ (2.5, 3.2, 1.8)	GD9 7/ (2.5, 3.2, 1.8)	GD9 40/ (2.5, 3.2, 1.8)
GSK1278863	AUC_{0-t} (μg.h/mL)	98.4	613	1620	146	855	2240
	C_{max} (μg/mL)	8.12	47.6 ± 3.1	92.6 ± 17.2	8.51 ± 0.62	52.0 ± 2.9	128 ± 4.7
	Tmax (h)	4.00	4.00	6.00	6.08	6.00	6.00
GSK2391220 (M2)	AUC_{0-t} (μg.h/mL)	2.72	2.24	3.03	2.65	2.50	2.51
	C_{max} (μg/mL)	1.55 ± 0.26	1.23 ± 0.07	1.56 ± 0.24	1.97 ± 0.26	1.55 ± 0.47	1.63 ± 0.23
	Tmax[a] (h)	6.25	6.25	0.250	0.250	0.250	6.25
GSK2506104 (M3)	AUC_{0-t} (μg.h/mL)	2.91	2.28	2.90	2.73	2.47	2.36
	C_{max} (μg/mL)	1.74 ± 0.37	1.37 ± 0.06	1.76 ± 0.27	2.19 ± 0.27	1.72 ± 0.51	1.75 ± 0.24
	Tmax[a] (h)	6.25	6.25	0.250	0.250	0.250	6.25

Rat 38 day study with Daprodustat (Continued)

PK Parameters:		LD21 0.8/ (2.5, 3.2, 1.8)	LD21 7/ (2.5, 3.2, 1.8)	LD21 40/ (2.5, 3.2, 1.8)	GD9 0.8/ (2.5, 3.2, 1.8)	GD9 7/ (2.5, 3.2, 1.8)	GD9 40/ (2.5, 3.2, 1.8)
GSK2531401 (M13)	AUC_{0-t} (μg.h/mL)	2.15	1.64	2.16	2.16	1.94	1.88
	C_{max} (μg/mL)	1.23 ± 0.14	0.983 ± 0.08	1.21 ± 0.19	1.51 ± 0.21	1.19 ± 0.40	1.33 ± 0.18
	Tmax[a] (h)	6.25	6.25	0.250	0.250	0.250	6.25

Additional Information: Mean or Mean ± SD
Composite toxicokinetic parameters were derived from mean plasma concentration data.
HPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
a: Tmax was calculated from the first daily dose

Table 4.13 Rat 4 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: RD2007/01333 **Study No.:** R42197

Species (Strain):		**Rat (SD)**					
Test Article:		GSK1278863					
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension					
Method of Administration:		Oral (gavage)					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		3M/3F					
Dose (mg/kg/day):		2, 7, 20					
Duration of dosing (weeks):		4					
PK Parameters:							
		Male			Female		
		2	7	20	2	7	20
C_{max} (µg/mL)	Day1	12.7 ± 0.5 [12.1 –13.0]	34.8 ± 1.2 [34.1 – 36.1]	94.7 ± 10.3 [83.2 – 103]	16.8 ± 4.8 [13.5 – 22.3]	38.1 ± 8.3 [30.0 – 46.5]	90.4 ±15.0 [74.2 – 104]
	Day28	26.5 ± 0.8 [25.7 – 27.2]	75.1 ± 8.8 [69.0 – 85.2]	96.0 ± 18.5 [79.6 – 116]	31.3 ± 7.3 [23.1 – 37.1]	69.7 ± 6.7 [64.4 – 77.3]	110 ± 10.6 [97.7 – 117]
AUC_{0-24} (µg.h/mL)	Day1	191 ± 5 [185 – 193]	572 ± 54 [512 – 615]	1450 ± 170 [1301 – 1635]	231 ± 67 [178 – 306]	525 ± 61 [467 – 589]	1300 ± 152 [1128 – 1416]
	Day28	403 ± 51 [358 – 459]	1197 ± 142 [1089 – 1358]	1726 ± 324 [1370 – 2003]	495 ± 149 [350 – 648]	1086 ± 127 [953 – 1205]	1900 ± 112 [1828 – 2029]
t_{max} (h)[a]	Day1	2.00 [1.00 – 2.00]	2.00 [1.00 – 2.00]	1.00 [1.00 – 4.00]	1.00 [1.00 – 1.00]	1.00 [0.50 – 1.00]	1.00 [1.00 – 2.00]
	Day28	2.00 [2.00 – 2.00]	2.00 [2.00 – 2.00]	2.00 [1.00 – 2.00]	1.00 [1.00 – 1.00]	1.00 [1.00 – 1.00]	1.00 [1.00 – 2.00]

Additional Information: Mean or Mean ± SD [range] (n=3)
HPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
a: Median [range]

Table 4.14 Rat 13 week study with Daprodustat

Test Article: GSK1278863 **Location in CTD:** m4.2.2.2

Species (Strain): **Rat (SD)** **Report No.:** CD2009/00951 **Study No.:** G09057

Test Article:		GSK1278863							
Vehicle/Formulation:		1% aqueous methylcellulose/Suspension							
Method of Administration:		Oral (gavage)							
Sample:		Plasma							
Analyte:		GSK1278863							
Assay:		HPLC-MS/MS							
Gender (M/F)/Number of Animals:		3M/3F							
Dose (mg/kg/day):		0.8, 4, 20, 100							
Duration of dosing (weeks):		13							
PK Parameters:									
		Male				Female			
	Dose (mg/kg/day)	0.8	4	20	100	0.8	4	20	100
C_{max} (µg/mL)	Day1	5.95 [5.81-6.02]	29.3 [25.6-32.5]	83 [68.9 – 95.2]	128 [119-132]	6.37 [6.08-6.60]	27.8 [25.8-29.1]	92.2 [8.73 – 95.1]	134 [131-139]
	Week4	11.8 [11.3-12.6]	42.4 [31.0-49.2]	98 [93.7 - 102]	122 [119-125]	10.9 [9.25-12.6]	59.1 [55.9-61.6]	105 [97.0 - 115]	150 [145-155]
	Week13	12.0 [10.9-13.0]	48.9 [47.0-51.3]	102 [96.3 - 111]	-	11.4 [9.26-14.0]	55.2 [49.7-61.7]	138 [132 - 143]	-
AUC_{0-24} (µg.h/mL)	Day1	101 [90.5 - 107]	495 [420 - 560]	1480 [1370-1600]	2450 [2280-2530]	103 [98.2 - 107]	379 [168 - 508]	1620 [1520-1670]	2900 [2800-3010]
	Week4	170 [160 -176]	567 [454 - 648]	1370 [1140-1490]	2200 [1890-2390]	159 [127 -197]	734 [665 - 810]	1780 [1500-2010]	2780 [2640-2910]
	Week13	152 [140 - 161]	606 [509 - 685]	1040 [842-1310]	-	167 [125 - 223]	701 [594 - 843]	2140 [2020-2330]	-
t_{max} (h)[a]	Day1	4 [2-4]	2 [2-4]	2 [1-8]	4 [2-4]	2 [1-2]	1 [1-2]	2 [1-4]	4 [4-8]
	Week4	1	1	4 [2-4]	4 [1-4]	0.5 [0.5-1]	0.5 [0.5-1]	4 [1-8]	8 [0.5-8]
	Week13	1	1 [0.5-2]	1 [1-2]	-	1 [1-2]	1 [0.5-2]	1	-

Additional Information: Mean [range] (n=3)

HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry

a: Median [range]

Table 4.15 Rat 26 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2011N126130 **Study No.:** G10267

Species (Strain):		**Rat (SD)**					
Test Article:		GSK1278863					
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension					
Method of Administration:		Oral (gavage)					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		3 or 9M/3 or 9F					
Dose (mg/kg/day):		0.8, 4, 10					
Duration of dosing (weeks):		26					
PK Parameters:							
		Male			Female		
Dose (mg/kg/day)		0.8	4	10	0.8	4	10
C_{max} (µg/mL)	Week4	10.0 ± 1.0 [9.00 – 11.0]	37.7 ± 4.08 [33.4 – 41.5]	49.5 ± 2.28 [47.0 – 51.5]	11.0 ± 0.3 [10.9 – 11.4]	40.9 ± 1.9 [39.4 – 43.1]	58.5 ± 5.8 [54.5 – 65.2]
	Week13	13.1 ± 3.1 [10.2 – 16.4]	41.0 ± 5.2 [35.9 – 46.3]	45.1[a] [43.1, 47.1]	15.3 ± 2.3 [13.2 – 17.7]	50.8 ± 6.2 [45.6 – 57.7]	71.4 ± 1.4 [69.9 – 72.6]
	Week26	13.3 ± 2.6 [10.4 – 15.5]	38.4 ± 6.2 [31.8 – 44.2]	NA[b]	15.7 ± 0.8 [15.2 – 16.6]	55.3 ± 12.7 [46.5 – 69.9]	75.0 ± 5.9 [68.5 – 80.2]
AUC_{0-t} (µg.h/mL)	Week4	173 ± 20 [152 – 191]	639 ± 54 [576 – 672]	941 ± 46 [893 – 985]	179 ± 3 [176 – 181]	707 ± 88 [605 – 762]	1034 ± 10 [1022 – 1040]
	Week13	197 ± 21 [173 – 214]	636 ± 43 [599 – 684]	807[a] [800, 814]	258 ± 15 [241 – 269]	857 ± 56 [799 – 911]	1233 ± 135 [1088 – 1354]
	Week26	208 ± 34 [172 - 240]	607 ± 74 [522 - 684]	NA[b]	259 ± 13 [249 – 273]	964 ± 136 [859 – 1118]	1377 ± 82 [1312 – 1469]
t_{max} (h)[c]	Week4	1.00 [1.00]	1.00 [1.00]	2.00 [0.50 – 8.00]	1.00 [1.00]	1.00 [0.50 – 1.00]	1.00 [1.00]
	Week13	1.00 [1.00 – 2.00]	0.50 [0.50 – 1.00]	1.00[a] [1.00]	2.00 [0.50 – 2.00]	0.50 [0.50 – 2.00]	2.00 [1.00 – 2.00]
	Week26	1.00 [1.00]	1.00 [0.50 – 2.00]	NA[b]	1.00 [1.00]	1.00 [0.50 – 1.00]	0.50 [0.50 – 1.00]

Additional Information: Mean or Mean ± SD [range] (n=3)
HPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
a: Animals from one sub-group were either found dead or euthanized due to deteriorating clinical condition prior to Week 13 of sampling.
b: In view of the reduced survival rate, all remaining males given 10 mg/kg/day were prematurely euthanized in Week 20 (Day 135/134). The death on Day 22 of the female given 0.8 mg/kg/day was not considered test article-related.
c: Median [range]

Table 4.16 Rat 2 year study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2014N196080 **Study No.:** R70375G

Species (Strain):		**Rat (SD)**							
Test Article:		GSK1278863							
Vehicle/Formulation:		1% (w/v) methyl cellulose with 0.2% Tween 80 (v/v) with 5 mM NaOH							
Method of Administration:		Oral (gavage)							
Sample:		Plasma							
Analyte:		GSK1278863							
Assay:		HPLC-MS/MS							
Gender (M/F)/Number of Animals:		3 – 9/sex/group							
Dose (mg/kg/day):		0.02, 0.1, 0.8, 4							
Duration of dosing (years):		2							
PK Parameters:									
		Male				Female			
Dose (mg/kg/day)		0.02	0.1	0.8	4	0.02	0.1	0.8	7
C_{max} (µg/mL)	Week4	0.335 ± 0.076	2.16 ± 0.45	12.3 ± 2.0	54.9 ± 5.0	0.473 ± 0.089	1.87 ± 0.29	13.8 ± 0.9	75.0 ± 3.2
		[0.254, 0.406]	[1.44, 2.73]	[10.7, 14.5]	[49.3, 58.7]	[0.397, 0.570]	[1.44, 2.21]	[13.1, 14.8]	[71.9, 78.3]
	Week26	0.398 ± 0.060	2.67 ± 0.26	17.5 ± 1.23	58.0 ± 8.33	0.569 ± 0.081	2.60 ± 0.44	18.5 ± 0.7	83.5 ± 9.9
		[0.357, 0.467]	[2.33, 3.04]	[16.1, 18.5]	[49.0, 65.4]	[0.490, 0.652]	[1.86, 3.12]	[18.0, 19.3]	[76.3, 94.8]
$AUC_{0\text{-}t}$ (µg.h/mL)	Week4	5.01 ± 0.70	31.2 ± 5.3	182 ± 20	844 ± 65	7.03 ± 0.89	26.4 ± 2.8	213 ± 32	1270 ± 132
		[4.42, 5.78]	[23.1, 38.2]	[163, 203]	[783, 912]	[6.28, 8.02]	[23.5, 30.8]	[192, 249]	[1120, 1370]
	Week26	6.53 ± 1.03	42.8 ± 5.0	242 ± 20	889 ± 76	10.4[a]	38.9 ± 5.6	306 ± 38	1430 ± 111
		[5.48, 7.54]	[35.3, 48.0]	[223, 263]	[831, 976]	[10.2, 10.5]	[31.3, 44.2]	[269, 345]	[1330, 1550]
t_{max} (h)[b]	Week4	1.00	1.00	1.00	2.00	2.00	1.00	1.00	2.00
		[1.00, 2.00]	[0.500, 1.00]	[1.00, 1.00]	[0.630, 2.00]	[0.500, 2.00]	[0.500, 2.00]	[0.500, 1.00]	[2.00, 2.00]
	Week26	2.00	1.00	1.00	1.00	1.00	0.500	1.00	2.00
		[2.00, 2.00]	[1.00, 2.00]	[1.00, 1.00]	[0.500, 1.00]	[0.500, 1.00]	[0.500, 1.00]	[0.500, 2.00]	[0.500, 2.00]

Additional Information: Mean or Mean ± SD [range] (n= 3 - 6)
HPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
a: Female Animal 2573 in the 0.02 mg/kg/day group did not have a blood sample collected at 24 hours postdose during Week 26 and was excluded from descriptive statistics.
b: Median [range]

Table 4.17 Rabbit 5 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2012N140611 **Study No.:** G12109

Species (Strain):	**Pregnant Rabbit (Dutch Belted)**
Test Article:	GSK1278863
Vehicle/Formulation:	1% (w/v) aqueous methylcellulose/Suspension
Method of Administration:	Oral (gavage)
Sample:	Plasma
Analyte:	GSK1278863, GSK2391220 (M2), GSK2531403 (M3), GSK2499166 (M4), GSK2531399 (M5), GSK2531398 (M6), and GSK2531400 (M13)
Assay:	UHPLC-MS/MS
Gender (M/F)/Number of Animals:	4F
Dose (mg/kg/day):	60
Duration of dosing (days):	5 (Day7 to 11 pc)

PK Parameters:

Analyte	GSK1278863	GSK2391220 (M2)	GSK2531403 (M3)	GSK2499166 (M4)	GSK2531399[a] (M5)	GSK2531398 (M6)	GSK2531400 (M13)
C_{max} (µg/mL)	17.7 ± 2.2 [15.7 – 20.5]	1.02 ± 0.12 [0.918 – 1.20]	0.222 ± 0.10 [0.126 – 0.332]	0.118 ± 0.028 [0.0900 – 0.157]	0.0432 [0.0307 ,0.0556]	0.0167 ± 0.0150 [0.00436 – 0.0363]	0.0158 ± 0.0028 [0.0118 – 0.0180]
AUC_{0-t} (µg.h/mL)	188 ± 17 [171 – 207]	11.7 ± 0.3 [11.2 – 12.0]	2.69 ± 1.57 [1.35 – 4.46]	1.37 ± 0.48 [1.04 – 2.07]	0.594 [0.466 ,0.723]	0.210 ± 0.217 [0.0300 – 0.492]	0.194 ± 0.055 [0.149 – 0.263]
t_{max} (h)[b]	3 [0.5 – 4]	3 [2 – 4]	3 [2 – 4]	3 [2 – 4]	2.3 [0.5 ,4]	3 [2 – 4]	3 [2 – 4]

Additional Information: Mean or Mean ± SD [range] (n=2 - 4), pc = postcoitum
UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
a: n=2, GSK2531399 plasma concentrations for animals 201 and 205 were not reportable.
b: Median [range]

Table 4.18 Rabbit 13 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: CD2008/01041 **Study No.:** D08163

Species (Strain):		**Pregnant Rabbit (Dutch Belted)**				
Test Article:		GSK1278863				
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension				
Method of Administration:		Oral (gavage)				
Sample:		Plasma				
Analyte:		GSK1278863				
Assay:		HPLC-MS/MS				
Gender (M/F)/Number of Animals:		3 or 4F				
Dose (mg/kg/day):		4, 30, 60, 125, 250				
Duration of dosing (days):		13 (Day7 to 19 pc)				
PK Parameters:						
Dose (mg/kg/day)		4	30	60[a]	125	250
C_{max} (µg/mL)	Dosing Day5	1.61	7.40	12.0	21.7	50.5
		[0.909–2.14]	[4.67–9.40]	[8.64–17.2]	[16.8–29.9]	[36.6–75.1]
AUC_{0-t} (µg.h/mL)	Dosing Day5	17.9	83.3	148	323	849
		[13.6–21.2]	[63.3–101]	[123–178]	[249–389]	[626–1170]
t_{max} (h)[c]	Dosing Day5	4.00	4.00	4.00	8.00	8.00
		[4.00–8.00]	[2.00–4.00]	[4.00–8.00]	[4.00–8.00]	[1.00–24.00]
C_{trough}	Dosing Day5	0.207	1.18	1.57	6.83	28.2
		[0.181–0.228]	[0.527–1.77]	[1.28–1.86]	[4.35–12.6]	[8.77–45.8]
	Dosing Day13	0.315	1.94	2.38	10.6	NA[b]
		[0.184–0.510]	[0.550–3.89]	[1.43–3.85]	[4.47–23.5]	

Additional Information: Mean [range] (n=4), pc = postcoitum
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: n=3 for the 60 mg/kg/day dose group. Rabbit 10186 was found to be not pregnant at necropsy and was excluded from sample analysis.
b: All rabbits given 250 mg/kg/day were euthanized on Dosing Day 6 (Day 12 pc).
c: Median [range]

Table 4.19 Rabbit 13 day study with Daprodustat (sampled after a duration of dosing of 5 days)

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: CD2009/00390 **Study No.:** G09061

Species (Strain):	**Pregnant Rabbit (Dutch Belted)**
Test Article:	GSK1278863
Vehicle/Formulation:	1% (w/v) aqueous methylcellulose/Suspension
Method of Administration:	Oral (gavage)
Sample:	Plasma
Analyte:	GSK1278863
Assay:	HPLC-MS/MS
Number of Animals:	22F
Dose (mg/kg/day):	4, 30, 60
Duration of dosing (days):	5 (GD 7 to 11)

PK Parameters:

	GD11		
Dose (mg/kg/day)	4	30	60
C_{max} (µg/mL)	1.49	9.24	11.8
AUC_{0-t} (µg.h/mL)	22.8	133	197
t_{max} (h)	4	4	8

Additional Information: GD = Gestation Day
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry.

Table 4.20 Rabbit 14 day study with Daprodustat

Study Type: Repeat Dose Dermal Irritation

Report Title: GSK1278863A: 2-Week Dermal Irritation Study in Rabbits (GlaxoSmithKline Study No. L42845)

Test Article: GSK1278863 (parent compound)

Batch No.: 111280742

Location in CTD: m4.2.2.2

Species/Strain: Rabbit/Hra (NZW)SPF

Duration of Exposure: approximately 23 hours/day

Study No.: L42845

Initial Age: 5 months

Route: Dermal (occluded, non-abraded and abraded sites

GSK Document Number: 2011N125481

Date of First Dose: 11 October 2011

Vehicle/Formulation: petrolatum/suspension

Study in Compliance with GLP: Yes

Data Collected: Toxicokinetics, clinical observations, body weight, food consumption, dermal irritation scores, hematology, macroscopic and microscopic (skin only) pathology

Conclusion: GSK1278863 formulations caused no dermal irritation. Treatment with GSK1278863 was associated with attenuation in the severity, pattern of distribution, and/or incidence of the skin microscopic changes (acanthosis, orthokeratotic hyperkeratosis, dermal mixed inflammatory cell infiltrate, and dermal edema) seen with the vehicle.

	Male				
Dose (%)[1]	0	0.5	1	2	
Number of Animals:	3[b]	3[b]	3[b]	6[c]	
AUC $_{(0\text{-}t)}$ (ng.h/mL)				Non-Abraded	Abraded
Day 1	NA	NA	NA	169[d]	219
Day 14	NA	NA	NA	2492	3332
C_{max} (ng/mL)					
Day 1	NA	NA	NA	23.4	22.3
Day 14	NA	NA	NA	248	319
Noteworthy Findings	GSK1278863 formulations caused no dermal irritation				

Key:

a. Doses are expressed in terms of the parent compound.

b. Each animal had both non-abraded and abraded application sites.

c. The first 3 animals in the group had non-abraded application sites, and the last 3 animals in the group had abraded application sites.

d. N=1

NA Not applicable

Table 4.21 Dog 3 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: RD2007/01367 **Study No.:** D42209

Species (Strain):	**Dog (Beagle)**			
Feeding Condition:	Fasted and non-fasted			
Test Article:	GSK1278863			
Vehicle/Formulation:	Gelatin/capsule (fasted) or 1% (w/v) methylcellulose/ suspension (non-fasted)			
Method of Administration:	Oral (gavage/capsule)			
Sample:	Plasma			
Analyte:	GSK1278863			
Assay:	HPLC-MS/MS			
Gender (M/F)/Number of Animals:	2M/2F			
Dose (mg/kg/day):	90			
Duration of dosing (weeks):	Single			
PK Parameters:				
	Capsule/Fasted		Suspension/Non fasted	
	Male	Female	Male	Female
C_{max} (µg/mL)	16.9	22.3	14.0	14.7
	[10.2, 23.5]	[15.3, 29.4]	[12.4, 15.6[a]]	[11.6[a], 17.7]
AUC_{0-24} (µg.h/mL)	249	250	188	185
	[126, 373]	[224, 275]	[157, 219[a]]	[145[a], 225]
t_{max} (h)[b]	6.00	4.00	4.00	4.00
	[4.00, 8.00]	[4.00, 4.00]	[4.00, 4.00[a]]	[4.00[a], 4.00]

Additional Information: Mean [range] (n=2)
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: One male (Dog 9) and one female (Dog 8) in Group 2 (gavage/suspension) vomited food-like substance within half an hour of dosing.
b: Median [range]

Table 4.22 Dog 7 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: CD2007/00353 **Study No.:** D07078

Species (Strain):		**Dog (Beagle)**					
Test Article:		GSK1278863					
Vehicle/Formulation:		1% (w/v) methylcellulose/ suspension					
Method of Administration:		Oral (gavage)					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		1M/1F					
Dose (mg/kg/day):		20, 60, 120					
Duration of dosing (days):		7					
PK Parameters:							
		Male			Female		
		20[a]	60	120[b]	20	60	120
C_{max} (µg/mL)	Day1	5.32	5.89	21.1	7.38	7.55	19.0
	Day7	4.29	4.17	7.73	6.04	7.53	8.39
AUC_{0-24} (µg.h/mL)	Day1	46.5	55.8	336	58.8	86.1	255
	Day7	49.5	57.5	93.7	67.0	114	135
t_{max} (h)	Day1	2.00	2.00	8.00	2.00	2.00	4.00
	Day7	4.00	2.00	2.00	2.00	4.00	4.00

Additional Information: Individual (n=1)

HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry

a: The male animal in this dose group (Animal D07M-1395) experienced a single episode of emesis at time of dosing on Day 7.

b: Both the male (Animal D07M-1399) and the female (Animal D07F-1400) experienced multiple episodes of emesis containing drug-like material between dosing and the 0.5 hr time point on Day 7.

Table 4.23 Dog 4 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: RD2007/01336 **Study No.:** D42196

Species (Strain):		**Dog (Beagle)**					
Test Article:		GSK1278863					
Vehicle/Formulation:		Gelatin capsule					
Method of Administration:		Oral					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		3M/3F					
Dose (mg/kg/day):		3, 30, 90					
Duration of dosing (weeks):		4					
PK Parameters:							
		Male			Female		
		3	30	90	3	30	90
C_{max} (µg/mL)	Day1	1.87 ± 0.66 [1.19 –2.51]	6.64 ± 2.02 [5.19 – 8.95]	12.7 ± 2.07 [11.0 – 15.0]	2.20 ± 0.91 [1.16 – 2.89]	7.71 ± 1.93 [5.77 – 9.63]	17.8 ± 5.35 [14.4 – 23.9]
	Day28	1.86 ± 0.78 [1.00 – 2.51]	6.57 ± 1.59 [5.01 – 8.19]	NA[a]	2.60 ± 0.94 [1.51 – 3.15]	5.97 ± 2.25 [3.99 – 8.42]	NA[b]
AUC_{0-t} (µg.h/mL)	Day1	19.5 ± 3.8 [16.7 – 23.8]	82.7 ± 60.1 [47.0 – 152]	180 ± 27.8 [148 – 198]	20.9 ± 5.4 [15.2 – 25.9]	116 ± 37.0 [81.3 – 155]	272 ± 152.4 [164 – 446]
	Day28	18.2 ± 7.2 [10.6 – 24.9]	88.3 ± 31.1 [66.2 – 124]	NA[a]	23.4 ± 11.1 [10.6 – 30.3]	90.8 ± 34.9 [57.4 – 127]	NA[b]
t_{max} (h)[c]	Day1	2.00 [2.00 – 4.00]	4.00 [1.00 – 4.00]	4.00 [4.00 – 4.00]	2.00 [2.00 – 2.00]	2.00 [2.00 – 4.00]	4.00 [4.00 – 8.00]
	Day28	2.00 [1.00 – 2.00]	2.00 [1.00 – 8.00]	NA[a]	1.00 [0.00 – 1.00]	4.00 [1.00 – 4.00]	NA[b]

Additional Information: Mean or Mean ± SD [range] (n=3)
HPLC-MS/MS = ltra high performance liquid chromatography-tandem mass spectrometry
a: Animal No. 19 was euthanized on Day 12 and the remaining dogs in this dose group were euthanized on Day 19 due to deteriorating clinical condition
b: Animals were euthanized on Day 19 due to deteriorating clinical condition.
c: Median [range]

Table 4.24 Dog 13 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: CD2009/00952 **Study No.:** G09058

Species (Strain):		**Dog (Beagle)**					
Test Article:		GSK1278863					
Vehicle/Formulation:		Gelatin capsule					
Method of Administration:		Oral					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		4 or 6 M/4 or 6 F					
Dose (mg/kg/day):		1, 3, 30 or 30/15[b]					
Duration of dosing (weeks):		13					
PK Parameters:							
		Male			Female		
		1	3	30	1	3	30/15[b]
C_{max} (µg/mL)	Day1	1.05 ± 0.476	1.78 ± 0.548	7.90 ± 3.71	0.981 ± 0.856	1.79 ± 0.192	8.12 ± 4.17
	Day17	-	-	-	-	-	5.94 ± 0.845
	Week7	1.11 ± 0.449	2.62 ± 0.719	5.89 ± 2.20	1.41 ± 0.357	4.24 ± 2.08	6.23 ± 1.70
	Week13	1.30 ± 0.749	1.79 ± 0.402	-	1.32 ± 0.325	2.89 ± 1.11	-
AUC_{0-t} (µg.h/mL)	Day1	10.4 ± 2.65	18.8 ± 0.768	104 ± 36.1	9.08 ± 6.28	26.7 ± 5.42	108 ± 41.6
	Day17	-	-	-	-	-	74.3 ± 18.5
	Week7	9.10 ± 2.58	28.7 ± 5.91	94.9 ± 24.2	12.1 ± 3.00	36.0 ± 13.0	67.1 ± 17.9
	Week13	7.39 ± 2.55	17.8 ± 4.93	-	8.06 ± 2.79	20.3 ± 10.4	-
t_{max} (h)[a]	Day1	1 to 2	1 to 2	4 to 8	1 to 4	1 to 4	4 to 24
	Day17	-	-	-	-	-	0.5 to 24
	Week7	1 to 4	1 to 2	2 to 8	1 to 2	1 to 2	0.5 to 4
	Week13	1	0 to 2	-	1	1	-

Additional Information: Mean ± SD (n=4 - 6)
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: Range, b: the dose was reduced to 15 mg/kg/day on Day 17 for the high dose females

Table 4.25 Minipig 14 day study with Daprodustat

Test Article: GSK1278863 **Location in CTD:** m4.2.2.2
Study Number: D12086 **Report No.:** 2012N148386
Study Title: GSK1278863A: A 14-Day Dose Range-Finding Dermal Irritation Study in Minipigs

Parameter (n = 1/sex/group)	Period	Dose (% w/w)		
		0.5	1.0	2.0
Male/Female				
AUC_{0-t} (ng.h/mL)	Day 1	NC / 47.0	84.3 / 35.0	73.2 / 177
	Day 14	2.13 / 143	568 / 97.0	469 / 1320
C_{max} (ng/mL)	Day 1	1.02 / 4.29	5.96 / 3.35	6.07 / 19.8
	Day 14	1.25 / 15.8	76.2 / 22.9	38.0 / 169
T_{max} (h)	Day 1	4.0 / 1.0	1.0 / 2.0	4.0 / 2.0
	Day 14	2.0 / 0.0	24.0 / 0.5	0.0 / 24.0

Noteworthy Findings: There was no evidence of test article-related dermal irritation noted clinically and there were no test article-related microscopic changes at the dose site.

Key:

NC – Not Calculated; insufficient plasma concentration data; only one quantifiable plasma concentration >LLQ (>1.00 ng/mL). The male AUC value at 0.5 % on Day 1 was assigned as zero for gender-averaged AUC calculation.

Table 4.26 Minipig 14 day study with Daprodustat

Test Article: GSK1278863 **Location in CTD:** m4.2.2.2
Study Number: F70438N **Report No.:** 2013N185591
Study Title: GSK1278863A: A 14 Day Dose Range-Finding Dermal Irritancy Study in Gottingen Minipigs

Parameter (n = 1/sex/group)	Period	Dose (% w/w)		
		10	20	35
Male/Female				
AUC_{0-t} (ng.h/mL)	Day 1 Day 14	NC / NC 86.5 / 80.8	NC / NC 57.9 / 75.8	NC / NC 194 / 77.3
C_{max} (ng/mL)	Day 1 Day 14	1.93 / 2.75 7.93 / 6.38	NQ / 3.21 2.97 / 4.84	15.6 / 8.19 23.3 / 6.63
T_{max} (h)	Day 1 Day 14	24.0 / 21.0 24.0 / 24.0	NA / 24.0 8.0 / 4.0	24.0 / 24.0 24.0 / 0.0

Noteworthy Findings: There was no evidence of test article-related dermal irritation noted clinically and there were no test article-related microscopic changes at the dose site.

Key:
NC = Not Calculated; insufficient plasma concentration data; NA = Not Applicable; NQ = Not Quantifiable (<1.00 ng.mL)

Table 4.27 Minipig 28 day study with Daprodustat

Test Article: GSK1278863 **Location in CTD:** m4.2.2.2
Study Number: F70727N **Report No.:** 2015N231048
Study Title: GSK1278863A: A 28 Day Dose Range Finding Dermal Irritancy Study in Gottingen Minipigs

Parameter	Period		Male (n=3/group) Nominal Dose Concentration of GSK1278863 (% w/w)		
			2	10	35
AUC_{0-t} [a] (ng.h/mL)	Day 1	Mean	8.59	14.0	31.2
		Min	5.88	12.4	21.5
		Max	11.3	15.2	44.2
	Day 28	Mean	104	314	626
		Min	58.8	306	269
		Max	165	322	984
C_{max} (ng/mL)	Day 1	Mean	1.15	1.53	3.90
		Min	0.991	1.09	2.18
		Max	1.42	2.39	4.84
	Day 28	Mean	10.6	31.7	44.3
		Min	4.02	18.9	32.4
		Max	20.7	38.5	59.1
T_{max} (h)	Day 1	Median	24.0	24.0	24.0
		Min	24.0	24.0	0.5
		Max	24.0	24.0	24.0
	Day 28	Median	24.0	24.0	24.0
		Min	4.0	24.0	21.0
		Max	24.0	24.0	24.0
Noteworthy Findings:	There was no systemic toxicity based on clinical observations, body weight, food consumption and clinical pathology. Test article-related clinical or macroscopic findings of erythema and scabbing and microscopic findings of slight to mild epidermal exudates and superficial dermal edema were seen in the treated skin of minipigs given 35% (w/w) GSK1278863.				

Key:
a. For calculating AUC0-t on Day 28, the concentrations at time zero on Day 28 were assigned the same values as concentrations at 24 hours.

Table 4.28 Minipig 13 week study with Daprodustat

Study Type: Repeat Dose Toxicity
Species/Strain: Minipigs/Gottingen
Initial Age: 4 months
Date of First Dose: 02 March 2016

Report Title: GSK1278863A: A 3 Month Dermal Toxicity Study in the Minipig
Duration of Dosing: 91/92 days

Route: Dermal/once daily for approximately 21 hours
Vehicle/Formulation: petrolatum/suspension

Test Article: GSK1278863 (parent compound)
Batch No.: 152389102
Study No.: F70916G
Location in CTD: m4.2.2.2
GSK Document Number: 2016N273683
Study in Compliance with GLP: Yes

Study Type: Repeat Dose Toxicity

Formulation	**Male**				**Female**			
Concentration (%, w/w)	0	5	10	20	0	5	10	20
Estimated Achieved Daily	0	42	84	168	0	42	84	168
Numbers of Animals:	4	4	4	4	4	4	4	4
$AUC_{(0\text{-}t)}$ (ng.h/mL)								
Week 4 (Day 28)	NC	205	336	704	NC	429	335	473
Week 13 (Day 91)	NC	107	475	1410	NC	388	397	1860
C_{max}(ng/mL)								
Week 4 (Day 28)	5.17[b]	23.7	23.4	66.9	2.15[b]	43.2	27.2	38.4
Week 13 (Day 91)	4.41[b]	7.29	28.5	143	2.46[b]	27.5	32.6	142

Key:
NC = Not calculated.
a - Estimated achieved doses and formulation concentration strengths are expressed in terms of the parent compound.
b - The concentrations of GSK1278863 in most samples collected from the control animals were above the limit of quantitation (0.100 ng/mL). Quantifiable control group concentrations ranged from 0.119 to 5.17 ng/mL for males and from 0.108 to 2.46 ng/mL for females. With the exception of several control group males having comparable concentrations to those measured in GSK1278863-treated males on Day 91, these control results were generally lower than the concentrations measured in animals at the low dose (42 mg/kg/day), and are therefore not expected to have any impact on the overall toxicokinetic interpretations.

Table 4.29 Monkey 14 day study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: CD2009/00780 **Study No.:** D09121

Species (Strain):		Monkey (Cynomolgus)					
Test Article:		GSK1278863					
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension					
Method of Administration:		Oral (gavage)					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		1M/1F					
Dose (mg/kg/day):		10, 30, 100					
Duration of dosing (weeks):		Single					
PK Parameters:							
		Male			Female		
Dose (mg/kg/day)		10	30	100	10	30	100
C_{max} (µg/mL)	Day1	1.41	2.53	9.03	1.01	2.00	3.75
	Day14	1.88	3.43	5.43[a]	1.96	3.23	6.39
AUC_{0-t} (µg.h/mL)	Day1	5.71	22.5	109	3.40	14.1	52.4
	Day14	10.0	29.5	60.3[a]	8.97	16.1	91.6
t_{max} (h)	Day1	2.00	2.00	8.00	2.00	1.00	2.00
	Day14	1.00	1.00	1.00[a]	1.00	1.00	8.00

Additional Information: Individual
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: A single episode of emesis was observed in the male monkey following oral administration of 100 mg/kg/day on Day 14.

Table 4.30 Monkey 13 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2010N108482 **Study No.:** G10005

Species (Strain):		Monkey (Cynomolgus)					
Test Article:		GSK1278863					
Vehicle/Formulation:		1% (w/v) aqueous methylcellulose/Suspension					
Method of Administration:		Oral (gavage)					
Sample:		Plasma					
Analyte:		GSK1278863					
Assay:		HPLC-MS/MS					
Gender (M/F)/Number of Animals:		4-6M/4-6F					
Dose (mg/kg/day):		5, 20, 100					
Duration of dosing (weeks):		13					
PK Parameters:							
		Male			Female		
	Dose (mg/kg/day)	5	20	100	5	20	100
C_{max} (µg/mL)	Day1	1.00 ± 0.51 [0.493 – 1.71]	1.98 ± 0.64 [1.29 – 2.79]	5.02 ± 1.18 [3.67 – 6.66]	0.887 ± 0.416 [0.462 – 1.34]	2.30 ± 0.68 [1.38 – 3.32]	4.60 ± 1.48 [2.91 – 7.07]
	Week4	0.898 ± 0.158 [0.715 – 1.06]	1.89 ± 0.55 [1.34 – 2.81]	3.96 ± 1.55 [2.84 – 6.97]	0.914 ± 0.209 [0.698 – 1.20]	2.55 ± 0.89 [1.36 – 3.50]	4.00 ± 0.72 [3.29 – 5.03]
	Week13	0.753 ± 0.20 [0.468 – 0.900]	1.43 ± 0.45 [0.938 – 2.22]	3.85 ± 2.07 [1.72 – 7.11]	0.762 ± 0.297 [0.457 – 1.15]	1.38 ± 0.59 [0.868 – 2.42]	2.83 ± 0.26 [2.44 – 3.16]
$AUC_{0\text{-}t}$ (µg.h/mL)	Day1	3.02 ± 0.77 [2.01 – 3.87]	13.4 ± 6.3 [5.84 – 22.1]	54.2 ± 14.5 [37.7 – 75.9]	2.84 ± 0.65 [1.97 – 3.56]	15.6 ± 5.52 [9.93 – 24.0]	55.1 ± 22.4 [34.6 – 95.3]
	Week4	4.66 ± 2.62 [2.92 – 8.54]	13.9 ± 4.7 [7.45 – 21.7]	47.0 ± 18.3 [25.7 – 76.1]	3.77 ± 0.42 [3.25 – 4.16]	21.2 ± 7.5 [9.52 – 30.2]	50.2 ± 10.5 [37.2 – 66.7]
	Week13	5.44 ± 2.02 [3.08 – 7.78]	16.2 ± 5.2 [12.0 – 26.0]	52.2 ± 31.3 [22.8 – 102]	3.67 ± 0.90 [2.53 – 4.55]	13.3 ± 6.0 [6.89 – 22.7]	34.3 ± 11.8 [23.5 – 51.3]
t_{max} (h)[a]	Day1	0.50 [0.50 – 0.50]	1.50 [0.50 – 2.00]	3.00 [1.00 – 8.00]	1.00 [0.50 – 2.00]	1.50 [1.00 – 2.00]	6.00 [1.00 – 8.00]
	Week4	0.75 [0.50 – 2.00]	0.75 [0.50 – 4.00]	6.00 [2.00 – 8.00]	1.25 [0.50 – 2.00]	1.50 [0.50 – 4.00]	4.00 [2.00 – 8.00]
	Week13	0.50 [0.50 – 4.00]	3.00 [0.50 – 4.00]	6.00 [1.00 – 8.00]	0.50 [0.50 – 0.50]	3.00 [0.50 – 4.00]	2.00 [1.00 – 8.00]

Additional Information: Mean or Mean ± SD [range] (n=4 - 6)
HPLC-MS/MS = high performance liquid chromatography-tandem mass spectrometry
a: Median [range]

Table 4.31 Monkey 13 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2011N117143 **Study No.:** 10DMM033

Species (Strain):	**Monkey (Cynomolgus)**
Feeding Condition:	Non-fasted
Test Article:	GSK1278863
Vehicle/Formulation:	1% methylcellulose (400cps @ 2%) in water/Suspension
Method of Administration:	Oral (gavage)
Sample:	Plasma
Analyte:	M2, M3, M4, M5, M6, M13
Assay:	UHPLC-MS/MS
Gender (M/F)/Number of Animals:	4-6M/4-6F
Dose (mg/kg/day):	5, 20, 100
Duration of dosing:	13 weeks
PK Parameters :	Continue to the Table of Parameters for each metabolite

Additional Information: Mean or Mean ± SD [range] (n=4 - 6)
UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
GSK2391220 (M2), GSK2531403 (M3), GSK2499166 (M4), GSK2531399 (M5), GSK2531398 (M6) and GSK2531400 (M13) are metabolites of GSK1278863.
For each metabolite, the maximum plasma concentrations were mainly observed between 1 and 8 hours after dosing. Generally, all metabolites were quantifiable through
24 hours sampling time point. For each metabolite, there was no marked (> 2-fold) difference in systemic exposure (AUC0-t and Cmax values) between male and female monkeys at all doses and sampling periods.

Monkey 13 week study with Daprodustat (Continued)

GSK2391220 (M2)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		5 (n=4)	20 (n=6)	100 (n=6)	5 (n=4)	20 (n=6)	100 (n=6)
C_{max} (µg/mL)	Day 1	0.0390 ± 0.0182 [0.0217 - 0.0617]	0.0846 ± 0.0209 [0.0580 - 0.116]	0.244 ± 0.109 [0.119 - 0.438]	0.0416 ± 0.0099 [0.0298 - 0.0532]	0.102 ± 0.046 [0.0539 - 0.174]	0.171 ± 0.050 [0.111 - 0.261]
	Week 4	0.0338 ± 0.008 [0.0258 - 0.0446]	0.0738 ± 0.0126 [0.0548 - 0.0918]	0.211 ± 0.118 [0.0934 - 0.436]	0.0539 ± 0.0121 [0.0447 - 0.0716]	0.0827 ± 0.0324 [0.0442 - 0.124]	0.169 ± 0.053 [0.124 - 0.243]
	Week 13	0.0322 ± 0.0102 [0.0230 - 0.0415]	0.0740 ± 0.0130 [0.0578 - 0.0884]	0.206 ± 0.0971 [0.0805 - 0.338]	0.0384 ± 0.0086 [0.0260 - 0.0456]	0.0733 ± 0.0291 [0.0327 - 0.110]	0.119 ± 0.029 [0.0823 - 0.155]
AUC_{0-t} (µg.h/mL)	Day 1	0.258 ± 0.022 [0.231 - 0.282]	0.864 ± 0.147 [0.661 - 1.05]	3.23 ± 1.28 [1.61 - 5.53]	0.306 ± 0.044 [0.258 - 0.364]	0.928 ± 0.451 [0.447 - 1.57]	2.26 ± 0.66 [1.47 - 3.42]
	Week 4	0.349 ± 0.124 [0.236 - 0.516]	0.928 ± 0.173 [0.657 - 1.12]	2.97 ± 1.84 [1.33 - 6.53]	0.438 ± 0.076 [0.329 - 0.499]	1.01 ± 0.40 [0.469 - 1.55]	2.48 ± 0.65 [1.87 - 3.67]
	Week 13	0.351 ± 0.121 [0.188 - 0.479]	0.997 ± 0.171 [0.778 - 1.19]	3.41 ± 1.58 [1.29 - 5.46]	0.350 ± 0.078 [0.250 - 0.424]	0.805 ± 0.316 [0.375 - 1.17]	1.91 ± 0.38 [1.28 - 2.30]
Median t_{max} (h)	Day 1	1.00 [1.00 - 4.00]	4.00 [1.00 - 4.00]	6.00 [2.00 - 8.00]	2.00 [1.00 - 2.00]	4.00 [2.00 - 4.00]	8.00 [4.00 - 8.00]
	Week 4	1.50 [1.00 - 4.00]	4.00 [2.00 - 8.00]	8.00 [4.00 - 8.00]	2.00 [2.00 - 2.00]	4.00 [4.00 - 4.00]	6.00 [4.00 - 8.00]
	Week 13	2.50 [1.00 - 4.00]	4.00 [4.00 - 8.00]	8.00 [4.00 - 8.00]	3.00 [1.00 - 4.00]	4.00 [2.00 - 4.00]	4.00 [4.00 - 8.00]

a: Results are reported as mean unless stated otherwise and [range).

Monkey 13 week study with Daprodustat (Continued)

GSK2531403 (M3)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		5 (n=4)	20 (n=6)	100 (n=6)	5 (n=4)	20 (n=6)	100 (n=6)
C_{max} (µg/mL)	Day 1	0.0146 ± 0.0069 [0.00793 - 0.0239]	0.0284 ± 0.0049 [0.0207 - 0.0348]	0.0892 ± 0.0405 [0.0411 - 0.156]	0.0139 ± 0.0024 [0.0103 - 0.0153]	0.0346 ± 0.0172 [0.0157 - 0.0590]	0.0623 ± 0.0242 [0.0340 - 0.105]
	Week 4	0.0123 ± 0.0015 [0.0105 - 0.0142]	0.0261 ± 0.0068 [0.0168 - 0.0342]	0.0767 ± 0.0442 [0.0316 - 0.157]	0.0198 ± 0.0059 [0.0150 - 0.0284]	0.0283 ± 0.0127 [0.0119 - 0.0423]	0.0597 ± 0.0206 [0.0392 - 0.0876]
	Week 13	0.0117 ± 0.0038 [0.00762 - 0.0150]	0.0254 ± 0.0037 [0.0203 - 0.0296]	0.0746 ± 0.0389 [0.0272 - 0.127]	0.0138 ± 0.0033 [0.00907 - 0.0165]	0.0257 ± 0.0115 [0.00939 - 0.0358]	0.0416 ± 0.0113 [0.0301 - 0.588]
$AUC_{0\text{-}t}$ (µg.h/mL)	Day 1	0.0719 ± 0.0178 [0.0521 - 0.0950]	0.296 ± 0.050 [0.203 - 0.345]	1.19 ± 0.50 [0.551 - 2.07]	0.0917 ± 0.0354 [0.0599 - 0.128]	0.316 ± 0.167 [0.134 - 0.539]	0.815 ± 0.309 [0.459 - 1.34]
	Week 4	0.116 ± 0.046 [0.0622 - 0.0169]	0.328 ± 0.087 [0.204 - 0.440]	1.08 ± 0.69 [0.455 - 2.39]	0.150 ± 0.051 [0.0756 - 0.189]	0.347 ± 0.150 [0.131 - 0.530]	0.871 ± 0.239 [0.599 - 0.894]
	Week 13	0.124 ± 0.041 [0.0738 - 0.173]	0.341 ± 0.049 [0.276 - 0.399]	1.22 ± 0.62 [0.439 - 2.00]	0.119 ± 0.048 [0.0533 - 0.170]	0.285 ± 0.127 [0.110 - 0.435]	0.665 ± 0.142 [0.499 - 0.819]
Median t_{max} (h)	Day 1	1.00 [1.00 - 4.00]	3.00 [0.50 - 4.00]	6.00 [2.00 - 8.00]	2.00 [1.00 - 2.00]	3.00 [2.00 - 4.00]	8.00 [4.00 - 8.00]
	Week 4	2.50 [1.00 - 4.00]	4.00 [2.00 - 8.00]	8.00 [4.00 - 8.00]	2.00 [2.00 - 2.00]	4.00 [2.00 - 4.00]	6.00 [2.00 - 8.00]
	Week 13	2.50 [1.00 - 4.00]	4.00 [4.00 - 8.00]	8.00 [4.00 - 8.00]	1.50 [1.00 - 4.00]	4.00 [2.00 - 4.00]	6.00 [4.00 - 8.00]

a: Results are reported as mean unless stated otherwise and [range).

Monkey 13 week study with Daprodustat (Continued)

GSK2499166 (M4)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		5 (n=4)	20 (n=6)	100 (n=6)	5 (n=4)	20 (n=6)	100 (n=6)
C_{max} (µg/mL)	Day 1	0.0424 ± 0.0202 [0.0239 - 0.0704]	0.0833 ± 0.0226 [0.0611 - 0.112]	0.248 ± 0.095 [0.128 - 0.398]	0.0412 ± 0.0107 [0.0281 - 0.0537]	0.100 ± 0.039 [0.0506 - 0.152]	0.190 ± 0.067 [0.109 - 0.306]
	Week 4	0.0360 ± 0.0050 [0.0297 - 0.0414]	0.0749 ± 0.0226 [0.0484 - 0.112]	0.207 ± 0.103 [0.0929 - 0.385]	0.0531 ± 0.0118 [0.0404 - 0.0690]	0.0828 ± 0.0295 [0.0447 - 0.112]	0.179 ± 0.063 [0.113 - 0.263]
	Week 13	0.0350 ± 0.0124 [0.0221 - 0.0469]	0.0728 ± 0.0132 [0.0544 - 0.0903]	0.201 ± 0.092 [0.0861 - 0.316]	0.0386 ± 0.0105 [0.0235 - 0.0461]	0.0737 ± 0.0269 [0.0362 - 0.103]	0.126 ± 0.034 [0.0902 - 0.168]
AUC_{0-t} (µg.h/mL)	Day 1	0.286 ± 0.021 [0.267 - 0.308]	0.867 ± 0.211 [0.588 - 1.23]	3.31 ± 1.18 [1.68 - 5.28]	0.304 ± 0.048 [0.242 - 0.357]	0.94 ± 0.41 [0.444 - 1.41]	2.47 ± 0.87 [1.46 - 3.98]
	Week 4	0.372 ± 0.093 [0.273 - 0.483]	0.941 ± 0.274 [0.570 - 1.34]	2.91 ± 1.61 [1.36 - 5.83]	0.447 ± 0.097 [0.302 - 0.505]	1.02 ± 0.36 [0.517 - 1.43]	2.61 ± 0.74 [1.74 - 3.88]
	Week 13	0.382 ± 0.134 [0.224 - 0.547]	0.982 ± 0.183 [0.736 - 1.26]	3.30 ± 1.49 [1.37 - 5.21]	0.357 ± 0.106 [0.225 - 0.463]	0.846 ± 0.327 [0.420 - 1.33]	2.01 ± 0.44 [1.39 - 2.48]
Median t_{max} (h)	Day 1	1.00 [1.00 - 4.00]	3.00 [1.00 - 4.00]	6.00 [2.00 - 8.00]	2.00 [1.00 - 2.00]	4.00 [2.00 - 4.00]	8.00 [4.00 - 8.00]
	Week 4	1.50 [1.00 - 4.00]	4.00 [2.00 - 8.00]	8.00 [4.00 - 8.00]	2.00 [2.00 - 4.00]	4.00 [4.00 - 4.00]	6.00 [4.00 - 8.00]
	Week 13	2.50 [1.00 - 4.00]	4.00 [4.00 - 8.00]	8.00 [4.00 - 8.00]	3.00 [1.00 - 4.00]	4.00 [4.00 - 4.00]	4.00 [4.00 - 8.00]

a: Results are reported as mean unless stated otherwise and [range].

Monkey 13 week study with Daprodustat (Continued)

GSK2531399 (M5)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		5 (n=4)	20 (n=6)	100 (n=6)	5 (n=4)	20 (n=6)	100 (n=6)
C_{max} (µg/mL)	Day 1	0.00215 ± 0.00098 [0.00134 - 0.00354]	0.00429 ± 0.00062 [0.00333 - 0.00506]	0.0134 ± 0.00724 [0.00440 - 0.0246]	0.00228 ± 0.00054 [0.00170 - 0.00288]	0.00553 ± 0.00300 [0.00219 - 0.00947]	0.00813 ± 0.00277 [0.00451 - 0.0127]
	Week 4	0.00187 ± 0.00022 [0.00161 - 0.00214]	0.0037 ± 0.0010 [0.00230 - 0.00491]	0.0104 ± 0.0066 [0.00360 - 0.0221]	0.00288 ± 0.00068 [0.00210 - 0.00376]	0.00451 ± 0.00233 [0.00144 - 0.00683]	0.00786 ± 0.00309 [0.00425 - 0.0129]
	Week 13	0.00226[b] [0.00205, 0.00247]	0.00348 ± 0.00070 [0.00260 - 0.00422]	0.0100 ± 0.0057 [0.00339 - 0.0172]	0.00195 ± 0.00047 [0.00128 - 0.00240]	0.00488 ± 0.00291 [0.00169 - 0.00891]	0.00611 ± 0.00158 [0.00433 - 0.00847]
AUC_{0-t} (µg.h/mL)	Day 1	0.00736[b] [0.00577, 0.00896]	0.0262 ± 0.0029 [0.0211 - 0.0296]	0.161 ± 0.084 [0.0250 - 0.278]	0.00665 ± 0.00135 [0.00527 - 0.00808]	0.0338[d] ± 0.0178 [0.00772 - 0.0556]	0.0941 ± 0.0531 [0.0292 - 0.174]
	Week 4	0.00883 ± 0.00442 [0.00462 - 0.0128]	0.0285 ± 0.0136 [0.0160 - 0.0530]	0.145 ± 0.119 [0.0471 - 0.366]	0.0137 ± 0.0050 [0.00651 - 0.0182]	0.0413[d] ± 0.0239 [0.0133 - 0.0785]	0.108 ± 0.045 [0.0630 - 0.180]
	Week 13	0.0102[b] [0.00702, 0.0133]	0.0365 ± 0.0186 [0.0177 - 0.0598]	0.159 ± 0.090 [0.0496 - 0.272]	0.00927[c`] ± 0.00282 [0.00693 - 0.0124]	0.0292[d] ± 0.0102 [0.0112 - 0.0349]	0.0910 ± 0.0166 [0.0701 - 0.113]
Median t_{max} (h)	Day 1	1.00 [1.00 - 4.00]	4.00 [1.00 - 8.00]	4.00 [2.00 - 8.00]	2.00 [1.00 - 2.00]	4.00 [2.00 - 4.00]	8.00 [4.00 - 8.00]
	Week 4	1.50 [1.00 - 4.00]	4.00 [2.00 - 8.00]	8.00 [4.00 - 8.00]	2.00 [1.00 - 2.00]	4.00 [2.00 - 8.00]	6.00 [2.00 - 8.00]
	Week 13	2.50[b] [1.00, 4.00]	4.00 [4.00 - 8.00]	6.00 [0.50 - 8.00]	1.50 [1.00 - 4.00]	4.00 [2.00 - 4.00]	3.00 [2.00 - 8.00]

a: Results are reported as mean unless stated otherwise and [range).
b: n = 2 due to insufficient plasma concentration data to calculate AUC from 2 other animals.
c: n = 3 due to insufficient plasma concentration time point to calculate AUC from one animal.
d: n = 5 due to insufficient plasma concentration time point to calculate AUC from one animal.

Monkey 13 week study with Daprodustat (Continued)

GSK2531398 (M6)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		5 (n=4)	20 (n=6)	100 (n=6)	5 (n=4)	20 (n=6)	100 (n=6)
C_{max} (µg/mL)	Day 1	0.00821 ± 0.0033 [0.00458 - 0.0126]	0.0160 ± 0.0042 [0.0102 - 0.0213]	0.0451 ± 0.0231 [0.0179 - 0.0816]	0.00927 ± 0.00442 [0.00506 - 0.0153]	0.0219 ± 0.0146 [0.00735 - 0.0439]	0.0310 ± 0.0118 [0.0162 - 0.0488]
	Week 4	0.00737 ± 0.0025 [0.00503 - 0.0110]	0.0150 ± 0.0088 [0.00743 - 0.0320]	0.0402 ± 0.0271 [0.0133 - 0.0880]	0.0126 ± 0.0077 [0.00948 - 0.0240]	0.0187 ± 0.0122 [0.00520 - 0.0377]	0.0315 ± 0.0108 [0.0178 - 0.0452]
	Week 13	0.00677 ± 0.0026 [0.00368 - 0.00984]	0.0136 ± 0.0059 [0.00953 - 0.0253]	0.0367 ± 0.0228 [0.0117 - 0.0665]	0.00913 ± 0.00480 [0.00424 - 0.0157]	0.0166 ± 0.0105 [0.00454 - 0.0328]	0.0218 ± 0.0055 [0.0143 - 0.0279]
$AUC_{0\text{-}t}$ (µg.h/mL)	Day 1	0.0368 ± 0.0121 [0.0236 - 0.0519]	0.160 ± 0.069 [0.0666 - 0.272]	0.622 ± 0.296 [0.246 - 1.13]	0.0611 ± 0.0490 [0.0292 - 0.134]	0.196 ± 0.161 [0.0381 - 0.418]	0.426 ± 0.166 [0.226 - 0.658]
	Week 4	0.0548 ± 0.0250 [0.0273 - 0.0839]	0.197 ± 0.102 [0.104 - 0.395]	0.588 ± 0.419 [0.206 - 1.36]	0.0915 ± 0.0576 [0.0380 - 0.170]	0.231 ± 0.140 [0.0323 - 0.399]	0.483 ± 0.159 [0.295 - 0.703]
	Week 13	0.0660 ± 0.0205 [0.0385 - 0.0854]	0.198 ± 0.067 [0.138 - 0.326]	0.619 ± 0.366 [0.194 - 1.12]	0.0727 ± 0.043 [0.0262 - 0.125]	0.188 ± 0.113 [0.0278 - 0.321]	0.360 ± 0.090 [0.249 - 0.503]
Median t_{max} (h)	Day 1	1.00 [1.00 - 2.00]	4.00 [1.00 - 4.00]	8.00 [2.00 - 8.00]	2.00 [1.00 - 2.00]	4.00 [2.00 - 4.00]	8.00 [4.00 - 8.00]
	Week 4	2.50 [1.00 - 4.00]	4.00 [2.00 - 8.00]	8.00 [4.00 - 8.00]	2.00 [2.00 - 2.00]	4.00 [2.00 - 8.00]	8.00 [4.00 - 8.00]
	Week 13	2.50 [1.00 - 4.00]	4.00 [4.00 - 8.00]	8.00 [4.00 - 8.00]	1.50 [1.00 - 4.00]	4.00 [2.00 - 4.00]	8.00 [4.00 - 8.00]

a: Results are reported as mean unless stated otherwise and [range].

Monkey 13 week study with Daprodustat (Continued)

GSK2531400 (M13)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		5 (n=4)	20 (n=6)	100 (n=6)	5 (n=4)	20 (n=6)	100 (n=6)
C_{max} (µg/mL)	Day 1	0.00658 ± 0.0034 [0.00302 - 0.00969]	0.0179 ± 0.0083 [0.00736 - 0.0286]	0.0518 ± 0.0382 [0.0151 - 0.126]	0.00886 ± 0.0022 [0.00720 - 0.0119]	0.0231 ± 0.0141 [0.00880 - 0.0449]	0.0318 ± 0.0076 [0.0259 - 0.0457]
	Week 4	0.00597 ± 0.00231 [0.00583 - 0.00875]	0.0160 ± 0.0034 [0.0112 - 0.0206]	0.0465 ± 0.0333 [0.0137 - 0.111]	0.0364 ± 0.0036 [0.0207 - 0.0670]	0.0197 ± 0.0107 [0.00582 - 0.0328]	0.0364 ± 0.0166 [0.0207 - 0.0670]
	Week 13	0.00559 ± 0.00115 [0.00454 - 0.00707]	0.0142 ± 0.0062 [0.00726 - 0.0234]	0.0395 ± 0.0242 [0.0106 - 0.0804]	0.00929 ± 0.00258 [0.00564 - 0.0112]	0.0150 ± 0.0083 [0.00482 - 0.0253]	0.0208 ± 0.0063 [0.0108 - 0.0302]
AUC_{0-t} (µg.h/mL)	Day 1	0.0332 ± 0.0145 [0.0167 - 0.0505]	0.168 ± 0.044 [0.111 - 0.244]	0.730 ± 0.484 [0.227 - 1.66]	0.0483 ± 0.0101 [0.0400 - 0.0624]	0.198 ± 0.156 [0.0449 - 0.417]	0.434 ± 0.090 [0.347 - 0.600]
	Week 4	0.0509 ± 0.0425 [0.0202 - 0.113]	0.206 ± 0.061 [0.155 - 0.321]	0.698 ± 0.545 [0.208 - 1.77]	0.545 ± 0.045 [0.355 - 0.942]	0.251 ± 0.148 [0.0376 - 0.442]	0.545 ± 0.218 [0.355 - 0.942]
	Week 13	0.0536 ± 0.0334 [0.0206 - 0.0910]	0.202 ± 0.087 [0.106 - 0.322]	0.686 ± 0.396 [0.180 - 1.33]	0.0802 ± 0.0389 [0.0378 - 0.122]	0.179 ± 0.111 [0.0300 - 0.303]	0.363 ± 0.110 [0.184 - 0.499]
Median t_{max} (h)	Day 1	1.50 [1.00 - 4.00]	4.00 [4.00 - 4.00]	8.00 [4.00 - 8.00]	3.00 [1.00 - 4.00]	4.00 [4.00 - 4.00]	8.00 [4.00 - 8.00]
	Week 4	4.00 [2.00 - 4.00]	4.00 [4.00 - 8.00]	8.00 [4.00 - 8.00]	8.00 [2.00 - 8.00]	6.00 [4.00 - 8.00]	8.00 [2.00 - 8.00]
	Week 13	4.00 [2.00 - 8.00]	4.00 [4.00 - 8.00]	8.00 [4.00 - 8.00]	4.00 [4.00 - 4.00]	4.00 [4.00 - 4.00]	8.00 [4.00 - 8.00]

a: Results are reported as mean unless stated otherwise and [range].

Table 4.32 Monkey 39 week study with Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.2
Report No.: 2011N126131 **Study No.:** G10277

Species (Strain):	Monkey (Cynomolgus)
Test Article:	GSK1278863
Vehicle/Formulation:	1% (w/v) aqueous methylcellulose/Suspension
Method of Administration:	Oral (gavage)
Sample:	Plasma
Analyte:	GSK1278863, GSK2391220 (M2), GSK2531403 (M3), GSK2499166 (M4), GSK2531399 (M5), GSK2531398 (M6), and GSK2531400 (M13)
Assay:	HPLC-MS/MS and UHPLC-MS/MS
Gender (M/F)/Number of Animals:	4M/4F
Dose (mg/kg/day):	3, 10, 50
Duration of dosing (weeks):	39
PK Parameters:	

		Male			Female		
Dose (mg/kg/day)		3	10	50	3	10	50
C_{max} (µg/mL)	Day1	0.771 ± 0.457	1.66 ± 0.57	5.15 ± 2.04	0.960 ± 0.367	2.62 ± 0.81	5.52 ± 2.06
		[0.365 – 1.40]	[1.25 – 2.50]	[2.38 – 8.04]	[0.703 – 1.49]	[1.99 – 3.73]	[3.93 – 9.43]
	Week4	1.80 ± 0.69	1.78 ± 0.36	4.76 ± 1.98	0.887 ± 0.282	2.71 ± 1.32	4.30 ± 1.06
		[1.19 – 2.70]	[1.40 – 2.19]	[2.72 – 7.99]	[0.602 – 1.15]	[1.96 – 4.68]	[3.37 – 5.73]
	Week26	1.03 ± 0.76	1.01 ± 0.34	2.62 ± 0.76	0.566 ± 0.163	2.12 ± 0.47	3.01 ± 1.62
		[0.420 – 2.03]	[0.720 – 1.38]	[1.73 – 3.62]	[0.395 – 0.787]	[1.49 – 2.50]	[2.08 – 6.26]
	Week39	0.829[a] ± 0.309	0.972 ± 0.155	2.44 ± 0.59	0.524 ± 0.266	1.62 ± 0.34	2.72 ± 0.90
		[0.473 – 1.02]	[0.856 – 1.19]	[1.32 – 2.90]	0.282 – 0.899]	[1.21 – 2.01]	[1.38 – 4.20]
AUC_{0-t} (µg.h/mL)	Day1	2.37 ± 0.84	9.49 ± 5.59	43.5 ± 18.5	3.46 ± 1.46	12.4 ± 6.4	50.7 ± 9.3
		[1.60 – 3.56]	[4.51 – 17.0]	[18.3 – 75.7]	[1.40 – 4.86]	[7.65 – 21.3]	[40.3 – 61.2]
	Week4	4.76 ± 2.00	14.1 ± 2.49	53.5 ± 25.9	2.51 ± 0.42	14.1 ± 2.6	50.0 ± 13.7
		[2.63 – 7.28]	[12.0 – 17.7]	[22.6 – 100]	[2.06 – 3.02]	[10.8 – 17.0]	[35.2 – 76.0]
	Week26	5.96 ± 4.98	6.52 ± 2.51	41.7 ± 18.6	2.68 ± 0.67	13.0 ± 1.3	43.7 ± 19.8
		[1.01 – 12.8]	[4.19 – 8.71]	[13.6 – 60.9]	[1.82 – 3.45]	[11.2 – 14.1]	[29.8 – 82.3]
	Week39	4.55[a] ± 1.94	7.76 ± 2.29	30.2 ± 6.0	2.69 ± 0.911	11.2 ± 4.92	29.4 ± 11.7
		[2.31 – 5.83]	[4.74 – 10.00]	[23.3 – 36.2]	[1.67 – 3.58]	[7.05 – 18.3]	[20.3 – 51.3]

Monkey 39 week study with Daprodustat (Continued)

PK Parameters:

		Male			Female		
Dose (mg/kg/day)		3	10	50	3	10	50
t_{max} (h)[b]	Day1	0.75 [0.50 – 4.00]	1.00 [0.50 – 4.00]	2.00 [0.50 – 4.00]	0.50 [0.50 – 1.00]	1.50 [0.50 – 2.00]	3.00 [2.00 – 4.00]
	Week4	0.50 [0.50]	0.75 [0.50 – 1.00]	3.00 [1.00 – 8.00]	0.50 [0.50]	0.75 [0.50 – 2.00]	4.00 [0.50 – 8.00]
	Week26	1.00 [0.50 – 4.00]	2.00 [1.00 – 4.00]	8.00 [1.00 – 24.00]	0.50 [0.50 – 2.00]	1.00 [1.00 – 4.00]	4.00 [1.00 – 8.00]
	Week39	2.00[a] [0.50 – 4.00]	1.50 [0.50 – 2.00]	4.00 [2.00 – 8.00]	0.50 [0.50 – 4.00]	3.00 [2.00 – 4.00]	2.00 [1.00 – 4.00]

Additional Information: Mean or Mean ± SD [range] (n=3 - 6)
UHPLC-MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry
a: n = 3, male Animal 204 given 3 mg/kg/day was euthanized during Week 38 (Day 262) due to deteriorating clinical conditions
b: Median [range]

GSK2391220 (M2)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		3[b]	10	50	3	10	50
Cmax (µg/mL)	Week 39	0.0281 ± 0.0099 [0.0209 - 0.0394]	0.0747 ± 0.0303 [0.0367 – 0.0813]	0.0809 ± 0.0403 [0.0384 – 0.14]	0.0335 ± 0.0226 [0.0084 - 0.0606]	0.0692 ± 0.0516 [0.0296 – 0.142]	0.0986 ± 0.0502 [0.399 – 0.17]
AUC0-t (µg.h/mL)	Week 39	0.269 ± 0.069 [0.213 - 0.346]	0.757 ± 0.324 [0.331 – 1.12]	1.15 ± 0.93 [0.264 – 2.43]	0.264 ± 0.169 [0.241 - 0.498]	0.663 ± 0.350 [0.318 – 1.10]	1.39 ± 0.72 [0.618 – 2.33]
tmax[c] (h)	Week 39	- [1.00 - 4.00]	- [2.00 - 2.00]	- [2.00 - 8.00]	- [2.00 - 4.00]	- [4.00 - 4.00]	- [2.00 - 8.00]

a: Mean or Mean ± SD [range] (n=3 - 6).
b: n = 3, male Animal 204 given 3 mg/kg/day was euthanized during Week 38 (Day 262) due to deteriorating clinical conditions.
c: Median [range]

Monkey 39 week study with Daprodustat (Continued)

GSK2531403 (M3)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		3b	10	50	3	10	50
Cmax (µg/mL)	Week 39	0.0101 ± 0.0030 [0.0073 - 0.0132]	0.0247 ± 0.0099 [0.0119 – 0.0326]	0.0307 ± 0.0195 [0.0115 – 0.0646]	0.0117 ± 0.0075 [0.0036 - 0.0210]	0.0232 ± 0.0186 [0.0101 – 0.0499]	0.0352 ± 0.0179 [0.0132 – 0.0597]
AUC0-t (µg.h/mL)	Week 39	0.0571 ± 0.0115 [0.0481 - 0.0700]	0.235 ± 0.122 [0.065 – 0.326]	0.440 ± 0.406 [0.102 – 1.090]	0.0952 ± 0.0562 [0.0226 - 0.1590]	0.218 ± 0.148 [0.066 – 0.406]	0.495 ± 0.262 [0.207 – 0.858]
tmax[c] (h)	Week 39	- [1.00 - 4.00]	- [2.00 - 2.00]	- [4.00 - 8.00]	- [2.00 - 4.00]	- [4.00 - 4.00]	- [2.00 - 8.00]

a: Mean or Mean ± SD [range] (n=3 - 6).
b: n = 3, male Animal 204 given 3 mg/kg/day was euthanized during Week 38 (Day 262) due to deteriorating clinical conditions.
c: Median [range]

GSK2499166 (M4)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		3[b]	10	50	3	10	50
Cmax (µg/mL)	Week 39	0.0267 ± 0.0069 [0.0215 - 0.0345]	0.0704 ± 0.0232 [0.0427 – 0.0917]	0.0825 ± 0.0422 [0.0391 – 0.1480]	0.0383 ± 0.0247 [0.0109 - 0.0666]	0.0711 ± 0.0572 [0.0279 – 0.1530]	0.1019 ± 0.0521 [0.0407 – 0.1830]
AUC0-t (µg.h/mL)	Week 39	0.276 ± 0.060 [0.228 - 0.344]	0.703 ± 0.272 [0.352 – 0.934]	1.18 ± 0.97 [0.28 – 2.6]	0.350 ± 0.196 [0.109 – 0.559]	0.697 ± 0.414 [0.291 – 1.240]	1.475 ± 0.786 [0.626 – 2.120]
tmax[c] (h)	Week 39	- [1.00 - 4.00]	- [2.00 - 2.00]	- [2.00 - 8.00]	- [2.00 - 4.00]	- [4.00 - 4.00]	- [2.00 - 8.00]

a: Mean or Mean ± SD [range] (n=3 - 6).
b: n = 3, male Animal 204 given 3 mg/kg/day was euthanized during Week 38 (Day 262) due to deteriorating clinical conditions.
c: Median [range]

Monkey 39 week study with Daprodustat (Continued)

GSK2531399 (M5)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		3	10	50	3	10	50
Cmax (µg/mL)	Week 39	0.00165[b] [0.00115 – 0.00214]	0.00339 ± 0.00163 [0.00144 – 0.00478]	0.00373 ± 0.00274 [0.00125 – 0.00874]	0.00198[c] ± 0.00083 [0.00109 - 0.00274]	0.00348 ± 0.00307 [0.00138 – 0.00790]	0.00444 ± 0.00231 [0.00155 – 0.00725]
AUC0-t (µg.h/mL)	Week 39	0.00503[d] -	0.0219[e] ± 0.0045 [0.0169 – 0.0256]	0.0596[f] ± 0.0614 [0.0120 – 0.1500]	0.00741[c] ± 0.00384 [0.00336 - 0.0110]	0.0311[g] [0.0201 – 0.0421]	0.0527 ± 0.0412 [0.0102 – 0.0989]
tmax[h] (h)	Week 39	- [1.00 - 2.00]	- [2.00 - 4.00]	- [2.00 - 8.00]	- [2.00 - 4.00]	- [4.00 - 4.00]	- [4.00 - 8.00]

a: Mean or Mean ± SD [range] (n=1 - 6).
b: n = 2, two animals had non-quantifiable or insufficient plasma concentration data to calculate toxicokinetic parameters.
c: n = 3, one animal had non-quantifiable or insufficient plasma concentration data to calculate toxicokinetic parameters.
d: n = 1, three animals had non-quantifiable or insufficient plasma concentration data to calculate AUC.
e: n = 3, one animal had non-quantifiable or insufficient plasma concentration data to calculate AUC.
f: n = 5, one animal had non-quantifiable or insufficient plasma concentration data to calculate AUC.
g: n = 2, two animals had non-quantifiable or insufficient plasma concentration data to calculate AUC.
h: Median [range]

GSK2531398 (M6)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		3[b]	10	50	3	10	50
Cmax (µg/mL)	Week 39	0.00475 ± 0.00185 [0.00339 – 0.00214]	0.0165 ± 0.0119 [0.0052 – 0.0329]	0.0165 ± 0.0151 [0.0050 – 0.0452]	0.00585 ± 0.00364 [0.0018 - 0.0070]	0.0123 ± 0.0107 [0.0043 – 0.0275]	0.0173 ± 0.0089 [0.0055 – 0.0275]
AUC0-t (µg.h/mL)	Week 39	0.0278 ± 0.0097 [0.0218 – 0.0390]	0.155 ± 0.118 [0.027 – 0.308]	0.240 ± 0.281 [0.032 – 0.732]	0.0317 ± 0.0207 [0.0057 - 0.0527]	0.073 ± 0.055 [0.029 – 0.148]	0.244 ± 0.152 [0.062 – 0.421]
tmax[c] (h)	Week 39	- [1.00 - 4.00]	- [2.00 - 2.00]	- [2.00 - 8.00]	- [2.00 - 4.00]	- [4.00 - 4.00]	- [4.00 - 8.00]

a: Mean or Mean ± SD [range] (n=3 - 6).
b: n = 3, male Animal 204 given 3 mg/kg/day was euthanized during Week 38 (Day 262) due to deteriorating clinical conditions.
c: Median [range]

Monkey 39 week study with Daprodustat (Continued)

GSK2531400 (M13)

PK Parameters	Period	Male			Female		
		Dose of GSK1278863 (mg/kg/day)			Dose of GSK1278863 (mg/kg/day)		
		3[b]	10	50	3[c]	10	50
Cmax (µg/mL)	Week 39	0.00515 ± 0.00287 [0.00264 – 0.00828]	0.0177 ± 0.0109 [0.0048 – 0.0312]	0.0142 ± 0.0086 [0.0056 – 0.0242]	0.00771 ± 0.00431 [0.00392 - 0.01240]	0.0126 ± 0.0095 [0.0050 – 0.0248]	0.0201 ± 0.0115 [0.0049 – 0.0340]
AUC0-t (µg.h/mL)	Week 39	0.0312 ± 0.0175 [0.0154 – 0.0500]	0.188 ± 0.145 [0.028 – 0.370]	0.215 ± 0.192 [0.040 – 0.492]	0.0412 ± 0.0172 [0.0232 - 0.0574]	0.098 ± 0.079 [0.031 – 0.190]	0.278 ± 0.182 [0.063 – 0.522]
tmax[c] (h)	Week 39	- [1.00 - 4.00]	- [2.00 - 2.00]	- [2.00 - 8.00]	- [2.00 - 4.00]	- [4.00 - 4.00]	- [4.00 - 8.00]

a: Mean or Mean ± SD [range] (n=3 - 6).
b: n = 3, male Animal 204 given 3 mg/kg/day was euthanized during Week 38 (Day 262) due to deteriorating clinical conditions.
c: n = 3, one animal has no quantifiable plasma concentration data to calculate toxicokinetic parameters.
d: Median [range]

5. PHARMACOKINETICS: IN VITRO ABSORPTION

Table 5.1 Evaluatation of percutaneous absorption of daprodustat in human skin

Test Article:	GSK1278863	**Location in CTD:**	m4.2.2.3
Study Number:	2010-495-JL	**Report No.:**	2012N152036
Study Type:	In vitro human skin absorption	**Detection:**	HPLC MS

Test Article	Total Absorption (μg/cm²)	Total Absorption (%)
0.2% Dispersed Cream Lot# 770-49-02	0.028 ±0.014	0.282 ±0.141
0.2% Dispersed Gel Lot# 770-50-02	0.028 ±0.025	0.275 ±0.252
0.2% Ointment Lot# ee490071	0.022 ±0.011	0.214 ±0.105

Table 5.2 Evaluation of skin permeation and deposition by Daprodustat

Test Article:	GSK1278863	**Location in CTD:**	m4.2.2.3
Study Number:	GK1202-01	**Report No.:**	2012N152038
Study Type:	In vitro human skin permeation and deposition	**Detection:**	HPLC MS

Skin Type	Formulations	Flux (ng/cm/h)
Abraded	0.2% Stfl cream (BA/SA) Lot# ee564527	0.64 ±0.13
	0.2% water miscible cream Lot# ee564529	0.14 ±0.07
Non-abraded	0.2% Stfl cream (BA/SA) Lot# ee564527	0.18 ±0.06
	0.2% water miscible cream Lot# ee564529	0.01 ±0.01

Table 5.3 Rate and extent of absorption of micronized vs. non-micronized daprodustat

Test Article:	GSK1278863	**Location in CTD:**		m4.2.2.3	
Study Number:	795839	**Report No.:**		2015N230888	
Study Type:	In vitro human skin absorption	**Detection:**		HPLC MS	
		Mean Test Material Recovered at 24 Hours post dose (ng/cm²)			
		Intact		**Abraded**	
Test Item Concentration (% w/w)	**Sample**	**Non-Micronised**	**Micronised**	**Non-Micronised**	**Micronised**
1	Stratum Corneum	2683	3875	3288	4381
	Epidermis	275	578	984	1217
	Dermis	159	332	404	483
	Receptor Fluid	8	14	10	13
5	Stratum Corneum	24651	24546	53767	17528
	Epidermis	786	1402	4913	5872
	Dermis	747	1181	2406	1528
	Receptor Fluid	11	10	19	32
15	Stratum Corneum	57462	71581	31821	79201
	Epidermis	4012	9019	10470	20957
	Dermis	1810	3733	4240	13510
	Receptor Fluid	23	44	15	16
30	Stratum Corneum	224495	307479	198941	214844
	Epidermis	14050	38245	21800	30995
	Dermis	8998	14684	9094	14672
	Receptor Fluid	8	113	114	979

Key:

Mironised Lot# = EE757297-M; Non-micronised Lot# = EE757297-UM

Receptor fluid samples (ng/cm²) = ((LC-MS/MS 24 h value (ng/mL) x total volume of receptor chamber) + mass (ng) of test item removed by previous time point aliquots) / skin surface area (0.64 cm²)

Non receptor fluid samples (ng/cm²) = (LC-MS/MS value (ng/mL) x total sample volume) / skin surface area (0.64 cm²)

6. PHARMACOKINETICS: ORGAN DISTRIBUTION

Table 6.1 Pharmacokinetics: Organ Distribution

Test Article: GSK1278863

Location in CTD: m4.2.2.3
Report No.: CD2008/00271 **Study No.:** 85-0708

Species:	Rat (Long Evans partially Pigmented)
Gender (M/F)/Number of Animals:	1M/time point
Feeding Condition:	Fasted
Vehicle/Formulation:	1% aqueous methylcellulose
Method of Administration:	Oral
Dose (mg/kg):	10
Radionuclide:	14C
Specific Activity (µCi/mg):	22.3
Sampling Times:	35 days (1 h, 4 h, 8 h, 24 h, 3 days, 7 days, and 35 days after dosing)
Analysis:	QWBA
Results:	

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)						
		1 h	4 h	8 h	24 h	3 Days	7 Days	35 Days
Vascular/Lymphatic	Aorta	47.911	27.900	23.258	11.980	0.789	BLQ	BLQ
	Blood (cardiac)	62.510	34.957	24.806	12.682	1.358	BLQ	BLQ
	Bone Marrow	15.103	9.251	4.731	2.881	0.162	BLQ	BLQ
	Mandibular Lymph Nodes	8.733	9.749	5.902	2.640	0.468	BLQ	BLQ
	Spleen	8.730	5.513	3.459	1.960	0.192	BLQ	BLQ
	Thymus	4.566	5.119	4.402	2.193	0.232	BLQ	BLQ
Excretory/Metabolic	Kidney	27.963	17.491	7.765	3.332	0.598	BLQ	BLQ
	Renal Cortex	28.834	17.777	8.507	3.068	0.504	BLQ	BLQ
	Renal Medulla	23.809	15.397	6.247	3.165	0.500	BLQ	BLQ
	Liver	33.747	18.775	12.413	3.335	0.470	BLQ	BLQ

Pharmacokinetics: Organ Distribution (Continued)

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)						
		1 h	4 h	8 h	24 h	3 Days	7 Days	35 Days
Central Nervous System	Brain	0.778	0.661	0.502	0.290	BLQ	BLQ	BLQ
	Choroid Plexus	10.340	9.270	5.386	2.669	0.930	BLQ	BLQ
	Meninges	43.960	31.988	30.329	11.977	1.196	BLQ	BLQ
	Peripheral Nerve	3.437	1.713	1.995	3.363	1.299	1.035	BLQ
	Spinal Cord	0.767	0.529	0.573	0.253	BLQ	BLQ	BLQ
Endocrine	Adrenal Cortex	20.409	11.940	10.806	4.325	0.699	BLQ	BLQ
	Adrenal Medulla	27.211	14.809	15.180	6.628	0.686	BLQ	BLQ
	Pineal Gland	10.267	9.883	8.044	3.989	0.367	BLQ	BLQ
	Pituitary	11.824	9.945	7.120	2.965	0.605	BLQ	BLQ
	Thyroid	8.873	7.775	9.212	1.464	0.314	BLQ	BLQ
Secretory	Exorbital Lachrymal Gland	15.055	8.816	8.460	5.294	0.299	BLQ	BLQ
	Intra-Orbital Lachrymal Gland	9.217	8.323	6.110	2.085	0.263	BLQ	BLQ
	Harderian Gland	8.413	8.332	5.128	2.526	0.210	BLQ	BLQ
	Pancreas	10.468	7.248	4.269	2.925	0.241	BLQ	BLQ
	Salivary Gland	12.515	9.304	5.720	3.425	0.326	BLQ	BLQ
Fatty	Fat (brown)	16.834	12.397	12.999	3.255	0.368	BLQ	BLQ
	Fat (abdominal)	1.985	1.653	2.384	0.564	0.038	BLQ	BLQ
Dermal	Skin (non-pigmented)	5.734	8.421	8.011	4.142	0.446	BLQ	BLQ
	Skin (pigmented)	5.278	8.587	7.819	4.789	0.589	BLQ	BLQ
Reproductive	Prostate Gland	4.626	5.345	9.582	2.758	0.259	BLQ	BLQ
	Epididymis	4.253	9.087	5.532	3.159	0.281	BLQ	BLQ
	Testis	3.879	8.697	6.943	2.928	0.199	BLQ	BLQ
Skeletal/ Muscular	Muscle (skeletal)	3.770	4.807	3.668	1.649	0.116	BLQ	BLQ
	Myocardium (heart)	19.816	11.106	11.154	4.056	0.570	BLQ	BLQ
Respiratory Tract	Lung	48.703	25.153	17.798	8.337	1.359	BLQ	BLQ
	Nasal Turbinates	7.959	7.730	4.481	3.356	0.216	BLQ	BLQ

Pharmacokinetics: Organ Distribution (Continued)

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)						
		1 h	4 h	8 h	24 h	3 Days	7 Days	35 Days
Alimentary Canal	Cecum Mucosa	9.539	21.316	29.967	5.720	1.233	BLQ	BLQ
	Esophagus	8.859	16.551	19.385	8.458	0.602	BLQ	BLQ
	Large Intestine Mucosa	13.247	7.048	14.346	3.168	0.317	BLQ	BLQ
	Large Intestine Contents	5.737	7.624	344.504	44.188	5.713	0.110	BLQ
	Rectum Mucosa	10.373	9.425	11.730	5.079	0.291	BLQ	BLQ
	Small Intestine Mucosa	44.819	62.731	8.201	2.412	0.282	BLQ	BLQ
	Small Intestine Contents	341.308	401.630*	62.228	20.132	2.540	BLQ	BLQ
	Stomach Mucosa	11.666	4.980	4.672	1.828	0.291	BLQ	BLQ
Ocular	Lens	BLQ	BLQ	0.122	BLQ	BLQ	BLQ	BLQ
	Uveal Tract	5.503	20.304	7.290	3.397	0.828	BLQ	BLQ

Individual (n=1)

BLQ: Below the limit of quantitation (<0.038 µg equiv/g) or tissue could not be visually identified because of non-detectable radioactivity

*: above the limit of quantitation (>366.353 µg equiv/g)

Table 6.2 Pharmacokinetics: Kidney and Liver Distribution

Test Article: GSK2531401 (M13)
Study System: In vivo **Location in CTD:** m4.2.2.3
Report No.: 2012N132320 **Study No.:** 11DMM049
Species: Mouse (CD-1)
Gender (M/F)/Number of Animals: Male/15 per group (3 per timepoint)
Vehicle/Formulation: Saline, pH 8.5 (GSK2531401); 2% DMSO, 49% of 40% Captisol in water and 49% saline, pH 8.5 (GSK2212842)
Method of Administration: Intravenous (bolus)
Dose (mg/kg): 40 (GSK2531401) and 1 (GSK2212842)

Target Entity, Test System and Method: The objective of this study was to determine the systemic exposure and erythropoietin response following a single intravenous (bolus) administration of GSK2531401 and positive control GSK2212842 (EPO response is known) at nominal doses of 40 and 1 mg/kg, respectively, to male CD-1 mice.

Results: GSK2531401 plasma concentrations were quantifiable only up to the 8 hour sampling period, therefore pharmacokinetic analysis of GSK2531401 was not performed due to insufficient data points for accurate analysis. Based on concentrations of drug present in the plasma, liver and kidney over the 24-hour sampling period, GSK2531401 cleared more quickly than GSK2212842 from the mice. Because of lack of vehicle treated group, it is difficult to conclude whether or not either compound induced EPO or VEGF in this study.

Plasma concentration

Test Article (Dose)	Mean of Actual dose received (mg/kg)	AUC_{0-t} (μg.h/mL)	C_{max} (μg/mL)	T_{max} (h)
GSK2212842 (1 mg/kg)	1.09	25.7	1.55	4.00

Mean GSK2531401 and GSK2212842 tissue concentrations (ng/mL)

	Sampling Time (hours after dosing)				
Treatment (Tissue)	2	4	8	12	24
GSK2531401 (Plasma)	589	22.2	4.83	NQ	NQ
GSK2212842 (Plasma)	1030	1550	1260	1190	634
GSK2531401 (Liver)	879	62.8	23.8	NQ	NQ
GSK2212842 (Liver)	8340	8530	8390	7240	3900
GSK2531401 (Kidney)	986	467	65.8	16.6	NQ
GSK2212842 (Kidney)	812	797	899	939	504

Pharmacokinetics: Kidney Distribution (Continued)

Mean Plasma EPO or VEGF plasma concentrations (ng/mL)					
	Sampling Time (hours after dosing)				
Treatment (Plasma EPO or VEGF levels)	2	4	8	12	24
GSK2531401 (Plasma EPO)	135	127	54.4	36.9	62.0
GSK2531401 (Plasma VEGF)	15.1	11.8	9.82	9.27	10.4
GSK2212842 (Plasma EPO)	69.4	72.4	47.6	87.9	75.2
GSK2212842 (Plasma VEGF)	9.34	8.13	9.23	7.96	6.64

Key:
NQ = Not Quantitated, below the level of detection.

Table 6.3 Pharmacokinetics: Kidney Distribution of Daprodustat or GSK2531401 (M13) in Mice

Test Article: GSK1278863 or GSK2531401 (M13) **Report No.:** 2012N156583
Study System: In vivo **Study No.:** 12DMM041
Species: Mouse (CD-1) **Location in CTD:** m4.2.2.3
Gender (M/F)/Number of Animals: Male (2 or3 per timepoint)
Vehicle/Formulation: Saline [pH 7] (GSK2531401); 10% Captisol in 25 mM Tris buffer [pH 9])/10% Captisol in 25 mM Tris buffer [pH 9] (GSK1278863)
Method of Administration: Intravenous (bolus) (GSK2531401, GSK1278863) and Oral (GSK2212842)
Dose (mg/kg): 40 or 100 (GSK2531401), 20 (GSK1278863) and 30 (GSK2212842)

Target Entity, Test System and Method: The objective of this study was to determine the systemic exposure and erythropoietin (EPO) response following a single intravenous (bolus) administration of either GSK2531401 at 40 or 100 mg/kg or GSK1278863 at 20 mg/kg to male CD-1 mice. The effect of GSK2531401A on vascular endothelial growth factor (VEGF) response was also evaluated. GSK2212842 (positive control) was used to induce EPO and VEGF and was administered orally. Concentrations of GSK2531401 and GSK1278863 in kidney tissues at select time points were also determined.

Results: Both GSK2531401 and GSK1278863 were characterized by low plasma clearance and a low steady state volume of distribution in male CD-1 mice. GSK2531401 (M13) exhibited approximately 40-fold higher clearance than GSK1278863. GSK1278863 significantly elevated plasma EPO levels in mice. GSK2531401 did not elevate EPO to a significant extent in this study. VEGF levels in the plasma samples were near the lower limits of detection and therefore no conclusion can be made about its regulation by any of these compounds. Despite being administered at a 2 to 5-fold lower dose, GSK1278863 concentrations were higher (15 to 500-fold) than GSK2531401 concentrations in the kidney at all time points.

	GSK2531401 (M13)		GSK1278863
Parameter	**40 mg/kg**	**100 mg/kg**	**20 mg/kg[a]**
Mean actual dose received (mg/kg)	33.8	101	18.2
AUC_{0-t} (μg.h/mL)	27.8	92.8	614
AUC_{0-inf} (μg.h/mL)	27.9	92.9	685
C_{max} (μg/mL)	81.5	210	88.0
T_{max} (h)	0.083	0.083	0.083
CL (mL/h/kg)	1210	1090	26.6
$T_{1/2}$ (h)	4.61	1.04	7.28
V_{ss} (L/kg)	0.433	0.418	0.270
MRT (h)	0.357	0.384	10.2

Key: a = GSK1278863 concentrations did not decrease more than 1-log unit over the 24 hour time period. Pharmacokinetic parameters of T1/2 and MRT should be used with caution as the terminal phase of this molecule was not sufficiently captured.

Pharmacokinetics: Kidney Distribution of Daprodustat or GSK2531401 (M13) in Mice (Continued)

Treatment (Tissue)	Nominal Dose (mg/kg)	Actual dose (mg/kg)	Mean Plasma Concentration (ng/mL)							
			0.083 h	0.25 h	0.5 h	1 h	2 h	4 h	8 h	24 h
GSK2531401	40	33.8	81500	31200	13300[b]	2630[b]	472[b]	26.1[b]	12.3[b]	1.23[a]
GSK2531401	100	101	210000[b]	148000[b]	50500[b]	5250[b]	1290[b]	167[b]	20.2[b]	NQ[b]
GSK1278833	20	18.2	88000	NS	NS	64000	NS	48000	25400	7010

Treatment (Tissue)	Nominal Dose (mg/kg)	Actual dose (mg/kg)	Mean Plasma EPO or VEGF Concentration (ng/mL) at Sampling time							
			0.083 h	0.25 h	0.5 h	1 h	2 h	4 h	8 h	24 h
GSK2531401 (Plasma EPO)	40	33.8	11.7	16.7	9.30[b]	9.83[b]	40.0[b]	92.9[b]	21.6[b]	15.9[b]
GSK2531401 (Plasma VEGF)	40	33.8	10.8	13.8	8.96[b]	9.50[b]	16.2[b]	19.0[b]	11.0[b]	8.66[b]
GSK2531401 (Plasma EPO)	100	101	19.6[b]	21.4[b]	9.14[b]	17.3[b]	61.4[b]	124[b]	45.8[b]	23.6[b]
GSK2531401 (Plasma VEGF)	100	101	13.0[b]	14.3[b]	15.3[b]	13.3[b]	17.9[b]	16.0[b]	13.2[b]	10.3[b]
GSK1278833 (plasma EPO)	20	18.2	25.4	NS	NS	21.5	NS	323	179	179
GSK1278833 (plasma VEGF)	20	18.2	13.6	NS	NS	13.2	NS	18.0	17.7	15.3
GSK2212842 (plasma EPO)	30		NS	NS	NS	NS	NS	NS	15500	31700[b]
GSK2212842 (plasma VEGF)	30		NS	NS	NS	NS	NS	NS	18.7	23.00[b]

Key: Mean (n=2 - 3)

a = n=2 (one sample was below the limit of quantitation, therefore mean was calculated using 0).

b = n=2 (due to insufficient dose volumes available on day of dosing).

NQ = Not quantitated, all samples below the level of quantitation. NS = Time point was not sampled

7. PHARMACOKINETICS: PLASMA PROTEIN BINDING

Table 7.1 Plasma Protein Binding for Daprodustat

Test Article: GSK1278863
Study System: In vitro
Location in CTD: m4.2.2.3
Report No.: UH2008/00002 **Study No.:** 07CDUP0350

Target Entity, Test System and Method: The in vitro plasma protein binding of GSK1278863 was investigated by equilibrium dialysis in mouse, rat, dog, monkey and human plasma at target concentrations of 1,000 and 10,000 ng/mL. Concentrations of GSK1278863 were determined by LC-MS/MS.

Results:

Nominal concentration (ng/mL)	% Bound to Plasma Proteins				
	Mouse	Rat	Dog	Monkey	Human
1000	98.4 ± 0.2	99.4 ± 0.5	96.7 ± 1.5	96.3 ± 2.0	98.4 ± 0.6
10000	97.8 ± 0.7	99.8 ± 0.1	94.7 ± 2.0	96.2 ± 1.4	98.8 ± 0.1

Mean ± SD (n=2 - 4)

Test Article: GSK1278863
Study System: In vitro
Location in CTD: m4.2.2.3
Report No.: CD2010/00340 **Study No.:** 09DMM060

Target Entity, Test System and Method: Plasma protein binding of GSK1278863 was determined *in vitro* in the rat, mouse, rabbit, dog, monkey and human by equilibrium dialysis at target concentrations of 0.2, 1, 10, and 50 μg/mL. Concentrations of GSK1278863 were determined by LC-MS/MS.

Results:

Nominal Plasma concentration (μg/mL)	% Bound in Plasma					
	Rat	Mouse	Rabbit	Dog	Monkey	Human
0.2	>99.5 ± 0.1	>99.6 ± 0.0	>99.5 ± 0.0	98.3 ± 0.1	93.6 ± 1.6	99.4 ± 0.0
1	>99.9 ± 0.0	99.8 ± 0.0	99.9 ± 0.0	97.9 ± 0.8	98.4 ± 0.1	99.4 ± 0.0
10	100.0 ± 0.0	99.8 ± 0.0	99.9 ± 0.0	98.3 ± 0.2	98.9 ± 0.2	99.5 ± 0.1
50	97.7 ± 4.0	99.6 ± 0.0	99.9 ± 0.0	98.2 ± 0.1	98.8 ± 0.0	98.3 ± 2.0

Mean ± SD (n=2 - 3)

Table 7.2 Plasma Protein Binding for Metabolites of Daprodustat

Test Article: GSK2391220 (M2), GSK2531403 and GSK2506104 (M3), GSK2499166 (M4), GSK2531399 (M5), GSK2531398 (M6), and GSK2531400 and GSK2531401 (M13)

Study System: In vitro **Location in CTD:** m4.2.2.3

Report No.: 2012N131897 **Study No.:** 11DMM027

Target Entity, Test System and Method: Plasma protein binding of the metabolites of GSK1278863 [GSK2391220 (M2), GSK2531403 and GSK2506104 (M3), GSK2499166 (M4), GSK2531399 (M5), GSK2531398 (M6), and GSK2531400 and GSK2531401 (M13)] was determined *in vitro* in the human by equilibrium dialysis at target concentrations of 10, 100, 250 and 500 ng/mL. Concentrations of each metabolite were determined by UPLC-MS/MS.

Results:

Nominal Plasma concentration (ng/mL)	% Bound in Plasma							
	GSK2391220 (M2)	GSK2506104 (M3)	GSK2531403 (M3)	GSK2499166 (M4)	GSK2531399 (M5)	GSK2531398 (M6)	GSK2531401 (M13)	GSK2531400 (M13)
10	2.2	13.2	1.0	68.3	74.0	25.9	0.1	0.3
100	13.9	16.9	3.8	86.9	90.8	33.5	2.7	0
250	12.1	17.0	5.9	97.5	97.9	33.7	3.9	0.7
500	10.5	23.5	3.2	97.7	98.3	23.5	0	0.5

Mean ± SD (n=2 - 3)

Table 7.3 Pharmacokinetics: Binding to Human Serum Albumin and Human alpha1-Acid Glycoprotein

Test Article: GSK1278863
Study System: In vitro **Location in CTD:** m4.2.2.3
Report No.: CD2010/00340 **Study No.:** 09DMM060

Target Entity, Test System and Method: The protein binding of GSK1278863 was determined *in vitro* in 0.600 mM human serum albumin (HSA) and in 0.015 mM and 0.150 mM α1-acid glycoprotein (AAG) at target concentrations of 0.2, 1, 10, and 30 μg/mL. Concentrations of GSK1278863 were determined by LC-MS/MS.

Results:

Nominal Plasma concentration (μg/mL)	% Bound in Protein		
	0.600 mM HSA	0.015 mM AAG	0.150 mM AAG
0.2	99.4 ± 0.0	11.1 ± 7.3	8.0 ± 2.7
1	99.4 ± 0.0	9.2 ± 5.9	7.9 ± 7.3
10	99.4 ± 0.0	16.7 ± 21.2	2.4 ± 2.6
30	99.3 ± 0.1	4.4 ± 7.6	6.0 ± 7.5

Mean ± SD (n=3)
HSA: Human serum albumin, AAG: alpha1-acid glycoprotein

Table 7.4 Pharmacokinetics: Blood Cell Partitioning

Test Article: GSK1278863
Study System: In vitro **Location in CTD:** m4.2.2.3
Report No.: UH2008/00002 **Study No.:** 07CDUP0969

Target Entity, Test System and Method: The in vitro blood cell association of GSK1278863 was determined in blood from CD-1 mice, Sprague Dawley rats, beagle dogs, and cynomolgus monkeys, and human at target concentrations of 1 and 10 μg/mL. The spiked blood samples were incubated at approximately 37°C for approximately 30 minutes and were analysed by LC/MS/MS.

Results:

	Cb/Cp	
Species	1 μg/mL	10 μg/mL
Mouse	0.45 ± 0.10	0.71 ± 0.09
Rat	1.64 ± 0.23	1.15 ± 0.03
Dog	1.05 ± 0.24	0.85 ± 0.16
Monkey	0.84 ± 0.23	0.61 ± 0.01
Human	1.23 ± 0.11	0.75 ± 0.16

Mean ± SD (n=6)
Cb/Cp = Concentration in blood to Concentration in plasma ratio.

Table 7.5 Pharmacokinetics: Blood to plasma and liver to blood ratios of total radioactivity following single oral administration of [14C]daprodustat

Test Article: [^{14}C]GSK1278863

		Blood, Plasma, or Liver levels (μg.equiv/g)							
		Report No.: CD2008/00100 **Study No.:**7717-705 **Location in CTD:**m4.2.2.3			**Report No.:** CD2008/00025 **Study No.:**AFA00607 **Location in CTD:**m4.2.2.3			**Report No.:** 2018N355713 **Study No.:**8361764 **Location in CTD:**m4.2.2.3	
		Rat (Sprague Dawley)			**Dog (beagle)**			**Monkey (cynomolgus)**	
Matrix	**Sampling Time (hours)**	10 mg/kg (oral gavage; vehicle = 1% methylcellulose) n=3/sex/group			20 mg/kg (oral gavage; vehicle = 1% methylcellulose) n=3/sex/group (Intact) n=2 males/group (BDC)			10 mg/kg (oral gavage; vehicle = 1% methylcellulose) n=3 males/group (intact) n=2 males/group (BDC)	
		Male	**Female**	**BDC Males**	**Male**	**Female**	**BDC Males**	**Male**	**BDC Males**
Blood	**1**	37.4 ±3.9	41.4 ±6.0	-	3.29 ±0.37	4.83 ±0.22	-	2.59 ±0.73	4.12
	4	31.9 ±3.0	30.5 ±5.7	-	7.29 ±0.89	7.49 ±2.44	-	4.62 ±0.27	3.85
	8	23.1 ±2.4	22.6 ±2.8	-	-	-	-	1.65 ±0.51	1.93
	10	-	-	-	4.12 ±0.86	4.98 ±2.51	-	-	-
	24	12.2 ±0.49	10.9 ±3.9	-	1.34 ±1.0	1.69 ±1.23	-	0.0985 ±0.0257	0.0667
	72	-	-	-	-	-	-	BQL	BQL
	96	-	-	0.356[a]	-	-	0.321	-	-
	168	0.072 ±0.029	0.033 ±0.015	-	BQL	BQL	-	BQL	BQL
Plasma	**1**	72.1 ±3.9	76.1 ±11.3	-	4.38 ±0.83	6.34 ±0.72	-	3.91 ±1.25	6.40
	4	61.3 ±7.0	56.9 ±9.0	-	10.1 ±1.3	10.2 ±3.31	-	7.22 ±0.33	5.95
	8	43.3 ±3.4	40.3 ±4.8	-	-	-	-	2.36 ±0.67	3.09
	10	-	-	-	5.05 ±1.4	6.24 ±3.42	-	-	-
	24	23.3 ±0.7	19.7 ±6.2	-	1.66 ±1.3	2.17 ±1.66	-	0.129 ±0.042	0.142
	72	-	-	-	-	-	-	BQL	BQL
	96	-	-	1.04 ±0.19	-	-	0.381	-	-
	168	0.142 ±0.05	0.062 ±0.030	-	BQL	BQL	-	BQL	BQL
Liver	1	30.7 ±3.7	22.2 ±4.9	-	-	-	-	-	-
	4	22.4 ±5.5	15.9 ±4.4	-	-	-	-	-	-
	8	12.6 ±0.81	9.01 ±1.1	-	-	-	-	-	-
	24	3.89 ±0.22	4.10 ±2.0	-	-	-	-	-	-

Pharmacokinetics: Blood to plasma and liver to blood ratios of total radioactivity following single oral administration of [14C]daprodustat (Continued)

Matrix	Sampling Time (hours)	Ratios							
		Rat (Sprague Dawley)			Dog (beagle)			Monkey (cynomolgus)	
		10 mg/kg (oral gavage; vehicle = 1% methylcellulose) n=3/sex/group			20 mg/kg (oral gavage; vehicle = 1% methylcellulose) n=3/sex/group (Intact) n=2 males/group (BDC)			10 mg/kg (oral gavage; vehicle = 1% methylcellulose) n=3 males/group (intact) n=2 males/group (BDC)	
		Male	Female	BDC Males	Male	Female	BDC Males	Male	BDC Males
Blood/ Plasma	1	0.517 ±0.027	0.544 ±0.009	-	0.76 ±0.08	0.77 ±0.08	-	0.672 ±0.056	0.644
	4	0.522 ±0.011	0.535 ±0.020	-	0.72 ±0.03	0.73 ±0.01	-	0.639 ±0.021	0.648
	8	0.534 ±0.017	0.560 ±0.005	-	-	-	-	0.701 ±0.048	0.626
	10	-	-	-	0.83 ±0.06	0.81 ±0.04	-	-	-
	24	0.526 ±0.019	0.549 ±0.026	-	0.87 ±0.14	0.80 ±0.07	-	0.775 ±0.067	0.624
	72	-	-	-	-	-	-	NA	NA
	96	-	-	0.519[b]	-	-	0.84[c]	-	-
	168	0.505 ±0.027	0.542 ±0.022	-	NA	NA		NA	NA
Liver/ Blood	1	0.825 ±0.083	0.533 ±0.043	-	-	-	-		
	4	0.697 ±0.125	0.516 ±0.056	-	-	-	-		
	8	0.547 ±0.037	0.400 ±0.032	-	-	-	-		
	24	0.319 ±0.023	0.364 ±0.043	-	-	-	-		

Key:
a = At least one value was BLQ; therefore, the SD was not reported.
b = At least one value was NC; therefore the SD was not reported.
c = n=1.
BDC = Bile duct-cannulated.
BQL = Below the quantifiable limit.
NA = Not applicable.
- = Not determined.
Note: The quantitation limits were as follows: Rat (blood: 0.00606 μg equiv/g; plasma: 0.00434 μg equiv/g), Dog (blood: 0.0796 μg equiv/g; plasma: 0.0576 μg equiv/g) and Monkey (blood: 0.0707 μg equiv/g; plasma: 0.0745 μg equiv/g).

8. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS

Table 8.1 Pharmacokinetics: Study in Pregnant or Nursing Animals – Placental Transfer

No studies

Table 8.2 Pharmacokinetics: Study in Pregnant or Nursing Animals – Excretion into Milk

Test Article: GSK1278863A plus M2 (GSK2391220), M3 (GSK2506104), and M13 (GSK2531401)

Location in CTD: m4.2.2.5

Species (Strain): **Rat (SD)** **Report No.:** 2018N365151 **Study No.:** R71242G

Test Article: GSK1278863 + metabolites

Vehicle/Formulation: Daprodustat: 1% (w/v) aqueous methylcellulose/Suspension
Metabolite Cocktail (subcutaneous): Aqueous 25 mM Phosphate Buffer, pH 7.4/ Solution

Method of Administration: Daprodustat: Oral (gavage)
Metabolite Cocktail: Subcutaneous

Sample: Plasma in pups

Analyte: GSK1278863, GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13)

Assay: HPLC-MS/MS

Dose (mg/kg/day): Daprodustat: 0.8, 7, 40
Metabolites cocktail: 2.5, 3.2, 1.8 (GSK2391220 (M2), GSK2506104 (M3), GSK2531401 (M13) mg/kg/day [dosed as 1.25, 1.8 and 0.9 mg/kg/dose BID every 6 hrs])

Duration of dosing (days): Gestation Day(GD) 6 to Lactation Day 21 or GD6 to GD9

		Plasma concentrations ng/mL			
Daily Oral Dose (mg/kg/day) GSK1278863[a]		**0 (Control)**	**0.8**	**7**	**40**
PND 10 (F_1) males	**GSK1278863**	b	4590-9310	19300-31700	40000-44600
	GSK2391220 (M2)	b	1.31-2.93	4.01-17.9	8.15-51.1
	GSK2506104 (M3)	NQ	0.491-1.39	0.407-3.32	0.553-7.29
	GSK2531401 (M13)	NQ	NQ-0.595	0.203-1.21	NQ-4.05
PND 10 (F_1) females	**GSK1278863**	NQ	3570-7830	18600-38500	36500-59500
	GSK2391220 (M2)	NQ	0.754-6.07	3.51-12.7	7.38-24.1
	GSK2506104 (M3)	NQ	0.324-2.94	0.480-1.81	0.434-4.59
	GSK2531401 (M13)	NQ	NQ-1.30	NQ-1.17	NQ-2.63

Key: PND = Postnatal Day NQ = Not Quantifiable (<0.200 ng/ml)

a. GSK1278863 was administered once daily to F0 females by oral gavage, followed by twice daily subcutaneous injection (approximately 6 hours apart) administration of human metabolite cocktail [GSK2391220 (M2), GSK2506104 (M3), and GSK2531401 (M13)] at nominal doses of 2.5, 3.2, and 1.8 mg/kg/day.

b. 3 male control pups have quantifiable concentrations of GSK1278863 (212 – 2510 ng/mL) at 3 hrs post the first maternal dose on PND 10. One of these pups (3401/2) also had 0.903 ng/mL of M2 at 3 hrs. These maternal animals did not have quantifiable concentrations of GSK1278863 as measured on LD 21, and the values reported were lower than values observed in 0.8 mg/kg/day GSK1278863 + metabolites pups, therefore were not considered to have affected interpretation of these results.

9. PHARMACOKINETICS: OTHER DISTRIBUTION STUDIES

Table 9.1 Pharmacokinetics: Passive and absorptive membrane permeability

Test Article: GSK1278863

Location in CTD: m4.2.2.3

Report No.: CD2008/00421 **Study No.:** 08DMM021

Target Entity, Test System and Method: In vitro (polarized Madin-Darby canine kidney MDCKII-MDR1 cell line heterologously expressing human P-gp)
Passive and absorptive membrane permeability of [^{14}C]GSK1278863 and [^{3}H]amprenavir (positive control) at various pH in MDCKII-MDR1 cell monolayers in the presence of GF120918 (P-gp inhibitor) (A→B direction only) were assessed using radio-HPLC analysis.

Results:

Compound	Permeability	pH	Transport medium		Px (Permeability coefficient) (nm/s)	Permeability Class
			Apical well	Basolateral well		
3 μM [^{14}C]GSK1278863	Passive	7.4	DMEM	DMEM	21 ± 2	Moderate
	Absorptive	7.4	FaSSIF	DMEM + 1% HSA	191[a]	High
	Absorptive	5.5	FaSSIF	DMEM + 1% HSA	325 ± 6.9	High
3 μM [^{3}H]amprenavir	Passive	7.4	DMEM	DMEM	171 ± 13.6	High

Data are the mean ± SD from three monolayers unless noted
a: Results for absorptive permeability at pH 7.4 are the mean of two wells due to sampling error of one well.
The pH of DMEM in all investigations is pH 7.4
Permeability classification was as low (<10 nm/s), moderate (10 - 100 nm/s) or high (>100 nm/s).
FaSSIF: Fasted State Simulated Intestinal Fluid. DMEM: Dulbecco's Modified Eagle Medium. HSA: Human serum albumin

Table 9.2 Microdialysis

Test Article: GSK1278863

Location in CTD: m4.2.2.3

Report No.: 2015N228833 **Study No.:** 0588-14228

Target Entity, Test System and Method: Serial 30-minute microdialysate samples were collected in a Culex fraction collector from a BASi microdialysis probe immersed in Ringers Solution (0.2% Tween 20) or one of two test solution concentrations (2.5 and 25 ng/mL) of GSK1278863. Dialysate samples were collected for 30 minutes from test solutions at a flow rate of 1 µL/minute.

A recovery value could not be calculated from the low concentration test solution samples due to all values being below the limit of quantitation.

Results:

Actual Conc	Result	Comments
2.16	2.23	
2.16	2.32	
5.41	5.73	
5.41	5.35	
21.6	22.3	
21.6	22.2	
90.1	87.9	
90.1	89.5	
541	551	
541	559	

Actual Conc	Result	Comments
0.721	0.706	
0.721	0.737	
1.44	1.52	
1.44	1.33	
2.88	2.86	
2.88	3.01	
10.8	10.5	
10.8	10.8	
43.2	41.3	
43.2	44.5	
180	186	
180	182	
586	596	
586	593	
721	724	
721	690	

Result	Comments
NQ	<LLQ
NQ	<LLQ
NQ	<LLQ

Time(min)	Result	Comments	Extrapolated	CRO ID	PPD ID
0-30	NQ	<LLQ	0.0431		AHXN101-1
31-60	NQ	<LLQ			AHXN102-1
121-150	NQ	<LLQ	0.222		AHXN105-1
151-180	NQ	<LLQ, Label fell off - sample could be Subject 11 or 13.	0.375		AHXN106-1
181-210	NQ	<LLQ	0.319		AHXN107-1
271-300	2.29				AHXN110-1
301-330	2.14	Label fell off - sample could be Subject 6 or 13.			AHXN111-1
331-360	2.49				AHXN112-1
361-390	1.54	Label fell off - sample could be Subject 6 or 11.			AHXN113-1
391-420	NQ	<LLQ	0.355		AHXN114-1
NA	2.18				AHXN115-1
NA	2.32				AHXN116-1
NA	21.4				AHXN117-1
NA	20.9				AHXN118-1

Table 9.3 Pharmacokinetics: P-gp substrate

Test Article: GSK1278863

Location in CTD: m4.2.2.3

Report No.: UH2008/00018 **Study No.:** 06MCD1239

Target Entity, Test System and Method: In vitro (human MDR1-transfected Madin-Darby canine kidney type II cell monolayers)
MDCKII-MDR1 cell monolayers were pre-incubated in transport buffer with or without GF120918 (P-gp inhibitor) at 37°C for 30 minutes. Test compound working solutions was then added to wells of apical ([A→B] transport) and basolateral ([B→A] transport) donor compartments. The cells were incubated at 37°C for 90 min. Samples were analyzed by LC/MS/MS analysis. Amprenavir was used as the positive control.

Results:

Test Compound (5 μM)	Apical Efflux Ratio		Substrate for P-gp	Avg A>B Papp (nm/sec) in presence of 2 μM GF120918
	No inhibitor	+ GF120918 (2 μM)		
GSK1278863	0.6	0.4	No	20 ± 3.3
Amprenavir	32	1.1	Yes	504 ± 18

Mean ± SD (n=3)

Table 9.4 Pharmacokinetics: Murine Bcrp substrate

Test Article: GSK1278863

Location in CTD: m4.2.2.3

Report No.: UH2008/00018 **Study No.:** 07CDUP1017

Target Entity, Test System and Method: In vitro (murine Bcrp1-transfected Madin-Darby canine kidney type II cell monolayers)
MDCKII-Bcrp1 cell monolayers were pre-incubated in transport buffer with or without GF120918 (BCRP inhibitor) at 37°C for 30 minutes. Test compound working solutions was then added to wells of apical ([A→B] transport) and basolateral ([B→A] transport) donor compartments. The cells were incubated at 37°C for 90 min. Samples were analyzed by LC/MS/MS analysis. Ofloxacin was used as the positive control.

Results:

Test Compound (3 μM)	Apical Efflux Ratio		Substrate for Bcrp	Avg A>B Papp (nm/sec) in presence of 5 μM GF120918
	No inhibitor	+ GF120918 (5 μM)		
GSK1278863	60	5.5	Yes	79 ± 10
Ofloxacin	30	1.3	Yes	76 ± 10

Mean ± SD (n=3)

Table 9.5 Pharmacokinetics: Human BCRP substrate

Test Article: GSK1278863

Location in CTD: m4.2.2.3

Report No.: 2015N237800 **Study No.:** 15DMM007

Target Entity, Test System and Method: In vitro (The polarized Madin-Darby canine kidney cell line heterologously expressing human BCRP) MDCKII-BCRP cell monolayers were pre-incubated (at 37ºC) in transport medium, with and without 2 μM GF120918 (BCRP inhibitor), for 30 minutes prior to addition of test article. For [A→B] directional transport, donor working solution with test article or [14C]cimetidine (positive control) was then added to the A (apical) compartment and receiver working solution to the B (basolateral) compartment. For [B→A] directional transport, donor working solution with test article or [^{14}C]cimetidine was added to the B compartment and receiver working solution to the A compartment. The cells were incubated at 37ºC for 90 minutes. The amount of [^{14}C]cimetidine was determined by LSC. The amount of GSK1278863 was determined by LC/MS/MS analysis.

Results:

Compound	Rate A→B (nmoles/h/cm^2)	Rate B→A (nmoles/h/cm^2)	Apical Efflux Ratio	BCRP Substrate	A→B Mass Balance (%)	B→A Mass Balance (%)
3 μM GSK1278863	0.032 ± 0.0018	0.14 ± 0.0073	4.4	Y	93 ± 3.1	96 ± 6.8
3 μM GSK1278863 + 2 μM GF120918	0.042[a] ± 0.0024	0.027 ± 0.0036	0.65	-	85[a] ± 3.3	86 ± 1.6
3 μM [^{14}C]cimetidine	0.040 ± 0.0056	0.17 ± 0.0028	4.2	Y	99 ± 0.81	99 ± 0.19
3 μM [^{14}C]cimetidine + 2 μM GF120918	0.051 ± 0.0097	0.030 ± 0.0064	0.59	-	100 ± 1.8	100 ± 0.36

Data presented are the mean ± SD of 4 replicates, unless otherwise indicated

a: Mean of 3 replicates, one replicate failed mass balance criteria.

All donor compartments contained Lucifer yellow CH to determine monolayer integrity (pass criterion P7.4 ≤50 nm/s) and wells designated for BCRP inhibition contained 2 μM GF120918 in both donor and receiver compartments. [^{14}C]cimetidine was used as positive control (criterion for assay acceptability: apical efflux ratio for cimetidine ≥2 collapsing to approximately 1 in the presence of inhibitor). A compound is classified as a BCRP substrate if the apical efflux ratio in the absence of inhibitor GF120918 is ≥2 and this efflux collapses to ~1 in the presence of inhibitor, GF120918. Efflux ratios ≥2 in the presence of inhibitor GF120918 indicate incomplete inhibition of directional transport of a compound.

Mass balance within the range 80 - 120% is considered acceptable.

Table 9.6 Pharmacokinetics: Hepatic uptake

Test Article: GSK1278863

Location in CTD: mm4.2.2.3

Report No.: 2015N232657 **Study No.:** 14DMM039

Target Entity, Test System and Method: In vitro (human hepatocytes)

Cell monolayers were pre-incubated at 37ºC with buffer alone or buffer containing transporter inhibitor cocktail. After pre-incubation, cells were incubated in triplicate for the appropriate amount of time with GSK1278863 at each concentration. Concentrations of GSK1278863 were determined using LC-MS/MS.

Results:

Time (min)	Donor 1 (0.5 μM[a]) (pmol/million cells)		Donor 2 (0.3 μM[a]) (pmol/million cells)		Donor 3 (0.3 μM[a]) (pmol/million cells)	
	No inhibitor	+ inhibitor cocktail[b]	No inhibitor	+ inhibitor cocktail[b]	No inhibitor	+ inhibitor cocktail[b]
0.5	2.2 ± 0.17	3.1 ± 0.55	0.98 ± 0.070	1.0 ± 0.013	1.4 ± 0.033	1.2 ± 0.25
1	7.6 ± 0.73	4.6 ± 0.63	1.9 ± 0.069	1.7 ± 0.046	1.9 ± 0.084	2.0 ± 0.13
2	7.9 ± 0.46	6.5 ± 0.73	3.0 ± 0.29	2.8 ± 0.083	2.8 ± 0.13	3.7 ± 0.17
5	15 ± 1.1	17 ± 2.3	5.2 ± 0.34	4.9 ± 0.19	4.9 ± 0.061	6.5 ± 0.59
15	37 ± 2.5	39 ± 5.4	15 ± 0.28	10 ± 0.67	14 ± 0.68	13 ± 0.66
25	48 ± 4.1	56 ± 8.8	21 ± 0.95	15 ± 1.6	20 ± 1.6	17 ± 0.79
40	52 ± 3.5	78 ± 8.4	22 ± 0.96	20 ± 0.29	29 ± 2.2	20 ± 0.49
0.02 μM [^{3}H]EG (2 min)	0.37 ± 0.072	0.047 ± 0.0037	0.13 ± 0.014	0.037 ± 0.0037	0.23 ± 0.017	0.049 ± 0.0059

Each value represents the mean ± SD of three samples, mean of two samples, or single value.

a: Concentrations listed represent actual measured concentrations.

b: Inhibitor cocktail consists of montelukast, rifamycin, cyclosporine A, and imipramine.

Table 9.7 Pharmacokinetics: OATP1B1 and OATP1B3 substrate of Daprodustat and Metabolites of Daprodustat

Test Article: GSK1278863, GSK2391220, GSK2506104, GSK2487818, GSK2506102, GSK2531398 and GSK2531401

Location in CTD: mm4.2.2.3

Report No.: 2015N255319 **Study No.:** XS-0674

Target Entity, Test System and Method: In vitro (HEK293 cells expressing OATP1B1 or OATP1B3)

Cells were pre-incubated at 37ºC with buffer. After pre-incubation, cells were incubated at 37ºC in triplicate for the appropriate amount of time with GSK1278863 at the appropriate concentrations. The concentrations of each test article were determined using LC-MS/MS.

Results:

Substrate	Concentration (uM)	Incubation time (min)	Cleared volume (µL/mg protein)			S/N ratio	
			Control	OATP1B1	OATP1B3	OATP1B1	OATP1B3
GSK1278863	0.1	1	29.9 ± 5.1	42.4 ± 6.3	39.8 ± 4.8	1.4	1.3
		2	54.1 ± 3.1	164 ± 27	90.3 ± 5.6	3.0	1.7
		5	70.5 ± 4.3	122 ± 87	132 ± 10	1.7	1.9
	1	1	30.3 ± 3.4	52.5 ± 4.8	46.6 ± 6.0	1.7	1.5
		2	58.7 ± 5.1	176 ± 9	116 ± 7	3.0	2.0
		5	88.2 ± 0.2	239 ± 4	181 ± 7	2.7	2.1
	10	1	11.6 ± 1.5	16.6 ± 1.7	15.4 ± 1.6	1.4	1.3
		2	20.6 ± 3.9	35.2 ± 1.1	28.6 ± 2.4	1.7	1.4
		5	40.2 ± 2.8	66.7 ± 8.4	56.4 ± 2.8	1.7	1.4
	100	1	10.5 ± 1.0	10.1 ± 0.1	9.00 ± 1.42	1.0	0.9
		2	20.5 ± 1.5	22.3 ± 0.8	21.1 ± 0.9	1.1	1.0
		5	43.1 ± 6.4	38.8 ± 6.8	40.6 ± 2.5	0.9	0.9

Each value represents the mean ± SD of three samples, mean of two samples, or single value.

NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OATP1B1 and OATP1B3 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

Substrate	Concentration (uM)	Incubationtime (min)	Cleared volume (µL/mg protein)			S/N ratio	
			Control	OATP1B1	OATP1B3	OATP1B1	OATP1B3
GSK2391220 (M2)	0.01	1	NC	NC	NC	NC	NC
		2	NC	NC	NC	NC	NC
		5	NC	NC	NC	NC	NC
	0.1	1	NC	NC	NC	NC	NC
		2	NC	NC	NC	NC	NC
		5	NC	NC	NC	NC	NC
	1	1	NC	0.152 (n=1)	NC	NC	NC
		2	NC	0.139 (n=1)	0.190 (n=2)	NC	NC
		5	NC	0.182 ± 0.039	0.0669 (n=2)	NC	NC
	10	1	0.0455 ± 0.0211	0.0909 ± 0.0191	0.0916 ± 0.0026	2.0	2.0
		2	0.0621 ± 0.0123	0.0905 ± 0.0156	0.156 ± 0.038	1.5	2.5
		5	0.0646 ± 0.0055	0.171 ± 0.037	0.156 ± 0.009	2.6	2.4
GSK2506104 (M3)	0.01	1	NC	NC	NC	NC	NC
		2	NC	NC	NC	NC	NC
		5	NC	NC	NC	NC	NC
	0.1	1	NC	NC	NC	NC	NC
		2	NC	NC	NC	NC	NC
		5	NC	NC	NC	NC	NC
	1	1	0.128 (n=1)	0.142 (n=2)	NC	1.1	NC
		2	0.154 (n=2)	0.195 ± 0.053	0.187 ± 0.009	1.3	1.2
		5	NC	0.303 ± 0.011	0.279 ± 0.060	NC	NC
	10	1	0.0571 ± 0.0178	0.112 ± 0.015	0.132 ± 0.060	2.0	2.3
		2	0.107 ± 0.025	0.118 ± 0.009	0.157 ± 0.035	1.1	1.5
		5	0.0984 ± 0.0341	0.238 ± 0.004	0.201 ± 0.017	2.4	2.0

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OATP1B1 and OATP1B3 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

Substrate	Concentration (uM)	Incubationtime (min)	Cleared volume (µL/mg protein)			S/N ratio	
			Control	OATP1B1	OATP1B3	OATP1B1	OATP1B3
GSK2531401 (M13)	1	2	NC	0.199 ± 0.026	0.159 (n=2)	NC	NC
		5	0.159 ± 0.010	0.230 ± 0.034	0.300 ± 0.076	1.4	1.9
		15	0.166 (n=1)	0.209 ± 0.050	0.303 ± 0.016	1.3	1.8
	3	2	0.0756 ± 0.0178	0.109 ± 0.030	0.0842 ± 0.0172	1.4	1.1
		5	0.113 ± 0.008	0.186 ± 0.031	0.232 ± 0.059	1.6	2.1
		15	0.836 ± 0.0033	0.164 ± 0.028	0.312 ± 0.031	2.0	3.7
	10	2	0.0459 ± 0.0095	0.0767 ± 0.0192	0.0802 ± 0.0133	1.7	1.7
		5	0.100 ± 0.003	0.167 ± 0.013	0.209 ± 0.009	1.7	2.1
		15	0.0645 ± 0.0120	0.158 ± 0.005	0.235 ± 0.026	2.4	3.6
	30	2	0.0424 ± 0.0063	0.0848 ± 0.0227	0.0942 ± 0.0109	2.0	2.2
		5	0.0951 ± 0.0087	0.176 ± 0.040	0.245 ± 0.102	1.9	2.6
		15	0.0534 ± 0.0057	0.147 ± 0.004	0.286 ± 0.021	2.8	5.4
GSK2487818 (M4)	1	2	NC	0.327 ± 0.060	0.271 ± 0.024	NC	NC
		5	0.159 (n=1)	0.433 ± 0.002	0.416 ± 0.073	2.7	2.6
		15	0.151 (n=1)	0.447 ± 0.029	0.659 ± 0.083	3.0	4.4
	3	2	NC	0.269 ± 0.011	0.291 ± 0.015	NC	NC
		5	0.141 (n=1)	0.519 ± 0.046	0.485 ± 0.009	3.7	3.4
		15	0.0763 (n=2)	0.524 ± 0.040	0.748 ± 0.024	6.9	9.8
	10	2	0.0520 ± 0.0181	0.250 ± 0.020	0.308 ± 0.027	4.8	5.9
		5	0.0906 ± 0.0346	0.486 ± 0.025	0.546 ± 0.042	5.4	6.0
		15	0.0625 ± 0.0119	0.504 ± 0.018	0.804 ± 0.028	8.1	12.9
	30	2	0.0549 ± 0.0158	0.255 ± 0.024	0.347 ± 0.019	4.6	6.3
		5	0.0998 ± 0.0212	0.409 ± 0.007	0.632 ± 0.033	4.1	6.3
		15	0.0633 ± 0.0141	0.435 ± 0.040	0.842 ± 0.034	6.9	13.3

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OATP1B1 and OATP1B3 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

Substrate	Concentration (uM)	Incubationtime (min)	Cleared volume (µL/mg protein)			S/N ratio	
			Control	OATP1B1	OATP1B3	OATP1B1	OATP1B3
GSK2506102 (M5)	1	2	NC	0.215 ± 0.020	0.337 ± 0.105	NC	NC
		5	0.158 (n=2)	0.186 ± 0.018	0.260 ± 0.018	1.2	1.6
		15	NC	0.251 ± 0.026	0.386 ± 0.027	NC	NC
	3	2	0.0913 ± 0.0062	0.133 ± 0.019	0.223 ± 0.044	1.5	2.4
		5	0.102 ± 0.016	0.172 ± 0.006	0.244 ± 0.016	1.7	2.4
		15	0.0609 (n=2)	0.230 ± 0.006	0.387 ± 0.008	3.8	6.4
	10	2	0.0784 ± 0.0279	0.142 ± 0.039	0.211 ± 0.016	1.8	2.7
		5	0.0853 ± 0.0099	0.161 ± 0.006	0.236 ± 0.009	1.9	2.8
		15	0.0385 ± 0.0052	0.211 ± 0.002	0.370 ± 0.015	5.5	9.6
	30	2	0.0840 ± 0.0356	0.113 ± 0.009	0.267 ± 0.126	1.3	3.2
		5	0.0636 ± 0.0156	0.136 ± 0.008	0.203 ± 0.016	2.1	3.2
		15	0.0357 ± 0.0002	0.203 ± 0.018	0.343 ± 0.009	5.7	9.6
GSK2531398 (M6)	1	2	0.196 (n=1)	0.750 ± 0.066	0.557 ± 0.063	3.8	2.8
		5	0.343 ± 0.046	0.561 ± 0.025	0.632 ± 0.051	1.6	1.8
		15	0.224 (n=1)	0.632 ± 0.013	0.722 ± 0.068	2.8	3.2
	3	2	0.120 ± 0.017	0.667 ± 0.026	0.451 ± 0.018	5.6	3.8
		5	0.311 ± 0.133	0.566 ± 0.029	0.672 ± 0.053	1.8	2.2
		15	0.332 ± 0.062	0.643 ± 0.044	0.803 ± 0.089	1.9	2.4
	10	2	0.0747 (n=2)	0.537 ± 0.024	0.349 ± 0.025	7.2	4.7
		5	0.227 ± 0.052	0.491 ± 0.005	0.615 ± 0.038	2.2	2.7
		15	0.291 ± 0.105	0.552 ± 0.018	0.712 ± 0.011	1.9	2.4
	30	2	0.0841 ± 0.0082	0.555 ± 0.045	0.486 ± 0.130	6.6	5.8
		5	0.246 ± 0.036	0.576 ± 0.115	0.621 ± 0.018	2.3	2.5
		15	0.134 ± 0.027	0.666 ± 0.128	0.738 ± 0.049	5.0	5.5

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Table 9.8 Pharmacokinetics: Hepatic uptake of GSK2487818 Metabolite of Daprodustat

Test Article: GSK2487818

Location in CTD: m4.2.2.3

Report No.: 2015N259558 **Study No.:** 15DMM035

Target Entity, Test System and Method: Plated pooled cryopreserved human hepatocytes

Cell monolayers were pre-incubated at 37ºC with buffer alone or buffer containing a cocktail of OATP1B1, OATP1B3, OATP2B1 and OCT1 transporter inhibitors. After pre-incubation, cells were incubated in triplicate for the appropriate amount of time with GSK2487818 at each concentration

Results:

Time (min)	2 µM GSK2487818 (pmol/well)		15 µM GSK2487818 run 1 (pmol/well)		Time (min)	15 µM GSK2487818 run 2 (pmol/well)	
	No inhibitor	+ inhibitor cocktail[b]	No inhibitor	+ inhibitor cocktail[b]		No inhibitor	+ inhibitor cocktail[b]
0.5	0.26 ± 0.017	0.28 ± 0.048	2.2 ± 0.66	1.7 ± 0.30	0.5	2.5 ± 0.43	1.2 ± 0.42
1	0.33 ± 0.074	0.31 ± 0.036	3.3 ± 0.79	1.5 ± 0.23	1	3.3 ± 0.40	2.4 ± 1.0
1.5	0.43 ± 0.059	0.52 ± 0.25	4.3± 0.65	2.7 ± 0.42	2	2.8 ± 0.53	3.5 ± 0.50
2	0.30 ± 0.040	0.40 ± 0.066	2.6 ± 0.12	3.3 ± 0.54	5	3.9 ± 0.50	4.3 ± 0.63
5	0.69 ± 0.15	0.25 ± 0.064	3.8 ± 0.60	4.6 ± 0.34	10	3.7 ± 0.23	3.8 ± 0.60
15	0.37 ± 0.067	0.60 ± 0.11	5.9 ± 1.4	7.7 ± 0.27	15	3.4 ± 0.37	4.9[a]
30	0.91 ± 0.077	0.45 ± 0.090	-	-	-	-	-
0.02 µM [^{3}H]EG (3 min)	0.088± 0.0028	0.021 ± 0.0012	0.099 ± 0.0069	0.025 ± 0.00069	0.02 µM [^{3}H]EG (3 min)	0.13 ± 0.024	0.034 ± 0.0089
S:N[c]	4.2		4.0		S:N[c]	3.8	

Each value represents the mean ± SD of three samples

a: n=2, SD was not calculated.

b: Inhibitor cocktail consists of 100 µM rifamycin and 100 µM imipramine.

c: Signal:Noise (S:N) ratio was calculated using transport of substrate in absence of inhibitors over transport of substrate in presence of inhibitors

Table 9.9 Pharmacokinetics: OAT1, OAT3, OCT2 substrate of Daprodustat and Metabolites of Daprodustat

Test Article: GSK1278863, GSK2391220, GSK2506104, GSK2487818, GSK2506102, GSK2531398 and GSK2531401

Location in CTD: m4.2.2.3

Report No.: 2015N249257 **Study No.:** XS-0662

Target Entity, Test System and Method: In vitro (S_2 cells expressing OAT1 or OAT3, HEK293 cells expressing OCT2)
Each cell was pre-incubated at 37ºC. After pre-incubation, cells were incubated in triplicate for the appropriate amount of time with each test article at each concentration. The concentrations of each test article were determined using LC-MS/MS. Radioactivity of each positive control substrate was counted using an LSC.

Results:

GSK1278863

Conc. (μM)	Incubation time (min)	OAT1			OAT3			OCT2		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells		Control cells	Expressing cells	
0.1	1	20.7 ± 1.6	28.4 ± 2.6	1.4	20.7 ± 1.6	28.0 ± 2.2	1.4	5.95 ± 0.84	8.78 ± 0.83	1.5
	2	29.8 ± 1.1	35.4 ± 2.1	1.2	29.8 ± 1.1	41.4 ± 2.6	1.4	9.51 ± 0.60	10.4 ± 1.8	1.1
	5	43.7 ± 4.2	50.8 ± 6.9	1.2	43.7 ± 4.2	74.3 ± 2.5	1.7	5.51 ± 0.32	6.92 ± 0.81	1.3
1	1	16.2 ± 0.8	19.7 ± 0.7	1.2	16.2 ± 0.8	22.7 ± 3.6	1.4	5.18 ± 1.29	5.96 ± 1.06	1.2
	2	25.7 ± 1.4	29.9 ± 1.9	1.2	25.7 ± 1.4	34.6 ± 1.1	1.3	8.20 ± 1.66	9.28 ± 1.78	1.1
	5	36.6 ± 0.7	49.0 ± 8.6	1.3	36.6 ± 0.7	72.7 ± 3.9	2.0	6.62 ± 0.63	6.60 ± 0.73	1.0
10	1	24.9 ± 1.4	22.0 ± 2.2	0.9	24.9 ± 1.4	29.6 ± 4.0	1.2	12.9 ± 3.1	9.20 ± 0.62	0.7
	2	40.5 ± 5.0	39.0 ± 3.7	1.0	40.5 ± 5.0	38.2 ± 4.2	0.9	14.7 ± 2.6	12.8 ± 4.3	0.9
	5	54.1 ± 8.1	59.9 ± 2.1	1.1	54.1 ± 8.1	72.2 ± 1.2	1.3	17.4 ± 1.9	19.9 ± 0.9	1.1
100	1	16.1 ± 1.7	13.8 ± 1.0	0.9	16.1 ± 1.7	15.4 ± 1.4	1.0	6.79 ± 0.48	8.69 ± 0.50	1.3
	2	24.3 ± 0.7	33.1 ± 2.8	1.4	24.3 ± 0.7	29.7 ± 3.1	1.2	17.4 ± 0.5	16.1 ± 1.9	0.9
	5	43.8 ± 1.7	42.2 ± 4.2	1.0	43.8 ± 1.7	53.1 ± 2.3	1.2	25.8 ± 0.9	26.0 ± 2.9	1.0

Each value represents the mean ± SD of three samples.

Pharmacokinetics: OAT1, OAT3, OCT2 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2391220 (M2)

Conc. (μM)*	Incubation time (min)	OAT1			OAT3			OCT2		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells		Control cells	Expressing cells	
0.01	1	NC	NC	NC	NC	NC	NC	NC	NC	NC
	2	NC	NC	NC	NC	NC	NC	NC	NC	NC
	5	NC	NC	NC	NC	NC	NC	NC	NC	NC
0.1	1	NC	NC	NC	NC	NC	NC	NC	NC	NC
	2	NC	NC	NC	NC	NC	NC	NC	NC	NC
	5	NC	NC	NC	NC	NC	NC	NC	NC	NC
1	1	NC	0.375 (n=1)	NC	NC	NC	NC	0.400 (n=1)	0.267 ± 0.174	0.7
	2	NC	NC	NC	NC	NC	NC	NC	0.186 (n=1)	NC
	5	0.300 (n=1)	NC	NC	0.300 (n=1)	0.450 ± 0.078	1.5	NC	NC	NC
10	1	0.0975 ± 0.0117	0.217 ± 0.084	2.2	0.0975 ± 0.0117	0.242 ± 0.121	2.5	0.113 ± 0.053	0.126 ± 0.021	1.1
	2	0.0920 ± 0.0082	0.217 ± 0.018	2.4	0.0920 ± 0.0082	0.254 ± 0.129	2.8	0.0713 ± 0.0190	0.122 ± 0.015	1.7
	5	0.191 ± 0.022	0.190 ± 0.021	1.0	0.191 ± 0.022	0.315 ± 0.022	1.6	0.0870 ± 0.0155	0.0741 ± 0.0120	0.9

Each value represents the mean ± SD of three samples or single value.

NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OAT1, OAT3, OCT2 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2506104 (M3)

Conc. (μM)	Incubation time (min)	OAT1			OAT3			OCT2		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	0.285 (n=1)	NC	NC	0.285 (n=1)	0.392 ± 0.039	1.4	NC	0.218 (n=1)	NC
	5	0.377 ± 0.092	0.292 ± 0.065	0.8	0.377 ± 0.092	0.745 ± 0.071	2.0	NC	NC	NC
	15	0.317 ± 0.051	0.318 ± 0.036	1.0	0.317 ± 0.051	1.25 ± 0.09	3.9	NC	NC	NC
3	2	0.217 ± 0.034	0.164 ± 0.013	0.8	0.217 ± 0.034	0.418 ± 0.036	1.9	0.0834 (n=1)	0.0830 (n=2)	1.0
	5	0.332 ± 0.020	0.298 ± 0.078	0.9	0.332 ± 0.020	0.684 ± 0.042	2.1	0.0913 (n=2)	0.0812 ± 0.0248	0.9
	15	0.275 ± 0.051	0.338 ± 0.054	1.2	0.275 ± 0.051	1.24 ± 0.09	4.5	0.121 ± 0.074	0.0884 ± 0.0414	0.7
10	2	0.227 ± 0.010	0.139 ± 0.020	0.6	0.227 ± 0.010	0.332 ± 0.059	1.5	0.0555 ± 0.0067	0.0505 ± 0.0012	0.9
	5	0.364 ± 0.035	0.271 ± 0.084	0.7	0.364 ± 0.035	0.663 ± 0.038	1.8	0.0731 ± 0.0053	0.0992 ± 0.0208	1.4
	15	0.257 ± 0.025	0.307 ± 0.044	1.2	0.257 ± 0.025	1.10 ± 0.08	4.3	0.0840 ± 0.0158	0.0745 ± 0.0196	0.9
30	2	0.177 ± 0.048	0.142 ± 0.037	0.8	0.177 ± 0.048	0.369 ± 0.089	2.1	0.0390 ± 0.0075	0.0412 ± 0.0010	1.1
	5	0.334 ± 0.095	0.282 ± 0.078	0.8	0.334 ± 0.095	0.670 ± 0.034	2.0	0.0725 ± 0.0238	0.117 ± 0.050	1.6
	15	0.250 ± 0.025	0.271 ± 0.022	1.1	0.250 ± 0.025	0.994 ± 0.035	4.0	0.0881 ± 0.0270	0.0691 ± 0.0257	0.8

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OAT1, OAT3, OCT2 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2487818 (M4)

Conc. (μM)	Incubation time (min)	OAT1			OAT3			OCT2		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	NC	NC	NC	NC	NC	NC	0.241 (n=1)	0.255 (n=2)	1.1
	5	NC	NC	NC	NC	0.234 (n=1)	NC	0.191 (n=2)	0.239 ± 0.028	1.3
	15	NC	NC	NC	NC	0.251 (n=2)	NC	0.232 (n=2)	0.177 (n=2)	0.8
3	2	0.187 (n=2)	0.117 ± 0.010	0.6	0.187 (n=2)	0.142 ± 0.084	0.8	0.159 ± 0.052	0.116 ± 0.026	0.7
	5	0.210 ± 0.112	0.0854 (n=2)	0.4	0.210 ± 0.112	0.211 ± 0.020	1.0	0.212 ± 0.041	0.233 ± 0.027	1.1
	15	0.223 ± 0.082	0.158 ± 0.025	0.7	0.223 ± 0.082	0.233 ± 0.049	1.0	0.158 ± 0.031	0.169 ± 0.052	1.1
10	2	0.179 ± 0.034	0.121 ± 0.008	0.7	0.179 ± 0.034	0.141 ± 0.036	0.8	0.112 ± 0.036	0.0914 ± 0.0062	0.8
	5	0.256 ± 0.024	0.0850 ± 0.0113	0.3	0.256 ± 0.024	0.178 ± 0.025	0.7	0.141 ± 0.060	0.175 ± 0.056	1.2
	15	0.242 ± 0.029	0.169 ± 0.007	0.7	0.242 ± 0.029	0.261 ± 0.029	1.1	0.155 ± 0.038	0.135 ± 0.042	0.9
30	2	0.268 ± 0.128	0.126 ± 0.013	0.5	0.268 ± 0.128	0.125 ± 0.021	0.5	0.0924 ± 0.0319	0.0765 ± 0.0136	0.8
	5	0.300 ± 0.072	0.120 ± 0.017	0.4	0.300 ± 0.072	0.208 ± 0.030	0.7	0.145 ± 0.045	0.216 ± 0.074	1.5
	15	0.213 ± 0.047	0.162 ± 0.024	0.8	0.213 ± 0.047	0.258 ± 0.013	1.2	0.121 ± 0.024	0.140 ± 0.043	1.2

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OAT1, OAT3, OCT2 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2506102 (M5)

GSK2506102 (M5)

Conc. (μM)	Incubation time (min)	OAT1			OAT3			OCT2		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	NC	0.226 (n=1)	NC	NC	NC	NC	NC	NC	NC
	5	NC	NC	NC	NC	0.239 ± 0.024	NC	NC	0.266 (n=1)	NC
	15	NC	NC	NC	NC	0.272 ± 0.068	NC	NC	NC	NC
3	2	0.106 ± 0.011	0.142 ± 0.029	1.3	0.106 ± 0.011	0.128 ± 0.017	1.2	0.101 ± 0.009	0.0947 (n=2)	0.9
	5	0.196 ± 0.027	0.0830 (n=2)	0.4	0.196 ± 0.027	0.186 ± 0.025	0.9	0.0717 ± 0.0123	0.206 ± 0.034	2.9
	15	0.175 ± 0.038	0.150 ± 0.022	0.9	0.175 ± 0.038	0.287 ± 0.041	1.6	0.0663 (n=1)	0.124 ± 0.015	1.9
10	2	0.131 ± 0.014	0.161 ± 0.040	1.2	0.131 ± 0.014	0.129 ± 0.030	1.0	0.0863 ± 0.0233	0.0745 ± 0.0109	0.9
	5	0.190 ± 0.044	0.0806 ± 0.0166	0.4	0.190 ± 0.044	0.155 ± 0.010	0.8	0.0671 ± 0.0156	0.133 ± 0.016	2.0
	15	0.227 ± 0.027	0.129 ± 0.023	0.6	0.227 ± 0.027	0.329 ± 0.061	1.4	0.0551 ± 0.0028	0.0831 ± 0.0049	1.5
30	2	0.116 ± 0.023	0.182 ± 0.013	1.6	0.116 ± 0.023	0.107 ± 0.012	0.9	0.0718 ± 0.0232	0.0449 ± 0.0116	0.6
	5	0.222 ± 0.101	0.0841 ± 0.0066	0.4	0.222 ± 0.101	0.144 ± 0.008	0.6	0.0660 ± 0.0150	0.104 ± 0.016	1.6
	15	0.192 ± 0.063	0.137 ± 0.023	0.7	0.192 ± 0.063	0.263 ± 0.010	1.4	0.0518 ± 0.0103	0.0815 ± 0.0020	1.6

Each value represents the mean ± SD of three samples, mean of two samples, or single value.

NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OAT1, OAT3, OCT2 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2531398 (M6)

Conc. (μM)	Incubation time (min)	OAT1			OAT3			OCT2		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	NC	0.398 (n=2)	NC	NC	NC	NC	0.166 (n=1)	NC	NC
	5	NC	NC	NC	NC	NC	NC	NC	0.200 (n=1)	NC
	15	NC	NC	NC	NC	NC	NC	NC	0.161 (n=1)	NC
3	2	0.115 ± 0.004	NC	NC	0.115 ± 0.004	NC	NC	0.125 ± 0.009	0.0704 ± 0.0184	0.6
	5	0.141 ± 0.029	NC	NC	0.141 ± 0.029	NC	NC	0.0874 ± 0.0180	0.165 ± 0.016	1.9
	15	0.162 ± 0.021	0.161 ± 0.019	1.0	0.162 ± 0.021	0.200 ± 0.045	1.2	0.127 ± 0.027	0.122 ± 0.032	1.0
10	2	0.111 ± 0.010	0.115 ± 0.011	1.0	0.111 ± 0.010	0.101 ± 0.007	0.9	0.133 ± 0.041	0.0608 ± 0.0096	0.5
	5	0.148 ± 0.018	0.0982 ± 0.0166	0.7	0.148 ± 0.018	0.102 ± 0.011	0.7	0.111 ± 0.012	0.144 ± 0.022	1.3
	15	0.187 ± 0.055	0.206 ± 0.043	1.1	0.187 ± 0.055	0.174 ± 0.019	0.9	0.204 ± 0.077	0.109 ± 0.008	0.5
30	2	0.127 ± 0.009	0.138 ± 0.025	1.1	0.127 ± 0.009	0.131 ± 0.023	1.0	0.116 ± 0.042	0.0645 ± 0.0078	0.6
	5	0.0894 ± 0.0046	0.106 ± 0.010	1.2	0.0894 ± 0.0046	0.122 ± 0.032	1.4	0.127 ± 0.020	0.107 ± 0.017	0.8
	15	0.182 ± 0.038	0.214 ± 0.046	1.2	0.182 ± 0.038	0.225 ± 0.031	1.2	0.162 ± 0.048	0.105 ± 0.030	0.6

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: OAT1, OAT3, OCT2 substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2531401 (M13)

Conc. (μM)	Incubation time (min)	OAT1			OAT3			OCT2		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	NC	0.255 (n=1)	NC	NC	NC	NC	NC	0.205 (n=2)	NC
	5	0.402 ± 0.096	0.272 (n=1)	0.7	0.402 ± 0.096	0.650 ± 0.143	1.6	NC	NC	NC
	15	0.357 (n=2)	0.300 (n=2)	0.8	0.357 (n=2)	1.29 ± 0.41	3.6	NC	0.152 (n=1)	NC
3	2	0.163 ± 0.060	0.165 ± 0.082	1.0	0.163 ± 0.060	0.191 (n=2)	1.2	0.0816 (n=2)	0.0872 (n=2)	1.1
	5	0.233 ± 0.052	0.295 ± 0.075	1.3	0.233 ± 0.052	0.713 ± 0.022	3.1	0.146 (n=2)	0.0898 (n=2)	0.6
	15	0.274 ± 0.022	0.385 ± 0.057	1.4	0.274 ± 0.022	1.42 ± 0.32	5.2	0.0973 ± 0.0211	0.135 (n=2)	1.4
10	2	0.226 ± 0.057	0.120 ± 0.028	0.5	0.226 ± 0.057	0.161 ± 0.087	0.7	0.0762 ± 0.0492	0.0689 ± 0.0322	0.9
	5	0.310 ± 0.085	0.290 ± 0.018	0.9	0.310 ± 0.085	0.633 ± 0.046	2.0	0.100 ± 0.059	0.0782 ± 0.0109	0.8
	15	0.393 ± 0.043	0.306 ± 0.033	0.8	0.393 ± 0.043	1.62 ± 0.58	4.1	0.139 ± 0.012	0.0866 ± 0.0133	0.6
30	2	0.171 ± 0.014	0.107 ± 0.019	0.6	0.171 ± 0.014	0.390 ± 0.268	2.3	0.0507 ± 0.0165	0.0447 ± 0.0105	0.9
	5	0.242 ± 0.042	0.313 ± 0.024	1.3	0.242 ± 0.042	0.617 ± 0.031	2.5	0.0978 ± 0.0496	0.0773 ± 0.0373	0.8
	15	0.293 ± 0.018	0.304 ± 0.041	1.0	0.293 ± 0.018	1.54 ± 0.25	5.3	0.104 ± 0.011	0.0898 ± 0.0236	0.9

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Table 9.10 Pharmacokinetics: MATE1 and MATE2-K substrate of Daprodustat and Metabolites of Daprodustat

Test Article: GSK1278863, GSK2391220, GSK2506104, GSK2487818, GSK2506102, GSK2531398 and GSK2531401

Location in CTD: m4.2.2.3

Report No.: 2015N249257 **Study No.:** XS-0662

Target Entity, Test System and Method: In vitro (HEK293 cells expressing MATE1 or MATE2-K)

Each cell was pre-incubated at 37ºC. After pre-incubation, cells were incubated in triplicate for the appropriate amount of time with each test article at each concentration. The concentrations of each test article were determined using LC-MS/MS. Radioactivity of each positive control substrate was counted using an LSC.

Results:

GSK1278863

Conc. (μM)	Incubation time (min)	MATE1			MATE2-K		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells	
0.1	1	1.90 ± 0.17	1.88 (n=2)	1.0	1.52 ± 0.19	1.83 ± 0.25	1.2
	2	2.01 ± 0.27	2.61 ± 0.10	1.3	2.14 ± 0.09	2.68 ± 0.29	1.3
	5	3.94 ± 0.34	5.43 ± 1.46	1.4	3.65 ± 0.76	4.13 ± 0.46	1.1
1	1	1.31 ± 0.12	1.91 ± 0.13	1.5	1.55 ± 0.20	2.05 ± 0.11	1.3
	2	2.04 ± 0.38	2.14 ± 0.25	1.0	2.32 ± 0.55	2.37 ± 0.37	1.0
	5	2.72 ± 0.28	3.35 ± 0.31	1.2	4.09 ± 0.67	3.49 ± 0.70	0.9
10	1	1.23 ± 0.08	1.84 ± 0.24	1.5	1.72 ± 0.17	2.32 ± 0.50	1.3
	2	2.30 ± 0.38	2.10 ± 0.23	0.9	2.20 ± 0.33	2.86 ± 0.20	1.3
	5	2.53 ± 0.36	3.51 ± 0.07	1.4	3.64 ± 0.54	4.24 ± 0.50	1.2
100	1	1.60 ± 0.08	1.46 ± 0.40	0.9	2.13 ± 0.79	1.92 ± 0.25	0.9
	2	1.41 ± 0.30	1.31 ± 0.06	0.9	2.31 ± 0.86	2.24 ± 0.25	1.0
	5	1.87 ± 0.23	2.27 ± 0.36	1.2	2.80 ± 1.21	3.64 ± 0.66	1.3

Each value represents the mean ± SD of three samples or mean of 2 samples.

Pharmacokinetics: MATE1 and MATE2-K substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2391220 (M2)

Conc. (μM)	Incubation time (min)	MATE1			Conc. (μM)	Incubation time (min)	MATE2-K		
		Cleared volume (μL/mg protein)		S/N ratio			Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells				Control cells	Expressing cells	
1	1	NC	0.221 (n=1)	NC	0.01	1	NC	NC	NC
	2	NC	NC	NC		2	NC	NC	NC
	5	0.588 ± 0.289	NC	NC		5	NC	NC	NC
3	1	0.0851 ± 0.0199	0.117 ± 0.018	1.4	0.1	1	NC	NC	NC
	2	0.0912 ± 0.0182	0.0860 ± 0.0239	0.9		2	NC	NC	NC
	5	0.610 ± 0.291	0.180 ± 0.106	0.3		5	NC	NC	NC
10	1	0.116 ± 0.040	0.136 ± 0.056	1.2	1	1	0.453 ± 0.125	0.196 ± 0.093	0.4
	2	0.0882 ± 0.0225	0.124 ± 0.062	1.4		2	0.488 ± 0.134	0.175 ± 0.029	0.4
	5	0.634 ± 0.428	0.105 ± 0.003	0.2		5	1.64 ± 0.34	0.260 ± 0.138	0.2
30	1	0.0756 ± 0.0430	0.0875 ± 0.0204	1.2	10	1	0.796 ± 0.225	0.884 ± 0.291	1.1
	2	0.0602 ± 0.0025	0.0772 ± 0.0297	1.3		2	0.490 ± 0.260	0.618 ± 0.229	1.3
	5	0.370 ± 0.170	0.0694 ± 0.0152	0.2		5	2.28 ± 0.59	2.42 ± 0.38	1.1

Each value represents the mean ± SD of three samples or single value.

NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: MATE1 and MATE2-K substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2506104 (M3)

Conc. (μM)	Incubation time (min)	MATE1			MATE2-K		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	NC	NC	NC	0.164 ± 0.032	0.319 ± 0.022	1.9
	5	0.119 (n=2)	NC	NC	0.158 ± 0.007	0.268 ± 0.034	1.7
	15	NC	NC	NC	0.240 ± 0.083	0.329 ± 0.111	1.4
3	2	0.0641 (n=1)	0.0738 (n=1)	1.2	0.0947 ± 0.0306	0.134 ± 0.030	1.4
	5	0.0505 ± 0.0080	NC	NC	0.184 ± 0.067	0.149 ± 0.026	0.8
	15	0.0673 ± 0.0071	0.0595 ± 0.0193	0.9	0.183 ± 0.052	0.206 ± 0.011	1.1
10	2	0.0434 ± 0.0141	0.0356 ± 0.0062	0.8	0.139 ± 0.049	0.117 ± 0.055	0.8
	5	0.0410 ± 0.0087	0.0304 ± 0.0071	0.7	0.135 ± 0.060	0.114 ± 0.036	0.8
	15	0.0777 ± 0.0015	0.0531 ± 0.0050	0.7	0.182 ± 0.038	0.195 ± 0.041	1.1
30	2	0.0402 ± 0.0088	0.0449 ± 0.0200	1.1	0.122 ± 0.104	0.0758 ± 0.0160	0.6
	5	0.0352 ± 0.0090	0.0643 ± 0.0350	1.8	0.119 ± 0.045	0.116 ± 0.025	1.0
	15	0.0687 ± 0.0121	0.138 ± 0.111	2.0	0.133 ± 0.088	0.141 ± 0.008	1.1

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: MATE1 and MATE2-K substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2487818 (M4)

Conc. (μM)	Incubation time (min)	MATE1			MATE2-K		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	NC	0.231 (n=1)	NC	0.286 (n=2)	0.149 (n=2)	0.5
	5	0.196 (n=2)	NC	NC	NC	0.201 (n=2)	NC
	15	NC	0.248 (n=2)	NC	0.387 ± 0.419	0.210 ± 0.056	0.5
3	2	0.0962 (n=2)	0.0877 (n=2)	0.9	0.209 ± 0.053	0.105 ± 0.026	0.5
	5	0.0800 ± 0.0188	0.0859 ± 0.0066	1.1	0.268 ± 0.093	0.217 ± 0.059	0.8
	15	0.0941 ± 0.0134	0.108 ± 0.023	1.1	0.244 ± 0.091	0.208 ± 0.079	0.9
10	2	0.0744 ± 0.0068	0.0663 ± 0.0136	0.9	0.199 ± 0.075	0.199 ± 0.111	1.0
	5	0.0652 ± 0.0076	0.0954 ± 0.0245	1.5	0.203 ± 0.011	0.205 ± 0.081	1.0
	15	0.0837 ± 0.0393	0.0903 ± 0.0422	1.1	0.143 ± 0.055	0.212 ± 0.079	1.5
30	2	0.0724 ± 0.0151	0.0580 ± 0.0065	0.8	0.108 ± 0.040	0.0892 ± 0.0284	0.8
	5	0.0645 ± 0.0154	0.0936 ± 0.0087	1.5	0.125 ± 0.002	0.225 ± 0.052	1.8
	15	0.119 ± 0.022	0.0679 ± 0.0237	0.6	0.166 ± 0.025	0.152 ± 0.033	0.9

Each value represents the mean ± SD of three samples, mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: MATE1 and MATE2-K substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2506102 (M5)

Conc. (μM)	Incubation time (min)	MATE1			MATE2-K		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	NC	NC	NC	NC	0.112 ± 0.012	NC
	5	NC	NC	NC	0.145 ± 0.018	0.172 ± 0.013	1.2
	15	NC	NC	NC	0.180 ± 0.054	0.222 ± 0.049	1.2
3	2	NC	NC	NC	0.180 ± 0.062	0.282 ± 0.019	1.6
	5	NC	NC	NC	0.243 ± 0.056	0.350 ± 0.105	1.4
	15	0.0680 (n=1)	0.0708 (n=1)	1.0	0.210 ± 0.099	0.336 ± 0.082	1.6
10	2	0.0264 ± 0.0029	0.0320 ± 0.0099	1.2	0.185 ± 0.067	0.231 ± 0.020	1.2
	5	0.0331 ± 0.0067	0.0356 ± 0.0115	1.1	0.138 ± 0.015	0.262 ± 0.056	1.9
	15	0.0494 ± 0.0086	0.0484 ± 0.0137	1.0	0.216 ± 0.058	0.314 ± 0.037	1.5
30	2	0.0314 ± 0.0058	0.0246 ± 0.0022	0.8	0.103 ± 0.039	0.215 ± 0.029	2.1
	5	0.0366 ± 0.0020	0.0314 ± 0.0037	0.9	0.155 ± 0.033	0.249 ± 0.024	1.6
	15	0.0619 ± 0.0088	0.0506 ± 0.0099	0.8	0.205 ± 0.052	0.312 ± 0.073	1.5

Each value represents the mean ± SD of three samples or single value.

NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: MATE1 and MATE2-K substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2531398 (M6)

Conc. (μM)	Incubation time (min)	MATE1			MATE2-K		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	0.177 (n=2)	NC	NC	0.157 (n=2)	0.233 ± 0.009	1.5
	5	0.188 (n=2)	0.213 ± 0.012	1.1	NC	NC	NC
	15	NC	0.164 (n=2)	NC	NC	NC	NC
3	2	0.0985 ± 0.0106	0.102 ± 0.012	1.0	0.0737 ± 0.0008	0.116 ± 0.043	1.6
	5	0.0735 ± 0.0113	0.117 ± 0.033	1.6	0.0772 ± 0.0026	0.107 ± 0.013	1.4
	15	0.100 ± 0.012	0.102 ± 0.008	1.0	0.0866 ± 0.0185	0.155 ± 0.014	1.8
10	2	0.0611 ± 0.0054	0.0632 ± 0.0062	1.0	0.0606 ± 0.0103	0.0961 ± 0.0173	1.6
	5	0.0480 ± 0.0035	0.0589 ± 0.0111	1.2	0.0724 ± 0.0108	0.125 ± 0.033	1.7
	15	0.0888 ± 0.0092	0.0968 ± 0.0363	1.1	0.0766 ± 0.0073	0.133 ± 0.019	1.7
30	2	0.0725 ± 0.0020	0.0693 ± 0.0039	1.0	0.0399 ± 0.0025	0.0702 ± 0.0141	1.8
	5	0.0462 ± 0.0088	0.0627 ± 0.0191	1.4	0.0566 ± 0.0057	0.0927 ± 0.0309	1.6
	15	0.0798 ± 0.0198	0.0736 ± 0.0207	0.9	0.0534 ± 0.0073	0.116 ± 0.010	2.2

Each value represents the mean ± SD of three samples or mean of two samples.

NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Pharmacokinetics: MATE1 and MATE2-K substrate of Daprodustat and Metabolites of Daprodustat (Continued)

GSK2531401 (M13)

Conc. (μM)	Incubation time (min)	MATE1			MATE2-K		
		Cleared volume (μL/mg protein)		S/N ratio	Cleared volume (μL/mg protein)		S/N ratio
		Control cells	Expressing cells		Control cells	Expressing cells	
1	2	0.179 (n=1)	NC	NC	0.533 (n=2)	0.256 (n=2)	0.5
	5	0.114 (n=1)	NC	NC	0.497 ± 0.429	0.347 (n=2)	0.7
	15	0.166 (n=2)	0.138 (n=1)	NC	0.929 (n=2)	0.414 (n=1)	0.4
3	2	0.0583 (n=1)	0.0414 (n=2)	0.7	0.230 ± 0.134	0.206 ± 0.110	0.9
	5	0.0689 ± 0.0050	0.0474 ± 0.0143	0.7	0.340 ± 0.234	0.320 ± 0.269	0.9
	15	0.117 ± 0.013	0.0479 ± 0.0125	0.4	0.310 ± 0.192	0.346 ± 0.123	1.1
10	2	0.0639 ± 0.0276	0.0643 ± 0.0109	1.0	0.0933 ± 0.0367	0.134 ± 0.048	1.4
	5	0.0734 ± 0.0204	0.0505 ± 0.0166	0.7	0.121 ± 0.036	0.158 ± 0.035	1.3
	15	0.0971 ± 0.0319	0.0775 ± 0.0447	0.8	0.231 ± 0.026	0.220 ± 0.065	1.0
30	2	0.0441 ± 0.0208	0.0894 ± 0.0880	2.0	0.194 ± 0.107	0.149 ± 0.058	0.8
	5	0.0499 ± 0.0245	0.0759 ± 0.0382	1.5	0.136 ± 0.040	0.206 ± 0.032	1.5
	15	0.0687 ± 0.0159	0.0862 ± 0.0552	1.3	0.233 ± 0.054	0.313 ± 0.080	1.3

Each value represents the mean ± SD of three samples or mean of two samples, or single value.
NC: Not calculated since the observed value in cell solution sample was below lower limit of quantification (0.05 nM in the cell solution).

Table 9.11 Pharmacokinetics: P-gp inhibition

Test Article: GSK1278863

Location in CTD: m4.2.2.3
Report No.: CD2008/00669 **Study No.:** 08DMM040

Target Entity, Test System and Method: In vitro (polarized Madin-Darby canine kidney MDCKII-MDR1 cell)
The MDCKII-MDR1 cell monolayers were pre-incubated for 20 minutes (37°C) with receiver working solutions containing GSK1278863, GF120918 (positive control) or vehicle. At the end of the pre-incubation period, the apical and basolateral well media was removed and the wells were refilled with donor working solution containing [3H]digoxin (final target concentration 30 nM) (basolateral wells) or receiver working solution (apical wells) to initiate the experiment. Plates were incubated at 37ºC for 90 minutes. All samples were analysed for total radioactivity using a microplate scintillation and luminescence counter.

Results:

Compound	Conc. (μM)	Digoxin transport rate (pmole/cm^2/h) ± SD	Digoxin transport rate (% control) ± SD
GSK1278863	0.1	1.97 ± 0.11	98 ± 5.4
	0.3	2.05 ± 0.25	102 ± 13
	1	1.82 ± 0.20	91 ± 10
	3	1.82 ± 0.35	91 ±18
	10	2.16 ± 0.27	108 ± 13
	30	2.46 ± 0.41	123 ± 20
	100	2.07 ± 0.43	104 ± 21
Digoxin Only	-	2.0 ± 0.16	100 ± 7.9
GF120918	2	0.18 ± 0.01	9.1 ± 0.69

Data are the mean ± SD from sets of three wells, unless otherwise indicated.
Quality control parameters were within acceptable limits unless otherwise indicated (acceptable values: Lucifer yellow $P_{7.4}$ ≤50 nm/sec; digoxin mass balance 80 - 120%; digoxin transport rate ≥1.5 pmoles transported/cm^2/h; digoxin transport rate in the presence of 2 μM GF120918 ≤30% of uninhibited rate). No effects of GSK1278863 on these quality parameters were observed.

Table 9.12 Pharmacokinetics: BCRP inhibition

Test Article: GSK1278863

Location in CTD: m4.2.2.3
Report No.: 2012N151642 **Study No.:** 12DMM034

Target Entity, Test System and Method: In vitro (polarized Madin-Darby canine kidney MDCKII-BCRP cell line heterologously expressing human BCRP)
The MDCKII-BCRP cell monolayers were pre-incubated at 37ºC for approximately 20 minutes with working solutions containing GSK1278863, GF120918 (positive control) or vehicle. Pre-incubation solutions were removed and filter inserts were transferred to a plate preloaded with donor working solution containing [^{14}C]cimetidine (500 nM). The assay was initiated by adding receiver working solution to the apical compartments. Plates were incubated at 37ºC for 90 minutes. All samples were analysed for total radioactivity using a microplate scintillation and luminescence counter.

Results:

Compound	Conc. (μM)	Cimetidine transport rate (pmole/cm^2/h)	Cimetidine transport rate (% control)
GSK1278863	0.01	13 ± 0.98	129 ± 9.6
	0.1	13 ± 1.2	124 ± 12
	0.3	11[a]	111[a]
	1.0	14 ± 0.65	133 ± 6.4
	3.0	14 ± 0.84	139 ± 8.2
	10	14[a]	137[a]
	30	9.7 ± 0.51	114 ± 5.0
	100	12 ± 0.79	95 ± 7.8
Cimetidine Only	-	10 ± 0.75	100 ± 7.4
GF120918	2	2.8 ± 0.40	27 ± 3.9

Data are the mean ± SD from sets of three wells, unless otherwise indicated.
a: n=2
Quality control parameters were within acceptable limits unless otherwise indicated (acceptable values: Lucifer yellow $P_{7.4} \leq 50$ nm/sec; cimetidine mass balance 80 - 120%; cimetidine transport rate ≥1.5 pmoles transported/cm^2/h; cimetidine transport rate in the presence of 2 μM GF120918 ≤30% of uninhibited rate). No effects of GSK1278863 on these quality parameters were observed.

Table 9.13 Pharmacokinetics: OATP1B1 and OATP1B3 inhibition by Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.3

Report No.: CD2008/00231 **Study No.:** 07DMM163

Target Entity, Test System and Method: In vitro (Chinese Hamster Ovary cell line heterologously expressing human organic anion transporting polypeptide 1B1 (CHO-OATP1B1)) (Human Embryonic Kidney MSRII cell line, transduced with BacMam baculovirus containing the human organic anion transporting polypeptide 1B3 (HEK MSRII OATP1B3)) Cells monolayers were pre-incubated at 37°C with transport medium containing the GSK1278863 and rifamycin (OATP1B1 and OATP1B3 inhibitor) without [3H]EG. Following removal of pre-incubation solutions, the appropriate working solutions containing GSK1278863 at the target concentration plus [3H]EG were added to the wells. Separate sets of wells were designated for [3H]EG only and [3H]EG plus rifamycin. Cells were incubated for 5 minutes for OATP1B1 and 10 minutes for OATP1B3 at 37°C. For analysis of total radioactivity, the contents of each well were determined by liquid scintillation counting in glass vials containing scintillant.

Results:

Test Compound	Concentration (μM)	OATP1B1		OATP1B3	
		Uptake Rate of [3H]EG (fmoles/cm^2/min)	Uptake Rate of [3H]EG (% control)	Uptake Rate of [3H]EG (fmoles/cm^2/min)	Uptake Rate of [3H]EG (% control)
[3H]EG only	None	12[a] ± 0.083	100 ± 0.68	6.3[c] ± 0.28	100 ± 4.5
[3H]EG + rifamycin	10	0.49 ± 0.014	4.0[b] ± 0.12	0.27[c] ± 0.033	4.2[d] ± 0.53
[3H]EG + GSK1278863	0.1	13 ± 0.051	110 ± 0.42	5.6 ± 0.11	90 ± 1.8
	1	12 ± 0.21	96 ± 1.7	5.8 ± 0.20	92 ± 3.2
	3	9.3 ± 0.38	75 ± 3.1	5.4 ± 0.080	86 ± 1.3
	10	4.8 ± 0.28	39 ± 2.3	3.3 ± 0.18	52 ± 2.8
	30	0.94 ± 0.037	7.6 ± 0.30	0.62 ± 0.060	9.9 ± 0.95
	100	0.49 ± 0.065	4.0 ± 0.53	0.17 ± 0.008	2.7 ± 0.13
IC50 (μM) of GSK1278863		6.0		11	

Data is the mean ± SD from three wells after background correction.

a: Acceptance criteria ≥6 fmoles/cm^2/min

b: Acceptance criteria ≤15% of control [3H]EG value

c: Acceptance criteria ≥3-fold signal to noise (average probe substrate uptake rate in uninhibited control wells/average uptake in rifamycin inhibited wells)

d: Acceptance criteria ≤33% of control [3H]EG value

Table 9.14 Pharmacokinetics: OATP1B1 inhibition by Metabolites of Daprodustat

Test Article: GSK2391220, GSK2487818, GSK2506102, GSK2531398, GSK2531401 and GSK2531403

Location in CTD: m4.2.2.3

Report No.: 2013N166580 **Study No.:** None

Target Entity, Test System and Method: In vitro (Bacmam transduced HEK cells expressing the OATP1B1 transporter)
Test compounds (final concentration 400nM) was added to the cells and incubated for 30 mins at room temperature. Image analysis was used to determine the relative amounts of substrate taken up by the cells.

Results:

Compound (0.085 to 50 μM, n=8)	Median IC_{50} (μM)
GSK2391220 (M2) #	> 50
GSK2487818 (M4) #	> 50
GSK2506102 (M5)	> 50
GSK2531398 (M6)	> 50
GSK2531401 (M13)	> 50
GSK2531403 (M3)	> 50

Reported IC50 values are given as the median of concentration-response curves from eight replicates. Where replicates contain a mixture of active and inactive data the median result includes all replicates.

#: GSK2391220 and GSK2487818 reported IC50 values of 25 μM and 3 μM respectively on one test occasion but were inactive on the other seven test occasions.

Table 9.15 Pharmacokinetics: OAT1, OAT3, OCT2, MATE1 and MATE2-K Inhibition by Daprodustat and Metaboites of Daprodustat

Test Article: GSK1278863, GSK2391220, GSK2506104, GSK2487818, GSK2506102, GSK2531398 and GSK2531401

Location in CTD: m4.2.2.3

Report No.: 2015N242070 **Study No.:** XS-0661

Target Entity, Test System and Method: In vitro (S_2 cells expressing OAT1 or OAT3, HEK293 cells expressing OCT2 or MATE1, and HEK293 cells expressing MATE2-K). Cells monolayers were pre-incubated at 37°C with transport medium containing the test article cocktail or positive control inhibitor. Following removal of pre-incubation solutions, the appropriate working solutions containing each probe substrate was added to the wells. Cells were incubated for 2 (OAT1, OAT3 and OCT2) or 5 (MATE1 and MATE2-K) minutes at 37°C. Radioactivity in each sample was counted using an LSC.

Results:

Transporter	Probe substrate (concentration)	Test compound or inhibitor (concentration)	Cleared volume (µL/mg protein)		% of control
			Control cells	Transporter expressing cells	
OAT1	[^{3}H]PAH (1 µM)	0	0.672 ± 0.101	25.4 ± 0.8	100.0
		Cocktail compound*	0.832 ± 0.204	37.3 ± 13.7	147.5
		Probenecid (100 µM)	0.741 ± 0.023	3.16 ± 0.75	9.8
OAT3	[^{3}H]ES (0.05 µM)	0	2.07 ± 0.21	15.2 ± 0.9	100.0
		Cocktail compound*	2.22 ± 0.15	13.8 ± 0.3	88.2
		Probenecid (100 µM)	2.57 ± 0.77	4.70 ± 0.82	16.2
OCT2	[^{14}C]Metformin (10 µM)	0	0.838 ± 0.134	69.6 ± 4.1	100.0
		Cocktail compound*	0.862 ± 0.071	74.5 ± 0.6	107.1
		Quinidine (300 µM)	0.356 ± 0.032	0.822 ± 0.090	0.7
MATE1	[^{14}C]Metformin (10 µM)	0	0.863 ± 0.071	26.2 ± 1.8	100.0
		Cocktail compound*	1.05 ± 0.08	33.7 ± 5.5	128.9
		Cimetidine (10 µM)	0.639 ± 0.046	4.08 ± 0.43	13.6
MATE2-K	[^{14}C]Metformin (10 µM)	0	0.965 ± 0.086	19.8 ± 2.2	100.0
		Cocktail compound*	0.873 ± 0.163	17.6 ± 1.0	88.8
		Cimetidine (100 µM)	0.576 ± 0.139	3.97 ± 0.51	18.0

Each value represents the mean ± SD of three samples.

*: GSK1278863: 1.4 µM, GSK2391220 (M2): 20 µM, GSK2506104 (M3): 20 µM, GSK2487818 (M4): 10 µM, GSK2506102 (M5): 5 µM, GSK2531398 (M6): 10 µM, GSK2531401: 10 µM

10. PHARMACOKINETICS: METABOLISM IN VIVO

Table 10.1 Pharmacokinetics: Preliminary Characterization of Metabolites of Daprodustat in Mouse, Rat, Rabbit and Monkey Plasma

Test Article: GSK1278863

Location in CTD: m4.2.2.4

Report No.: 2010N109722 Study No.: 09DMM062

Species (strain):	Mouse (CD-1), Rat (Sprague Dawley), Rabbit (Dutch Belted), Monkey (cynomolgus)
Gender (M/F)/Number of Animals:	20M/20F (Mouse), 3M/3F (Rat), 9F (Rabbit), 3M/3F (Monkey)
Vehicle/Formulation:	1% methylcellulose/Suspension
Method of Administration:	Repeated Oral (gavage)
Dose (mg/kg/day):	30 (Mouse), 20 (Rat), 60 (Rabbit), 100 (Monkey)
Duration of dosing:	Day 14 (Mouse), Week 13 (Rat), Day 5 (Rabbit), Day 1 and Day 14 (Monkey)
Source of Biological Samples:	Select plasma samples were transferred from separate toxicity studies. Pooled plasma samples were combined for each species using volumes in proportion to the time interval between individual samples.

Pharmacokinetics: Preliminary Characterization of Metabolites of Daprodustat in Mouse, Rat, Rabbit and Monkey Plasma (Continued)

Results:

		% of Compound in Plasma						
		Mouse[a]		Rat (M)[b]	Rat (F)b	Rabbit (F)[c]	Monkey[d]	
	RT	0 - 8 h, Day 14	8 - 24 h, Day 14[e]	0 - 24 h, Week 13	0 - 24 h, Week 13	0 - 24 h, Week 13	0 - 24 h, Day 1	0 - 24 h, Day 14
GSK1278863 (Parent)	52.9 - 53.4	✓	✓	✓	✓	>15% DRM 100% P	>15% DRM 100% P	>15% DRM 100% P
M2 (Di-oxygenation)	19.5	ND	ND	ND	ND	5→15% DRM 5→15% P	5→15% DRM >15% P	5→15% DRM 5→15% P
M3 (Di-oxygenation)	21.4	ND	ND	ND	ND	1→5% DRM 1→5% P	1→5% DRM 5→15% P	1→5% DRM 1→5% P
M4 (Di-oxygenation)	22.4	ND	ND	ND	ND	1→5% DRM 1→5% P	5→15% DRM 5→15% P	5→15% DRM 5→15% P
M5 (Di-oxygenation)	23.0 - 23.1	ND	ND	ND	ND	<1% DRM <1% P	<1% DRM 1→5% P	<1% DRM <1% P
M6 (Di-oxygenation)	24.0	ND	ND	ND	ND	ND	1→5% DRM 1→5% P	<1% DRM 1→5% P
M7 (Di-oxygenation)	25.4	ND	ND	ND	ND	ND	<1% DRM 1→5% P	<1% DRM <1% P
M8 (Mono-oxygenation)	34.7	✓	ND	ND	ND	5→15% DRM 5→15% P	5→15% DRM 5→15% P	5→15% DRM >15% P
M9/M22 f (Mono-oxygenation)	35.6 - 35.9	✓	ND	✓	✓	1→5% DRM 5→15% P	1→5% DRM 5→15% P	5→15% DRM 5→15% P
M10 (Mono-oxygenation)	38.6 - 38.7	ND	ND	ND	ND	1→5% DRM 1→5% P	5→15% DRM 5→15% P	5→15% DRM 5→15% P
M13 (Tri-oxygenation)	17.0	ND	ND	ND	ND	ND	5→15% DRM >15% P	1→5% DRM 1→5% P

Pharmacokinetics: Preliminary Characterization of Metabolites of Daprodustat in Mouse, Rat, Rabbit and Monkey Plasma (Continued)

		% of Compound in Plasma						
		Mouse[a]		Rat (M)[b]	Rat (F)[b]	Rabbit (F)[c]	Monkey[d]	
	RT	0 - 8 h, Day 14	8 - 24 h, Day 14e	0 - 24 h, Week 13	0 - 24 h, Week 13	0 - 24 h, Week 13	0 - 24 h, Day 1	0 - 24 h, Day 14
M21 (Di-oxygenation)	30.6	ND	ND	ND	ND	ND	✓g	✓g

Additional Information: - = unknown; x = detected; NA = not applicable; ND = not detected; RT = retention time of Mass spectrometer (min), % DRM = percent drug related material; % P = mole percent of parent drug

<1 = estimated that the metabolite is less than 1% drug related material (or % Parent); 1→5 = estimated that the metabolite is ≥1% but <5% drug related material (or % Parent); 5→15 = estimated that the metabolite is ≥5% but <15% drug related material (or % Parent); >15 = estimated that the metabolite is ≥15% drug related material (or % Parent)

Metabolites identified in monkey plasma included products of mono-, di- and tri-oxygenation, while metabolites identified in rabbit plasma included products of mono- and di-oxygenation. Mouse and rat plasma only contained products of mono-oxygenation. Unchanged GSK1278863 was detected in the plasma of all species analyzed.

a: Mouse samples were obtained from a 14 day oral toxicity study (CD2009/00900/00) where male and female mice were dosed 30 mg/kg/day. Male and female samples were pooled together.

b: Rat samples were obtained from a 13 week oral toxicity study (CD2009/00951/00) where male and female rats were dosed 20 mg/kg/day.

c: Rabbit samples were obtained from an embryo fetal development study (CD2009/00390/00) where female rabbits were dosed 60 mg/kg/day.

d: Day 1 monkey samples were obtained from a single dose escalation (maximum tolerated dose) oral toxicity study (CD2009/00639/00) where male and female monkeys were dosed 100 mg/kg. Day 14 monkey samples were obtained from a 14 day oral dose range toxicity study (CD2009/00780/00) where a male and a female monkey were dosed 100 mg/kg/day. Male and female samples were pooled together.

e: This sample was not a time proportional pooled sample.

f: M9 and M22 are distinguishable by the MS3 spectrum. They have the same retention time, accurate mass, and MS/MS. The MS3 fingerprint indicates the presence of a mixture of M9 and M22. The ratio of M9 to M22 could not be determined because no standard for M22 was available. (MS3 = 3-stage mass spectrometry; MS/MS = tandem mass spectrometry)

g: Quantitative assessment estimation was not possible due to lack of a suitable biological reference standard.

Table 10.2 Metabolites in Isolated Perfused Rat Liver

Test Article: [14C]GSK1278863, GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2008/00941 **Study No.:** 07DMM151

Characterization of Circulating metabolites in fasted intact Sprague Dawley rats

Gender (M/F)/Number of Animals: 14M
Feeding Condition: Fasted
Vehicle/Formulation: 6% aqueous Cavitron™/Solution
Method of Administration: Single Oral (gavage)
Dose (mg/kg): 10
Radionuclide: [14C]
Specific Activity: 136 μCi/mg

Results:

Species	Sample	Sampling Time or Period	% Overall Recovery[b]	% of Compound in Sample[a]	
				Parent	Parent Related Compound
Rat (Sprague Dawley)	Plasma[c]	4 h	90.7	87.3	ND
		8 h	95.7	93.7	ND
		24 h	91.4	89.0	ND
		48 h	89.1	87.4	ND

Metabolites in Isolated Perfused Rat Liver (Continued)

Hepatic metabolites of daprodustat from isolated perfused BDC male Sprague Dawley rat livers		
Gender (M/F)/Number of Animals:	3M BDC	
Feeding Condition:	Not specified	
Vehicle/Formulation:	6% aqueous Cavitron™/Solution	
Method of Administration:	Single	
Dose (mg/kg):	30	
Radionuclide:	[14C]	
Specific Activity:	136 μCi/mg	
Results:		
	Bile	**Plasma**
Parent	✓	✓
M2	✓	ND
M8	✓	ND
M9	✓	ND
M10	✓	ND

Additional Information: ✓ = detected; ND = not detected

The only radiolabeled component detected in plasma at 4, 8, 24 and 48 hours post-dosing was unchanged GSK1278863 (87% to 94% plasma radioactivity).

a: Percent radioactivity recovered under each peak, corrected by the overall sample preparation recovery.

b: % recovery of radioactivity following 1:8 methanol: acetonitrile extraction, evaporation and reconstitution of plasma samples.

c: pooled plasma

Table 10.3 Metabolites in Bile Duct-Cannulated Rat

Test Article: [14C]GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2008/01663 **Study No.:** 07DMM161

Gender (M/F)/Number of Animals: 3M/3F (Intact), 3M (Bile duct-cannulated)
Feeding Condition: Fasted
Vehicle/Formulation: 1% methylcellulose/Suspension
Method of Administration: Single Oral (gavage)
Dose (mg/kg): 10
Radionuclide: [14C]
Specific Activity: 22.3 μCi/mg
Results:

Species	Sample	Gender	Sampling Time or Period	% of Dose in Sample	Mean % of Compound in Sample (Mean % of Dose)			
					Parent	M2 (di-oxygenation)	M8 (mono-oxygenation)	M9 (mono-oxygenation)
Rat (Sprague Dawley)	Plasma[a]	Male	1 h	NA	88.6	ND	ND	ND
			4 h	NA	87.5	ND	ND	ND
			8 h	NA	85.5	ND	ND	ND
			24 h	NA	85.8	ND	ND	ND
			168 h	NA	90.7	ND	ND	ND
	Plasma[a]	Female	1 h	NA	66.0	ND	ND	ND
			4 h	NA	88.5	ND	ND	ND
			8 h	NA	87.7	ND	ND	ND
			24 h	NA	87.8	ND	ND	ND
			168 h	NA	94.1	ND	ND	ND
	Plasma[a]	Male (BDC)	96 h	NA	90.8	ND	ND	ND

Additional Information: BDC = Bile duct-cannulated; NA = not applicable; ND = not detected; SD = Sprague Dawley
Following oral administration of [14C]GSK1278863 at 10 mg/kg, the only radiolabeled component detected in male and female rat plasma and liver was unchanged GSK1278863.
Elimination of GSK1278863 in intact rats occurred largely as oxidative metabolites and unchanged parent in faeces.
In bile duct-cannulated rats, unchanged parent (18% of dose) and 3 oxidative metabolites (together 20% of dose) were eliminated via the bile.
The metabolite profiles observed were similar in male and female animals.
a: pooled sample

Table 10.4 Metabolites in Bile Duct-Cannulated Dog

Test Article: [14C]GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2008/01662 **Study No.:** 07DMM160

Gender (M/F)/Number of Animals: (Intact) 3M/3F, (Bile duct-cannulated) 2M
Feeding Condition: Fasted
Vehicle/Formulation: 1% methylcellulose/Suspension
Method of Administration: Single Oral (gavage)
Dose (mg/kg): 20
Radionuclide: [14C]
Specific Activity: 1.92 μCi/mg
Results:

					Mean % of Compound in Sample (Mean % of Dose)		
Species	Sample	Gender	Sampling Time or Period	% of Dose in Sample	Parent	M8	M9
Dog (beagle)	Plasma[a]	Male	1 h	NA	82.3	ND	ND
			4 h	NA	93.0	ND	ND
			10 h	NA	92.6	ND	ND
			24 h	NA	95.1	ND	ND
		Female	1 h	NA	88.3	ND	ND
			4 h	NA	89.1	ND	ND
			10 h	NA	84.4	ND	ND
			24 h	NA	94.6	ND	ND

Additional Information: BDC =Bile duct-cannulated; NA = not applicable; ND = not detected
Following oral administration of [14C]GSK1278863, the only radiolabeled component detected in male and female dog plasma was unchanged GSK1278863. Elimination of GSK1278863 in dogs occurred largely as parent in faeces. After absorption, elimination in bile was largely as unchanged parent (6% dose). Urinary excretion was low and predominantly consisted of unchanged parent. The metabolite profiles observed were similar in male and female animals.
a: pooled plasma
b: n = 1

Table 10.5 Characterization of Metabolites in Monkeys

Test Article: [14C]GSK1278863

Location in CTD: m4.2.2.4

Report No.: 2018N386289 **Study No.:** 8361764

Gender (M/F)/Number of Animals: (Intact) 3M
Feeding Condition: Fasted overnight through 4 hr post-dose
Vehicle/Formulation: 1% (w/v) aqueous methylcellulose (400cps)
Method of Administration: Oral (gavage)
Dose (mg/kg): 10
Radionuclide: [14C]
Specific Activity: 2.42 μCi/mg
Results:

Metabolite ID	% Plasma Radioactivity (ng equivalents [^{14}C]GSK1278863/g plasma)[a]			
	1 hour	4 hour	8 hour	24 hour
P	82.7 (3234)	79.1 (5711)	56.4 (1331)	47.1 (60.8)
M2	0.6 (23.5)	0.8 (57.8)	3.3 (77.9)	NQ (NQ)
M4	0.6 (23.5)	0.8 (57.8)	3.3 (77.9)	NQ (NQ)
M8	1.9 (74.3)	2.2 (159)	3.0 (70.8)	NQ (NQ)
M9, M22[b]	1.3 (50.8)	1.1 (79.4)	1.7 (40.1)	NQ (NQ)
M10	4.3 (168)	4.9 (354)	2.9 (68.4)	NQ (NQ)

Characterization of Metabolites in Monkeys (Continued)

Metabolite ID	% Plasma Radioactivity (ng equivalents [^{14}C]GSK1278863/g plasma)[a]			
	1 hour	4 hour	8 hour	24 hour
M18	1.6 (62.6)	1.2 (86.6)	21.5 (507)	52.9 (68.2)
Total Quantified	93.0 (3636)	90.1 (6505)	92.1 (2174)	100.0 (129)
Conc. in Pooled Matrix[c]	(3910)	(7220)	(2360)	(129)
% Overall Recovery[d]	93	90	92	100

Key:
NQ = Not quantifiable (<15 cpm in peak height)
P = Parent (GSK1278863)
a = % radioactivity recovered under each peak, corrected by the overall recovery (expressed as ng equivalents of GSK1278863 per g of plasma)
b = Co-eluting radio components quantified as a sum total
c = Data from balance excretion study 2018N355713
d = %Recovery of radioactivity following extraction, evaporation, and re-constitution

Characterization of Metabolites in Monkeys (Continued)

Pharmacokinetics: Metabolism *In Vivo*
Species (strain): Monkey (Cynomolgus)
Gender/Number of Animals: 3 intact male, 2 bile duct-cannulated (BDC) male
Feeding Condition: Fasted overnight through 4 hr post-dose
Vehicle/Formulation: 1% (w/v) aqueous methylcellulose (400cps)
Method of Administration: Oral gavage
Dose (mg/kg): 10
Radionuclide: 14C
Specific Activity: 2.42 µCi/mg (5.4 DPM/ng)
Analysis: Radio-HPLC

Metabolite ID	Mean % Matrix Radioactivity (Mean % Dose)[a]				
	Urine		Bile	Feces	
	Intact Male	BDC Male	BDC Male	Intact Male	BDC Male
P	NQ (NQ)	1.3 (0.2)	33.1 (17.2)	53.5 (38.6)	79.1 (17.4)
M2	13.0 (1.5)	13.9 (2.1)	5.6 (2.9)	5.0 (3.6)	NQ (NQ)
M3	5.6 (0.7)	6.9 (1.0)	2.9 (1.5)	2.5 (1.8)	NQ (NQ)
M4	10.3 (1.2)	19.6 (2.9)	5.1 (2.7)	4.5 (3.2)	NQ (NQ)
M5	2.0 (0.2)	2.1 (0.3)	0.4 (0.2)	0.7 (0.5)	NQ (NQ)
M6	1.7 (0.2)	2.6 (0.4)	1.2 (0.6)	0.7 (0.5)	NQ (NQ)
M7	NQ (NQ)	0.6 (0.1)	NQ (NQ)	NQ (NQ)	NQ (NQ)
M8	27.9 (3.3)	20.1 (3.0)	10.8 (5.6)	10.7 (7.7)	2.0 (0.4)
M9, M22[b]	17.0 (2.0)	11.9 (1.8)	6.3 (3.3)	6.9 (5.0)	2.7 (0.6)

Characterization of Metabolites in Monkeys (Continued)

Metabolite ID	Mean % Matrix Radioactivity (Mean % Dose)[a]				
	Urine		Bile	Feces	
	Intact Male	BDC Male	BDC Male	Intact Male	BDC Male
M10	18.9 (2.3)	14.5 (2.2)	13.1 (6.8)	10.1 (7.3)	NQ (NQ)
M13	2.4 (0.3)	2.5 (0.4)	0.5 (0.3)	NQ (NQ)	NQ (NQ)
M18 + M28[c]	NQ (NQ)	0.3 (0.1)	3.3 (1.7)	NQ (NQ)	NQ (NQ)
M19 + M20	NQ (NQ)	NQ (NQ)	8.7 (4.5)	NQ (NQ)	NQ (NQ)
M21	0.6 (0.1)	1.1 (0.2)	NQ (NQ)	NQ (NQ)	NQ (NQ)
M25	NQ (NQ)	NQ (NQ)	2.3 (1.2)	NQ (NQ)	NQ (NQ)
M26	NQ (NQ)	NQ (NQ)	2.6 (1.3)	NQ (NQ)	NQ (NQ)
M27	0.6 (0.1)	0.8 (0.1)	NQ (NQ)	NQ (NQ)	NQ (NQ)
M28	NQ (NQ)	NQ (NQ)	3.3 (1.7)	NQ (NQ)	NQ (NQ)
M29	NQ (NQ)	NQ (NQ)	3.1 (1.6)	NQ (NQ)	NQ (NQ)
Total Quantified	100 (11.9)	98.3 (14.8)	99.0 (51.4)	94.5 (68.2)	83.8 (18.4)
% Dose in Pooled Matrix[d]	11.9	14.9	52.0	72.1	22.0
% Dose Excreted in Matrix[e]	13.4	15.8	53.0	77.5	24.1
% Overall Recovery[f]	100	99	100	96	86

Key:
NQ = Not quantifiable (<15 cpm in peak height)
a = % radioactivity recovered under each peak, corrected for centrifugation/extraction recovery
b = Co-eluting radiocomponents quantified as a sum total
c = M18 co-eluted with M28 in urine, however M18 was not present in bile.
d = % dose pooled across time points (not corrected for centrifugation/extraction recovery)
e = Data from balance excretion study 2018N355713
f = % Recovery of radioactivity following centrifugation (urine and bile) or extraction, evaporation, and re-constitution (feces)

Table 10.6 Preliminary Characterization of Metabolites in Human

Test Article: GSK1278863

Location in CTD: m4.2.2.4

Report No.: 2010N109720 **Study No.:** 08DMM117

Species: **Human**
Gender (M/F)/Number of Animals: 4M
Feeding Condition: Fasted
Vehicle/Formulation: Tablets
Method of Administration: Single Oral
Dose (mg): 300
Radionuclide: NA
Specific Activity: NA
Source of Biological Samples: Samples of human plasma and urine from subjects having received 300 mg of GSK1278863 were transferred from study PHX111427 (Phase I study) and used in this study.
Plasma samples from subjects 1052, 1054, 1055 and 1056 at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hours post-dose were combined using volumes in proportion to the time interval between individual samples. This resulted in a single pool of human plasma representative of the AUC over the range 0 to 8 hours across all volunteers. Equal volumes (0.5 mL) of the 8 to 24 hour sample from each subject were pooled to create a single 8 to 24 hour plasma sample. Urine pools were created by combining equal volumes (approximately 50 mL) from the 0 to 12 and 12 to 24 hour collections from subjects to give a single representative 0 to 24 hour sample for analysis.
Results: GSK1278863 and 19 metabolites were detected in 0-24 h urine

			% of Compound in Human Plasma and Urine		
		RT	Plasma 0 - 8 h	Plasma 8 - 24 h	Urine 0 - 24 h
GSK1278863	(Parent)	53.0	>15% DRM, 100% P	✓	✓
M2	(Di-oxygenation)	19.2	5→15% DRM, >15% P	✓a	✓
M3	(Di-oxygenation)	21.1	5→15% DRM, >15% P	✓	✓
M4	(Di-oxygenation)	22.1	5→15% DRM, 5→15% P	ND	✓
M5	(Di-oxygenation)	22.7	1→5% DRM, 5→15% P	ND	✓

Preliminary Characterization of Metabolites in Human (Continued)

			% of Compound in Human Plasma and Urine		
		RT	Plasma 0 - 8 h	Plasma 8 - 24 h	Urine 0 - 24 h
M6	(Di-oxygenation)	23.9	1→5% DRM, 5→15% P	ND	✓
M7	(Di-oxygenation)	25.2	<1% DRM, 1→5% P	ND	✓
M8	(Mono-oxygenation)	34.3	1→5% DRM, 1→5% P	ND	✓
M9/M22 d	(Mono-oxygenation)	35.5	1→5% DRM, 1→5% P	ND	✓
M10	(Mono-oxygenation)	38.2	1→5% DRM, 1→5% P	ND	✓
M13	(Tri-oxygenation)	16.6	5→15% DRM, 5→15% Pb, c	✓	✓
M14, M15, M16	(Mono-oxygenated plus mono-oxygenation +dehydrogenation)	22.8, 24.4, 26.0	✓	ND	✓
M17	(Mono-oxygenation + dehydrogenation)	39.6	<1% DRM, 1→5% Pc	ND	✓
M18	(Undefined glucuronide)	40.9	ND	ND	✓
M19	(Undefined glucuronide)	42.9	ND	ND	✓
M20	(Undefined glucuronide)	42.4	ND	ND	✓
M21	(Di-oxygenation)	30.3	✓	ND	✓

Additional Information: x = detected, NA = not applicable; ND = not detected; RT = retention time of Mass spectrometer from human urine (min), % DRM = percent drug related material; % P = mole percent of parent drug; <1 = estimated that the metabolite is less than 1% drug related material (or % Parent); 1→5 = estimated that the metabolite is ≥1% but <5% drug related material (or % Parent); 5→15 = estimated that the metabolite is ≥5% but <15% drug related material (or % Parent); >15 = estimated that the metabolite is ≥15% drug related material (or % Parent)

Unchanged GSK1278863 and metabolites resulting from oxidation (mono-, di- and tri-oxygenation) were detected in human plasma extracts following single oral dosing of 300 mg.

a: No MSn (multistage mass spectrometry) data was obtained due to weak signal.

b: MS (mass spectrometer) response factor could not be obtained for M13 from the reference sample due to poor signal-to-noise ratio. An average response factor from six closely eluting, structurally-similar metabolites (M2 through M7) was used.

c: The radiolabeled reference spectra used to estimate M13 and M17 had low signal-to-noise ratio. Consequently, the error of the M13 and M17 estimation in human plasma is expected to be larger in these cases.

d: M9 and M22 are distinguishable by the MS3 spectrum. They have the same retention time, accurate mass and MS/MS. The MS3 fingerprint indicates the presence of a mixture of M9 and M22. The ratio of M9 to M22 could not be determined because no standard for M22 was available. (MS3 = 3-stage mass spectrometry; MS/MS = tandem mass spectrometry)

Table 10.7 Characterization of Metabolites in Human

Pharmacokinetics: Metabolism In Vivo	**Test Article:** GSK1278863	**Report No.:** 2018N376203	**Study No.:** 17DMM043
Species: Human (healthy)	**Gender:** Male	**Number of Subjects:** 6	**Location in CTD:** m4.2.2.4

Feeding Condition:	Overnight fast of at least 8 hours
Vehicle/Formulation:	IV solution of [^{14}C]-GSK1278863 was prepared aseptically in sterile isotonic phosphate buffered saline at pH 7.5; Oral solution of [^{14}C]-GSK1278863 was prepared aseptically in sterile phosphate buffered saline at pH 7.5.
Method of Administration:	Treatment Period 1 (TP1): A microtracer IV concomitant with non-radiolabelled oral tablet Treatment Period 2 (TP2): A radiolabelled oral solution two weeks after the administration in TP1
Dose:	50 µg IV concomitant with 6 mg oral in TP1, and 25 mg oral in TP2
Radionuclide:	^{14}C
Specific Activity:	2.51 µCi/mg
Analysis:	Metabolite profiles of plasma, urine and feces were generated by HPLC-TopCount; a metabolite profile of duodenal bile was generated by HPLC-AMS; structural assignment of metabolites was conducted using LC/MS^n.

Results:

	% Sample Radioactivity (ng equivalents GSK1278865/g Sample)		**Mean % Sample Radioactivity (Mean % of Administered Dose in Sample)**		**% Sample Radioactivity**
Matrix	**Plasma**		**Urine**	**Feces**	**Duodenal Bile[b]**
Time Range	**0-8 hour**	**10-12 hour**	**0-24 hour**	**24-120 hour**	
P (GSK1278863)	39.5 (106)	<LLQ (<LLQ)	ND (ND)	0.7 (0.5)	0.5
M2 (GSK2391220) and M33 (only M2 in feces and bile)	10.4[a] (27.7)	14.4 (3.1)	15.8 (3.2)	19.8 (14.4)	12.3
M3 (GSK2506104)	7.6 (20.2)	11.8 (2.6)	16.0 (3.3)	14.1 (10.3)	20.0
M4 (GSK2487818)	5.7 (15.2)	ND (ND)	7.9 (1.6)	17.0 (12.3)	15.5
M5 (GSK2506102) and M14 (only M5 in bile)	4.5 (11.9)	<LLQ (<LLQ)	10.4 (2.1)	7.7 (5.6)	11.1
M6 (GSK2531398)	3.6 (9.8)	<LLQ (<LLQ)	5.8 (1.2)	6.2 (4.5)	6.3

Characterization of Metabolites in Human (Continued)

	% Sample Radioactivity (ng equivalents GSK1278865/g Sample)		Mean % Sample Radioactivity (Mean % of Administered Dose in Sample)		% Sample Radioactivity
Matrix	**Plasma**		**Urine**	**Feces**	**Duodenal Bile[b]**
Time Range	**0-8 hour**	**10-12 hour**	**0-24 hour**	**24-120 hour**	
M7	<LLQ (<LLQ)	ND (ND)	0.5 (0.1)	2.2 (1.6)	3.1
M8	0.8 (2.2)	ND (ND)	0.2 (0.04)	3.3 (2.4)	3.9
M9 and M22	1.3 (3.5)	ND (ND)	1.5 (0.3)	1.9 (1.4)	2.4
M10	1.5 (4.0)	ND (ND)	<LLQ (<LLQ)	3.4 (2.5)	3.7
M13 (GSK2531401)	8.3 (22.1)	16.1 (3.5)	16.5 (3.4)	4.9 (3.6)	2.4
M15	2.5 (6.8)	<LLQ (<LLQ)	4.8 (1.0)	1.9 (1.4)	ND
M16	0.9 (2.5)	ND (ND)	0.9 (0.2)	1.1 (0.8)	ND
M30	ND (ND)	ND (ND)	0.4 (0.1)	ND (ND)	ND
M31	ND (ND)	ND (ND)	0.4 (0.1)	1.3 (0.9)	1.2
M32	1.0 (2.6)	ND (ND)	3.2 (0.7)	0.5 (0.4)	ND
A	ND (ND)	ND (ND)	ND (ND)	1.3 (0.9)	ND
B	1.0 (2.5)	ND (ND)	2.2 (0.4)	2.5 (1.8)	ND

Characterization of Metabolites in Human (Continued)

	% Sample Radioactivity (ng equivalents GSK1278865/g Sample)		Mean % Sample Radioactivity (Mean % of Administered Dose in Sample)		% Sample Radioactivity
Matrix	**Plasma**		**Urine**	**Feces**	**Duodenal Bile[b]**
Time Range	**0-8 hour**	**10-12 hour**	**0-24 hour**	**24-120 hour**	
C	0.8 (2.2)	ND (ND)	ND (ND)	0.8 (0.6)	ND
D	1.2 (3.2)	ND (ND)	0.2 (0.04)	1.4 (1.1)	ND
E	ND (ND)	ND (ND)	0.2 (0.1)	ND (ND)	ND
F	ND (ND)	ND (ND)	ND (ND)	ND (ND)	2.3
G	ND (ND)	ND (ND)	ND (ND)	ND (ND)	2.4
H	ND (ND)	ND (ND)	ND (ND)	0.6 (0.4)	ND
Un-Retained Radio-Peak	2.4 (6.4)	7.8 (1.7)	ND (ND)	ND (ND)	ND
Total Quantified	92.8 (249)	50.0 (10.9)	86.7 (17.7)	92.5 (67.4)	87.1
Concentration (ng eq./g Sample)	268	21.7	NA	NA	NA
(% Dose in Sample Analyzed)	(NA)	(NA)	(20.5)	(72.8)	(NA)
(% Dose in Total Sample)	(NA)	(NA)	(21.1)	(73.7)	(NA)
% Recovery of Radioactivity	85.4	69.3	99.8	99.8	NA

Note: All metabolites identified were oxidative products of GSK1278863.

a = The levels of M2 and M33 in the 0-8 h plasma pool from this study were estimated to be 8.1% and 2.3%, respectively in referencing to the validated bioanalysis data [2017N350026]

b = Absolute quantification of drug related components was not possible due to the qualitative nature of the bile string sampling and extraction procedure. Bile strings withdrawn 1 hour post 1-hour IV infusion

ND – Not Detected; NA – Not Applicable; LLQ – Lower Limit of Quantification

Table 10.8 Pharmacokinetics: Metabolites in Urine, Faeces and Bile from Bile Duct-Cannulated Rats

Test Article: [14C]GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2008/01663 **Study No.:** 07DMM161

Species: **Rat (Sprague Dawley)**
Gender (M/F)/Number of Animals: 3M/3F (Intact), 3M (Bile duct-cannulated)
Feeding Condition: Fasted
Vehicle/Formulation: 1% methylcellulose/Suspension
Method of Administration: Single Oral (gavage)
Dose (mg/kg): 10
Radionuclide: 14C
Specific Activity: 22.3 μCi/mg
Results:

Sample	Gender	Sampling Time or Period	% of Dose in Sample	Mean % of Compound in Sample (Mean % of Dose)			
				Parent	M2 (di-oxygenation)	M8 (mono-oxygenation)	M9 (mono-oxygenation)
Urine	Male	0 - 12 or 24 h	6.9	11.2 (0.8)	10.1 (0.7)	55.4 (4.0)	19.7 (1.4)
	Female	0 - 12 h	4.9	13.9 (0.7)	9.6 (0.5)	55.3 (2.8)	17.4 (0.9)
	Male (BDC)	0 - 12 h	2.5	13.6 (0.3)	8.5 (0.2)	54.1 (1.4)	18.6 (0.5)
Faeces	Male	0 - 96 h	78.4	38.9 (32.8)	6.5 (5.4)	36.5 (30.6)	11.4 (9.6)
	Female	0 - 96 h	77.2	43.4 (37.4)	4.8 (4.1)	32.2 (27.6)	9.4 (8.1)
	Male (BDC)	0 - 48 h	33.4	77.9 (33.4)	ND	ND	ND
Bile	Male (BDC)	0 - 72 h	37.1	41.8 (17.5)	5.3 (2.1)	34.9 (13.8)	9.5 (3.7)

Additional Information: BDC = Bile duct-cannulated; NA = not applicable; ND = not detected
Following oral administration of [14C]GSK1278863 at 10 mg/kg, the only radiolabeled component detected in male and female rat plasma and liver was unchanged GSK1278863.
Elimination of GSK1278863 in intact rats occurred largely as oxidative metabolites and unchanged parent in faeces.
In bile duct-cannulated rats, unchanged parent (18% of dose) and 3 oxidative metabolites (together 20% of dose) were eliminated via the bile.
The metabolite profiles observed were similar in male and female animals.
a: pooled sample

Table 10.9 Pharmacokinetics: Metabolites in Urine, Faeces and Bile from Bile Duct-Cannulated Dogs

Test Article: [14C]GSK1278863

Report No.: CD2008/01662 **Location in CTD:** m4.2.2.4 **Study No.:** 07DMM160

Species:	**Dog**
Gender (M/F)/Number of Animals:	(Intact) 3M/3F, (Bile duct-cannulated) 2M
Feeding Condition:	Fasted
Vehicle/Formulation:	1% methylcellulose/Suspension
Method of Administration:	Single Oral (gavage)
Dose (mg/kg):	20
Radionuclide:	14C
Specific Activity:	1.92 μCi/mg

Results:

Sample	Gender	Sampling Time or Period	% of Dose in Sample	Mean % of Compound in Sample (Mean % of Dose)		
				Parent	M8	M9
Urine[a]	Male	0 - 12 h	2.71	96.47 (2.61)	1.70 (0.05)	1.79 (0.05)
	Female	0 - 12 h	2.23	90.08 (2.16)	1.20 (0.03)	1.51 (0.04)
Faeces	Male	0 - 48 or 72 h, 24 - 72 h	84.12	98.09 (84.12)	ND	ND
	Female	0 - 48 or 72 h	78.64	97.93 (78.64)	ND	ND
	Male (BDC)	0 - 48 h	73.89	97.07 (73.89)	ND	ND
Bile	Male (BDC)	0 - 12 h or 12 - 24 h	5.97	93.22 (5.97)	ND	ND

Additional Information: BDC =Bile duct-cannulated; NA = not applicable; ND = not detected

Following oral administration of [14C]GSK1278863, the only radiolabeled component detected in male and female dog plasma was unchanged GSK1278863. (Approximately equal volumes of plasma were pooled to give one representative sample per sex for each time point (1, 4, 10 and 24 hours)).

Elimination of GSK1278863 in dogs occurred largely as parent in faeces. After absorption, elimination in bile was largely as unchanged parent (6% dose). Urinary excretion was low and predominantly consisted of unchanged parent. The metabolite profiles observed were similar in male and female animals.

a: n = 1

Table 10.10 Pharmacokinetics: Metabolites from Isolated Perfused Rat Liver

Test Article: [14C]GSK1278863, GSK1278863

Location in CTD: m4.2.2.4
Report No.: CD2008/00941 **Study No.:** 07DMM151

Study System: In situ isolated perfused rat liver model
Species (Strain): Rat (Sprague Dawley)
Gender (M/F)/Number of Animals: 3M
Feeding Condition: NA
Vehicle/Formulation: Perfusate/Solution
Method of Administration: Perfusion
Dose (mg/kg): 30
Radionuclide: [14C]
Specific Activity: 136 μCi/mg
Results:

[14C]GSK1278863-Related Metabolites Identified in Bile and Plasma[c]

	Parent	M2	M8	M9	M10
Retention time[a] (min)	52.9	19.3	34.4	35.7	38.5
Proposed structure[b]	NA	Di-oxygenation	Mono-oxygenation	Mono-oxygenation	Mono-oxygenation
Bile	✓	✓	✓	✓	✓
Plasma	✓	ND	ND	ND	ND

Additional Information: NA = not applicable; ND = not detected; x = detected
Liver perfusion continued for 4 hours after dosing. Bile samples were analysed for definitive structural identification of metabolites.
GSK1278863 was the predominant component observed in isolated perfused rat livers following a nominal dose of 30 mg/kg [14C]GSK1278863. In addition to parent, three mono-oxygenated and one di-oxygenated metabolite were detected.
a: Retention time from IPRL (isolated perfused rat liver) bile
b: The cyclohexyl groups appear coincident in the NMR data (obtained in deuterated methanol) due to tautomerism.
c: The circulating metabolites were characterized in intact male rats following a single oral administration of [14C]GSK1278863 at 10 mg/kg (in the same study).

Table 10.11 Pharmacokinetics: Stereoisomeric Conversion

Test Article: GSK1278863, M2 (GSK2391220), M3 (GSK2506104) and M13 (GSK2531401) (Mouse), GSK1278863 (Human)

Location in CTD: m4.2.2.4

Report No.: 2014N221116 **Study No.:** 14DMM007

Sample:	Human plasma samples (1, 3 and 8 hour samples for subject 10, and 1 hour sample for subject 1) were transferred from a clinical study (PPO116097 [2014N19471100]). Mouse plasma (acidified) samples (0.5 hour samples from animals 3061, 3062, 3063, 3561, 3562 and 3563) were transferred from a 13-week toxicity study in the mouse (M70456G [2014N19903100]).
Gender (M/F)/Number of Animals:	3M/3F (Mouse), 2 (Human, Gender is unknown)
Feeding Condition:	- (Mouse), - (Human)
Vehicle/Formulation:	Mouse: 1% methylcellulose (400cP @ 2%) in water/Suspension (Oral), Aqueous 25 mM Phosphate buffer/Solution (SC) Human: -
Method of Administration:	Mouse: Oral (gavage) (GSK1278863), SC (M2, M3, M13); Human: Oral
Dose (mg/kg):	Mouse: 20 mg/kg/day (GSK1278863) and 2.0, 2.5 and 1.3 mg/kg/day (M2, M3 and M13), 4 weeks Human: -
Radionuclide:	NA
Specific Activity:	NA
Methods:	Human plasma samples were analyzed for M2, M3, M4, M5, M6 and M13 metabolites using an analytical method based on solid phase extraction followed by UHPLC/MS/MS analysis. Metabolites M3 and M13 were isolated by fraction collection and re-analyzed using UPC2-MS/MS for separation of stereoisomeric forms. Mouse plasma (acidified) samples were analyzed for stereoisomeric forms of M3 and M13 metabolites using an analytical method based on protein precipitation followed by UPC2-MS/MS analysis.

Pharmacokinetics: Stereoisomeric Conversion (Continued)

Results:

Sample		Sampling Time (h)	GSK2506104 Peak Area	GSK2531403 Peak Area	%GSK2531403[a]
Human M3	Subject 10	1	358386	22404	6.25
	Subject 10	3	854085	54279	6.36
	Subject 10	8	662158	42872	6.47
	Subject 1	1	2150213	141899	6.60
Mouse M3	Animal 3061	0.5	2681591	NQ	NA
	Animal 3062	0.5	5254332	NQ	NA
	Animal 3063	0.5	4250135	NQ	NA
	Animal 3561	0.5	4983992	NQ	NA
	Animal 3562	0.5	5298161	NQ	NA
	Animal 3563	0.5	6592205	NQ	NA
			GSK2531401 Peak Area	GSK2531400 Peak Area	%GSK2531400[a]
Human M13	Subject 10	1	32609	NQ	NA
	Subject 10	3	95248	NQ	NA
	Subject 10	8	73470	NQ	NA
	Subject 1	1	87672	NQ	NA
Mouse M13	Animal 3061	0.5	1085766	NQ	NA
	Animal 3062	0.5	1983791	NQ	NA
	Animal 3063	0.5	1354233	NQ	NA
	Animal 3561	0.5	1591404	NQ	NA
	Animal 3562	0.5	1635528	NQ	NA
	Animal 3563	0.5	2245499	NQ	NA

Additional Information: - = unknown; NA = not applicable; NQ = non quantifiable; UHPLC/MS/MS = Ultra high performance liquid chromatography-tandem mass spectrometry; UPC2-MS/MS = Ultra performance convergence chromatography-tandem mass spectrometry

GSK2531403 is a stereoisomer of M3 (GSK2506104). GSK2531400 is a stereoisomer of M13 (GSK2531401).

The predominant circulating stereoisomers of the major human metabolites M3 and M13 are GSK2506104 and GSK2531401, respectively. Furthermore, there was no change in the peak area ratio of the two M3 stereoisomers over the time course, which suggests a lack of stereoisomeric interconversion in human plasma. Analysis of mouse plasma samples following dosing with GSK1278863 and metabolites M2, M3 (GSK2506104) and M13 (GSK2531401) showed the presence of only the dosed stereoisomers. There were no detectable amounts of GSK2531403 and GSK2531400 in mouse plasma, which is indicative of no stereoisomeric conversion of the dosed stereoisomers.

a: Peak area ratio of GSK2531403 or GSK2531400 to GSK2506104 expressed as percentage.

11. PHARMACOKINETICS: METABOLISM IN VITRO

Table 11.1 Blood Stability of Daprodustat

Test Article: GSK1278863

Location in CTD: m4.2.2.4

Report No.: UH2008/00002 **Study No.:** 07CDUP0349

Target Entity, Test System and Method: The in vitro blood stability of daprodustat was determined in human blood at 1 or 10 μg/mL (0.5% dimethylsulfoxide in blood at 37°c) and incubated for up to 2 hours (sampled at 0.25, 0.5, 1 and 2 hours), with analysis for daprodustat by LC/MS/MS

Results:

Time (minutes)	Blood (1.0 μg/mL)[a]		Blood (10.0 μg/mL)[a]	
	Recovery (%)	CV (%)	Recovery (%)	CV (%)
0	100.0	NA	100.0	NA
15	74.8	15.0	97.8	5.0
30	81.1	13.1	105.3	5.9
60	80.7	12.3	98.8	6.8
120	85.8	10.8	104.3	9.0

Key:

a = Nominal concentration of GSK1278863 in human blood.

Table 11.2 Pharmacokinetics: Clearance in Liver Microsomes and Hepatocytes

Test Article: GSK1278863

Location in CTD: m4.2.2.4

Report No.: UH2008/00018 **Study No.:** 06MCD1510, 06MCD1511, 06MCD1564, 06MCD1585, 06MCD1595

Target Entity, Test System and Method: In vitro metabolism in liver microsomes and hepatocytes

In Vitro rate constants and CLi were determined following incubation of GSK1278863 in Mouse, Rat, Dog, Monkey and Human liver microsomes and hepatocytes.

Results:

Species	Matrices	Rate Constant (min^{-1})	CLi (mL/min/g liver)
Mouse	Microsomes	-0.0017	<0.5
	Hepatocytes	0.0003	<0.5
Rat	Microsomes	-0.0031	<0.5
	Hepatocytes	0.0008	0.5
Dog	Microsomes	-0.0012	<0.5
	Hepatocytes	-7.4^{e-05}	<0.5
Monkey	Microsomes	-0.0026	<0.5
	Hepatocytes	0.0008	0.5
Human	Microsomes	0.0020	<0.5
	Hepatocytes	0.0018	1.1

Table 11.3 Pharmacokinetics: Identification of Metabolites in CYP and Hepatocytes

Test Article: GSK1278863
Location in CTD: m4.2.2.4
Report No.: UH2008/00018 **Study No.:** 06MCD1512, 06MCD1565, 06MCD1586, 06MCD1596, 07CDUP0362
Target Entity, Test System and Method: Identification of metabolites in human CYP enzymes and hepatocytes
Metabolites of GSK1278863 were detected following incubations in Mouse, Rat, Dog, Monkey and Human hepatocytes and rh-CYP450 isozymes.
Results:

		Hepatocytes					Human CYP isozymes		
		Mouse	Rat	Dog	Monkey	Human	2C9	2D6	3A4
PC1	Ketone formation	ND	✓	ND	ND	ND	ND	ND	ND
PC2	Ketone formation	ND	✓	ND	✓	✓	ND	ND	ND
PC3	Mono-oxygenation	✓	✓	✓	✓	✓	✓	✓	✓
PC4	Mono-oxygenation	✓	✓	✓	✓	✓	✓	✓	✓
PC5	Mono-oxygenation	✓	✓	✓	✓	✓	✓	✓	✓
PC6	Ketone formation and mono-oxygenation	ND	ND	ND	ND	✓	ND	ND	ND
PC7	Ketone formation and mono-oxygenation	ND	ND	ND	ND	✓	ND	ND	ND
PC8	Ketone formation and mono-oxygenation	ND	ND	ND	ND	✓	ND	ND	ND
PC9	Di-oxygenation	✓	✓	ND	✓	✓	ND	ND	ND
PC10	Di-oxygenation	ND	ND	ND	✓	✓	ND	ND	ND
PC11	Di-oxygenation	ND	ND	ND	✓	✓	ND	ND	ND
PC12	Di-oxygenation	ND	ND	ND	✓	✓	ND	ND	ND
PC13	Di-oxygenation	ND	ND	ND	✓	✓	ND	ND	ND
PC14	Di-oxygenation	ND	ND	ND	✓	✓	ND	ND	ND
PC15	Tri-oxygenation	ND	ND	ND	✓	✓	ND	ND	ND
PC16	Glucuronidation	ND	ND	ND	✓	ND	ND	ND	ND
PC17	Glucuronidation	ND	ND	ND	✓	ND	ND	ND	ND
PC18	Mono-oxygenation and glucuronidation	ND	✓	ND	✓	ND	ND	ND	ND
PC19	Mono-oxygenation and glucuronidation	ND	✓	ND	ND	ND	ND	ND	ND

ND = Not Detected

Table 11.4 Pharmacokinetics: Identification of Metabolites in Animal and Human Hepatocytes

Test Article: [14C]GSK1278863 or GSK1278863

Location in CTD: m4.2.2.4

Report No.:	CD2008/01286	2011N125815	2013N160562
Study No.:	07DMM139	11DMM021	12DMM025
Target Entity, Test System and Method:	[14C]GSK1278863 was incubated at a concentration of 12.5 μM in the presence of male CD-1 mouse, Sprague Dawley rat, Beagle dog, Cynomolgus monkey and human (1M/2F) and female Dutch Belted rabbit hepatocytes for up to 24 hours.	GSK1278863 was incubated at a concentration of 19 μM in the presence of male Gottengen minipig hepatocytes for 24 hours.	GSK1278863 was incubated at a concentration of 20 μM in the presence of male Syrian hamster hepatocytes (1 x 106 cells/mL) for 24 hours.

Results:

Parent and Metabolites	RT[a] (min)	Proposed structure	Human	Mouse	Rat	Rabbit	Dog	Monkey	Minipig	Hamster
Parent (P)	53.3	GSK1278863	✓	✓	✓	✓	✓	✓	✓	✓
M1	18.7	Di-oxygenation	✓	ND	ND	✓	ND	✓	ND	ND
M2	19.4	Di-oxygenation	✓	ND	NR	✓	ND	✓	✓	ND
M3	21.3	Di-oxygenation	✓	ND	ND	✓	ND	✓	✓	ND
M4	22.2	Di-oxygenation	✓	ND	ND	✓	ND	✓	✓	ND
M5	22.9	Di-oxygenation	✓	ND	ND	ND	ND	✓	ND	ND
M6	23.9	Di-oxygenation	✓	ND	ND	ND	ND	✓	ND	ND
M7	25.2	Di-oxygenation	✓	ND	ND	ND	ND	✓	ND	ND
M8	34.5	Mono-oxygenation	✓	✓	✓	✓	ND	✓	✓	✓
M9[b]	35.7	Mono-oxygenation	✓	ND	✓	✓	ND	✓	✓	✓
M10	38.5	Mono-oxygenation	✓	ND	ND	✓	ND	✓	✓	✓
M12	44.0	Undefined glucuronide	ND	ND	ND	ND	ND	✓	ND	ND
M21	-	Di-oxygenation	ND	ND	ND	ND	ND	ND	✓	ND
M23	-	Di-oxygenation	ND	ND	ND	ND	ND	ND	✓	ND
M24	-	Di-oxygenation	ND	ND	ND	ND	ND	ND	✓	ND

Additional Information: ✓ = detected; ND = not detected; SD = Sprague-Dawley

In human hepatocytes, the notable routes of metabolism were mono- and di-oxygenation. In general, metabolite profiles in hepatocytes of nonclinical species (excluding dog) and human were qualitatively similar; however, only the monkey hepatocytes produced all primary human metabolites.

All GSK1278863 biotransformations identified in minipig and hamster hepatocyte incubates were oxygenation products similar to findings in human, monkey and rabbit hepatocytes [CD2008/01286]. Two new di-oxygenation products (M23 and M24) detected in minipig hepatocyte incubates are stereo- or regioisomers of previously identified metabolites. All metabolites detected in minipig and hamster hepatocytes were detected in human urine and plasma [2010N109720] with the exception of the new di-oxygenation products detected in minipig.

a: RT (Retention time) from human hepatocytes except M12 which was from monkey hepatocytes.

b: M9 and M22 are distinguishable by their MS3 spectra. They have the same retention time, accurate mass, and MS/MS. The observed MS3 finger print indicates the presence of a mixture of M9 and M22. (MS3 = 3-stage mass spectrometry; MS/MS = tandem mass spectrometry)

Table 11.5 Pharmacokinetics: Identification of Metabolites in Animal and Human Hepatocytes

Test Article: [14C]GSK1278863
Location in CTD: m4.2.2.4
Report No.: 2011N114076 **Study No.:** 10DMM007
Target Entity, Test System and Method: [^{14}C]GSK1278863 was incubated in vitro using conditions similar to those used in the microbial mutagenicity assay (AMES) and the mouse lymphoma assay (MLA). The in vitro incubations were performed using Aroclor 1254 induced rat liver S9 fraction (S9). To mimic conditions used during the AMES assay for GSK1278863, [^{14}C]GSK1278863 was incubated at 4, 40 and 400 μM GSK1278863 for 3 hours at 37°C in sodium phosphate buffer in 1.6% S9 in the presence of oxidative co-factor. To mimic conditions used during the MLA assay for GSK1278863, [^{14}C]GSK1278863 was incubated at 25 and 180 μM for 3 hours at 37°C in RPMI culture media in 2% S9 in the presence of oxidative co-factor. The reaction was terminated by adding an equal volume of acetonitrile/ethanol/acetic acid (80:20:1). The incubation mixture was centrifuged, and the resulting supernatant was analyzed by radio-HPLC.
Results: Following in vitro incubation of [^{14}C]GSK1278863 with Aroclor-1254 induced rat S9 fraction, under conditions similar to those used during the AMES and MLA assays for GSK1278863, unchanged GSK1278863 was the only detectable radio-component, and no metabolites were observed in the radio-chromatograms. Due to the lack of metabolism, HPLC-MSn analysis was not conducted.

Table 11.6 Pharmacokinetics: Metabolite Formation by Human Liver Microsomes and CYP Enzyme

Test Article: [14C]GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2010/00300 **Study No.:** 09DMM051

Target Entity, Test System and Method: [14C]GSK1278863 and metabolites in human liver microsomes and recombinant CYP enzymes were investigated.

Test Concentration: [14C]GSK1278863 at 5 μM

Results:

ID	MS RTa (min.)	Proposed structure	CYP2C8	CYP3A4	HLM Azamulin	HLM Montelukast	HLM NIC	HLM NCF
Parent	49.7	GSK1278863	✓	✓	✓	✓	✓	✓
M2	18.7	Di-oxygenation	✓	ND	ND	ND	ND	ND
M3	20.4	Di-oxygenation	✓	ND	ND	ND	ND	ND
M4	21.3	Di-oxygenation	✓	ND	ND	ND	ND	ND
M5	21.9	Di-oxygenation	✓	ND	ND	ND	ND	ND
M6	22.7	Di-oxygenation	✓	ND	ND	ND	ND	ND
M7	23.9	Di-oxygenation	✓	ND	ND	ND	ND	ND
M8	32.8	Mono-oxygenation	✓	✓	✓	✓	✓	ND
M9 and M22[b]	33.7	Mono-oxygenation	✓	✓	✓	✓	✓	ND
M10	36.2	Mono-oxygenation	✓	ND	✓	✓	✓	ND
M17	37.3	Keton-formation	✓	ND	ND	ND	ND	ND

Key:

✓ = detected by radio and MS, NA = no associated significant radio peak

a: RT from 2C8

b: M9 and M22 are distinguishable by the MS3 spectrum. They have the same retention time, accurate mass, and MS/MS. The MS3 finger print indicates the presence of a mixture of M9 and M22. The ratio of M9 to M22 cannot be determined because no standard for M22 is available.

NIC = No inhibitor control.

NCF = No cofactor control.

Table 11.7 Pharmacokinetics: Metabolite identification Formation by Human Liver Microsomes and CYP Enzyme using [^{14}C]Daprodustat

Test Article: [14C]GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2010/00300 **Study No.:** 09DMM051

Target Entity, Test System and Method: Inhibition of [14C]GSK1278863 Metabolite Formation in Human Liver Microsomes in the Presence of Selective CYP Inhibitors were investigated.

Test Concentration: [14C]GSK1278863 at 5 μM

Target Entity, Test System and Method: Inhibition of [14C]GSK1278863 Metabolite Formation in Human Liver Microsomes

Inhibition of [14C]GSK1278863 Metabolite Formation in Human Liver Microsomes in the Presence of Selective CYP Inhibitors were investigated.

Results:

	Mean % Inhibition			
Inhibitor	M8	M9 and M22	M10	X[a]
Furafylline (CYP1A2)	4.2	0.0	0.0	0.0
Montelukast (CYP2C8)	45	38	43	33
Sulphaphenazole (CYP2C9)	1.5	0.0	0.0	2.5
Benzylnirvanol (CYP2C19)	4.4	0.0	0.0	ND[b]
Quinidine (CYP2D6)	7.4	0.0	0.0	ND*
Azamulin (CYP3A4)	0.0	17	14	0.0

a: This peak (labeled metabolite X) was not detected by LC/MS and therefore was not structurally characterized, but its rate of inhibition was calculated

b: ND: peaks were not detected in chromatogram

Table 11.8 Pharmacokinetics: Oxidative Bioactivation

Test Article: [14C]GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2007/01464 **Study No.:** 07DMM137

Target Entity, Test System and Method: The potential of GSK1278863 for oxidative bioactivation was assessed in vitro following incubation of [^{14}C]GSK1278863 (10 μM) with human liver microsomes in the presence and absence of NADPH.

Results:

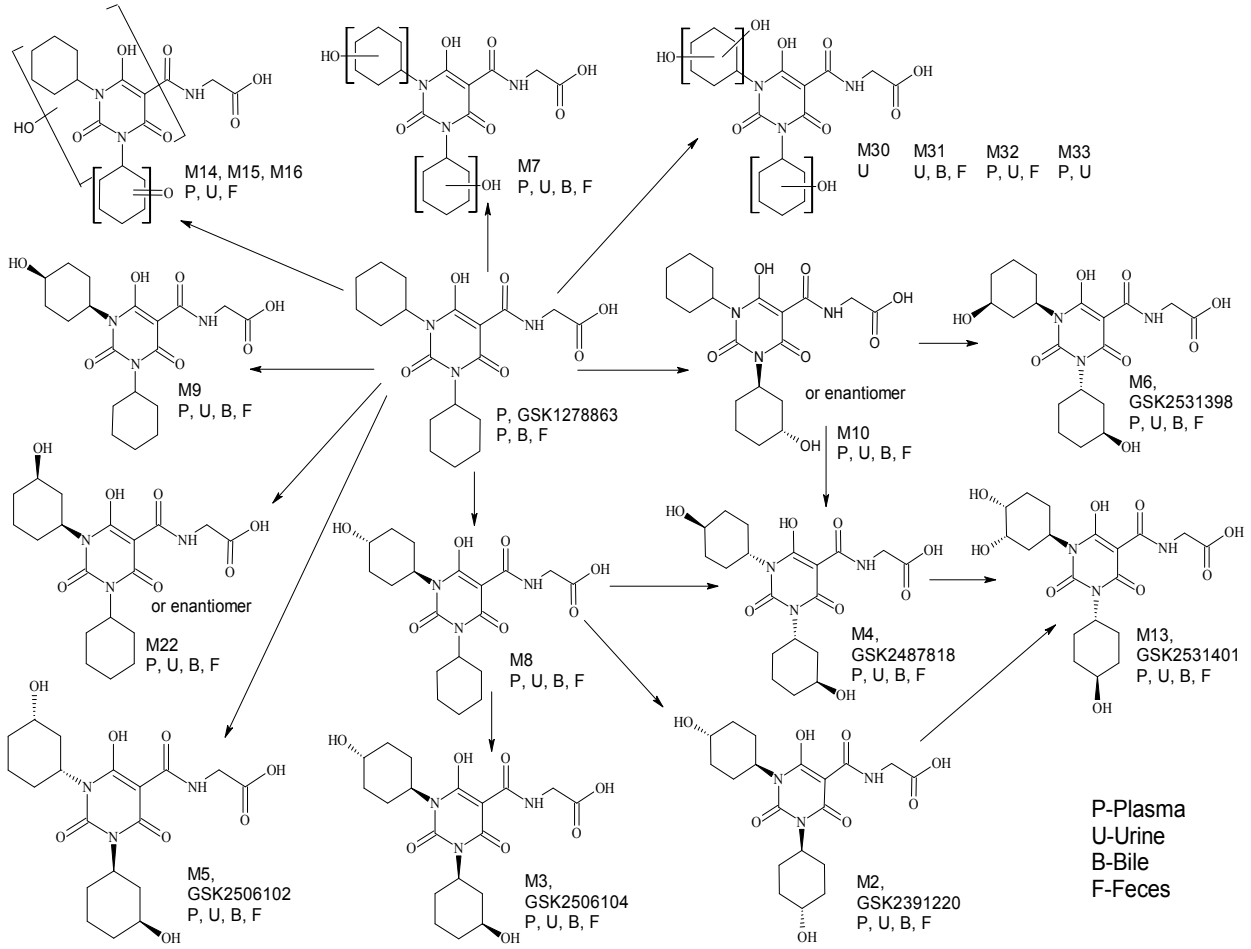
	Binding in the absence of NADPH (pmol/mg)		Binding in the presence of NADPH (pmol/mg)		NADPH-Dependent binding (pmol/mg)	
Incubation Time (min)	30	60	30	60	30	60
GSK1278863	0	1.74 ± 1.05	7.99 ± 8.17	0	7.99	0
Acetaminophen Control	NA[a]	1.50 ± 5.65	NA[a]	212 ± 14.7	NA[a]	210

Mean ± SD (n=3)

a: Acetaminophen control incubations were performed for 60 minutes only.

12. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

Table 12.1 Pharmacokinetics: Possible Metabolic Pathways



13. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLISING ENZYMES

Table 13.1 Induction of Drug Metabolising Enzymes

Test Article: GSK1278863

Location in CTD: m4.2.2.4

Report No.: CD2008/01088 **Study No.:** 08MM042

Target Entity, Test System and Method: In vitro induction of CYP mRNA levels by quantitative reverse transcriptase polymerase chain reaction using human hepatocytes. The effects of GSK1278863 on the messenger RNA (mRNA) levels of cytochrome P450 (CYP) genes (CYP1A2, 2B6 and 3A4) were evaluated at concentrations from 0.1 to 100 μM in cultured human hepatocytes from three donors. After 48-hours incubation, the mRNA level for each specific CYP was determined using quantitative real-time polymerase chain reaction technology (TaqMan™).

Results:

		Effect on mRNA Level (Mean ratio of treated over control)		
Treatment	Concentration (uM)	CYP1A2[a] Ratio	CYP2B6[b] Ratio	CYP3A4[b] Ratio
GSK1278863	100	0.40	0.98	0.34
	30	0.63	0.68	0.36
	10	0.77	0.85	0.42
	3	0.67	0.82	0.33
	1	0.81	0.83	0.39
	0.3	0.73	0.85	0.33
	0.1	0.75	1.0	0.43
Prototypical Inducer[c]	50 μM Omeprazole, 50 μM Phenytoin, 10 μM Rifampicin[d]	240	20	53

Additional Information: Mean ratio (n = 3)

Controls are defined as 0.1% (v/v) DMSO. Data presented to 2 significant figures.

Following exposure of cultured human hepatocytes to GSK1278863 for 48 hours, no notable increases in the mean mRNA levels of CYP1A2, CYP2B6 or CYP3A4 were observed.

a: Increase in mRNA level of ≥5 mean ratio of treated over control is considered as a notable induction response for both omeprazole and GSK test compound.

b: Increase in mRNA level of ≥3 mean ratio of treated over control is considered as a notable induction response for phenytoin and rifampicin. Increase in mRNA level of ≥2 mean ratio of treated over control is considered as a notable induction response for GSK test compound.

c: When a notable induction is observed, the induction response is also reported as a percentage induction response of the appropriate prototypic inducer.

d: Prototypical CYP Inducers: 50 μM Omeprazole (CYP1A2), 50 μM Phenytoin (CYP2B6), 10 μM Rifampicin (CYP3A4).

Table 13.2 Daprodustat: Inhibition of Drug Metabolising Enzymes

Test Article: GSK1278863

Location in CTD: m4.2.2.4

Report No.: UH2008/00018 **Study No.:** 07CDUP0390

Target Entity, Test System and Method: In vitro CYP Inhibition in Human Liver Microsomes

IC50 Values for GSK1278863 was calculated in Concentration-Dependent Cytochrome P450 Inhibition Assays in Human Liver Microsomes

Results:

P450 enzymes	1A2 phenacetin	2C9 diclofenac	2C19 S-mephenytoin	2D6 bufuralol	3A4 midazolam	3A4 nifedipine	3A4 atorvastatin
P450 IC50 values (μM)	>100	>100	>100	>100	>100	>100	>100

Test Article: GSK1278863

Location in CTD: 4.2.2.4

Report No.: UH2008/00018 **Study No.:** 07CDUP0403, 07CDUP0518,07CDUP0629

Target Entity, Test System and Method: In vitro CYP Inhibition in Human Liver Microsomes

IC50 Values and Fold Changes for GSK1278863 was calculated in Metabolism-Dependent Cytochrome P450 Inhibition Assays in Human Liver Microsomes

Results:

Enzyme (substrate)	Test compound	IC50, μM – NADPH pre- incubation	IC50, μM + NADPH pre-incubation	IC50 fold decrease
CYP1A2	GSK1278863	>33	29	No change
(phenacetin)	Furafylline	1.9	0.08	24
CYP2C9	GSK1278863	>33	>33	No change
(diclofenac)	Tienilic acid	3.1	0.55	5.6
CYP2C19	GSK1278863	>100	>100	No change
(S-mephenytoin)	Ticlopidine	0.45	0.09	5.0
CYP2D6	GSK1278863	>33	>33	No change
(bufuralol)	Paroxetine	1.02	0.13	7.8
CYP3A4	GSK1278863	>100	>100	No change
(Nifedipine)	Troleandomycin	39	15	2.6

Table 13.3 Daprodustat: Inhibition of Drug Metabolising Enzymes

Test Article: GSK1278863

Location in CTD: m4.2.2.4
Report No.: CD2008/01013 **Study No.:** 07DMM149

Target Entity, Test System and Method: In vitro CYP Inhibition in Human Liver Microsomes
Cryopreserved human liver microsomes from a pooled sample of 15 donors were used to assess the ability of GSK1278863 to inhibit major human CYP isoforms. Two sets of incubations performed in duplicate at each concentration (0, 0.1, 0.33, 1, 3.3, 10, 33, 100 μM) were prepared ('co-factor-pre-incubation' and 'control pre-incubation'). All samples were incubated at 37°C in a shaking water bath. To examine metabolism-dependent inhibition, the 'co-factor-pre-incubations' were performed by incubating GSK1278863 and microsomes with a NADPH regenerating system for 20 minutes prior to initiation of reaction by addition of probe substrate. To examine the direct inhibition, the 'control pre-incubations' were performed for 20 minutes with GSK1278863, microsomes and probe substrate and the reaction initiated by the addition a NADPH regenerating system. Positive control incubations (replacing GSK1278863 with an appropriate concentration of a known cytochrome P450 inhibitor) and control incubations without inhibitor (containing 2% v/v solvent only) were also performed. Reactions were terminated after 5 or 10 minutes by the addition of 250 μL acetonitrile. Incubations were analysed by LC-MS/MS.
Results:

CYP	Substrate	Direct Inhibition IC50 (μM)	Metabolism-Dependent Inhibition		
			Control pre-inc[a] IC50 (μM)	NADPH pre-inc[b] IC50 (μM)	Fold Change in IC50
1A2	Phenacetin	>100	>100	>100	1.0
2A6	Coumarin	>100	>100	>100	1.0
2B6	Bupropion	>100	>100	>100	1.0
2C8	Rosiglitazone	21	25	26	0.96
2C9	Diclofenac	>100	>100	>100	1.0
2C19	S-mephenytoin	>100	>100	>100	1.0
2D6	Bufuralol	>100	>100	>100	1.0
3A4	Atorvastatin	>100	>100	>100	1.0
3A4	Midazolam	>100	>100	>100	1.0
3A4	Nifedipine	>100	>100	>100	1.0

Additional Information: NADPH solution = NADPH regenerating system (1.7 mg NADP, 7.8 mg glucose-6-phosphate and 6 units of glucose-6-phosphate dehydrogenase per mL of 2% w/v sodium bicarbonate); LC-MS/MS = liquid chromatography-tandem mass spectrometry
GSK1278863 inhibited CYP2C8 with a calculated IC50 value of 21 μM, in human liver microsomes. There was no metabolism-dependent inhibition of CYP2C8 in human liver microsomes.
a: Microsomes, buffer and GSK1278863 pre-incubated for 20 minutes with probe substrate prior to initiation of reaction with NADPH.
b: Microsomes, buffer and GSK1278863 pre-incubated for 20 minutes with NADPH prior to initiation of reaction with probe substrate.

Table 13.4 Metabolites: Inhibition of Drug Metabolising Enzymes

Test Article: GSK2391220, GSK2531403, GSK2487818, GSK2506102, GSK2531398 and GSK2531401

Location in CTD: m4.2.2.4

Report No.: 2013N167801 **Study No.:** 12DMM032

Target Entity, Test System and Method: In vitro CYP Inhibition in Human Recombinant CYP Enzyme using Fluorescence Measurement System
A range of concentrations of GSK2391220, GSK2531403, GSK2487818, GSK2506102, GSK2531398 and GSK2531401 in methanol were pre-incubated in duplicate at 37°C for 5 minutes in 50 mM potassium phosphate buffer (pH 7.4) containing 0.1 mg/mL recombinant human CYP450 microsomal protein (Cypex) and the appropriate probe substrate. Following pre-incubation, 25 μL of an NADPH generating system (7.8 mg glucose-6-phosphate, 1.7 mg NADP and 6 units glucose-6-phosphate dehydrogenase/mL of 2% w/v NaHCO3) was added to each well to start the reaction. Production of fluorescent metabolite was measured over 30 minute using a Cytofluor (Applied Biosciences) plate reader. Appropriate negative and positive control incubations (replacing GSK compounds with an appropriate concentration range of a time-dependent CYP inhibitor) and control incubations without inhibitor (containing 2% v/v methanol only) were also performed.

Results:

Recombinant CYP	Compound	Final Concentration (μM)	Time-Dependent Inhibition		
			Initial 1-5 min Direct IC50 (μM)	Final 16-20 min IC50 (μM)	Fold Change in IC50
CYP1A2	GSK2487818	0.1-100 μM	>100	>100[b]	No change
	GSK2531401	0.1-100 μM	>100	>100[b]	No change
	GSK2531403	0.1-100 μM	>100	>100[b]	No change
	GSK2391220	0.1-100 μM	>100	>100[b]	No change
	GSK2531398	0.1-100 μM	>100	>100[b]	No change
	GSK2506102	0.1-100 μM	>100	>100[b]	No change
	Fluconazole (-) control	0.01-10 μM	>10	>10[b]	No change
	Furafylline[a]	0.01-10 μM	>10	5.1[b]	>2.0
CYP2C8	GSK2487818	0.1-100 μM	>100	10	>10
	GSK2531401	0.1-100 μM	>100	>100	No change
	GSK2531403	0.1-100 μM	>100	>100	No change
	GSK2391220	0.1-100 μM	>100	>100	No change
	GSK2531398	0.1-100 μM	>100	>100	No change
	GSK2506102	0.1-100 μM	>100	>100	No change
	Fluconazole (-) control	0.01-10 μM	>10	>10	No change
	Phenelzine[a]	0.01-10 μM	>10	0.2	>81

Metabolites: Inhibition of Drug Metabolising Enzymes (Continued)

Recombinant CYP	Compound	Final Concentration (μM)	Time-Dependent Inhibition		
			Initial 1-5 min Direct IC50 (μM)	Final 26-30 min IC50 (μM)	Fold Change in IC50
CYP3A4 (DEF as Substrate)	GSK2487818	0.1-100 μM	>100	35[b]	>2.9
	GSK2531401	0.1-100 μM	>100	>100[b]	No change
	GSK2531403	0.1-100 μM	>100	>100[b]	No change
	GSK2391220	0.1-100 μM	>100	>100[b]	No change
	GSK2531398	0.1-100 μM	>100	>100[b]	No change
	GSK2506102	0.1-100 μM	>100	>100[b]	No change
	Fluconazole (-) control	0.01-10 μM	6.2	>10[b]	No change
	Troleandomycin[a]	0.01-10 μM	0.58	0.16[b]	3.6
CYP3A4 (7-BQ as Substrate)	GSK2487818	0.1-100 μM	>100	>100	No change
	GSK2531401	0.1-100 μM	>100	>100	No change
	GSK2531403	0.1-100 μM	>100	>100	No change
	GSK2391220	0.1-100 μM	>100	>100	No change
	GSK2531398	0.1-100 μM	>100	>100	No change
	GSK2506102	0.1-100 μM	>100	>100	No change
	Fluconazole (-) control	0.01-10 μM	>10	>10	No change
	Troleandomycin[a]	0.01-10 μM	9.8	1.8	5.4

Metabolites: Inhibition of Drug Metabolising Enzymes (Continued)

Recombinant CYP	Compound	Final Concentration (μM)	Time-Dependent Inhibition		
			Initial 1-5 min Direct IC50 (μM)	Final 26-30 min IC50 (μM)	Fold Change in IC50
CYP2C9	GSK2487818	0.1-100 μM	>100	>100	No change
	GSK2531401	0.1-100 μM	>100	>100	No change
	GSK2531403	0.1-100 μM	>100	>100	No change
	GSK2391220	0.1-100 μM	>100	>100	No change
	GSK2531398	0.1-100 μM	>100	>100	No change
	GSK2506102	0.1-100 μM	>100	>100	No change
	Fluconazole (-) control	0.01-10 μM	>10	>10	No change
	Tienilic Acid[a]	0.01-10 μM	1.8	0.68	2.6
CYP2C19	GSK2487818	0.1-100 μM	>100	1.3[b]	>77
	GSK2531401	0.1-100 μM	>100	>100[b]	No change
	GSK2531403	0.1-100 μM	>100	>100[b]	No change
	GSK2391220	0.1-100 μM	>100	>100[b]	No change
	GSK2531398	0.1-100 μM	>100	>100[b]	No change
	GSK2506102	0.1-100 μM	>10	>10[b]	No change
	Fluconazole (-) control	0.01-10 μM	9.8	>10[b]	No change
	Ticlodipine[a]	0.01-10 μM	0.35	0.10[b]	3.5

Additional Information: DEF = Diethoxyfluorescein; 7-BQ = 7-Benzyloxyquinoline

GSK2487818 was not a direct inhibitor of the CYP enzymes tested, but was a time-dependent inhibitor of CYP2C8, CYP2C19 and CYP3A4 (DEF) under these in vitro conditions.

a: Selective time-dependent inhibition positive control

b: Due to the lack of the linearity after 20 min of solvent control samples, 16-20 min IC50 values were reported as final IC50.

Table 13.5 GSK2506104: Metabolites: Inhibition of Drug Metabolising Enzymes

Pharmacokinetics: Inhibition of Drug Metabolising CYP450 Enzymes by M3 (GSK2506104)		**Test Articles:** GSK2506104
Report No: 2018N382188		**Study No.:** 180538
Study System:	Human Liver Microsomes	**Location in CTD**: m4.2.2.4
Concentration Range:	0.0729, 0.243, 0.81, 2.7, 9, 30, and 100 μM	
Method:	To determine the inhibitory potential of GSK2506104, two sets of incubations performed in duplicate at each concentration listed above were prepared ('reversible inhibition' and 'metabolism-dependent inhibition' incubations). All samples were incubated at 37°C on a heated Jitterbug™ Microplate Incubator Shaker/96-well plate. To examine metabolism-dependent inhibition, the 'co-factor-pre-incubations' were performed by incubating GSK2506104 and microsomes with NADPH for 30 minutes prior to initiation of reaction by addition of probe substrate. To examine the direct inhibition, the 'control pre-incubations' were performed for 30 minutes with GSK2506104 and microsomes prior to addition of probe substrate. The reaction is initiated by the addition of NADPH.	
Analysis:	LC-MS/MS	

Results:

Direct and Metabolism-Dependent Inhibition of Cytochrome P450 Enzymes by GSK2506104

CYP	Substrate	Reversible Inhibition IC_{50} (μM)[a]	Metabolism-dependent inhibition IC_{50} (μM)[b]	Fold Change in IC50[c]
1A2	Phenacetin	>100	>100	None
2B6	Efavirenz	>100	>100	None
2C8	Amodiaquine	>100	>100	None
2C9	Diclofenac	>100	>100	None
2C19	S-Mephenytoin	>100	>100	None
2D6	R-Bufuralol	>100	>100	None
3A4	Midazolam	>100	>100	None
3A4	Testosterone	>100	>100	None

Key:

a = Microsomes, buffer and compound pre-incubated for 30 minutes prior to initiation of reaction with probe substrate and NADPH.

b = Microsomes, buffer and compound pre-incubated for 30 minutes with NADPH prior to initiation of reaction with probe substrate.

c = Data obtained from direct inhibition assay conditions performed on a separate plate from control pre-incubation plate.

Table 13.6 GSK2487818: Metabolites: Inhibition of Drug Metabolising Enzymes

Test Article: M4 (GSK2487818)

Location in CTD: m4.2.2.4

Report No.: 2014N223000 **Study No.:** 13DMM003

Target Entity, Test System and Method: In vitro CYP Inhibition in Human Liver Microsomes

To determine the inhibitory potential of GSK2487818, two sets of incubations performed in duplicate at each concentration (0, 0.1, 0.33, 1, 3.3, 10, 33, 100 μM) were prepared ('co-factor-pre-incubation' and 'control pre-incubation'). All samples were incubated at 37°C in a shaking water bath. To examine metabolism-dependent inhibition, the 'co-factor-pre-incubations' were performed by incubating GSK2487818 and microsomes with a NADPH regenerating system for 20 minutes prior to initiation of reaction by addition of probe substrate. To examine the direct inhibition, the 'control pre-incubations' were performed for 20 minutes with GSK2487818, microsomes and probe substrate and the reaction initiated by the addition a NADPH regenerating system.

Results:

		Direct and Metabolism-Dependent Inhibition of Cytochrome P450 Enzymes by GSK2487818			Direct and Metabolism-Dependent Inhibition of Cytochrome P450 Enzymes by Positive Control Inhibitors			
CYP	Substrate	Direct/Control Pre-inc[a], IC50 (μM)	NADPH Pre-inc[b], IC50 (μM)	Fold change in IC50	Positive Control Inhibitor	Direct/Control Pre-inc[a], IC50 (μM)	NADPH Pre-inc[b], IC50 (μM)	Fold change in IC50
2C8	Rosiglitazone	>100	>100	1.0	Phenelzine	140	37	3.8
2C19	Mephenytoin	>100	>100	1.0	Ticlopidine	0.88	0.25	3.5
3A4	Atorvastatin	>100	>100	1.0	Troleandomycin	30	9.5	3.2
3A4	Midazolam	>100	>100	1.0	Troleandomycin	13	1.3	9.9
3A4	Nifedipine	>100	>100	1.0	Troleandomycin	24	3.2	7.5

Additional Information: NADPH regenerating system = 1.7 mg NADP, 7.8 mg glucose-6-phosphate and 6 units of glucose-6-phosphate dehydrogenase per mL of 2% w/v sodium bicarbonate

GSK2487818 was not a direct inhibitor of CYP2C8, 2C19 or 3A4 (atorvastatin, midazolam, nifedipine) over the concentration range tested (0.1 to 100 μM).

GSK2487818 did not show metabolism-dependent inhibition of CYP2C8, 2C19 or 3A4 (atorvastatin, midazolam, nifedipine) following pre-incubation with NADPH. a: Microsomes, buffer and compound pre-incubated for 20 minutes with probe substrate prior to initiation of reaction with NADPH.

b: Microsomes, buffer and compound pre-incubated for 20 minutes with NADPH prior to initiation of reaction with probe substrate.

14. PHARMACOKINETICS: EXCRETION

Table 14.1 Pharmacokinetics: Excretion

Test Article: [14C]GSK1278863 **Location in CTD:** m4.2.2.5

Report No.:	CD2008/00100	CD2008/00025	2018N355713
Study No.:	7717-705	AFA00607	8361764
Species:	Rat	Dog	Monkey
Gender (M/F)/Number of Animals:	15M15F	3M/3F	3M
Feeding Condition:	Fasted	Fasted	Fasted
Vehicle/Formulation:	1%(w/w) aqueous methylcellulose	1%(w/w) aqueous methylcellulose	1%(w/w) aqueous methylcellulose
Method of Administration:	Oral (gavage)	Oral (gavage)	Oral (gavage)
Dose (mg/kg):	10	20	10
Analyte:	[^{14}C]GSK1278863	[^{14}C]GSK1278863	[^{14}C]GSK1278863
Assay:	LSC	LSC	LSC

Pharmacokinetics: Excretion (Continued)

Results:

Collection Interval (h)	Mean Recovery of Radioactivity (Percent of Administered Dose)									
	Male rat		Female rat		Male dog		Female dog		Male monkey	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
0-8	-	-	-	-	-	-	-	-	4.9 ± 1.6	-
0-12	6.37 ± 1.15	-	5.12 ± 0.59	-	1.26 ± 1.37	-	1.42 ± 0.94	-	-	-
8-24	-	-	-	-	-	-	-	-	7.0 ± 1.3	-
12-24	1.92 ± 0.26	-	1.60 ± 0.16	-	0.20 ± 0.26	-	0.22 ± 0.09	-	-	-
0-24	-	59.7 ± 2.45	-	52.4 ± 3.55	-	51.33 ± 44.52	-	67.18 ± 5.77	-	44.3 ± 7.7
24-48	1.10 ± 0.38	15.3 ± 1.88	1.16 ± 0.11	24.3 ± 3.17	0.22 ± 0.14	32.62 ± 47.34	0.19 ± 0.12	11.33 ± 6.24	1.1 ± 0.4	27.9 ± 11.3
48-72	0.57 ± 0.04	5.91 ± 0.49	0.47 ± 0.01	6.35 ± 1.24	0.06 ± 0.04	1.85 ± 1.59	0.13 ± 0.16	1.93 ± 1.29	0.3 ± 0.2	4.6 ± 0.9
72-96	0.28 ± 0.05	3.17 ± 0.23	0.22 ± 0.03	2.92 ± 0.83	0.02 ± 0.00	0.38 ± 0.50	0.03 ± 0.02	0.73 ± 0.79	0.1 ± 0.1	0.6 ± 0.3
96-120	0.15 ± 0.02	1.29 ± 0.29	0.10 ± 0.02	0.92 ± 0.31	0.01 ± 0.01	0.11 ± 0.14	0.02 ± 0.02	0.16 ± 0.15	0.0	0.1 ± 0.1
120-144	0.07 ± 0.02	0.65 ± 0.04	0.05 ± 0.01	0.45 ± 0.16	0.01 ± 0.01	0.05 ± 0.05	0.02 ± 0.01	0.05 ± 0.02	0.1	0.1
144-168	0.03 ± 0.00	0.30 ± 0.13	0.02 ± 0.01	0.15 ± 0.04	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.0	0.0
Sub-total	10.5 ± 1.70	86.3 ± 1.30	8.74 ± 0.84	87.5 ± 1.08	1.79 ± 1.49	86.38 ± 3.76	2.03 ± 0.80	81.41 ± 6.49	13.4 ± 1.2	77.5 ± 3.9

Matrix	Mean Recovery of Radioactivity (Percent of Administered Dose)				
	Male rat	Female rat	Male dog	Female dog	Male monkey
Cage Rinse	0.29 ± 0.13	0.29 ± 0.10	1.82 ± 2.16	2.69 ± 2.56	0.5 ± 0.4
Cage Debris/Hair	-	-	0.13 ± 0.19	0.50 ± 0.66	1.1 ± 0.5
Cage Wash	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.04	0.82 ± 1.11	0.1 ± 0.1
Cage Wipe	0.07 ± 0.02	0.11 ± 0.04	0.00 ± 0.01	0.49 ± 0.61	0.3 ± 0.0
Carcass	0.42 ± 0.13	0.21 ± 0.07	-	-	-
Total	97.6 ± 0.61	96.9 ± 0.36	90.16 ± 1.11	87.95 ± 1.06	93.1 ± 3.2

Values are the mean ± SD (n=3).

-: Not determined

15. PHARMACOKINETICS: EXCRETION INTO BILE

Table 15.1 Pharmacokinetics: Excretion into Bile

Test Article: [14C]GSK1278863 **Location in CTD:** m4.2.2.5

Report No.:	CD2008/00100	CD2008/00025	2018N355713
Study No.:	7717-705	AFA00607	8361764
Species:	Rat	Dog	Monkey
Gender (M/F)/Number of Animals:	3M(BDC)	3M(BDC)	2M(BDC)
Feeding Condition:	Fasted	Fasted	Fasted
Vehicle/Formulation:	1% methylcellulose	1%(w/w) aqueous methylcellulose	1%(w/w) aqueous methylcellulose
Method of Administration:	Oral (gavage)	Oral (gavage)	Oral (gavage)
Dose (mg/kg):	10	20	10
Analyte:	[14C]GSK1278863	[14C]GSK1278863	[14C]GSK1278863
Assay:	LSC	LSC	LSC

Results:

Collection Interval (h)	Mean Recovery of Radioactivity (Percent of Administered Dose)								
	Male rat			Male dog			Male monkey		
	Urine	Feces	Bile	Urine	Feces	Bile	Urine	Feces	Bile
0-8	-	-	-	-	-	-	9.0	-	27.8
0-12	2.61 ± 0.56	-	23.2 ± 7.11	0.01	–	5.16	-	-	-
8-24	-	-	-	-	-	-	6.0	-	24.1
12-24	1.45 ± 0.19	-	7.40 ± 1.37	0.31	–	2.56	-	-	-
0-24	-	40.8 ± 9.53	-	–	72.02	–		10.5	-
24-48	1.57 ± 0.21	3.13 ± 0.93	6.93 ± 0.89	0.16	4.08	0.06[a]	0.5	11.4	1.0
48-72	0.63 ± 0.08	1.30 ± 0.34	3.00 ± 0.54	0.05	0.19	0.00[a]	0.2	1.9	0.1
72-96	0.34 ± 0.02	0.48 ± 0.15	1.42 ± 0.25	-	-	-	0.1	0.1	0.0
96-120	-	-	-	-	-	-	0.0	0.0	0.0
120-144	-	-	-	-	-	-	0.0	0.1	0.0
144-168	-	-	-	-	-	-	0.0	0.0	0.0
Sub-total	6.61 ± 0.62	45.7 ± 9.23	42.0 ± 10.0	0.52	76.29	7.77	15.8	24.1	53.0

Pharmacokinetics: Excretion into Bile (Continued)

Matrix	Mean Recovery of Radioactivity (Percent of Administered Dose)		
	Male rat	Male dog	Male monkey
Cage Rinse	0.21 ± 0.02	1.20	0.3
Cage Debris/Hair	-	0.70	0.4
Cage Wash	0.11 ± 0.01	0.28	0.1
Cage Wipe	0.15 ± 0.08	0.06	0.2
Carcass	1.56 ± 0.31	-	-
Bile Cannula	0.00*	0.00	0.0
Bile Jacket	0.01 ± 0.00	0.00	0.1
Total	96.4 ± 1.34	86.80	93.9

Values are the mean ± SD (rat: n=3, dog and monkey: n =2, data for one of the BDC animals were excluded from mean calculations because the dose for this animal was less than 75% of the target dose due to emesis.).
Values of zero represent <0.005% of the dose.
-: Not determined
BDC: Bile duct-cannulated
*: At least one value was below the limit of quantitation; therefore, the SD was not reported.

16. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS

Table 16.1 Pharmacokinetics: Drug-Drug Interactions

No studies.

17. PHARMACOKINETICS: OTHER

Table 17.1 Pharmacokinetics: Other

No studies.