Once daily atazanavir/cobicistat and darunavir/cobicistat exposure over 72 hours post dose in plasma, urine and saliva: contribution to drug pharmacokinetic knowledge.

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Abstract

Background: We investigated the pharmacokinetics (PK) of atazanavir/cobicistat and darunavir/cobicistat once-daily over 72h following drug intake cessation in plasma, saliva and urine.

Materials and methods: Healthy volunteers received a fixed-dose-combination of atazanavir/cobicistat 300/150mg once-daily for 10 days, followed by a 10-day washout period and then a fixed-dose-combination of darunavir/cobicistat 800/150mg once-daily for 10 days. Full PK profiles were assessed for each phase for 72h following day 10 and parameters determined to the last measurable concentration in plasma, saliva and urine by non-compartmental methods.

Results: Sixteen subjects completed the study. Geometric mean (GM) terminal elimination half-life to 72h of atazanavir and darunavir were 6.77h and 6.35h.

All subjects had atazanavir concentrations above the suggested minimum effective concentration of 150ng/mL 24h post-dose and 14/16 subjects had concentrations higher than this target at 30h post-dose (GM of 759 and 407ng/mL). Thirteen/16 subjects had darunavir concentrations higher than the target of 550ng/mL at 24h post-dose, and 5/16 subjects had concentrations higher than the target at 30h post-dose (GM of 1033 and 382ng/mL). Cobicistat half-life to 72h was 4.21h with atazanavir and 3.62h with darunavir. GM saliva and urine atazanavir and darunavir C_{24h} were 141ng/mL and 43ng/mL, and 24857ng/mL and 11878ng/mL. Concentrations decay in saliva/urine mirrored plasma concentrations for both drugs.

Conclusions: Different concentration decay patterns were seen for atazanavir and darunavir, which may be partially explained by cobicistat half-life (longer with atazanavir than darunavir). For the first time, we also measured drug PK forgiveness in saliva and urine, which represent easier markers of adherence.

Introduction

Ritonavir-boosted protease inhibitors such as atazanavir and darunavir have been used for many years and are an instrumental option as third agents in the management of HIV.[1] Advantages of pharmacological boosting include increased drug exposure and a prolonged half-life thereby reducing pill burden, allowing once daily dosing, and in the case of PIs, achieving a high genetic barrier to resistance.[2]

The use of ritonavir as a boosting agent, however, presents a number of disadvantages. Consequently, cobicistat, a structural analogue without antiviral activity, is now available as an alternative pharmacokinetic enhancer. Unlike ritonavir, good solubility lends cobicistat to co-formulation and a lack of enzyme-inducing activity, potentially offers a better drug interaction profile.[3] It inhibits cytochrome P450 3A4 (CYP3A4) with a potency similar to that of ritonavir and at a dosage of 150 mg once daily, provides bioequivalent exposures of atazanavir (300 mg once daily) and darunavir (800 mg once daily)[4] compared with those observed with 100 mg of ritonavir once daily.[3]

We previously presented data on the pharmacokinetic forgiveness of once-daily ritonavir-boosted darunavir and atazanavir, showing, a favorable atazanavir pharmacokinetic tail (PK) tail and a slight increase in decline rate for both protease inhibitors as ritonavir concentrations decrease.[5]

In vivo data for atazanavir/cobicistat and darunavir/cobicistat (both available in fixed dose combination) concentration decay after intake cessation have not, however, been previously described.

PK forgiveness is important in clinical practice in order to understand the management of late and missed doses, particularly with protease inhibitors as their use is increasingly targeted to complex cases of viral resistance, poor adherence and extensive antiretroviral treatment experience.[1] Drug persistence in plasma is dependent on its half-life (which itself depends on clearance and volume of distribution).[6] As such, antiretroviral agents with longer half-lives may be more forgiving and allow for forgotten doses, especially if drug

concentrations remain therapeutic until the patient reinitiates drug intake.

In addition to the above, there is a paucity of data in the literature on protease inhibitor exposure in other matrices such as saliva and urine, with one report available[7] and no study on drug levels in saliva or urine post cessation of drug intake. Sampling of saliva and urine is significantly less invasive than venipuncture and therefore may be a valuable measure of adherence in clinical practice in future. This has been shown to be useful in other infectious diseases where long-term treatment, optimal drug absorption and adherence are fundamental, like tuberculosis (TB), where urine colorimetry can detect low rifampicin plasma concentrations in HIV/TB co-infected individuals.[8]

The object of this study was therefore to investigate the steady-state PK of atazanavir/cobicistat and darunavir/cobicistat once daily dosing over 72 hours following drug intake cessation in plasma, saliva and urine.

Methods

Participants

Eligible participants were male and non-pregnant and non-lactating female healthy volunteers aged between 18 and 65 years with a BMI between 18 and 35 kg/m^2. Participants were excluded if they had any significant acute or chronic medical illness; abnormal physical examination, ECG or clinical laboratory determinations; positive screens for HIV, hepatitis B or C; current or recent (within three months) gastrointestinal disease; clinically relevant alcohol or drug use that the investigator felt would adversely affect compliance with trial procedures; exposure to any investigational drug or placebo within three months of the first dose of the study drug; use of any other drugs, including over the counter medications and herbal preparations, within two weeks of the first dose of the study drug; and previous allergy to any of the constituents of the pharmaceuticals administered during the trial.

Study design

This was an open-label, two-phase, 33-day PK trial carried out at the Clinical Trial Unit of the St. Stephen's Centre, Chelsea, and Westminster Hospital, London, United Kingdom.

At screening, participants had a clinical assessment and routine laboratory investigations performed. The safety and tolerability of study medications were evaluated throughout the trial (on days 1, 5, 10, 21, and 30, and at follow-up) using the NIAID Division of AIDS table for grading the severity of adult and pediatric adverse events to characterize abnormal findings (published 2004), vital signs, physical examinations and clinical laboratory investigation. After successful screening, during the first study phase, volunteers were administered fixed-dose combination atazanavir/cobicistat at 300/150 mg once daily (Evotaz®) in the morning for 10 days. On study days 10 to 13, atazanavir and cobicistat plasma concentrations were assessed pre-dose and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, and 72 hours post dose. After a washout period of seven days, all subjects were administered fixed-dose combination darunavir/cobicistat at 800/150 mg once daily (Rezolsta®) for 10 days. On study days 30 to 33, darunavir and cobicistat plasma

concentrations were assessed pre-dose and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, and 72 hours post dose. On the PK days, study medication intake with a standardized breakfast (626 kcal) and 240 mL of water was witnessed.

Analytical and PK methods

Blood samples were collected into lithium heparin-containing blood tubes (12 mL) at each time-point, immediately inverted several times and then kept on ice or refrigerated until centrifugation. Within 30 min of blood collection, each blood sample was centrifuged for 10 min at 2000 g at 4C. Plasma was then aliquoted equally into three 2.0 mL tubes (Sarstedt, Germany) and stored at -20C.

On the PK days, saliva (study subjects were asked to spit) and a minimum of 5 mL of urine were collected into universal containers at each scheduled sampling time (24, 30, 36, 48, 60, and 72 hours post-dose).

Samples were shipped on dry ice to the Liverpool Bioanalytical Facility for analysis. The laboratory participates in an external quality assurance scheme (KKGT, the Netherlands).

Quantification of atazanavir, darunavir, and cobicistat

Concentrations of atazanavir, darunavir and cobicistat in plasma, saliva and urine were measured using validated high-pressure liquid chromatography–tandem mass spectrometry methods.[9] The lower limits of quantification (LLQ) for the plasma analyses was 10 ng/mL for atazanavir, cobicistat and 15 ng/mL for darunavir. For concentrations below the assay limit of quantification, a value of one-half of the quantification limit was used.

The saliva assay was validated over a calibration range of 3.7-500 ng/mL for all three analytes. Accuracy (percentage bias) was between 99.5% and 108.2% (atazanavir), 94.2% and 101.2% (darunavir) and 92.3% and 104.0% (cobicistat), and precision was between 2.8% and 5.4% (atazanavir), 4.4% and 6.0% (darunavir) and 3.1% and 6.5% (cobicistat).

Data analysis

The calculated PK parameters for plasma atazanavir, darunavir and cobicistat were the plasma concentration measured 24 hours after the observed dose (C_{24h}), the maximum observed plasma concentration (C_{max}) and the area under the plasma concentration curve

from 0 to 24 hours (AUC_{0-24}). The half-life was determined from the elimination phase within the normal dosing interval of 0–24 hours and as a terminal elimination half-life to the last measurable concentration within 72 hours. All PK parameters were calculated using actual blood sampling time and non-compartmental modeling techniques (WinNonlin Phoenix, version 6.1; Pharsight, Mountain View, CA). Descriptive statistics, including geometric mean (GM) and 95% confidence intervals (95% CI) were calculated for atazanavir, darunavir and cobicistat plasma PK parameters. GMs were compared with the suggested therapeutic targets that were established in vivo (atazanavir) and in vitro (darunavir) and are available in the current literature for each drug.[10,11] These targets estimate the minimum effective concentration to be equivalent to 10 times the protein binding corrected inhibitory concentration at 50% [IC_{50}] for wild -type virus for atazanavir and for darunavir at150 ng/mL and 550 ng/mL, respectively.

Inter individual variability in drug PK parameters was expressed as a percentage coefficient of variation [CV, (standard deviation/mean)×100].

Urine and saliva concentrations were described as GM and 95% CI at each sampling time-point over the concentration decay curves. Plasma drug concentrations were correlated to saliva and urine concentrations by linear regression analysis.

Ethics

The study protocol was approved by the Bloomsbury Research Ethics Committee, London, United Kingdom (REC reference: 15/LO/1596, IRAS project ID: 184771) as well as by the Medicines and Healthcare products Regulatory Agency (MHRA) in the United Kingdom and was conducted according to Good Clinical Practice and the Declaration of Helsinki (EudraCT Number: 2015-002956-28). Written informed consent was obtained from eligible participants after screening and counseling, on the same day.

Results

Study population

Sixteen volunteers completed all phases of the study. Median (range) age and median body mass index (BMI) were 38 (24 to 54) years, and 25 (22 to 31) kg/m^2, respectively. Nine were female, nine described themselves as Caucasians, six as black, and two as Asian.

Atazanavir plasma pharmacokinetics

Atazanavir GM plasma concentration versus time curves when combined with cobicistat are shown in Figure 1 and PK parameters are summarized in Table 1.

The GM terminal elimination half-life to 72 hours of atazanavir was 6.77 hours (95% CI 6.2-7.5). This value was lower than the half-life measured over the dosing interval of 24 hours (GM 9.69 hours; 95% CI 9.2-12.8).

All subjects had atazanavir concentrations above the suggested target 24 hours post-dose (GM 759.2 ng/mL). Two/16 and 13/16 subjects had concentrations below the target at 30 and 48 hours post-dose, respectively (GM 407.0 and 65.9 ng/mL, Table 2).

The inter-individual variability in atazanavir C_{24h} was 73%.

Darunavir plasma pharmacokinetics

Darunavir GM plasma concentration versus time curves when combined with cobicistat are shown in Figure 1 and PK parameters are summarized in Table 1.

Darunavir GM terminal elimination half-life was 6.4 hours (95% CI 5.9-7.0). This value was lower than the half-life measured over the dosing interval of 24 hours (GM 10.4h; 95% CI 9.2-12.9).

Three/16 subjects had darunavir concentrations below the suggested target 24 hours post-dose and 11/16 had concentrations lower than the target at 30 hours (GM 1032.6 and 381.7 ng/mL, Table 2).

The inter-individual variability in darunavir C_{24h} values was 65%.

Cobicistat plasma pharmacokinetics

Steady-state cobicistat PK parameters when combined with atazanavir and darunavir are reported in Table 3.

When combined with atazanavir, the GM terminal elimination half-life to the last measurable

concentration for cobicistat was 4.2 hours (95% CI 3.9-4.7) and over the dosing interval of 24 hours was 4.4 hours, 95% CI 4.0–5.2).

These were higher than when cobicistat was combined with darunavir, where the GM terminal elimination half-life to the last measurable concentration was 3.6 hours (95% CI 3.3-4.0) and over the dosing interval of 24 hours was 3.8 (95% CI 3.5-4.3).

Saliva and urine concentrations

GM saliva and urine concentrations measured between 24 and 72 hours post-dose are illustrated in Figure 2.

Although saliva concentrations of atazanavir and darunavir are lower than those measured in plasma (GM C_{24h} saliva:plasma ratios were 0.19 and 0.04, respectively, saliva PK profiles showed the same concentration decay trends of plasma.

On the other hand, urine concentrations were higher than in plasma with urine:plasma ratios of 32.7 for atazanavir and 11.5 for darunavir.

Plasma-saliva correlation coefficients were R^2=0.533 for atazanavir and R^2=0.64 for darunavir.

Plasma atazanavir concentrations correlated significantly with urine atazanavir concentrations (R^2=0.78). Plasma darunavir concentrations also correlated with urine darunavir (R^2=0.65) even though the relationship did not appear as strong as for atazanavir.

Safety and tolerability

Treatment was generally well tolerated, and no serious adverse events occurred during the study. As expected because extensively described in the literature,[12] the most common adverse events observed throughout the study were scleral icterus and hyperbilibirubinaemia (during the atazanavir/cobicistat phase). No other clinically relevant changes in laboratory parameters were reported.

Discussion

We report for the first time the steady state PK of atazanavir and darunavir in plasma, saliva and urine over 72 hours following drug intake cessation in HIV negative healthy volunteers to describe the

PK forgiveness of these two commonly used PIs when boosted by cobicistat.

Concentrations of atazanavir were measurable in all subjects 48 hours post dose and in 11 and 2 subjects 60 and 72 hours post dose. Importantly 14/16 subjects had concentrations above the suggested MEC of 150 ng/mL and the remaining two had concentrations equal to 148 ng/mL 30 hours post dose, suggesting that a six hour drug intake delay would not compromise optimal drug exposure and efficacy. Similarly, darunavir concentrations were measurable in 13/16, 6/16, and 2/16 subjects 48, 60, and 72 hours post dose, respectively. However, 3/16 study individuals had concentrations below the suggested 550 ng/mL cut-off 24 hours post dose, and only five had concentrations above 550 ng/mL 30 hours post dose. Whether this is clinically significant is unclear and more data in patients who are poorly adherent to darunavir/cobicistat will hopefully emerge in the near future to help clinicians prescribing the optimal booster in certain complex clinical situations (e.g. suboptimal viral replication suppression).

Notably, measurements of atazanavir PK forgiveness in the presence of cobicistat are similar to those in the presence of ritonavir [6], where atazanavir terminal elimination half-life was 6.77 hours with cobicistat versus 6.74 hours with ritonavir. Darunavir terminal elimination half-life was measured to be 6.35 hours with cobicistat versus 6.48 hours with ritonavir.

While there is no doubt of protease inhibitor robustness in antiretroviral naïve people living with HIV (PLWH), in patients who are inclined to poor compliance or harbor viral resistance, PK forgiveness knowledge may be particularly important.

However, the clinical significance of our findings is unclear as pharmacodynamics (PD) data on what concentrations are needed to ensure long-term viral suppression maintenance in PLWH are unavailable and it is often unclear how delayed a dose is or how many doses can be omitted before efficacy is lost.

A further study limitation is that it was conducted in HIV negative healthy volunteers not to impose antiretroviral dose delays in PLWH. Data on drug exposure potential differences between PLWH and HIV negative volunteers are controversial but must be taken into consideration.[13]

Cobicistat terminal half-life was 4.21 hours with atazanavir and 3.62 hours with darunavir, therefore shorter than ritonavir terminal half-life with atazanavir (5.03 hours) and darunavir (6.30 hours),

247 respectively.

248 Both cobicistat and ritonavir inhibit the cytochrome P450 3A4 (CYP3A4), thereby reduce the
249 metabolism of concomitantly administered protease inhibitors and lead to enhanced drug exposure.[3]
250 Although very similar, the two drugs are not identical and their relationship with the therapeutic agent
251 they enhance may explain concentration decay patterns. Importantly, the rates of decline of both
252 atazanavir and darunavir slightly increased as cobicistat concentrations declined. Cobicistat itself is
253 metabolized by CYP3A4 and when given with atazanavir, a moderate CYP 3A4 inhibitor,[14] it achieves
254 slightly higher concentrations than when co-administered with darunavir, which on the other hand is
255 an inducer of CYP3A4.[14]

256 The inter individual variability (CV) in atazanavir and darunavir C_{24h} was 73% and 65% with cobicistat,
257 therefore similar to those previously measured with ritonavir (81% and 62%, respectively).[5]
258 Drug saliva and urine pharmacokinetic elimination profiles mirrored that of plasma, suggesting
259 potential use of these matrices' as a marker of adherence/PK forgiveness.
260 Atazanavir/cobicistat and darunavir/cobicistat were well tolerated, with adverse events limited to
261 expected increases in indirect bilirubin levels during the atazanavir/cobicistat study phase.
262 In conclusion, our data report the PK forgiveness of atazanavir/cobicistat and darunavir/cobicistat and
263 contribute to the understanding of the extent of whether drug doses can be delayed or missed. This is
264 important in the context of chronic diseases, where sub-optimal compliance to medications may be
265 common[15] and therefore within the HIV medicine field where repercussions of insufficient drug
266 exposure can be serious since if drug concentrations drop to sub-therapeutic levels after missed
267 doses, there is a risk of emergence of drug resistant HIV strains, which limit future therapeutic
268 options.

269

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Transparency declarations

EE has received speaking and travel grants from Janssen, ViiV, Bristol-Myers Squibb, Merck Sharp & Dohme, and Gilead.

AA no conflicts.

NP has received a travel grants from MSD, Gilead, Astella.

AS no conflicts.

CH no conflicts.

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Legends to Tables and Figures

Table 1: Plasma atazanavir (ATV) and darunavir (DRV) steady state pharmacokinetic (PK) parameters, expressed as geometric mean (GM) and 95% confidence intervals (CI), range (minimum, Min and maximum, Max) and coefficient of variation (CV), measured over 24 and 72 hours.

C_{max} = maximum concentration, AUC = area under the curve, C_{24} = 24 hour post-dose concentration, C_{last} = last measurable concentration, $t_{1/2}$ = half-life.

Table 2: Plasma concentrations of atazanavir and darunavir measured at 24, 30, 36, 48 hours post dose, expressed as geometric mean (GM) and range, and number (N) of subjects below target per time-point.

Table 3: Cobicistat steady state plasma pharmacokinetic (PK) parameters, expressed as geometric mean (GM) and 95% confidence (CI), range (minimum, Min and maximum, Max) and coefficient of variation (CV), measured over 24 and 72 hours with atazanavir (ATV) and darunavir (DRV).

C_{max} = maximum concentration, AUC = area under the curve, C_{24} = 24 hour post-dose concentration, C_{last} = last measurable concentration, $t_{1/2}$ = half-life.

Figure 1: Geometric mean steady state plasma concentrations of atazanavir (ATV, black) and darunavir (DRV, grey) when boosted by 150 mg of cobicistat over 72 hours (black dashed with ATV and grey dashed with DRV).

Figure 2: A. Saliva atazanavir (ATV) and cobicistat (COBI) B. Saliva darunavir (DRV) and cobicistat (COBI) C. Urine atazanavir (ATV) and cobicistat (COB) D. Urine darunavir (DRV) and cobicistat (COBI) concentration decay between 24 and 72 hours post-dose expressed as geometric mean (GM - continuous lines) and 90% confidence interval (90%CI – dashed lines).

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347 Table 1

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	PK parameters ATV 300mg OD						
	$t_{1/2}$ (0-24 h)	**$t_{1/2}$ (0-C_{last} h)**	**C_{max} (ng/ml)**	**C_{24} (ng/ml)**	**C_{last} (ng/ml)**	**AUC_{0-24} (ng.h/ml)**	**$AUC_{0-Clast}$ (ng.h/ml)**
Geomean	**9.69**	**6.77**	**3718.85**	**759.20**	**6.36**	**37713.15**	**46128.91**
low 95%	9.24	6.22	3308.00	612.57	1.29	32661.47	38592.12
up 95%	12.83	7.54	4940.55	1290.07	19.00	51555.93	67844.20
Min	6.32	5.42	844.97	256.10	5.00	11413.66	14057.77
Max	19.26	9.96	7282.82	2666.54	77.28	83763.28	128322.91
CV (%)	33	20	40	73	178	46	56
	PK parameters DRV 800mg OD						
	$t_{1/2}$ (0-24 h)	**$t_{1/2}$ (0-C_{last})**	**C_{max} (ng/ml)**	**C_{24} (ng/ml)**	**C_{last} (ng/ml)**	**AUC_{0-24} ng.h/ml)**	**$AUC_{0-Clast}$ (ng.h/ml)**
Geomea	**10.41**	**6.35**	**5515.02**	**1032.56**	**8.80**	**58099.81**	**66710.08**
low 95%	9.18	5.88	4949.07	837.92	6.01	51464.12	58145.46
up 95%	12.94	7.03	6566.03	1625.74	14.44	70391.27	83214.29
Min	5.23	4.25	2855.55	372.96	7.50	26404.49	29317.22
Max	19.15	8.48	8365.97	3359.34	41.13	111312.19	141982.09
CV	35	18	29	65	84	32	36

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350 Table 2

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	Hours post dose			
	24	30	36	48
Darunavir (ng/mL) GM (range)	1033 (373-3359)	381 (97-257)	109 (7.5-594)	45 (7.5-149)
N of subjects below target (550 ng/mL)	3/16	11/16	15/16	16/16
Atazanavir (ng/mL) GM (range)	759 (249-2667)	407 (148-1679)	201 (65-1093)	66 (14-949)
N of subjects below target (150 ng/mL)	0/16	2/16	5/16	13/16

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354 Table 3

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with ATV	$t_{1/2}$ (0-24h)	$t_{1/2}$ (0-Clast)	C_{max} (ng/ml)	C_{24} (ng/ml)	C_{last} (ng/ml)	AUC_{0-24} (ng.h/ml)	$AUC_{0-Clast}$ (ng.h/ml)
Geomean	**4.43**	**4.21**	**1408.02**	**49.59**	**5.00**	**10553.97**	**10923.56**
low 95%	3.95	3.87	1293.37	42.07	5.00	9589.47	9904.56
up 95%	5.19	4.69	1577.76	79.63	5.00	12058.87	12535.27
Median	4.32	4.17	1381.49	56.35	5.00	10569.53	10735.04
Min	3.14	3.21	929.72	14.15	5.00	7825.70	8145.20
Max	8.39	6.13	1986.37	156.24	5.00	14680.79	15068.30
CV	28	19	20	63	0	23	24
with DRV	$t_{1/2}$ (0-24h)	$t_{1/2}$ (0-Clast)	C_{max} (ng/ml)	C_{24} (ng/ml)	C_{last} (ng/ml)	AUC_{0-24} (ng.h/ml)	$AUC_{0-Clast}$ (ng.h/ml)
Geomean	**3.81**	**3.62**	**1250.25**	**27.56**	**5.00**	**9532.06**	**9681.21**
low 95%	3.49	3.34	1149.77	22.29	5.00	8677.55	8790.87
up 95%	4.29	3.98	1392.73	51.37	5.00	10857.17	11078.72
Min	2.59	2.59	932.46	5.00	5.00	6167.33	6254.42
Max	5.60	5.55	1867.32	120.90	5.00	14425.77	14933.25
CV	21	18	20	81	0	23	23

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357 Figure 1

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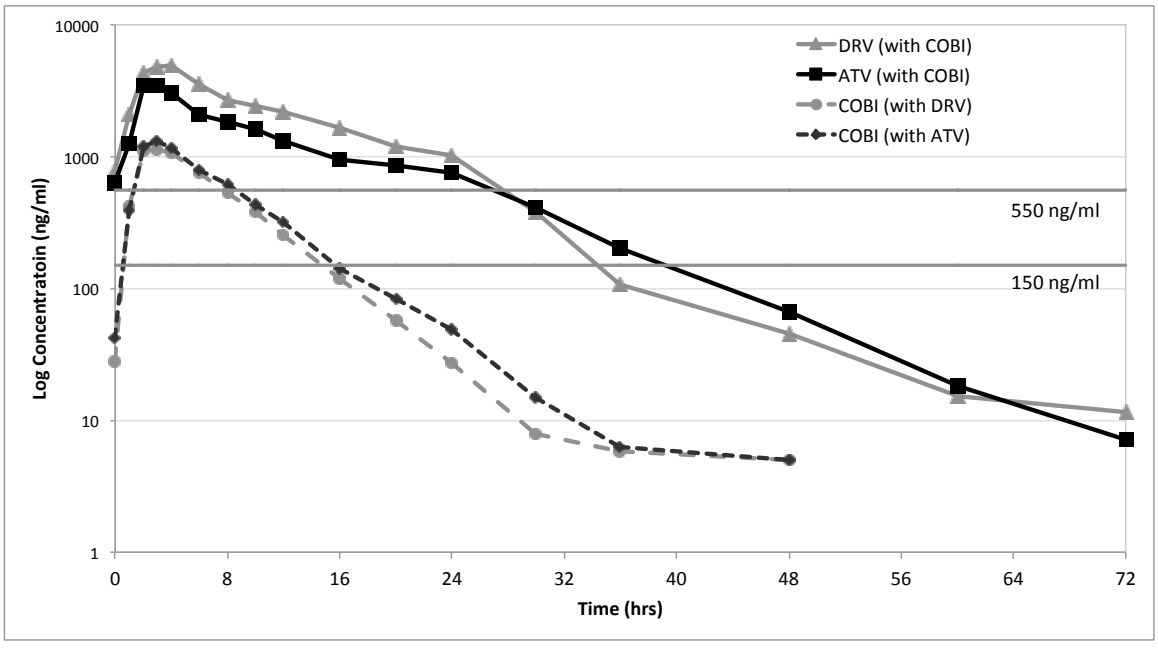

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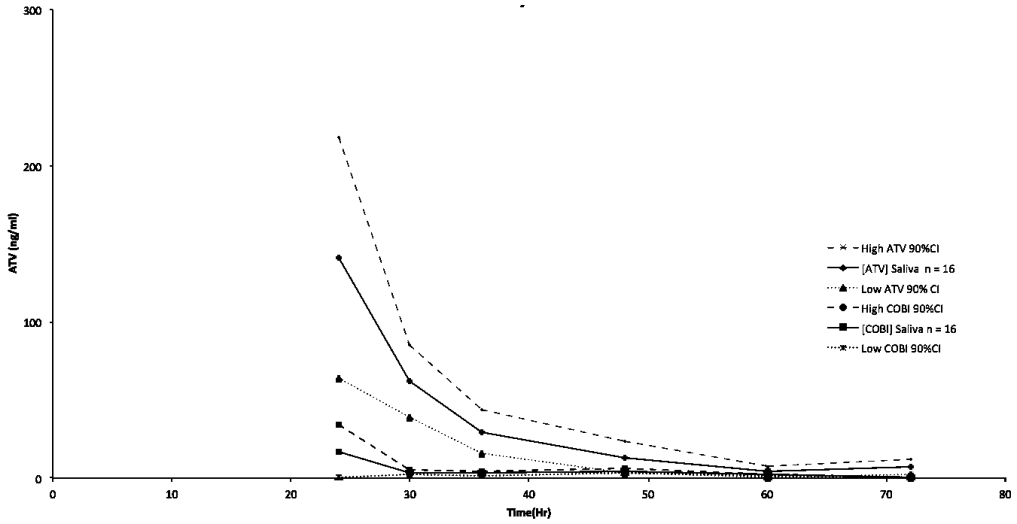
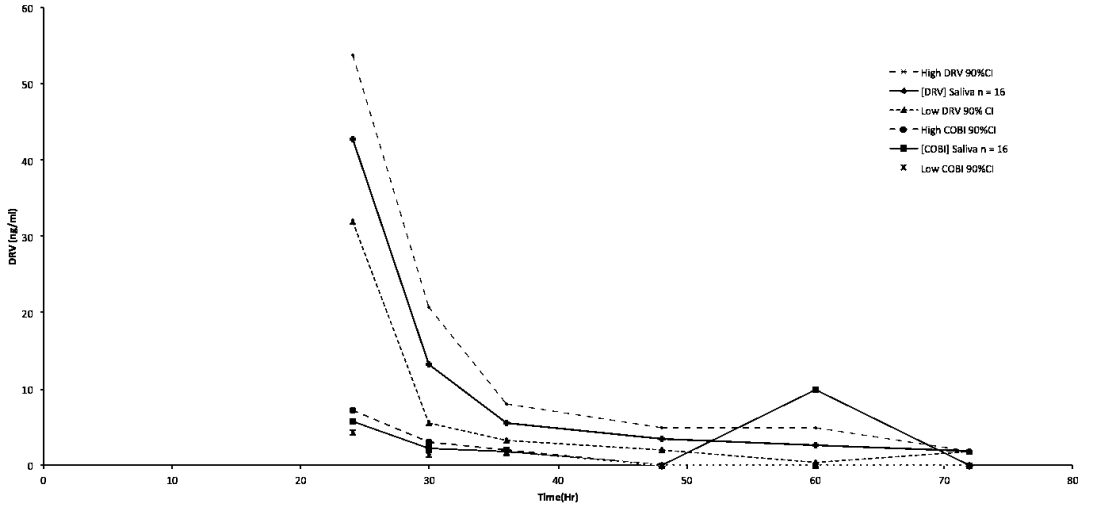
Figure 2

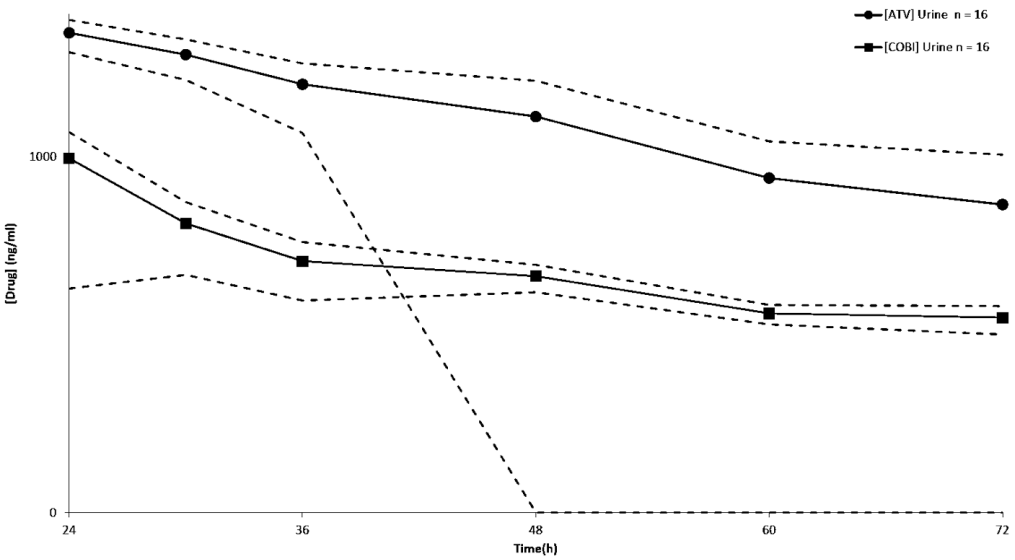
A


ATV (ng/ml)
Time(Hr)
High ATV 90%CI
[ATV] Saliva n = 16
Low ATV 90% CI
High COBI 90%CI
[COBI] Saliva n = 16
Low COBI 90%CI

B


DRV (ng/ml)
Time(Hr)
High DRV 90%CI
[DRV] Saliva n = 16
Low DRV 90% CI
High COBI 90%CI
[COBI] Saliva n = 16
Low COBI 90%CI

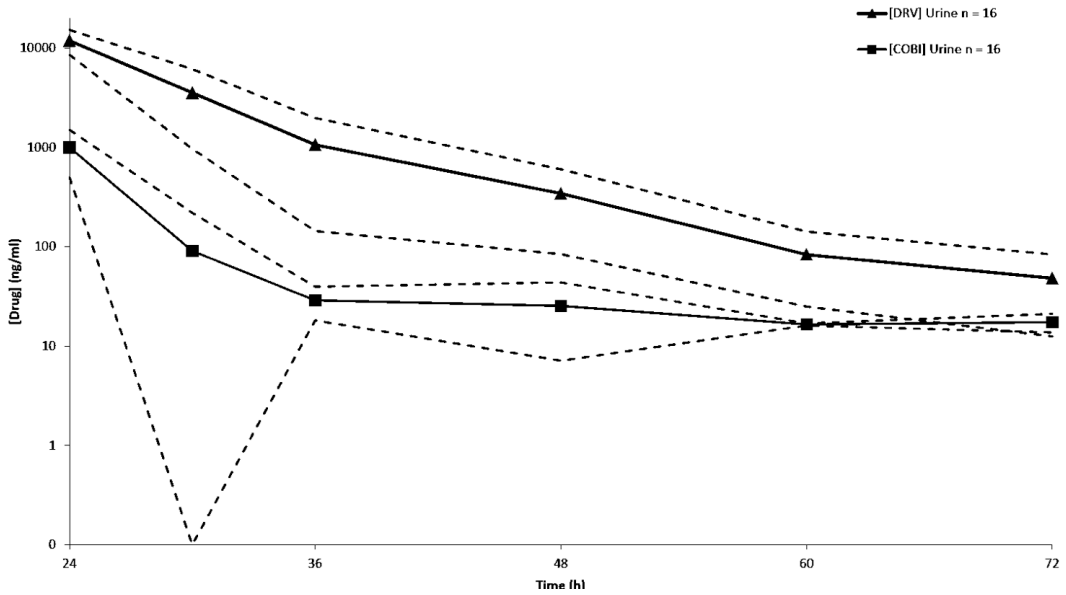
376 C



377

378

379 D



380

381