

**Stereoselective Metabolism of Prasugrel in Humans Using a Novel
Chiral Liquid Chromatography-Tandem Mass Spectrometry**

Enaksha R. Wickremsinhe, Ye Tian, Kenneth J. Ruterbories, Elizabeth M. Verburg, Govinda J. Weerakkody, Atsushi Kurihara, and Nagy A. Farid

ERW, YT, KJR, EMV, GJW, NAF at Lilly Research Laboratories, Eli Lilly and Company,
Indianapolis, IN, 46285, USA

AK at Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd.
Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

Primary laboratory of origin: Lilly Research Laboratories, Lilly Corporate Center, Indianapolis,
IN 46285, USA

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Corresponding Author:

Enaksha R. Wickremsinhe, Ph.D.

Lilly Research Laboratories

Eli Lilly and Company

Lilly Corporate Center

Indianapolis, IN, 46285

Tel: 317-433-9290

Fax: 317-433-6432

e-mail: enaksha@lilly.com

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Nonstandard Abbreviations: EDTA, ethylenediamine tetraacetate acid ; HPLC, high performance liquid chromatography; LC/MS/MS, liquid chromatography/tandem mass spectrometry; QC, quality control; SPE, solid phase extraction; R-95913, 2-[2-oxo-6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl]-1-cyclopropyl-2-(2-fluorophenyl)ethanone; R-138727, 2-[1-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4-mercapto-3-piperidinylidene]acetic acid;

Abstract

An LC-MS/MS method was developed to chromatographically separate the four stereoisomers of the active metabolite of prasugrel, R-138727, in human plasma following derivatization with bromo-methoxy acetophenone to stabilize the molecule. This technique was designed to determine the relative contribution of each stereoisomer, based on statistical analyses of each stereoisomer's chromatographic peak areas. The methodology was validated and used for the analysis of clinical samples in which the R-138727 had been derivatized at the time of blood collection. This technique can be useful to determine ratios of stereoisomers in biological samples (e.g. plasma) especially in situations where authentic standards of each individual stereoisomer are scarce or unavailable. In humans, the metabolic formation of R-138727 from prasugrel was found to be stereoselective where 84% of R-138727 was present as RS and RR, the two most pharmacologically potent isomers, while the SR and SS enantiomers accounted for about 16%. The ratios of the R-138727 stereoisomers were consistent among subjects, regardless of the dose, time of sample collection, or whether the blood was sampled after the first dose or after 4 weeks of therapy.

Introduction

Prasugrel, a novel thienopyridine antiplatelet compound, is a prodrug that is rapidly hydrolyzed *in vivo* to the pharmacologically inactive thiolactone R-95913, which is then metabolized by various cytochrome P450 enzymes to the pharmacologically active R-138727 (Fig. 1) (Farid et al., 2005; Rehmel et al., 2006). The pharmacological action of prasugrel is the result of R-138727 binding irreversibly to the P2Y₁₂ platelet adenosine diphosphate receptor, thus inhibiting platelet activation and aggregation (Jakubowski et al., 2006). R-138727 possesses two chiral centers, and it has been reported that the four stereoisomers possess differing antiplatelet activity, with the two stereoisomers with the R-configuration at the thiol group (R-125689 and R-125690) as the most potent (Hasegawa et al., 2005).

Xenobiotics that contain multiple chiral centers (stereocenters) pose a challenging dimension to the overall drug development process. The chemistry and pharmacological significance of multiple stereocenters have been reviewed (Testa et al., 1993; Tomaszewski and Rumore, 1994; Reist et al., 1995; Van Miert, 2003). A compound with two chiral centers can exist in four stereoisomeric forms: RR, SS, RS, and SR. Enantiomers are stereoisomers that are mirror images (RR and SS are enantiomers as are SR and RS). Diastereomers are stereoisomers but are not mirror images, e.g. RR is a diastereomer of both RS and SR; likewise, SS is a diastereomer of both RS and SR. Such molecules pose a significant challenge for the bioanalytical chemist when analyzing plasma samples for pharmacokinetic analyses (Marzo and Heftman, 2002). Typically, “pure” standards of these stereoisomers are not available; therefore, the molecule is quantified as a single peak (achiral assay) or as doublets (RR/SS and RS/SR) using reversed-phase chromatography.

An achiral assay that determines the concentration of R-138727 in human plasma samples (following derivatization to stabilize the molecule 2-bromo-3'-methoxyacetophenone) has been validated and used to support clinical studies (Farid et al., 2007). However, the extent of formation of each of R-138727 stereoisomers in animals or humans has not been previously investigated. The objective of this research was to develop an assay to separate and determine the relative contribution of each of the four stereoisomers to the total R-138727 present in human plasma after administration of varying single and multiple doses of prasugrel. The chiral assay was also designed to statistically identify divergence in the individual peak area ratios, if any, compared with the "standard" comparators (quality control [QC] samples). The method used a stable isotope labeled internal standard and the sample preparation was similar to that of the achiral method. Theoretically, the ratio of the four peaks comprising R-138727 in the standards used in the assay should be equimolar (1:1:1:1); therefore, the calculated ratio is considered equivalent to 1:1:1:1.

Materials and Methods

Reagents and Materials. All chemicals were of analytical grade and used without further purification. Methanol and acetonitrile were purchased from Burdick & Jackson (Muskegon, MI, USA), formic acid (96%), ammonium acetate (97%), ammonium hydroxide (28–30%), ammonium chloride, 2-bromo-3'-methoxy-acetophenone (98%) from Sigma-Aldrich (St. Louis, MO, USA), and dimethylsulfoxide (99.9%) from Mallinckrodt (Hazelwood, MO, USA). A Waters MilliQ system (Billerica, MA, USA) was used to obtain purified water. ScreenMates™ 96-well plate vials (1.4 mL) and ScreenMates™ 96 Cap strips were purchased from Matrix Technologies (Cheshire, UK). Human EDTA plasma was purchased from Biochemed Pharmacologicals (Winchester, VA, USA) and was used to prepare all control, standard curve, QC, and validation samples.

Analytical Standards. R-138727 and [²H₄]-R-138727 were obtained from Sankyo Co., Ltd., Tokyo, Japan and stored at –20°C (Fig. 2). Additionally, small amounts of each of R-138727 stereoisomers (R-125687, R-125688, R-125689, and R-125690) were also provided by Sankyo Co. for positive identification after chromatographic separation.

LC-MS/MS. A PE Sciex API 4000 mass spectrometer (Applied Biosystems, Foster City, CA, USA) was used with a TurboIonSpray interface operated in positive ion mode. Nitrogen for use as the curtain and collision gases was from an Air Liquide in-house system. The high performance liquid chromatography (HPLC) system was comprised of two Shimadzu LC-10ADvp pumps, a Shimadzu DGU-14A solvent degasser, a Shimadzu SCL-10Avp system controller (Kyoto, Japan), a Jones Chromatography column heater (Barnstaple, UK), and a Gilson 215 autosampler (Middleton, WI, USA). Samples (10 µL) were injected onto a 10×2mm BetaBasic C4 Javelin Guard (Thermo Electron Corp., Waltham, MA, USA) connected to two

(150×2mm and 250×2mm) ProntoSIL Chiral AXQN columns in series (MAC-MOD Analytical, Chadds Ford, PA, USA) (Fig. 3). An isocratic mobile phase consisting of 200/1.2/0.6/0.0025 methanol/acetic acid/water/0.1 M ammonium acetate was used at a flow rate of 170 $\mu\text{L}/\text{min}$. The column temperature was maintained at 30°C. Methanol was used as the autosampler wash solution. The total cycle time was approximately 20 min per injection.

R-138727 was monitored as its derivatized form by following the selected reaction monitoring transition from m/z 498.2 to m/z 348.3 using a dwell time of 1000 msec (Fig. 4). The deuterated internal standard ($[^2\text{H}_4]$ -R-138727) was similarly monitored using the selected reaction monitoring transition from m/z 502.2 to m/z 352.3 using a dwell time of 200 msec. The following mass spectrometer settings were used: source temperature of 500°C, IonSpray voltage of 5500 volts, gas 1 setting at 50, gas 2 setting at 50, curtain gas setting at 10, collision gas setting at 4, declustering potential at 75 v, entrance potential at 10 v, and collision energy at 29 v.

Derivatization of R-138727 and the Internal Standard for Analysis. A 50 $\mu\text{g}/\text{mL}$ stock solution of R-138727 or $[^2\text{H}_4]$ -R-138727 in the derivatized form was prepared by the addition of 4.0 mL of 0.1 M ammonium hydroxide:0.1 M ammonium chloride (1:4, v/v) to 1.25 mg of R-138727, followed by 0.5 mL of 0.1 M 2-bromo-3'-methoxyacetophenone (the derivatizing reagent) for at least ten minutes, and diluting to 25 mL with acetonitrile as described previously (Farid et al., 2007). The internal standard was further diluted with acetonitrile to yield a 100 ng/mL working solution. All solutions were stored at -20°C in polypropylene vials.

Standard Curve and Quality Control (QC) Samples. Standard curve and QC samples were prepared in plasma at R-138727 concentrations from 4 to 256 ng/mL. Each standard curve point was prepared in duplicate for the validation. Stability and QC samples were prepared at 4,

16, 64, and 256 ng/mL. Each concentration of QC samples was prepared in replicates of six for validation experiments and in triplicate for routine analyses.

Stability Experiments. Room temperature, freeze/thaw, and frozen-storage stability experiments were conducted using triplicates of each QC concentration. Long-term frozen stability was conducted at -20°C . The objective of these stability experiments was to establish that there were no changes in the ratio of the four stereoisomers upon storage.

96-Well Extraction Procedure. All samples were vortex mixed and centrifuged (3,500 rpm for 10 min at 15°C) to remove any particulate matter, following which 200 μL aliquots of each (blanks, standard curve samples, QC samples—also used as validation samples, and study samples) were pipetted into designated 96-well polypropylene tubes. The internal standard (20 μL of the 100 ng/mL solution) was added to all vials except the plasma blanks, to which 20 μL of 1:1 methanol/water (v/v) was added. To all samples, 300 μL of 1% formic acid was added, the vials were capped, vortex mixed, and centrifuged (3,000 rpm for 15 min at room temperature). 3M Empore C8-SD96 well SPE plates (3M, St. Paul, MN, USA) were used for solid-phase extraction (SPE) and was performed on a Tomtec Quadra 96 liquid-handling station (Hamden, CT, USA). The SPE plate was conditioned with 100 μL of methanol followed by 200 μL of water. The entire sample supernatant was loaded onto the SPE plate using the vacuum manifold, washed with 500 μL of 1% formic acid, 500 μL of 50 mM ammonium acetate, and 500 μL of 30% methanol/water, respectively. The dry SPE plate was placed on a 96-well polypropylene collection plate, and the sample was slowly eluted with 150 μL of methanol. The plate was capped and centrifuged, and the supernatant was transferred to a 96-well plate for chiral electrospray LC-MS/MS analysis.

Plasma Samples. R-138727 was derivatized with 2-bromo-3'-methoxyacetophenone at the time of blood collection to ensure stabilization of the thiol moiety of the compound (Farid et al., 2007).

Method Validation. Validation of this methodology consisted of analyzing three separate batches, each containing two standard curves (range 4 to 256 ng R-138727/mL) to establish the linearity, and six replicates of each QC sample (4, 16, 64, and 256 R-138727/mL) to establish accuracy and precision of the assay. Calibration curves were obtained by plotting the total peak area ratio (from all four derivatized R-138727 stereoisomers) and their internal standards versus the concentration. A weighted ($1/\text{concentration}^2$) least-squares-regression analysis was used to define the assay range. Accuracy and precision of the technique were calculated using the total peak area at each of the four QC concentrations. Although the theoretical value for each stereoisomer is 25% (total of 100%), the absolute peak areas generated could be different. The technique for the analyses of the individual stereoisomers (RR, RS, SR, and SS) and the ability to identify any divergence in the ratio of the four peaks was based on the statistical criteria described below using the individual peak areas of each isomer.

Data Analysis and Statistical Methodology. Each individual peak was reported as a percent of the total peak area using equation 1. The overall mean of the peak area ratio for each stereoisomer was then used to define low and high limits that would be considered equimolar. The low and high limits ($\alpha = 0.05/2$ to account for two-tailed test) were defined using equation 2 based on the Student's *t*-distribution (Milliken and Johnson, 1992). Since there are four mutually exclusive outcomes (stereoisomers) resulting from the two independent chiral centers yielding equimolar concentrations (25% of each), a Bonferroni correction (Milliken and Johnson, 1992) for multiplicity was also applied. Thus, an α value of $0.05/4$ was used.

Equation 1.

$$[\text{individual peak area}/\text{total IS peak area} \times 100] / [\text{total individual peak area}/\text{total IS peak area}]$$

Equation 2. $\text{Mean} \pm t_{\alpha} \times \text{SD} \times (1 + 1/n)^{1/2}$

where $\alpha = 0.0125$.

Unknown samples (stability samples, clinical samples, etc.) with individual peak-area ratio percentages that fell outside the low and high limits determined for each batch (the acceptance criteria based on the QC samples) for at least two stereoisomers (peaks) were considered to be nonconforming with the theoretical ratio of 1:1:1:1.

Analysis of Plasma Samples after Prasugrel Administration. Plasma samples were analyzed to determine the ratio of R-138727 stereoisomer after single oral doses of prasugrel of 40 mg or 60 mg, and multiple doses ranging from 5 mg to 15 mg for 1 month. Triplicate injections of each QC concentration were simultaneously analyzed. Standard curve samples were not included since there was no intent to generate actual concentrations in the absence of individual standards for each stereoisomer. All plasma samples were positioned within each batch in a manner that they were bracketed by the QC samples. Acceptance of the batch was based on at least five of the six replicates at each validation QC concentration falling within the above-defined low and high limits and the overall precision of the individual peak-area ratio percentages being less than 15% relative standard deviation.

Results

Characteristics of the New Chiral LC/MS/MS Methodology. The identity of each stereoisomer of R-138727 was determined by individually analyzing a small authentic sample of each stereoisomer (following the same derivatization procedure). The four stereoisomers eluted in the following sequence SR, SS, RS, and RR. Chromatograms representing the lowest standard and a blank plasma sample along with the identification of the individual stereoisomer peaks are presented as Fig. 5.

Typical retention times for the four peaks were approximately 11.0 (R-125688), 12.0 (R-125687), 15.0 (R-125690), and 17.0 (R-125689) min. The assay lower and upper limits of quantification for R-138727 were 4 ng/mL and 256 ng/mL, respectively. The intra-batch accuracy (relative error) for R-138727 ranged from 0.24% to 7.3%, and the intra-batch precision ranged from 0.96% to 5.2% at the concentrations tested (Table 1), thus demonstrating validation of the methodology. The correlation coefficient (r^2) for derivatized R-138727 (quantified as the total of all four stereoisomers) was 0.9986 or greater during validation.

Analysis of a plasma blank injected following each injection of the highest standard resulted in no quantifiable peaks, indicating no detectable system carryover and enabled the use of this methodology for routine analyses without additional method development to counter carryover. The individual stereoisomers peak-area ratios of the validation QC samples were within the low and high limits defined based on the standard curve samples, indicating that the ratios of the stereoisomers were similar between the standards and QCs.

The results of the stability experiments demonstrated that the ratio of the four stereoisomers of R-138727 remained constant in human plasma when exposed to room

temperature for 24 h, subjected to four freeze/thaw cycles, or during long-term frozen storage for 31 days at -20°C .

Analysis of Human Plasma Samples after Prasugrel Administration. Ninety samples obtained from multiple subjects from different prasugrel treatment groups (doses) were analyzed to determine the R-138727 stereoisomer ratios. The percent peak area limits for each stereoisomer corresponding to a 1:1:1:1 ratio was calculated using the QC samples and the data are shown in Table 2. A summary of the results is shown in Table 3, and a representative chromatogram depicting a subject plasma concentration is shown as Figure 6.

The stereoisomer ratios of R-138727 in plasma samples of subjects given prasugrel diverged from the 1:1:1:1 ratio obtained in the QC samples. Overall, the four stereoisomers, R-125688, R-125687, R-125690, and R-125689 exhibited a 1:1:4:4 ratio. On average, the diastereomers RS (R-125690) and RR (R-125689) comprised about 84%, while the diastereomers SR (R-125688) and SS (R-125687) accounted for about 16% of the R-138727 present in plasma (Table 3).

Discussion

This is the first report describing the separation of the enantiomers of prasugrel's active metabolite and its clinical application. The data show the successful development and validation of a novel chiral liquid chromatography - tandem mass spectrometric methodology that can be used to identify divergence in diastereomer peaks in the absence of authentic standards. This technique can also be extremely valuable in situations where selective disposition of diastereomers can be implicated in understanding clinical pharmacology. The use of two HPLC columns in tandem was required to enable the separation of the R-138727 stereoisomers. There was no divergence in peak area ratios observed during the conduct of stability studies.

Plasma samples obtained from multiple subjects from different prasugrel treatment groups (doses) were selected such as to include various time-points after the first (loading dose) and/or 1 month of daily maintenance doses of prasugrel. The data showed that regardless of the prasugrel dose administered, the time of blood sampling after the dose (15 min to 12 h), or the duration of treatment, the peak area ratios for stereoisomers R-125688 and R-125687 ranged from 3.1 to 14.5%, and from 33.6 to 47.6% for stereoisomers R-125690 and R-125689 (Table 3). These data represent the first report that the metabolism of thienopyridines to form the active metabolite is a stereoselective process.

Prasugrel is administered as a racemate. Hydrolysis of prasugrel by esterases produces a 2-hydroxythiophene moiety, which can be transiently tautomerized to a 1,4-enone, which in turn rearranges to the more thermodynamically stable R-95913. Formation of R-95913 introduces a second chiral center in the molecule, and, considering the postulated route of its formation, all R-95913 diastereomers would be formed in equal proportion. The observed stereoselectivity in the active metabolite formation in humans from R-95913 suggests that either the opening of the thiolactone ring in R-95913, an enzyme-mediated process (Rehmel et al., 2006), is stereoselective, and the chirality is preserved up to the step leading to R-138727 formation, or the ultimate step leading to R-138727 formation (e.g. reduction of a thioketone) is stereoselective. The results also showed that the two most pharmacologically potent antiplatelet stereoisomers R-125690 and R-125689 represent 84% of the R-138727 circulating in human plasma. The data showed that the ratios of the R-138727 stereoisomers are consistent among subjects, regardless of the dose, time of sample collection, or whether the blood was sampled after the first dose of the drug or after a few weeks of therapy.

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References

- Farid NA, Smith RL, Rash TJ, Blair PE, Gillespie TA, Turik MA, Naganuma H, and Goldberg MJ (2005) The disposition of prasugrel (CS-747, LY640315), a novel thienopyridine, in humans. *Drug Metab Reviews* **37(Suppl 2)**:86.
- Farid NA, McIntosh M, Garofolo F, Wong E, Shwajch A, Kennedy M, Young M, Sarkar P, Kawabata K, Takahashi M, and Pang H (2007) Determination of the active and inactive metabolites of prasugrel in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* **21**:169-179.
- Hasegawa M, Sugidachi A, Ogawa T, Isobe T, Jakubowski JA, and Asai F (2005) Stereoselective inhibition of human platelet aggregation by R-138727, the active metabolite of CS-747 (prasugrel, LY640315), a novel P2Y₁₂ receptor inhibitor. *Thromb Haemost* **94**:593-598.
- Jakubowski JA, Payne CD, Brandt JT, Weerakkody GJ, Farid NA, Small DS, Naganuma H, Li GY, and Winters KJ (2006) The platelet inhibitory effects and pharmacokinetics of prasugrel after administration of loading and maintenance doses in healthy subjects. *J Cardiovasc Pharmacol* **47**:377-384.
- Marzo A and Heftman E (2002) Enantioselective analytical methods in pharmacokinetics with specific reference to genetic polymorphic metabolism. *J Biochem Biophys Methods* **54**:57-70.
- Milliken GA and Johnson DE (1992) Designed experiments, in *Analysis of Messy Data, Volume I* Chapman & Hall/CRC, New York.

- Rehmel JLF, Eckstein JA, Farid NA, Heim JB, Kasper SC, Kurihara A, Wrighton SA, and Ring BJ (2006) Interactions of two major metabolites of prasugrel, a thienopyridine antiplatelet agent, with the cytochromes P450. *Drug Metab Dispos* **34**:600-607.
- Reist M, Testa B, Carrupt PA, Jung M, and Schurig V (1995) Racemization, enantiomerization, diastereomerization, and epimerization – their meaning and pharmacological significance. *Chirality* **7**:396-400.
- Testa B, Carrupt PA, and Gal J (1993) The so-called interconversion of stereoisomeric drugs – an attempt at clarification. *Chirality* **5**:105-111.
- Tomaszewski J and Rumore MM (1994) Stereoisomeric drugs: FDA's policy statement and the impact on drug development. *Drug Dev Ind Pharm* **20**:119-139.
- Van Miert ASJPAM (2003) Stereoselectivity in drug development. A clinical perspective? *The Veterinary J* **165**:3-4.

Figure Legends

FIG. 1. Metabolism of prasugrel to R-138727.

FIG. 2. Structures of R-138727, derivatized R-138727, internal standard [$^2\text{H}_4$]-R-138727, and derivatized [$^2\text{H}_4$]-R-138727.

FIG. 3. Schematic of the set-up using tandem HPLC columns for stereoisomeric separation.

FIG. 4. Product ion spectrum for derivatized R-138727.

FIG. 5. Chromatograms representing the lowest standard concentration (4 ng/mL) and a blank plasma sample. The identity of each peak was assigned following the analyses of authentic standards of each stereoisomer.

FIG. 6. Chromatogram of a subject sample depicting the differences in the individual stereoisomer peaks.

TABLE 1

Inter- and intra-batch accuracy and precision for R-138727 quantified as the summation of all four peaks (RS, RR, SR, and SS)

	R-138727 Concentration (ng/mL)			
	4	16	64	256
Intra-batch assay:				
Validation batch #1				
Mean (<i>n</i> =6)	3.65	15.65	64.63	238.67
Accuracy (%RE)	-8.75	-2.19	0.99	-6.77
Precision (%RSD)	3.38	3.61	4.44	6.53
Validation batch #2				
Mean (<i>n</i> =6)	4.06	16.60	68.02	262.83
Accuracy (%RE)	1.37	3.75	6.28	2.67
Precision (%RSD)	4.02	1.20	4.40	2.69
Validation batch #3				
Mean (<i>n</i> =6)	3.84	15.97	66.05	272.67
Accuracy (%RE)	-3.96	-0.21	3.20	6.51
Precision (%RSD)	3.44	2.59	5.50	2.51
Inter-Assay Mean (<i>n</i> =18)	3.85	16.07	66.23	258.06
Inter-Assay Accuracy (%RE)	-3.78	0.45	3.49	0.80
Inter-Assay Precision (%RSD)	5.60	3.52	5.00	6.89

TABLE 2

Calculation of low and high limits for percent peak-area ratios, for individual R-138727 stereoisomers, using quality control (QC) samples from validation

QC Sample Concentration (ng R-138727/mL)	Percentage of individual stereoisomers based on total peak area			
	SR	SS	RS	RR
4	22.7	27.2	26.2	23.9
16	21.7	26.9	25.4	26.0
64	22.5	26.6	26.2	24.7
256	22.1	26.8	25.6	25.5
4	21.7	27.1	26.3	24.9
16	21.9	27.1	25.8	25.2
64	22.1	27.0	25.5	25.4
256	21.4	27.0	25.5	26.1
4	21.7	27.2	25.9	25.3
16	21.6	27.2	25.3	25.9
64	21.8	27.2	25.5	25.5
256	21.3	27.5	25.6	25.6
Mean	21.9	27.1	25.7	25.3
SD	0.4	0.2	0.4	0.6
% RSD	1.8	0.8	1.4	2.4
Limits (\pm)*	1.06	0.60	0.95	1.6
Low Limit	20.8	26.5	24.8	23.7

High Limit	22.9	27.7	26.7	27.0
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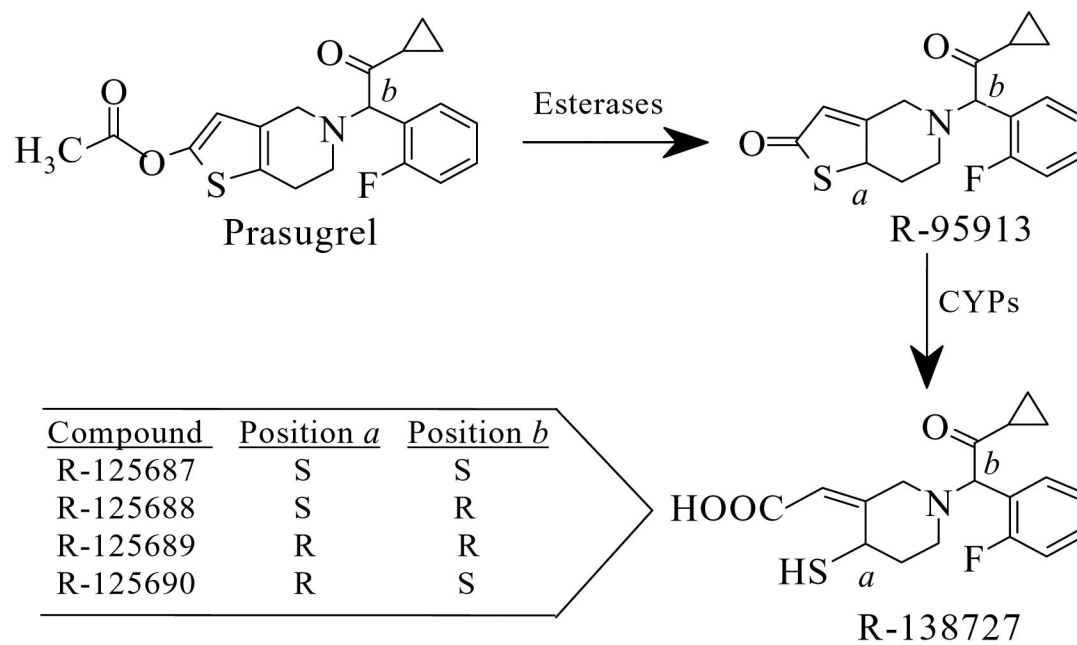
*Limits (\pm) = Mean $\pm t_{\alpha} \times SD \times (1 + 1/n)^{1/2}$, $n = 12$, $t = 2.593$, $\alpha = 0.0125$

TABLE 3

Ratio of R-138727 stereoisomers in plasma samples from subjects dosed with prasugrel

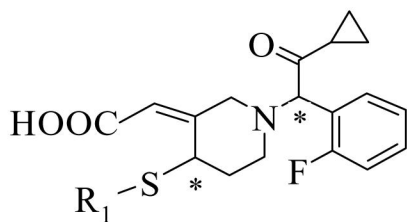
	SR	SS	RS	RR
	(R-125688)	(R-125687)	(R-125690)	(R-125689)
Mean percent of peak area (Mean \pm SD, $n=90$)	7.3 \pm 2.4	9.1 \pm 3.0	41.2 \pm 2.7	42.4 \pm 2.9
Percent peak area range	3.1 – 12.8	3.9 – 14.5	33.6 – 46.3	34.9 – 47.6
Mean percent of peak area of QC samples	21.9	27.1	25.7	25.3

Figure 1

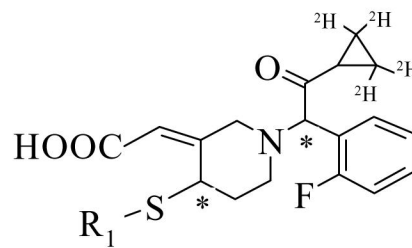


Chiral centers are denoted as *a* and *b*

Figure 2



Analyte



Internal standard

* denotes chiral centers

<u>Molecule</u>	<u>R₁</u>	<u>Molecular Weight</u>
R-138727 (analyte)	H	349.4
R-138727 (derivatized)]		497.6
[² H ₄]-R-138727 (internal standard)	H	353.4
[² H ₄]-R-138727 (derivatized)		501.6

Figure 3

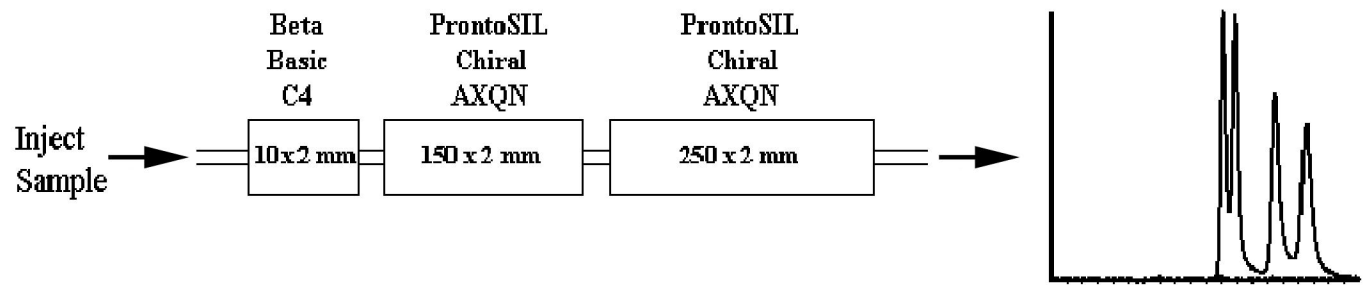


Figure 4

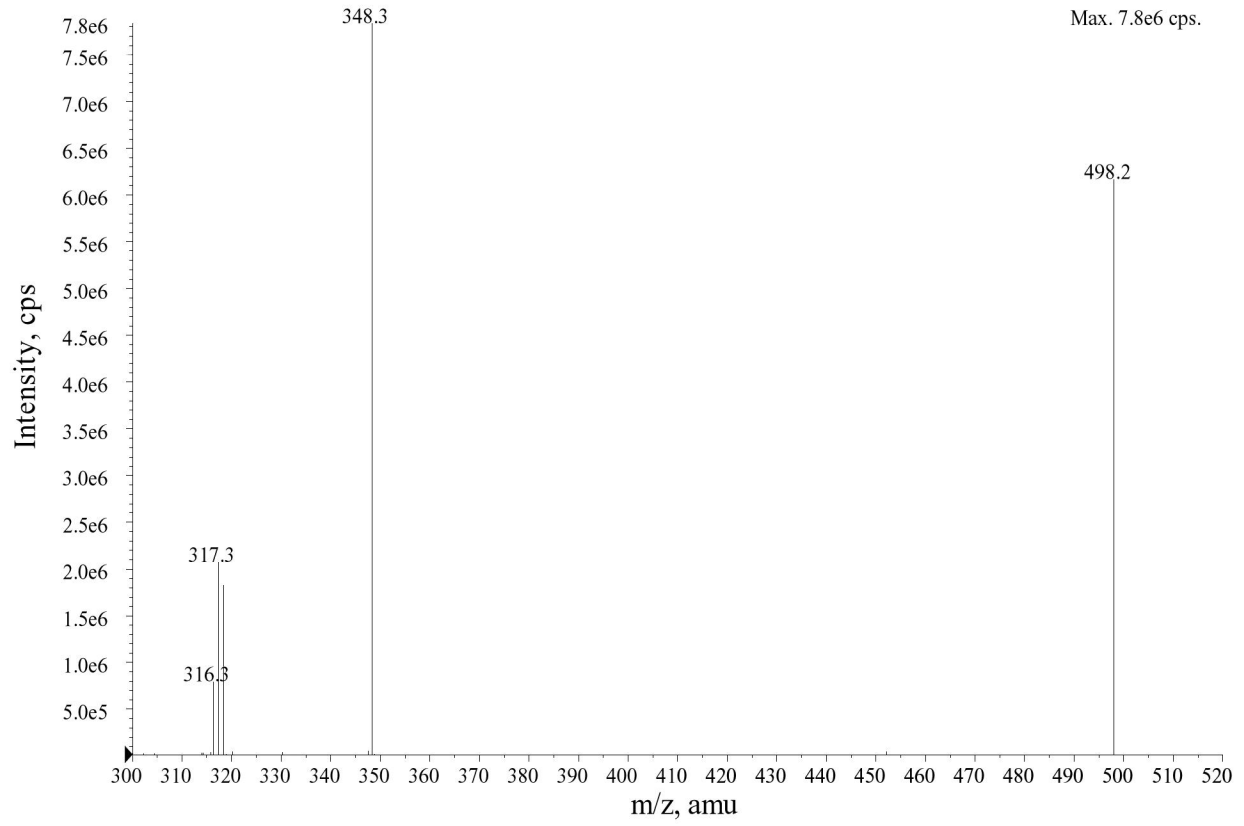


Figure 5

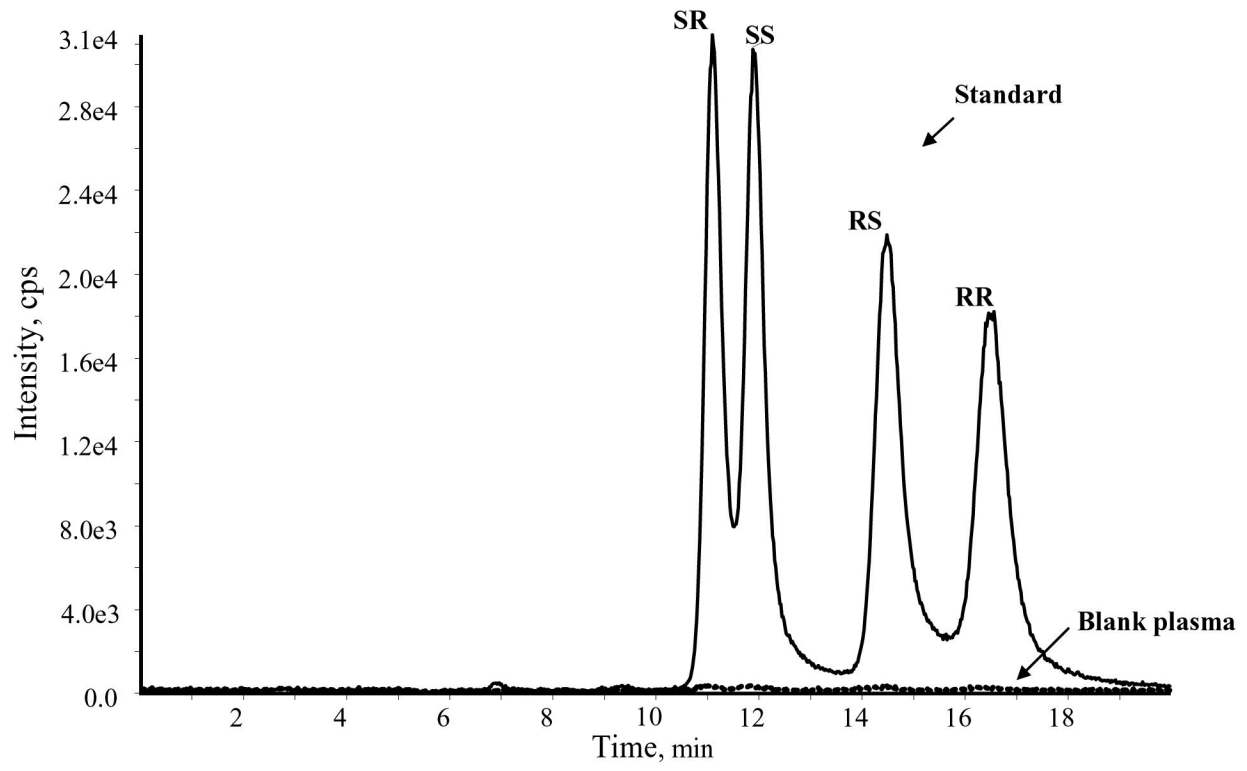


Figure 6

