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**Absorption, Distribution, Metabolism, and Excretion of Ticagrelor in Healthy
Subjects**

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Running Title: Pharmacokinetics and metabolic pathways of ticagrelor (AZD6140)

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Number of text pages: 23

Number of tables: 4

Number of figures: 8

Number of references: 21

Abstract Word count [Max. 250]: 250

Introduction Word count [Max. 750]: 358

Discussion Word count [Max. 1500]: 828

Non-standard abbreviations:

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ABSTRACT :

Ticagrelor (AZD6140) is a reversibly binding oral P2Y₁₂ receptor antagonist in development for the prevention of thrombotic events in patients with acute coronary syndromes. The pharmacokinetics, metabolism, and excretion of ticagrelor were investigated over 168 h in 6 healthy male subjects receiving a single oral suspension dose of [¹⁴C]-ticagrelor 200 mg. Ticagrelor was rapidly absorbed with a maximum plasma concentration at 1.5 h. The major active metabolite, AR-C124910XX, is formed by O-deethylation. Exposure to AR-C124910XX was 29% of peak and 40% of overall exposure to ticagrelor. In most subjects, radioactivity was undetectable in plasma after 20 h and whole blood after 12 h (half-life values of 6.3 h and 4.6 h, respectively). The ratio of radioactivity in plasma:whole blood was 1.69, suggesting ticagrelor and its metabolites are largely restricted to the plasma space. Mean radioactivity recovery was 26.5% in urine and 57.8% in feces. Major circulating components in the plasma and feces were identified as ticagrelor and AR-C124910XX, whereas in urine the major components were metabolite M5 (AR-C133913XX) and its glucuronide conjugate M4. Levels of unchanged ticagrelor and AR-C124910XX were <0.05% in the urine, indicating that renal clearance of ticagrelor and AR-C124910XX is of minor importance. Inter-individual variability was small in both urine and fecal extracts with only small quantitative differences. All ten of the metabolites were fully or partially characterized and a full biotransformation pathway proposed for ticagrelor, in which oxidative loss of the hydroxyethyl sidechain from ticagrelor forms AR-C124910XX and a second oxidative pathway leads to N-dealkylation of ticagrelor forming AR-C133913XX.



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Introduction

Standard therapy to reduce the risk of thrombotic complications in patients with acute coronary syndromes (ACS) is currently aspirin in combination with clopidogrel (Bassand et al. 2007; Yusuf 2001). However, there is still a need for treatment regimens with improved efficacy. New therapies with more optimal and consistent inhibition of platelet aggregation (IPA) are in development to address this need.

Ticagrelor (AZD6140) is a reversibly binding, non-competitive, orally administered P2Y₁₂ receptor antagonist that is in clinical development for reduction of clinical thrombotic events in patients with ACS (James et al. 2009. Springthorpe et al. 2007, van Giezen and Humphries 2005). It is one of a chemical class of antiplatelet agents termed the cyclopentyltriazolopyrimidines, which act directly on the P2Y₁₂ receptor without requiring metabolic activation. In comparative trials, ticagrelor has produced greater and more consistent levels of IPA and a favorable trend in reducing risk for myocardial infarction compared with clopidogrel, without increasing the risk of major bleeding (Cannon et al. 2007, Husted et al. 2006, Storey et al. 2007). In the phase III PLATelet inhibition and patients Outcomes (PLATO) trial, treatment with ticagrelor versus clopidogrel significantly reduced the rate of death from vascular causes, myocardial infarction, or stroke (9.8% vs 11.7% [hazard ratio, 0.84; 95% CI, 0.77–0.92; P<0.001]) (Wallentin et al. 2009).

Ticagrelor has one active metabolite, AR-C124910XX, which is at least as potent at the P2Y₁₂ receptor as ticagrelor; it is present in the circulation at approximately one third of the concentration of the parent drug (Husted et al. 2006). In healthy human subjects and patients with stable atherosclerosis the pharmacokinetics of ticagrelor are linear and predictable over a wide dose range (Butler and Teng 2010; Husted et al.

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2006; Teng and Butler 2008; Teng and Butler 2010). Data from preclinical studies (rat and marmoset) have shown that ticagrelor is predominantly excreted via feces with minor renal elimination (Astra Zeneca, data on file).

To elucidate the exact disposition and metabolism of ticagrelor in humans, the present study was undertaken in healthy volunteers, using a single oral dose of radiolabelled ticagrelor. Pharmacokinetic data are reported for ticagrelor and its active metabolite AR-C124910XX. In addition, ticagrelor's metabolic profile and the main metabolites present in plasma and excreted in urine and feces are identified.

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Methods

Radiolabeled Drugs and Chemicals. Ticagrelor (1S,2S,3R,5S)-3-[7-[[[(1R,2S)-2-(3,4-Difluorophenyl)cyclopropyl]amino]-5-(propylthio)-3H-1,2,3-triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)-1,2-cyclopentane-1,2-diol), AR-C124910XX and AR-C133913XX were supplied by AstraZeneca Pharmaceuticals (Wilmington, Delaware, USA or Loughborough, Leicestershire, UK). [¹⁴C]-ticagrelor oral suspension (Fig. 1) (radiochemical purity 97%) was manufactured by Amersham (Buckinghamshire, UK). All reagents were of analytical or high performance liquid chromatography (HPLC) grade.

Study Design and Subjects. This was an open-label, single-dose, non-randomized study carried out at a single center (Study D5130C000130; Alderley Park Clinical Pharmacology Unit, AstraZeneca R&D Alderley Park, Macclesfield, Cheshire, UK). Written, informed consent was obtained from all volunteers prior to initiation of the study. The study was performed in accordance with the ethical principles established by the Declaration of Helsinki and was consistent with ICH/Good Clinical Practice, applicable regulatory requirements (including local ethical review board approval) and the AstraZeneca policy on bioethics.

Six healthy male subjects (aged 40–55 years) were enrolled in the study. The health status of each volunteer was determined during a screening visit (Visit 1) based on medical history, physical examination, ECG, and clinical laboratory test results. Subjects were excluded if they had participated in studies involving radiolabelled substances in the past 12 months or were monitored for radioactivity as part of their job; had symptoms of clinically relevant illness; had any disease or condition known to interfere with the absorption, distribution, metabolism or excretion of drugs; had severe allergic disease; or

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had demonstrated hypersensitivity to any drugs related structurally or mechanistically with ticagrelor.

Within 21 days of the screening visit, fasted subjects (no food for 12 h overnight) received a single oral dose of 200 mg [¹⁴C]-ticagrelor as a 10 g oral suspension (222.7 kBq/g). Subjects remained in an upright position (standing, sitting or semi-recumbent) without food for at least 4 h following administration of the dose. Subjects remained onsite for the next 168 h (7 days). Subjects were monitored for the incidence of any causally-related or serious adverse events, and for clinically significant changes in electrocardiogram (ECG), blood pressure (BP), heart rate (HR) or laboratory safety variables in blood or urine throughout the treatment period and at follow-up (7–14 days post-dose).

Collection and Storage of Blood and Plasma Samples. Blood samples (6 ml), for measurement of total radioactivity in whole blood and plasma and of ticagrelor and AR-C1249910XX in plasma, were collected predose and at various times up to 168 h postdose. For metabolite profiling, additional 20 ml plasma samples were collected in lithium heparin tubes at 1, 3, 12, and 24 h post-dose. Pooled samples (all subjects) of plasma at 1, 1.5, 2, 3, 4, 6, 8, and 10 h (1–1.2 ml) post-dose were also analyzed. Plasma samples were stored at –20°C or below and whole blood samples at 4°C.

Collection and Storage of Urine and Fecal Samples. Urine samples were collected for determination of ticagrelor, AR-C124910XX, and total radioactivity. Urine collection intervals were at predose and 0–6, 6–12, 12–24, daily for 7 days postdose. Fecal samples were collected for determination of total radioactivity predose and then at 24-hour intervals postdose. Samples were collected until 2 successive samples were

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shown to be <3 times background radiation, or total recovery was $\geq 90\%$. Urine samples were stored at room temperature (15–25°C) and fecal samples were stored at -20°C.

Samples for metabolic profiling analysis were selected based on the level of radioactivity. The 6-h urine samples from each of the 6 volunteers were analysed and fecal subsamples (~5 g) were selected for volunteers 4 and 6 at 48 h, volunteers 3 and 5 at 72 h and volunteers 1 and 2 at 96 h. Pooled samples (all subjects) of urine from 6 to 24 h (18.31 g), feces at various time points (time-point selected if sample had dpm >20,000) (15.70 g), and plasma at 1, 1.5, 2, 3, 4, 6, 8, and 10 h (1-1.2 ml) post dose were also analyzed.

Sample preparation for LSC and HPLC analysis. Urine samples were centrifuged at 3000 rpm for 10 min. Fecal samples were extracted 3 times with 3 ml/g acetonitrile:water (1:1 v/v), centrifuged (3000 rpm, 10 min), then decanted. An aliquot of each extract (20 ml) was partitioned with hexane (3 x 20 ml) to remove fat. The hexane layers were removed and the extracts were concentrated under nitrogen, centrifuged (3000 rpm, 10 min) and duplicate aliquots analyzed for liquid scintillation counting (LSC). Plasma samples were extracted with 3 ml/g of acetonitrile:methanol (1:1 v/v), vortex mixed and stored at -20°C for 10 min to aid precipitation. Samples were centrifuged (3000 rpm, 10 min) and the supernatant concentrated under nitrogen, and decanted into Eppendorf tubes. Duplicate aliquots of all samples were taken for LSC prior to HPLC.

Bioanalytical Assay of Ticagrelor and AR-C124910XX. Ticagrelor and AR-C124910XX concentrations in urine and plasma were analyzed by York Bioanalytical Solutions (York, UK) using a separately validated liquid chromatography technique with tandem mass spectrometric detection (LC/MS/MS). For plasma analysis, mean intra-

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batch accuracy was 91.9–109.0% and 86.8–109.2%, for ticagrelor and AR-C124910XX, respectively; intra-batch precision was 4.0–8.4% and 5.2–16.9%, respectively. For urine analysis, mean intra-batch accuracy was 86.8–97.0% and 83.4–92.8%, for ticagrelor and AR-C124910XX, respectively; intra-batch precision was 2.9–9.1% and 2.6–6.7%, respectively. Assay lower limits of quantification were 5.0 ng/ml for ticagrelor and 2.5 ng/ml for AR-C124910XX in plasma and 1.0 ng/ml and 2.5 ng/ml, respectively, in urine.

Measurement of total Radioactivity by Liquid Scintillation Counting. Total [^{14}C]-radioactivity in samples of blood and plasma (measured directly), feces (mechanically homogenized with water), and urine (diluted with acetonitrile (4:1 urine:acetonitrile [v/v], to ensure complete solubility of ticagrelor) was determined by liquid scintillation counting (LSC; Packard Tricarb). Duplicate (or triplicate for feces) oxidized samples were analyzed for [^{14}C]-radioactivity, corrected for background activity using [^{133}Ba] as external source, and disintegrations per minute (dpm) values calculated. A nominal 25 dpm above background was set as the limit of detection.

Pharmacokinetic Analysis. Pharmacokinetic analysis was done using WinNonlin (Pharsight, Mountain View, CA, USA). Primary pharmacokinetic assessments for total radioactivity in plasma and blood included: peak concentration (C_{max}); time to peak concentration (t_{max}); elimination half-life ($t_{1/2}$); area under the concentration-time curve from zero to the time of the last concentration above the limit of quantification (AUC_{0-t}); area under the concentration curve to infinity (AUC), and plasma-to-blood ratio, % dose excreted in urine and feces, and total recovery (%) of [^{14}C]-radioactivity. For ticagrelor and AR-C124910XX, pharmacokinetic measurements included: C_{max} , t_{max} , $t_{1/2}$, AUC_{0-t} , AUC, total amount of drug/metabolite excreted in urine ($\text{Ae}_{(\infty)}$), and % dose excreted in urine. In addition, renal clearance (CL_R) was calculated or estimated for

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ticagrelor. For AR-C124910XX, metabolite:parent C_{max} and AUC ratios were also calculated. The terminal elimination rate constant (λ_z) was calculated by log-linear regression of the terminal portion of the plasma concentration-time profile using at least 3 time points, and $t_{1/2}$ was calculated as $0.693/\lambda_z$. AUC_{0-t} was calculated using the linear trapezoidal method, and AUC was derived by extrapolation of the terminal elimination phase to infinity. CL_R was estimated as the ratio of total amount of ticagrelor or AR-C124910XX excreted unchanged in urine to ticagrelor or AR-C124910XX AUC.

Determination of Ticagrelor and its Metabolites – HPLC Chromatographic Conditions for Radioactivity Profiling. Metabolic profiling and metabolite identification were performed by Charles River Laboratories (Tranent, Scotland). The plasma, urine and fecal samples were analyzed for ticagrelor and its metabolites by HPLC (Agilent Hewlett Packard 1100 series) using on-line radiodetection (Packard Radiomatic™ Flo-One® 505TR flow scintillation analyzer) for urine or feces, or by fraction collection every 60 s (Gilson 202 fraction collector) and analysis by LSC (Packard 1600-TR) for plasma. Ultraviolet detection was set at 254 nm. The HPLC column was an Ace 3 C18 (250 x 4.6 mm, 3 μ m particle size), with a variable mobile phase consisting of 0.1% formic acid in milliQ water and acetonitrile; the percentage of acetonitrile was increased from 5% to 50% over 40 min and then to 95% at 45 min, where it was held for 3 min. The flow rate was 1.0 ml/min.

The retention time of ticagrelor (~42.5 min) was determined by running an aliquot of unlabelled ticagrelor (~1 mg/ml in 1:3 (v/v) acetonitrile:water) immediately prior to each batch of samples. The limit of quantification was defined as background level (mean of the first or last 2 fractions in each analytical run) plus 30 dpm of radioactivity,

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and plasma sample fractions containing less than this were not included in further calculations.

Validation of the HPLC System. The linearity of response of the radiodetector was considered acceptable (correlation coefficient 0.9991), as established by conducting a series of dilutions of [¹⁴C]-ticagrelor in mobile phase over the range 9000–135,000 dpm. The coefficient of variation (3.3%; acceptance criteria of <15%) for the reproducibility of the HPLC method was established by carrying out 9 consecutive injections of [¹⁴C]-ticagrelor at approximately 50% of the linear range. An injection of mobile phase immediately following these injections demonstrated that there was no carry over of radioactivity. The recovery of radioactivity was 94.3% in urine, 95.7% in plasma and 96.9% in feces. No significant degradation of [¹⁴C]-ticagrelor was observed during these analyses.

LC/MS/MS Analysis of ticagrelor and its metabolites. The 6–24 h pooled urine samples, pooled fecal extract samples, and 3 h pooled plasma samples were analyzed for ticagrelor and its metabolites by LC/MS/MS (Agilent Hewlett Packard 1100 series liquid chromatograph and Waters/Micromass Q-TOF micro mass spectrometer operating MassLynx software [version 4.0, SP 2]). Radiochemical detection was performed with the Radiomatic 500TR series flow scintillation analyzer using Flo-One software (version 3.65). For LC, an injection volume of 10–250 µl was used with a split to the mass spectrometer of ~200 µl. MS was performed in positive ion electrospray ionization mode. Ticagrelor, AR-C124910XX and AR-C133913XX were used as reference compounds.

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For determination of sample radioactivity, duplicate aliquots of liquid samples were transferred to separate scintillation vials, diluted with water if necessary, and analyzed by LSC. Solid sample of fecal post-extracted solids were weighed in duplicate into Combusticones[®] (Packard Instruments) with pads for combustion analysis (Perkin Elmer; Model 307 Tri-Carb Automatic Sample Oxidiser). Combustion efficiency and carry-over were assessed at the start of each run of 30 samples by combusting blanks and quality control standards containing Spec-Check[™] -¹⁴C. Throughout the analysis, combustion efficiency was >97%. All samples were counted for 5 min with representative blanks using a Packard 1600-TR liquid scintillation analyzer with automatic quench correction by external channel ratio. The representative blank sample values were subtracted from sample dpm to give net dpm per sample.

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Results

Six healthy male subjects with mean age of 45.7 years (range 41–54 years), mean body weight 78.9 kg (63.3–91.0 kg), and mean body mass index 25.8 kg/m² (23.7–29.4 kg/m²) enrolled and completed the study. The 200 mg oral dose of [¹⁴C]-ticagrelor was well tolerated, with no serious adverse events reported. There were no clinically important changes in laboratory parameters, vital signs, electrocardiograms, or physical findings.

Pharmacokinetics. Ticagrelor was rapidly absorbed, with median t_{\max} observed at 1.5 h. The active metabolite AR-C124910XX appeared rapidly in the plasma, with median t_{\max} observed at 3.0 h (Fig. 2; Table 1). Plasma half-life was approximately 8 and 12 h for ticagrelor and AR-C124910XX, respectively. Exposure to AR-C124910XX was 29% that of the parent compound at peak levels and 40% overall. Amounts of unchanged ticagrelor and AR-C124910XX excreted in the urine accounted for 0.02% and 0.04% of the total dose, respectively. Median t_{\max} for total radioactivity was 2.5 h in plasma and 3.0 h in whole blood (Fig. 3). Radioactivity concentrations declined steadily thereafter and in most subjects were not quantifiable in plasma after 20 h and in whole blood after 12 h. The geometric mean AUC_{0-t} ratio of total radioactivity in plasma:whole blood was 1.69, indicating that ticagrelor and its metabolites are largely restricted to the plasma space. The mean recovery of total radioactivity from both urine and feces was $84.3 \pm 5.5\%$ (\pm S.D.) of the dose, consisting of $57.8 \pm 4.4\%$ in the feces and $26.5 \pm 4.1\%$ in the urine. The majority of radioactivity was recovered in urine by 12 h and in feces by 96 h. (Fig. 4).

Radioprofiling of Individual and Pooled Samples. HPLC chromatograms of the pooled plasma samples from all 6 subjects showed that the major peak at all time-

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points up to 6 h (1, 1.5, 2, 3, 4, 6 h) was the parent compound ticagrelor (retention time: 43 min) (Fig. 5a). However, LC/MS/MS analysis confirmed that the peak comprised both ticagrelor and the major metabolite AR-C124910XX (M8) (Table 2). This same peak was also present in the 10 h pooled plasma sample (0.132 $\mu\text{g Eq/g}$), although an additional peak (M2; retention time 18 min) was larger (0.168 $\mu\text{g Eq/g}$) (Fig. 5b). Minor peaks of radioactivity (≤ 0.354 $\mu\text{g Eq/g}$) were also identified at 1.5, 2 and 3h, which LC/MS/MS analysis confirmed as being metabolites M1, M2, M5 (AR-C133913XX) and M7 (Table 2). Individual radiochromatograms from individual subjects were qualitatively and quantitatively consistent for all subjects and time points.

Radiochromatograms obtained from the 6–24 h pooled urine sample are shown in Fig. 6 and quantitative distributions of the metabolites in the samples are shown in Table 3. Radioactivity excreted in urine accounted for 22.7% of the dose. Chromatograms for 6 h urine samples from each subject and the pooled urine sample were qualitatively consistent, with minor quantitative differences. In urine, the major peak was identified as M5 (AR-C133913XX; retention time 24.3 min), which accounted for 9.21% of the total dose, and a second major peak was identified as M4 (retention time 22.4 min), accounting for 6.64% of the dose. Additional peaks between 14 and 21 min were identified as M1, M2 and M3, (each <2% of the dose) and a small peak at 37.7 min representing M6, M9, and M10 combined (<6.64% of dose).

Radiochromatograms obtained from the pooled fecal sample are shown (Fig. 6); with quantitative distributions of the metabolites in Table 3. Radioactivity excretion in feces accounted for 54.8% of the dose. Chromatograms for fecal samples were qualitatively and quantitatively consistent for all subjects and time points. The major peaks in feces had retention times of 42.7 and 42.0 min, representing ticagrelor (27.09%

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of dose), and AR-C124910XX (M8, 21.73% of dose), respectively. Additional minor peaks were present at 24.4 min (M5) and 33.4 min (M7).

Identification of Metabolites in Urine, Feces and Plasma. LC/MS/MS analysis identified 10 discrete metabolites from pooled urine (6–24 h), pooled feces and pooled plasma (3 h) matrices samples (Table 4). Analysis of ticagrelor (m/z 523), AR-C124910XX (M8, m/z 479), and AR-C133913XX (M5, m/z 371) reference standards demonstrated that the structures were consistent with those previously elucidated. Product ion spectra of each radiolabeled peak were used to characterize the probable structure of each metabolite.

In urine there were two major radiolabeled peaks designated M4 (22.3 min, m/z 547) and M5 (24.3 min, m/z 371) (Fig. 7). The product ion spectrum of the ion m/z 547 showed major ions at 371, 343, 183 and 141 (Fig 7a). An intense ion at m/z 371 indicated the loss of glucuronide (-176 Da), suggesting that the metabolite M4 is a glucuronide in which the difluorophenylcyclopropyl moiety has been lost. The exact position of glucuronidation is unknown, but the proposed structure is illustrated in Fig. 7a. The metabolite M5 had the same retention time (24.3 min) and full scan and product ion mass spectra properties ($[M+H]^+$ at m/z 371) as reference standard AR-C133913XX (fig 7b).

Several minor urinary metabolites were also identified; M1 (13.9 min, m/z 387), M2 (14.3 min, m/z 387) and M3 (20.2 min, m/z 503) and their tentative structures are identified in the proposed metabolic profile for ticagrelor (Fig. 8). These data suggest that M2 is formed by the loss of the difluorophenylcyclopropyl side chain from ticagrelor followed by oxidation and that M1 may be an isomer of M2. M3 is proposed as a glucuronide of

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ticagrelor in which both the difluorophenylcyclopropyl side chain and the hydroxyethyl side chain have been cleaved, although the exact site of glucuronidation remains unknown. MS analysis of the radiopeak at ~36.7 min showed multiple related urinary metabolites (designated M6, M9 and M10). The full spectra for metabolites M6 (36.8 min, m/z 655) and M9 (37.1 min, m/z 699) showed other intense ions at m/z 479, and m/z 523, respectively, indicating loss of glucuronide, suggesting that M6 and M9 were glucuronidated parent compound after cleavage of the hydroxyethyl side chain. M10 (37.3 min, m/z 539) was considered to be the parent molecule that has undergone monohydroxylation in the cyclopentoxy ethanol moiety. This characterization is supported by presence of an ion at m/z 363, indicating intact triazolopyrimidine core, propyl side chain, and difluorophenylcyclopropyl group, and an ion at m/z 153, also indicating an intact difluorophenylcyclopropyl moiety. The proposed structures of metabolites M6, M9, and M10 are identified in the proposed metabolic profile for ticagrelor (Fig. 8).

Spectral analysis of the pooled fecal sample showed 2 major radiolabeled components at retention times 41.5 min (m/z 523) and 40.9 min (m/z 479), corresponding to the reference standards ticagrelor and AR-C124910XX (M8), respectively (Table 4). One minor peak at 24.3 min (m/z 371) was identified as corresponding to the reference standard AR-C133913XX (M5). Another minor peak was designated M7 (32.7 min, m/z 495). Based on other ions identified in the spectrum (m/z 477, indicating a facile loss of H₂O, m/z 449, indicating loss of N₂ from the triazolopyrimidine core, and m/z 153, indicating an intact difluorophenylcyclopropyl moiety) this metabolite was assigned as the parent molecule that had lost the hydroxyethyl side chain and undergone further oxidation. Oxidation may occur in the propyl side chain, possibly β to the sulfur, as suggested by the facile loss of H₂O.

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Radioactivity levels in the 3 h plasma samples were not high enough to permit a concurrent radiochromatogram. Reconstructed chromatograms based on HPLC fraction collection/LSC data from the radioprofiling phase showed 2 peaks at ~43 min and ~25 min that had similar retention times, full scan and ion spectra properties as ticagrelor/AR-C124910XX (M8) and AR-C133913XX (M5) peaks, respectively, identified in other sample matrices and in the reference standards.

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Discussion

The present study characterizes the pharmacokinetics and proposed metabolic pathways involved in the excretion of a single oral dose of [¹⁴C]-ticagrelor 200 mg in healthy male subjects. Our findings showed that ticagrelor is rapidly absorbed and extensively metabolized in humans, with a total of 10 metabolites characterized by LC-MS from plasma, urine, and feces.

Absorption of ticagrelor was rapid, concordant with previous data showing that the onset of ticagrelor's antiplatelet effect is approximately 30 minutes after oral administration (Gurbel et al. 2009; Tapp et al. 2010). The major circulating components in plasma were ticagrelor and its active metabolite AR-C124910XX (M8), consistent with previous plasma concentration data and PK parameters of ticagrelor and AR-C124910XX (Butler and Teng 2010; Husted et al. 2006; Teng and Butler 2008; Teng and Butler 2010). Ticagrelor reached a maximal mean concentration at 1.5 h, then being rapidly metabolized, with a terminal elimination half-life of approximately 8 h. AR-C124910XX reached a maximal mean concentration at 3 h, with a mean plasma half-life of 12 h. Both ticagrelor and its active metabolite, AR-C124910XX, had undetectable concentrations by 48 h post-dose.

Previous studies have shown that the extent of platelet inhibition is dependent on the concentration of drug/metabolite available to bind to platelets, closely reflecting plasma concentrations of ticagrelor + AR-C124910XX (Husted et al. 2006; Peters et al. 2004; Peters et al. 2006). Thus, changes in the plasma concentrations of ticagrelor and AR-C124910XX would be expected to affect antiplatelet activity. However, since the drug and the metabolite are equipotent (AstraZeneca, data on file), it is considered that the majority of ticagrelor's antiplatelet effect is due to the parent compound since AR-

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C124910XX is present at a concentration approximately 40% (29% at peak concentration) of the parent drug.

In contrast to the thienopyridines, which require metabolic activation to exert their antiplatelet effect, the consequence of the parent compound having most of ticagrelor's activity will have different implications for drug interactions. It is proposed that AR-C124910XX, the major and active metabolite, is formed from ticagrelor via probable oxidative loss of the parent hydroxyethyl sidechain. In-vitro experiments with human liver microsomes have shown that ticagrelor is metabolized by CYP3A4/5 isoforms (AstraZeneca, data on file). Thus, there is a potential for drug-drug interactions with ticagrelor. However, whilst ticagrelor and ARC124910XX plasma levels are likely to change during concomitant administration of CYP3A inhibitors, it is anticipated that the overall antiplatelet activity would not be decreased (but may be increased) since ticagrelor acts directly. In contrast, the antiplatelet activity of the pro-drug clopidogrel is diminished in the presence of CYP3A inhibitors, since conversion to its active metabolite occurs via a series of cytochrome P450 enzymes, including CYP2C19 and CYP3A (Farid et al. 2007).

Radiochromatograms of both urine and fecal samples showed low inter-subject variability with only small quantitative differences in the metabolic profiles across each of the six volunteers. Although this small study did not examine if any genetic polymorphisms exist in the metabolic biotransformation of ticagrelor, our findings combined with those from earlier studies (Husted et al. 2006, Peters et al. 2006) suggest there is likely to be low variability in antiplatelet response with ticagrelor. In contrast, variability in the response to clopidogrel (Mega et al. 2009), partially due to the genetic variation of cytochrome P450 2C19 (Varenhorst et al. 2009), has led to an FDA-boxed

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warning for poor metabolizers (Food and Drug administration, 2010). Since ticagrelor and ARC124910XX are equipotent (AstraZeneca, data on file), and ticagrelor does not require metabolism for activity, genetic polymorphisms in cytochrome P450s are unlikely to impact its activity.

Overall recovery of total radioactivity from a single dose of 200 mg oral dose was 84.3%. Most of this radioactivity was recovered (as ticagrelor and AR-C124910XX [M8]) in the feces, suggesting low absorption of the parent ticagrelor or that these components have undergone either biliary or intestinal excretion. Ticagrelor and AR-C124910XX may have passed directly into the bile from the liver, or may have been transported by intestinal P-glycoprotein and thereby secreted from the systemic circulation into the intestine. In this study we are unable to differentiate between these two proposed mechanisms. Measurement of the AUC_{0-t} ratio of total radioactivity in plasma relative to whole blood was 1.69, indicating that ticagrelor and its metabolites are largely restricted to the plasma space and unlikely to extensively penetrate or bind to erythrocytes.

Metabolism was extensive as indicated by the minor amounts of unchanged ticagrelor and AR-C124910XX excreted in urine compared with total radioactivity recovered in urine (less than 0.05% of the dose compared with total radioactivity recovery of 26.5% in the urine). Although AR-C133913XX (M5) was only present at lower levels and was undetectable in plasma after 8 h, this metabolite was the major component in the pooled urine sample. Our findings suggest that an oxidative pathway leads to N-dealkylation of ticagrelor resulting in the major urinary metabolite AR-C133913XX (M5) by loss of a difluorophenylcyclopropyl group; the enzyme involved in this pathway has not been identified.

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Glucuronidation of ticagrelor, AR-C124910XX, and AR-C133913XX by uridine 5'-diphospho-glucuronosyltransferase formed the more polar M9, M6, and M4 metabolites, respectively. An additional glucuronidated metabolite was formed from AR-C133913XX by initial loss of the hydroxyethyl sidechain followed by conjugation with glucuronic acid to form metabolite M3. In line with these metabolites being highly polar, they are expected to be rapidly excreted in urine, and are, therefore, unlikely to have any significant pharmacological activity.

The minor metabolites M1 and M2 (found in urine) were formed via hydroxylation of AR-C133913XX, and metabolite M10 (a minor metabolite in urine) was formed via hydroxylation of ticagrelor. In addition a minor metabolite in feces (M7) was formed by hydroxylation of AR-C124910XX. The isozymes responsible for these oxidative reactions have not been characterized. However, given that these metabolites are minor, the pharmacological relevance of these biotransformations is likely to be minimal. Furthermore, since there were minimal amounts of the active ticagrelor and AR-C124910XX in urine, our data therefore suggest that renal impairment may have minimal effect on systemic exposure to ticagrelor and its active metabolite, and, thus, the antiplatelet effect of ticagrelor.

Total radioactivity recovery was 84.3%, with recovery from 2 subjects falling below 80%. The reason for the lower recovery of the radiolabeled drug is unknown. Previous ADME studies with a low recovery have been explained by the long half-life of the drug. However, given that ticagrelor and AR-C124910XX have a half-life of no more than 12 h, it may be that lower recovery is the result of limitations of the procedure rather than the pharmacology of the drug.

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The metabolite profile of ticagrelor in humans is similar to that in rats and marmosets with comparable levels of circulating metabolites and the two major metabolites being AR-C124910XX and AR-C133913XX (AstraZeneca, data on file). Preclinical studies evaluating the toxicology of ticagrelor in mice, rats and marmosets also showed that AR-C124910XX was observed as a major metabolite in these models. Exposures to ticagrelor and AR-C124910XX in these models exceeded those observed in humans, and provided adequate safety margins for the toxicities seen.

In the pooled plasma samples, five metabolites were identified: AR-C124910XX (M8), AR-C133913XX (M5), and metabolites M1, M2 and M7 (metabolites M3, M4, M6 and M9 were only identified in urine, and M10 was only identified in feces). However, previous data have shown that AR-C133913XX plasma concentrations have only been quantifiable at the maximum tolerated dose or higher (ticagrelor 900 and 1200 mg) (AstraZeneca, data on file). Furthermore, in this study concentrations of M1, M2 and M7 were much lower than AR-C133913XX. Consequently, since plasma concentrations of M1, M2 and M7 are not expected to be quantifiable at the proposed therapeutic dose (ticagrelor 90mg bd), pharmacological activity has only been evaluated for metabolites AR-C124910XX and AR-C133913XX (showing that AR-C124910XX, but not AR-C133913XX has activity) (AstraZeneca, data on file) In addition, to aid further characterization of ticagrelor and its metabolites, AR-C124910XX and AR-C133913XX, a rapid and sensitive analytical method using liquid chromatography with tandem mass spectrometry has been developed (Sillén et al, manuscript submitted).

In conclusion, in humans ticagrelor is rapidly absorbed and extensively metabolized. The majority of the drug was detected in feces, as ticagrelor and AR-

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C124910XX whereas in urine, these were very minor components, with the major metabolite being AR-C133913XX. Inter-individual variability was small in both urine and fecal extracts with only small quantitative differences. All ten of the detected metabolites were fully or partially characterized, and a full biotransformation pathway proposed.

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Acknowledgements

Financial support for the conduct of the research was provided by AstraZeneca.

Colleagues at Clinical Pharmacology & DMPK involved in this project are acknowledged for their dedicated work. Colleagues at York Bioanalytical Solutions, Syngenta Ltd are acknowledged for their assistance with sample analysis, and Charles River Laboratories (Tranent, Scotland) for metabolic profiling and metabolite identification.

The authors would also like to acknowledge Louise Profit PhD (Gardiner-Caldwell Communications, Macclesfield, UK) for assistance in the preparation of the draft and collating author contributions, funded by AstraZeneca.

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FIGURE LEGENDS

Fig. 1 Structure of [¹⁴C]-radiolabelled ticagrelor (the asterix indicates the site of the radiolabel).

Fig. 2. Mean (\pm S.D.) plasma concentrations of ticagrelor and AR-C124910XX in healthy subjects ($n = 6$) after a single oral dose of [¹⁴C]-ticagrelor 200 mg.

Fig. 3. Mean (\pm S.D.) concentrations of total [¹⁴C]-radioactivity in plasma and whole blood in healthy subjects ($n = 6$) after a single oral dose of [¹⁴C]-ticagrelor 200 mg.

Fig. 4. Mean recovery (\pm S.D.) of total radioactivity in urine and feces (0-168 h) and total recovery from healthy subjects ($n = 6$) after a single oral dose of [¹⁴C]-ticagrelor 200 mg.

Fig. 5. HPLC radiochromatograms of pooled plasma at 3 h (A), and 10 h (B) in healthy subjects ($n = 6$) after a single oral dose of [¹⁴C]-ticagrelor 200 mg.

Fig. 6. On-line radiochromatograms of pooled 6–24 h urine sample (A) and pooled fecal extract sample (B) from healthy subjects ($n = 6$) after a single oral dose of [¹⁴C]-ticagrelor 200 mg.

Fig. 7. Mass spectrum of the two major metabolites M4 (A) and M5 (B) from a pooled 6–24 h urine sample from healthy subjects ($n = 6$) after a single oral dose of [¹⁴C]-ticagrelor 200 mg.

A Metabolite M4

Fragment ions derived from M4 (m/z 547):371, 343, 183, 141

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B Metabolite M5 (AR-C133913XX)

Fragment ions derived from M5 (m/z 371):301, 221, 183, 169, 141

Fig. 8. Proposed metabolic pathway for the formation and elimination of ticagrelor metabolites (shadowed areas indicate possible sites of biotransformation). The proportion of the dose of [¹⁴C]-ticagrelor identified as these metabolites in urine or feces is indicated.

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TABLES

TABLE 1. Pharmacokinetic parameters for ticagrelor, AR-C124910XX and total radioactivity in plasma and whole blood in healthy male subjects ($n = 6$) after a single oral dose of [^{14}C]-ticagrelor 200 mg

| Parameter | Ticagrelor | AR-C124910XX | Plasma ^{14}C | Blood ^{14}C |
|--|----------------------|---------------|------------------------|-----------------------|
| t_{\max} (h) | 1.5 (1.0–3.0) | 3.0 (2.0–3.0) | 2.5 (2.0–3.0) | 3.0 (2.0–4.0) |
| C_{\max} (ng/ml) | 923 (35.6) | 264 (22.0) | 1534 (21.0) | 1129 (17.0) |
| AUC_{0-t} (ng.h/ml) | 6591 (44.8) | 2477 (28.4) | 9007 (34.1) | 5331 (15.2) |
| AUC (ng.h/ml) | 6675 (44.7) | 2538 (28.4) | 11,042 (35.4) | 7132 (18.1) |
| $t_{1/2}$ (h) | 8.4 (2.3) | 11.5 (4.5) | 6.3 (4.1) | 4.6 (2.2) |
| $\text{Ae}_{(\infty)}$ (μg) | 41.5 (22.9) | 81.3 (21.4) | -- | -- |
| CL_R (l/h) | 0.00584 (0.00252) | NQ -- | | -- |
| Metabolite:parent | -- 0.29 | (0.064) | -- | -- |
| C_{\max} ratio | | | | |
| Metabolite:parent | -- | 0.40 (0.124) | -- | -- |
| AUC ratio | | | | |

*Units for radioactivity are ng Eq/ml for C_{\max} and ng Eq.h/ml for AUC_{0-t} and AUC.

Data for t_{\max} are median (range); data for C_{\max} , AUC_{0-t} , and AUC are geometric mean (coefficient of variation %); all other data are mean (standard deviation).

NQ= non-quantifiable (below limit of quantification)

TABLE 2. Concentration of ticagrelor and metabolites over time in pooled plasma samples from healthy male subjects ($n = 6$) after a single oral dose of [^{14}C]-ticagrelor 200 mg

| Metabolite ($\mu\text{g Eq/g}$) | Hour Post-dose | | | | | | | |
|-----------------------------------|----------------|-------|-------|-------|-------|-------|----|-------|
| | 1 | 1.5 | 2 | 3 | 4 | 6 | 8* | 10 |
| M1 | 0.185 | | | | | | | |
| M2 | | | 0.146 | | | 0.168 | | |
| M5 (AR-C133913XX) | | | 0.354 | | | | | |
| M7 | 0.101 | | | | | | | |
| Ticagrelor + M8 (AR-C124910XX) | 0.754 | 0.319 | 0.710 | 0.546 | 0.715 | 0.433 | | 0.132 |

*Below limit of quantification for the 8 h sample.

TABLE 3. Retention times of ticagrelor and its metabolites and percentage of radioactive dose in pooled urine and feces samples from healthy subjects ($n = 6$) after a single oral dose of [^{14}C]-ticagrelor 200 mg

| Metabolite | Urine (22.7 % of Dose) | | Feces (54.8% of Dose) | |
|-------------------|------------------------|-----------|------------------------|-----------|
| | Retention Time, min | % of Dose | Retention Time, min | % of Dose |
| M1 13.8 | | 1.32 | | |
| M2 14.0 | | 1.32 | | |
| M3 20.6 | | 1.91 | | |
| M4 22.4 | | 6.64 | | |
| M5 (AR-C133913XX) | 24.3 9.21 | | 24.4 2.77 | |
| M7 | | | 33.4 | 0.61 |
| M8 (AR-C124910XX) | | | 42.0 | 21.73 |
| Ticagrelor | | | 42.7 | 27.09 |

TABLE 4. Metabolites of ticagrelor identified by LC/MS analysis of reference standards and metabolites in pooled urine (6–24 h), pooled feces, and pooled plasma (3 h) from healthy subjects ($n = 6$) after a single oral dose of [^{14}C]-ticagrelor 200 mg

| Metabolite | [M+H] ⁺ | Product ions <i>m/z</i> |
|------------------------------------|--------------------|--|
| Reference standards | | |
| Ticagrelor ^{b,c} | 523 | 495, 453, 363, 321, 335, 293, 153, 127 |
| M5 ^{a,b,c} (AR-C133913XX) | 371 | 301, 263, 221, 183, 169, 141 |
| M8 ^{b,c} (AR-C124910XX) | 479 363 | , 335, 321, 293, 153, 127 |
| Other metabolites | | |
| M1 ^a 387 | | ND |
| M2 ^a 387 | | ND |
| M3 ^a | 503 | 327, 211, 141 |
| M4 ^a | 547 | 371, 343, 183, 141 |
| M6 ^a | 655 | 479 |
| M9 ^a | 699 | 523, 495 |
| M10 ^a | 539 | 363, 153 |
| M7 ^b | 495 | 477, 449, 361, 153 |

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^apresent in urine; ^bpresent in feces; ^cpresent in plasma

Bolded ions represent those only identified with ticagrelor as reference standard

ND = no data

Figure 1

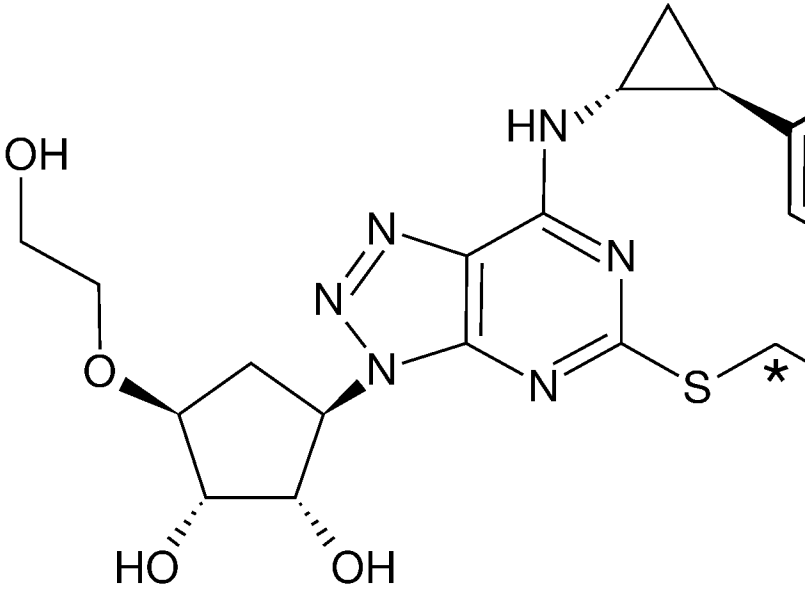


Figure 2

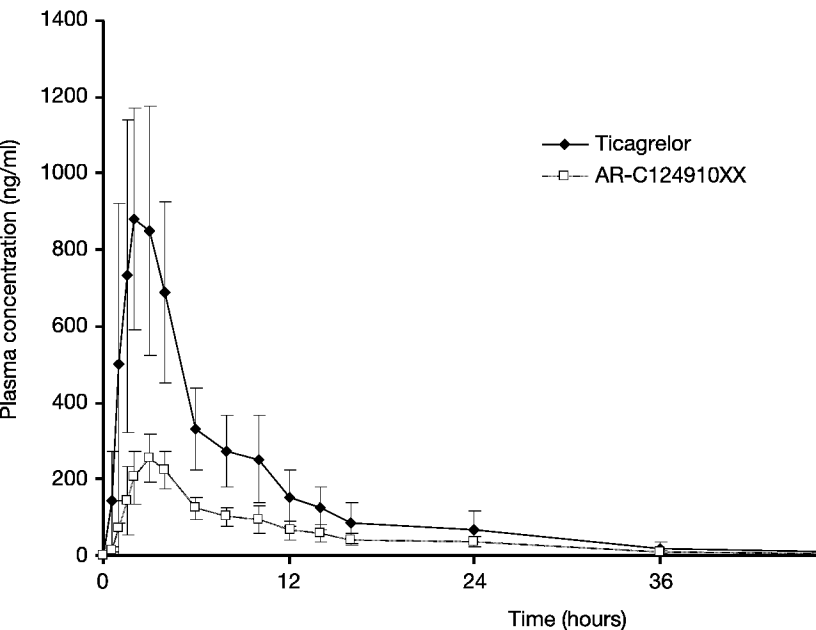


Figure 3

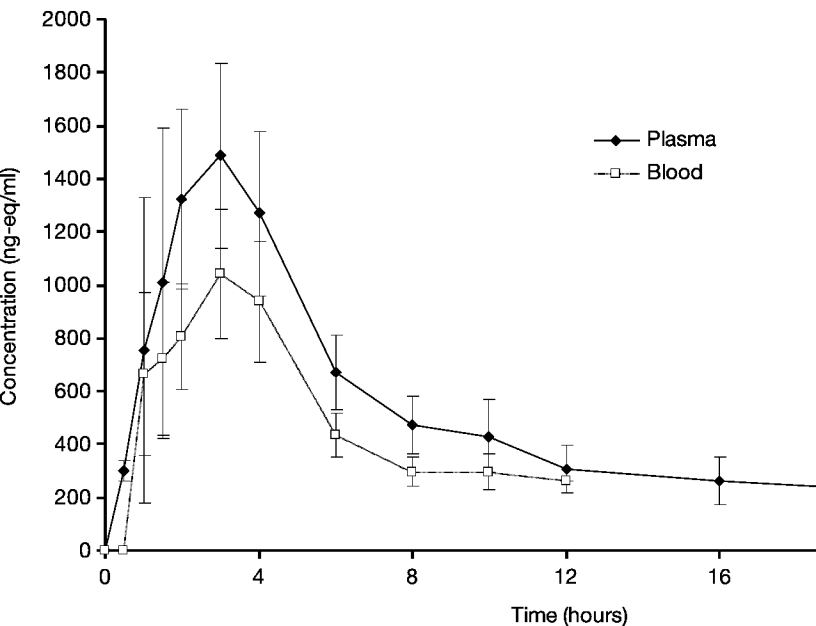


Figure 4

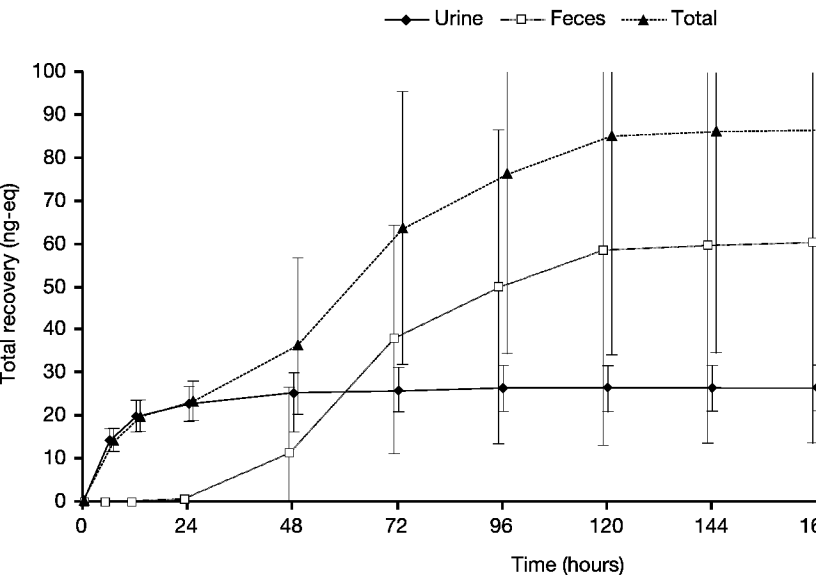


Figure 5

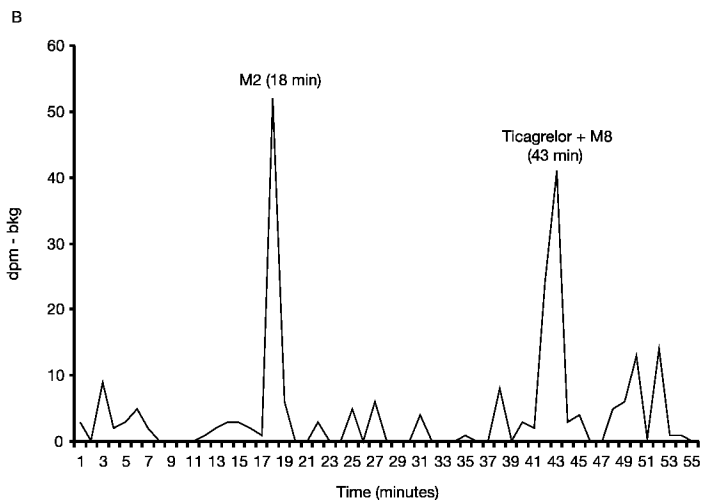
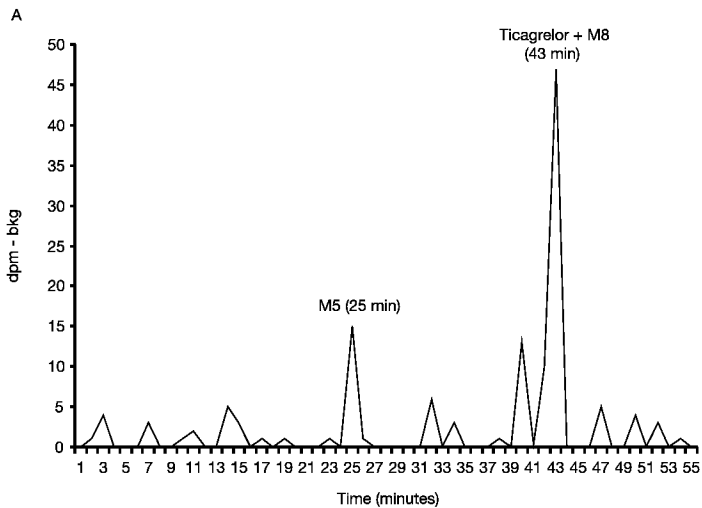


Figure 6

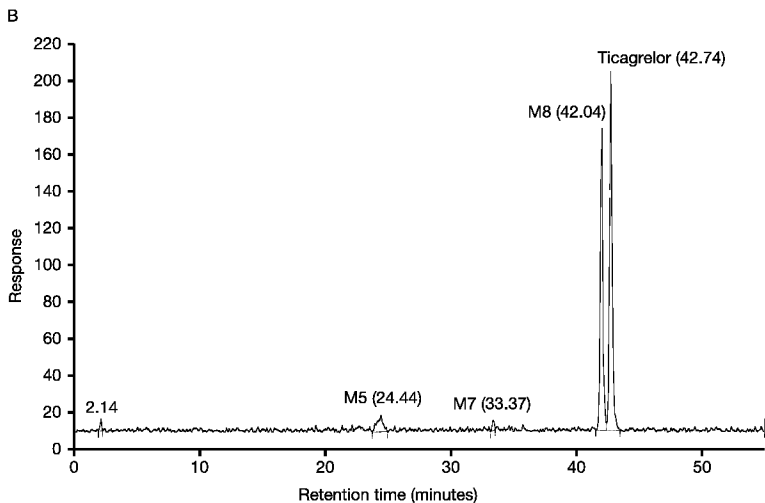
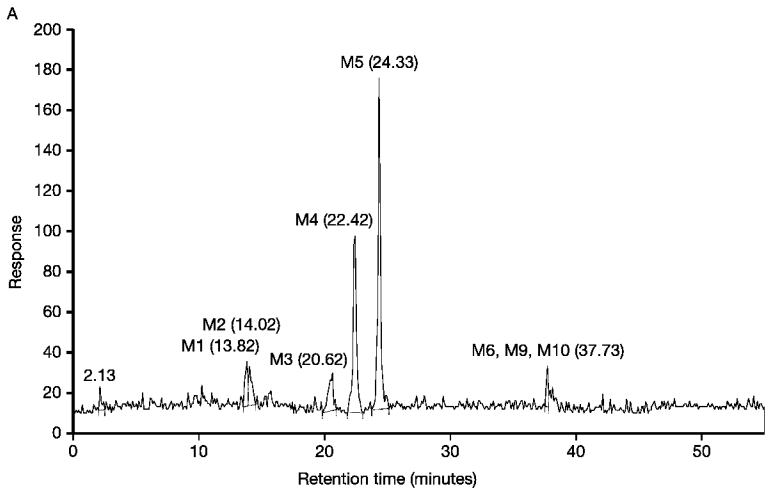
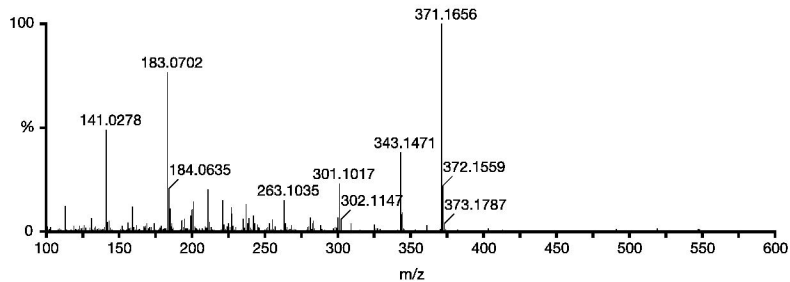
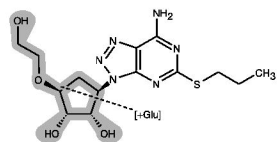


Figure 7

A



B

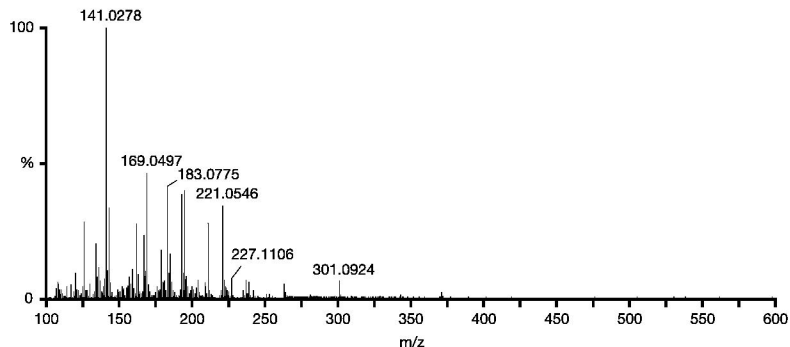
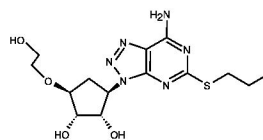
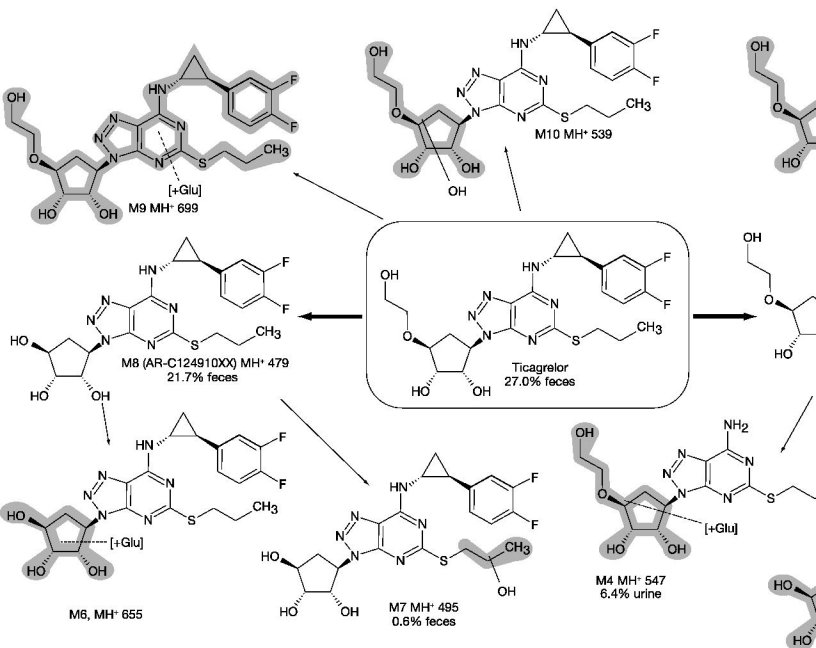


Figure 8



M6, M9, M10 0.5-1.4% urine