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Simultaneous LC-MS/MS Determination of Racemic Warfarin and Etravirine in Rat Plasma and Its Application in Pharmacokinetic Studies

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1. Introduction

Warfarin, one of the most commonly used oral anticoagulant in the US and across the globe, is a drug of choice for millions. It's a unique drug of its kind due to the multiple pharmacological and pharmacokinetic properties. The posology of warfarin cannot be generalized. Having the right dose for the right patient makes this drug follow a pattern that is unique among the lot. Warfarin has a high interpatient variability (Min-Jung Kwon et al., 2009) and narrow therapeutic index which requires continuous monitoring of its plasma concentration, the prothrombin time and the international normalized ratio followed by a dosage adjustment. Warfarin in pure form exists as a racemic mixture consisting of equal amounts of R and S enantiomers (Porter et al., 1986). The S enantiomer is more potent than the R form (Breckenridge et al., 1974). Warfarin is highly metabolized in the body in a stereo specific pathway catalyzed by cytochrome P450. R-warfarin is metabolized primarily by CYP1A2 to 6- and 8-hydroxy warfarin, and by CYP3A4 to 10-hydroxy warfarin, while S-warfarin is metabolized primarily by CYP2C9 to 7-hydroxy warfarin (Kaminsky and Zhang., 1997). The properties of warfarin, such as narrow therapeutic index, high protein binding, CYP dependent metabolism and a very high elimination half life render to be prone to many drug interactions (Chan et al., 2009). Elimination half life of warfarin is relatively long (10-16 hours in animals and 40-46 hours in humans), causing a dramatic increase in the anticoagulant effect upon concomitant administration of warfarin with other drugs causing drug-drug interactions (Alexander and Areg et al., 2009).

Etravirine is the first drug in the second generation of non-nucleoside reverse transcriptase inhibitors (NNRTIs) for HIV/AIDS. The drug was recently marketed for the treatment of HIV infection. It is of great advantage in combination with other antiretrovirals in the treatment of patients who are on this regimen for a considerable period of time. (Martha Boffito et al., 2009). Etravirine is highly bound to plasma proteins and is primarily metabolized by CYP450; 3A4, 2C9 and 2C19 iso-enzymes. Potential drug interactions of warfarin and etravirine are expected due to their high protein binding and similar hepatic metabolic characteristics. For most antiretrovirals it's critical that drug concentrations are maintained above the suggested minimum effective concentration throughout the dosing interval. Suboptimal antiretroviral exposure may permit viral replication and predispose to

the selection of drug-resistant virus. Resistant strains may hence lead to disease progression and treatment failure (Martha Boffito et al., 2009). On the other hand, long term use of NNRTIs can potentially cause HIV related thromboembolic events, which requires the use of the anticoagulant warfarin. A clinically significant etravirine-warfarin interaction could occur after their concomitant administration (Welzen et al., 2011).

Several LC-MS/MS methods have been developed for the analysis of etravirine in human plasma samples. For example, (Heine et al., 2009) developed an LC-MS/MS method quantifying etravirine in human plasma, dry blood spot, and peripheral blood mononuclear cell lysate. LC-tandem MS methods were also available for simultaneous analysis of etravirine with other NNRTIs (Fayet et al., 2009; Else et al., 2010) in human plasma and with protease inhibitors (Quaranta et al., 2009). All the above referenced methods used a direct plasma protein precipitation using organic co-solvent and had etravirine detection limit of ≥ 10 ng/mL, except for a study by (Rezk et al., 2009), where a 2 ng/mL detection limit was reported. Most of these LC-MS/MS methods were aimed for and been used for clinical drug monitoring. Few have been used for pharmacokinetic characterization of potential drug-drug interactions between etravirine and other concomitantly administered drugs. For example, Scholler-Gyure et al., 2008 validated and applied an LC/MS/MS method of etravirine for a pharmacokinetic drug-drug interaction study between etravirine and acid-suppressing agents in healthy human volunteers. However, very limited analytical methods are currently available for the determination of etravirine in rat plasma. Furthermore, there are no analytical methods till date for the simultaneous determination of racemic warfarin and etravirine in the rat plasma. Developing such analytical method is necessary for further characterization and evaluation of factors that affect the absorption and disposition of etravirine and warfarin. Hence it is a highly significant to develop an analytical method for the simultaneous determination and validation of racemic warfarin and etravirine to extrapolate its applicability in pharmacokinetic studies of these compounds. In the current study, we were successful in coming up with an LC-MS/MS determination of racemic warfarin and etravirine in rat plasma which can be of high relevance in the pharmacokinetic profiling of both etravirine and warfarin. The limit of detection was as low as 1ng/mL and the injection volume used was 10ul without any interference with the sensitivity of the assay. The total run time of 11 minutes permitted the assay to be carried out in a relatively short time period with minimal consumption of the solvents and reagents.

Therefore, the objective of this study is to develop a simultaneous LC-MS/MS method suitable for determination and quantification of R-warfarin, S-warfarin, and etravirine in rat plasma. The method has been validated and applied to a pharmacokinetic evaluation of the drug-drug interaction between warfarin and etravirine using rat as an animal model.

2. Materials and methods

2.1 Chemicals

Racemic warfarin standard powder was purchased from Sigma Aldrich Co. (Missouri, St. Louis, USA). Etravirine as the standard powder was purchased from Toronto research chemicals Inc. (Canada). Acetaminophen and acetic acid was purchased from Sigma Aldrich Co. (Missouri, St. Louis, USA). HPLC graded water was purchased from Mallinckrodt Baker, Inc. (New Jersey, Phillipsburg, USA). Acetonitrile HPLC grade was purchased from VWR international Chemicals (Pennsylvania, West Chester, USA). The chemical structures

and fragmentation patterns for racemic warfarin, etravirine, and the internal standard acetaminophen are presented in Figure 1.

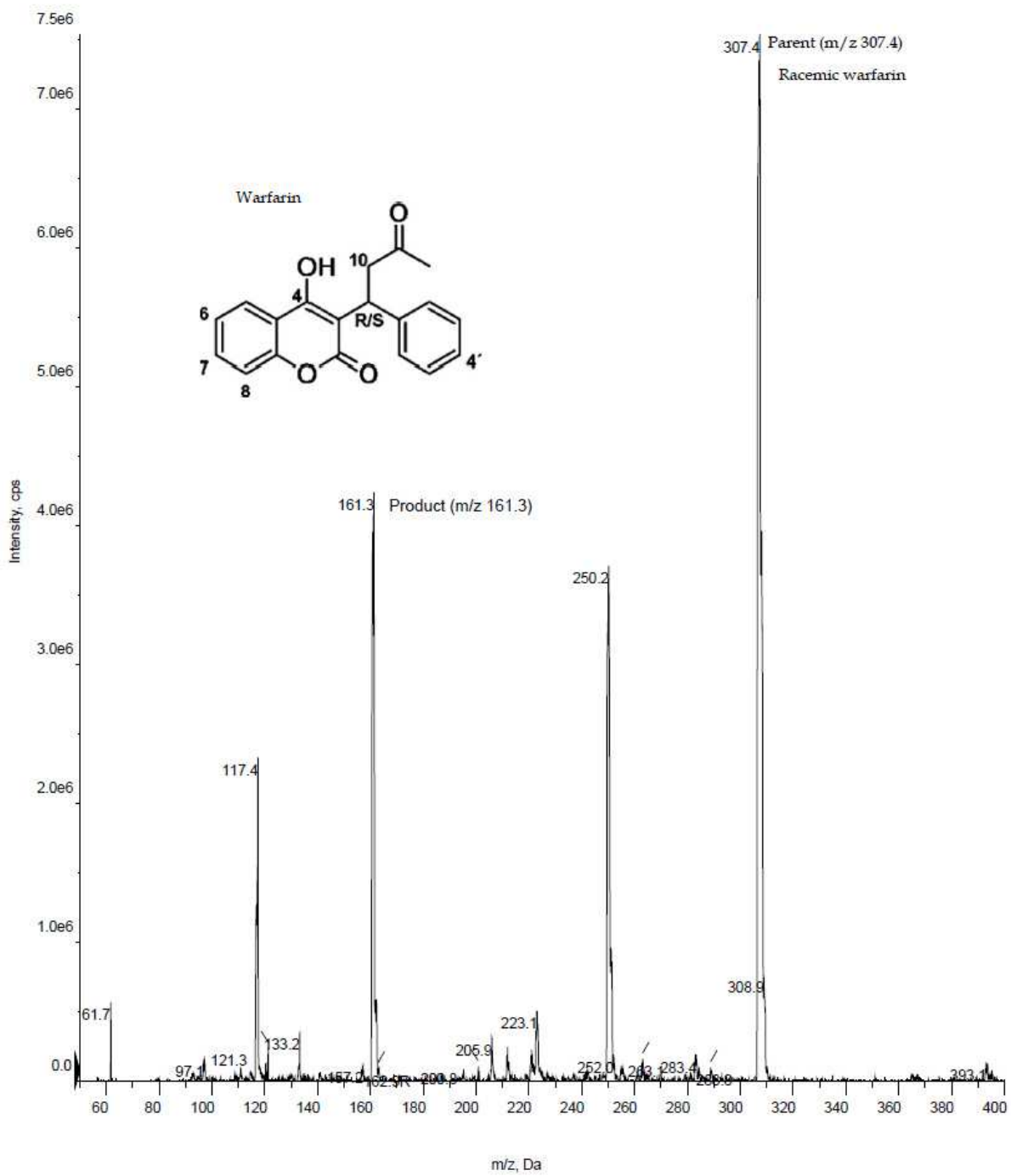


Fig. 1. Part I.

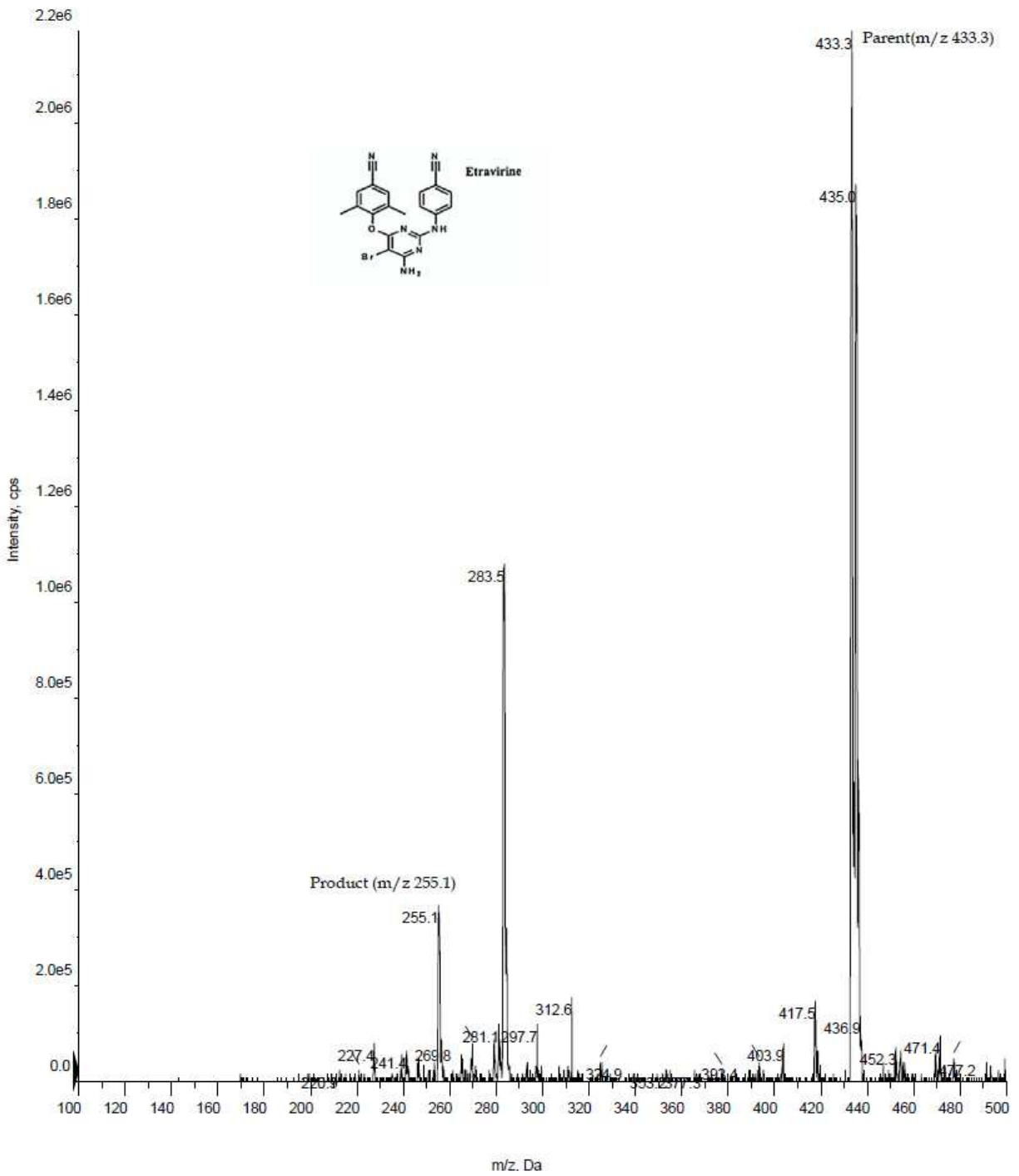
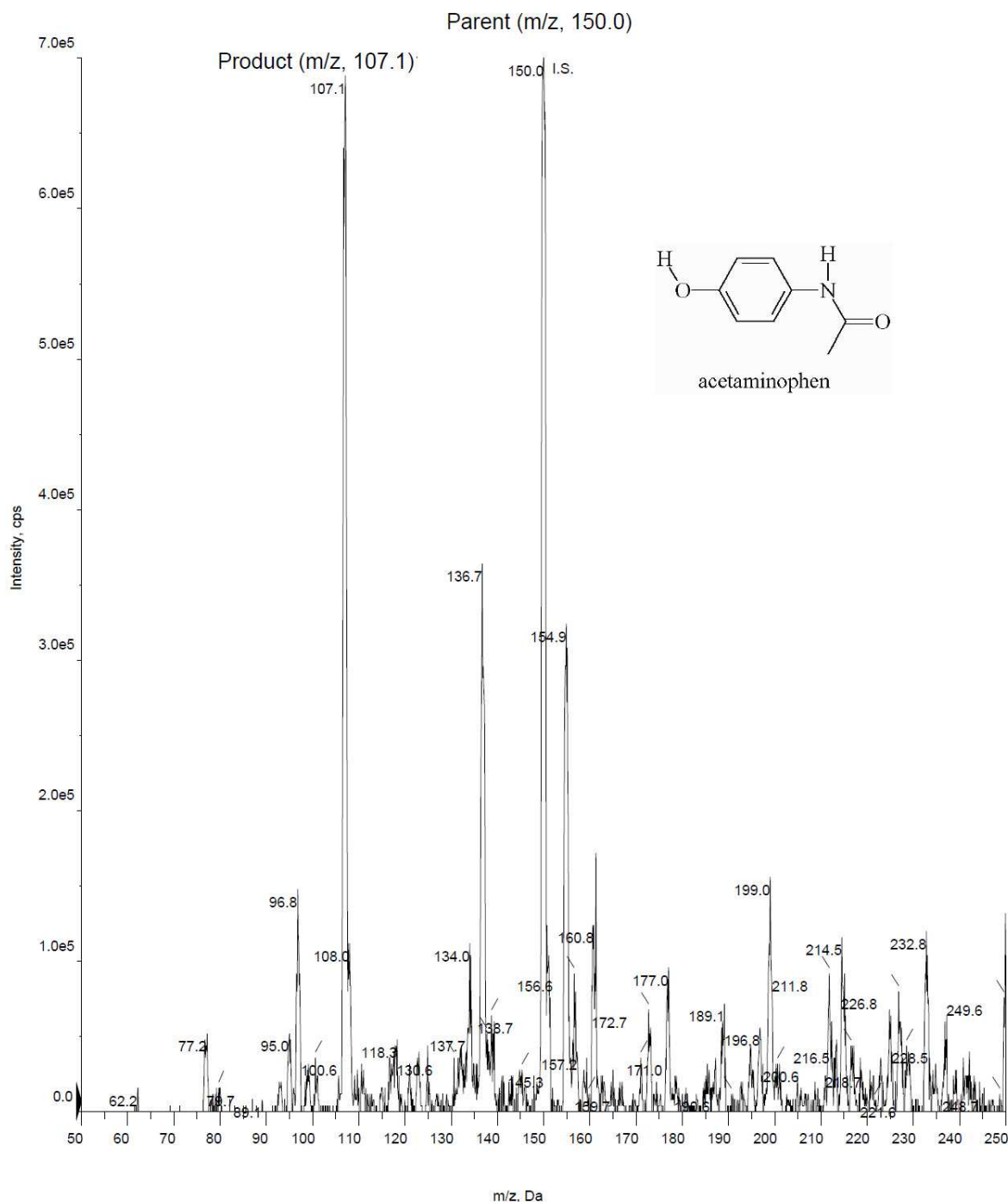


Fig. 1. Part II.



Part III.

Fig. 1. Chemical structures and fragmentation patterns for racemic warfarin (A), etravirine (B) and internal standard, acetaminophen (C).

2.2 LC/MS/MS conditions

Warfarin, etravirine and the IS were analyzed by using an Agilent 1200 series HPLC system (Foster city, CA) using a commercially available chiral column, Lux cellulose -1 with a

dimension of 250X4.6mm i.d. packed with 5 μ m particles (Phenomenex, Torrance, CA, USA) in conjunction with a Lux Cellulose-1 guard column with a dimension of 4 x 3.0mm (Phenomenex, Torrance, CA, USA). A gradient elution was used, consisting of 0.1% acetic acid in water (mobile phase A) and 100% acetonitrile (mobile phase B). The gradient started with mobile phase A and B being mixed at a ratio of 40:60 %v/v respectively, at a flow rate of 1.5mL/min till 4.5 minutes. At time 4.5-6 minutes, A and B were mixed at a ratio of 40:60 % v/v respectively, at a flow rate of 1.3 mL/min. From 6-9.1 minutes, the mobile phase was solely comprised of B, at a flow rate of 1.5mL/min. At 9.1-11 minutes, A and B were mixed at a ratio of 40:60 %v/v, at a flow rate of 1.5 mL/min. Two minutes was allowed at the end of each run for equilibration. The injection volume was 10 μ l and the total run time was 11 minutes. The column effluent was monitored using a 3200 QTRAP[®] LC-MS/MS (AB Sciex, Foster city, CA) which is a hybrid triple quadrupole linear ion trap equipped with a TurboIonSpray ion source. A Parker Balston Source 5000Tri Gas generator was used to generate pure nitrogen. The nebulizer gas and the heater gas were maintained at 80psi and the ion spray heater was set at 650 $^{\circ}$ C. Ion spray needle voltage was 4500 V, the curtain gas was 30psi and the collision gas was set to medium. The mass spectrophotometer was set at the negative mode. The transition ions were detected using multiple reaction monitoring from a specific parent ion to product ion for etravirine (m/z 433.3 \rightarrow 142.1), racemic warfarin 307.4 \rightarrow 161.3 and acetaminophen 150 \rightarrow 107.1. Peak areas and other compound parameters were determined by Analyst^R software, ver.1.5.

2.3 Preparation of standards and calibration curves

Individual stock solutions of racemic warfarin, acetaminophen and etravirine (1mg/mL) were prepared by dissolving 25mg of each substance in 25mL of respective solvents (acetonitrile for racemic warfarin and acetaminophen, DMSO for etravirine) and stored in refrigerator at 4 $^{\circ}$ C. All the working solutions were freshly prepared daily. For quantitative analysis, a series of working standard mixtures were prepared by mixing and diluting the stock solutions of racemic warfarin and etravirine with mobile phase to yield concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5 and 5 μ g/mL. A working solution of IS was prepared by diluting the stock solution of acetaminophen with acetonitrile to yield a final concentration of 500ng/mL. In vivo standard curves were prepared by spiking blank plasma with warfarin and etravirine standard solutions to yield calibration standards in plasma at concentrations of 2.5, 5, 10, 25, 50, 100, 250 and 500 ng/mL.

2.4 In vivo sample preparation

The rat plasma was collected from male Sprague Dawley rats in our laboratory and stored at -80 $^{\circ}$ C until its use. Each warfarin and etravirine containing rat plasma sample (100 μ l) was extracted and deproteinized by mixing it with 100 μ l of IS working solution containing 500ng of acetaminophen per mL of acetonitrile. The mixture was briefly vortexed for 1 minute and centrifuged at 13,000 rpm for 10 minutes. A 10 μ l of the supernatant was directly injected in to the HPLC column. Calibration curves were plotted by peak area ratio of each analyte and internal standard vs. concentrations in rat plasma.

2.5 Assay validation

The "Guidance for Industry – Bioanalytical Method Validation" document from FDA was used as a guide for the assay validation described as follows (FDA. 2001).

2.5.1 Linearity, accuracy, precision, and recovery

Linear calibration curves in rat plasma were generated by plotting peak area ratios of racemic warfarin and etravirine to the IS versus seven known plasma racemic warfarin and etravirine concentrations over the range of 2.5-500 ng/mL. Slope, intercept, and coefficient of determination values were estimated using least square regression analysis. Quality control plasma samples containing low (10ng/mL), medium (100ng/mL), and high (400ng/mL) racemic warfarin and etravirine concentrations were used to evaluate the precision and accuracy of the assay method. The intra-day assay precision and accuracy were obtained by analyzing six replicates of the quality control samples using calibration curves constructed on the same day. The inter-day assay precision and accuracy were obtained by analyzing six quality control samples using calibration curves constructed on 3 different days. The assay precision was reflected by the relative standard deviation and the assay accuracy was reflected by the relative percentage error from the theoretical drug concentrations. The limit of detection (LOD) was defined as the plasma concentration that yielded a peak height equal to three times that of baseline noise. The lowest limit of quantification (LLOQ) was selected as the lowest racemic warfarin and etravirine plasma level on the calibration curve. The extraction recoveries of racemic warfarin and etravirine from rat plasma (expressed as a percentage) were calculated as the ratio of the slope of a calibration curve for racemic warfarin and etravirine in spiked plasma to that of spiked mobile phase.

2.5.2 Stability

Three aliquots of the low, medium, high concentration QC samples were used to conduct each of the following sets of stability tests: three freeze-thaw cycles, storage under refrigeration conditions (4°C), and storage at room temperature in autosampler.

2.5.3 Matrix effects

Matrix effects from endogenous substances present in extracted rat plasma may cause ion suppression or enhancement of the signal. Matrix effects were assessed by comparing the peak areas of racemic warfarin and etravirine after addition of low, medium, and high (n=3 each) concentrations of racemic warfarin and etravirine to (A) mobile phase and (B) and supernatant of extracted blank plasma. The peak area ratio of B/A (as a percentage) or the percentage matrix factor was used as a quantitative measure of matrix effect. The absolute matrix effect was quantified by using the "post extraction spike method" which compares the average peak area ratios of a group of standard samples diluted in mobile phase (group A) and another group spiked into plasma after plasma extraction (group B). Concentrations of the standard solutions were established low, medium and high values (n=3 each). The formula utilized to compare the peak area ratios was "B" PAR/ "A" PAR = %Matrix effect.

2.5.4 Pharmacokinetic study

The animal experiment and protocol were reviewed and approved by the Institutional Animal Care and Use Committee at Texas Southern University. The jugular veins of six male adult Sprague Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 250-300g were cannulated under anesthesia the day before the study. Each rat was given a 1mg/kg of racemic warfarin intravenous followed by 25mg/kg etravirine intravenous.

Serial heparinized blood samples (approximately 250 μ l) were collected from the jugular vein cannula for up to 144h. After centrifugation, the plasma samples were collected, immediately stored in -80°C and analyzed within a week.

3. Results and discussion

3.1 Chromatographic conditions

The chromatographic run times for the protein precipitated plasma samples were 11 minutes. Retention times 2, 6.02, 7.76, and 9.5 minutes were observed for internal standard, R-warfarin, S-warfarin and etravirine, respectively. Figure 2 illustrates chromatograms obtained from blank rat plasma, spiked drug concentrations in plasma and after administration of the drug at 8 hours. These chromatograms demonstrate peak separation at the baseline excluding in vivo matrix interference.

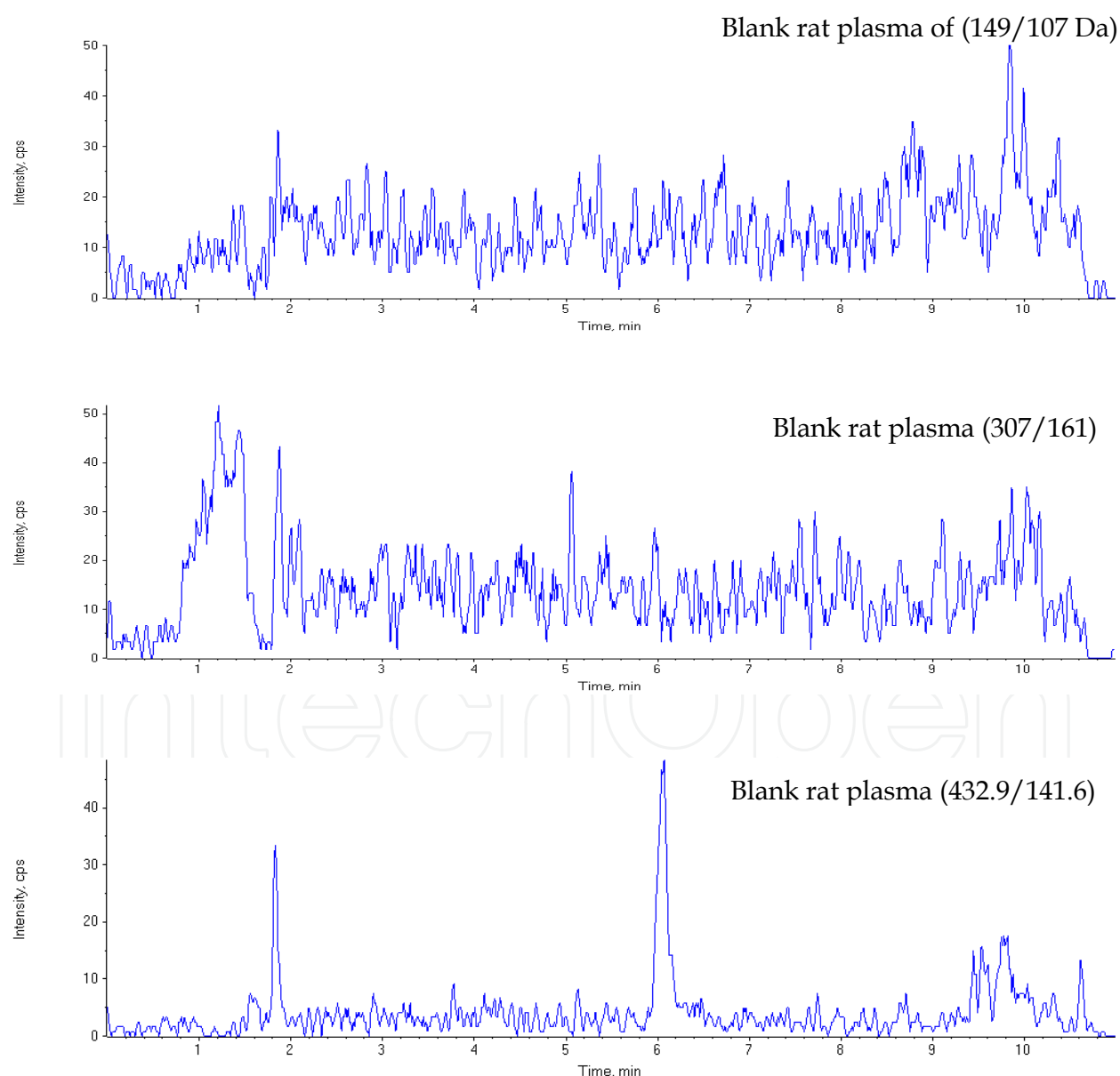


Fig. 2. (A)

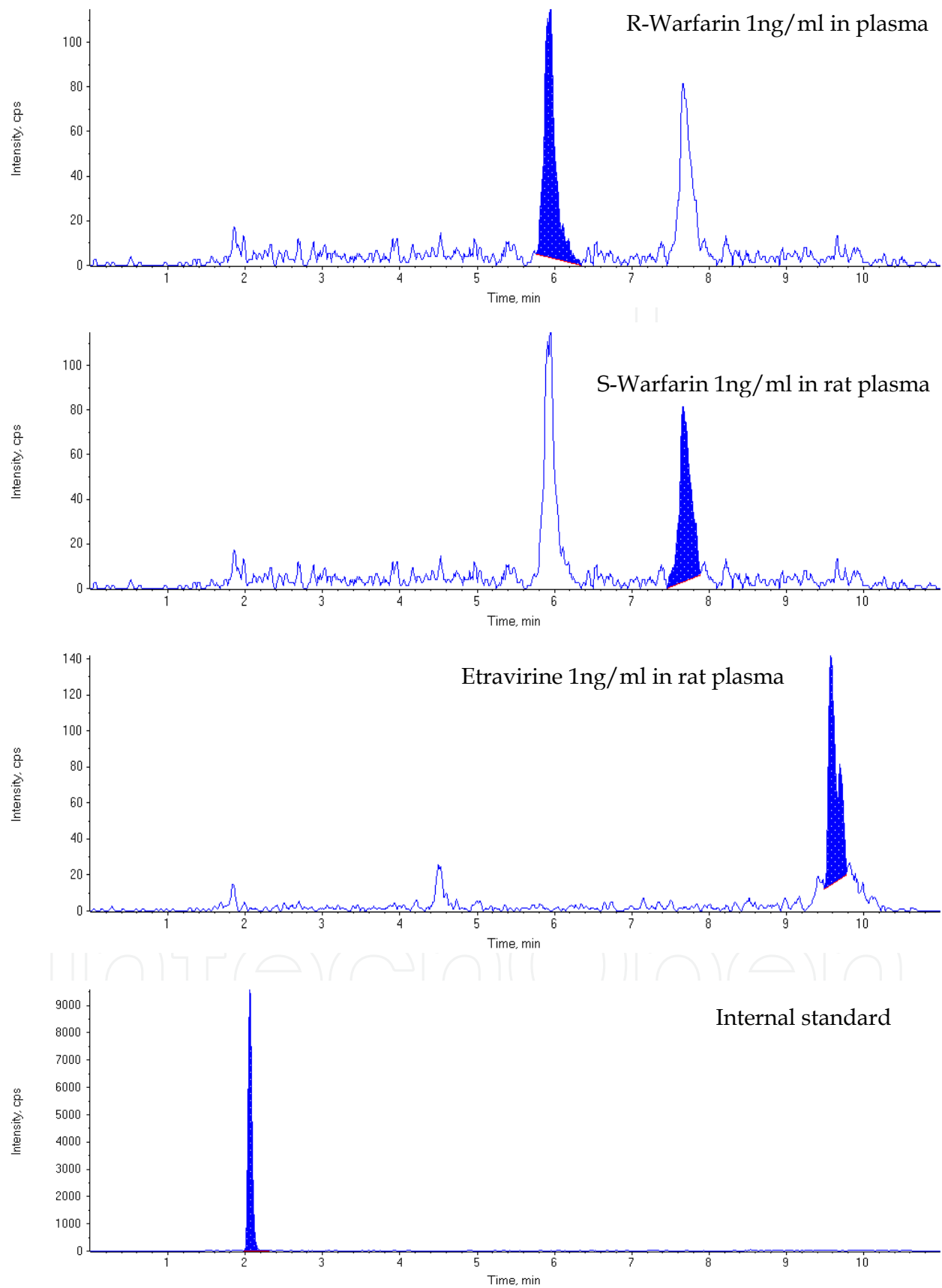


Fig. 2. (B)

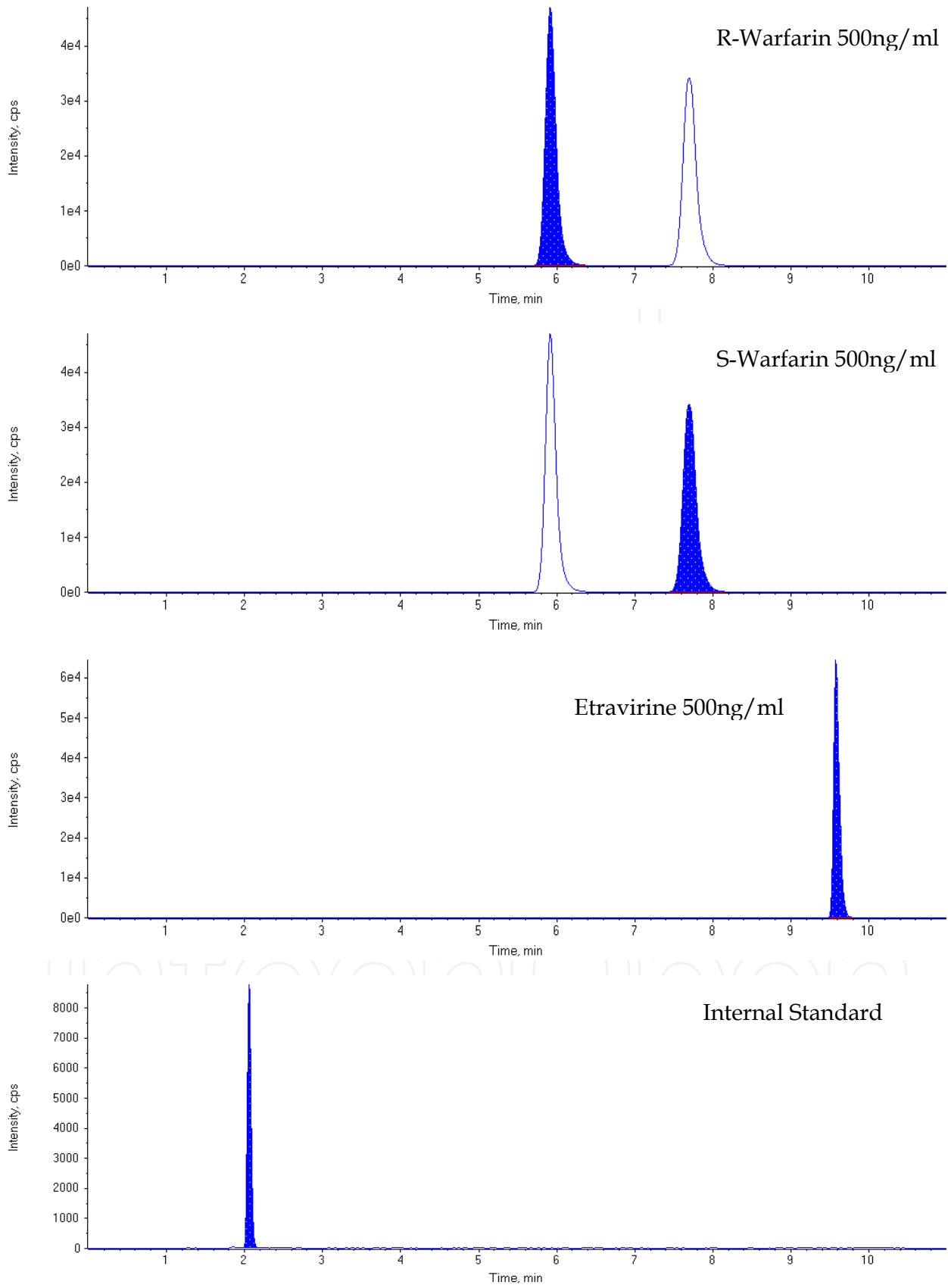


Fig. 2. (C)

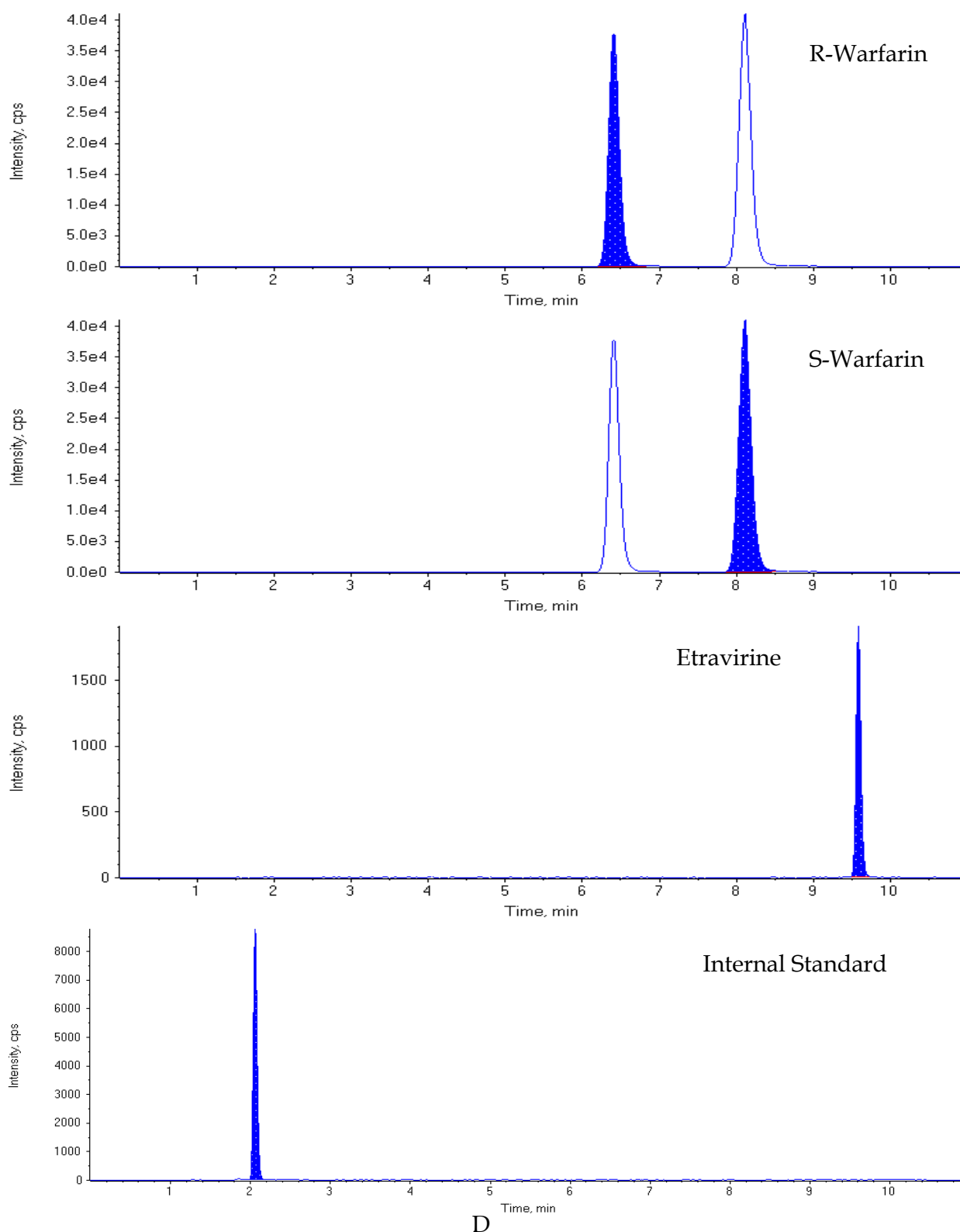


Fig. 2. Chromatograms of: (A) blank rat plasma; (B) Blank rat plasma spiked with 1ng/mL racemic warfarin, etravirine and the internal standard; (C) Blank rat plasma spiked with 500ng/mL racemic warfarin, etravirine and the internal standard; (D) Plasma sample from a rat at 8 hours after concomitant intravenous administration of 1mg/kg dose of racemic warfarin and intravenous 25mg/kg dose of etravirine.

3.2 Linearity, sensitivity and detection limit of the assay

Linear relationships were determined by the ratios of the peak areas of analytes to that of the internal standard over the range of 2.5-500ng/mL. Table 1 shows the intra- and inter-day assay changeability of the calibration curves. The lowest limits of detection for all analytes were 1 ng/mL. The lowest limit of quantitation for R-warfarin, S-warfarin, and etravirine were 5, 5 and 2.5 ng/mL, respectively.

	Y-intercept	Slope	R ²
R-Warfarin			
Intra-day (n=3)			
Mean	0.0360	0.0640	0.9990
SD	0.0090	0.0020	0.0010
Precision (CV%)	25.73	5.6	0.1
Inter-day (n=5)			
Mean	0.0256	0.0707	0.998
SD	0.0031	0.0029	0.002
Precision (CV%)	12.06	4.0	0.2
S-Warfarin			
Intra-day (n=3)			
Mean	0.1681	0.0580	0.9980
SD	0.0503	0.0019	0.0010
Precision (CV%)	30	6.4	0.1
Inter-day (n=5)			
Mean	0.0255	0.0634	0.9970
SD	0.0216	0.0046	0.0040
Precision (CV%)	84.4	7.3	0.4
Etravirine			
Intra-day (n=3)			
Mean	0.0453	0.0062	0.9970
SD	0.0012	0.0029	0.0030
Precision (CV%)	2.6	23.4	0.3
Inter-day (n=3)			
Mean	0.0160	0.0059	0.998
SD	0.0055	0.0005	0.003
Precision (CV%)	34.58	9.3	0.3

CV% = (standard deviation/ mean)×100.

Table 1. Linearity of calibration curves for warfarin-R in rat plasma.

Theoretical concentration			
	Low (10 ng/mL)	Medium (100ng/mL)	High (400 ng/mL)
R-Warfarin			
Intra-day (n=6)			
Mean	9.84	94.19	435.68
SD	0.41	6.60	33.70
Precision (CV%)	4.1%	7%	7.7%
Accuracy (% bias)	1.6%	5.8%	8.9%
Inter-day (n=18)			
Mean	8.90	97.34	406.44
SD	0.89	10.0	5.65
Precision (CV%)	10.0%	10.27%	1.40%
Accuracy (% bias)	11%	2.7%	1.6%
S-Warfarin			
Intra-day (n=6)			
Mean	9.93	97.7	409.18
SD	0.59	6.15	34.5
Precision (CV%)	6%	6.3%	8.4%
Accuracy (% bias)	0.7%	2.3%	2.3%
Inter-day (n=18)			
Mean	9.20	101.55	403.11
SD	0.74	3.84	5.26
Precision (CV%)	8.08%	3.7%	1.31%
Accuracy (% bias)	8%	1.2%	0.78%
Etravirine			
Intra-day (n=6)			
Mean	12.7	109.18	438.3
SD	1.42	11.5	63.73
Precision (CV%)	11.07%	10.5%	14.5%
Accuracy (% bias)	27%	9.1%	9.5%
Inter-day (n=18)			
Mean	10.88	98.87	416.00
SD	0.47	8.99	23.15
Precision (CV%)	4.32%	9%	5.6%
Accuracy (% bias)	8.8%	1.13%	4%

CV% = (standard deviation/mean)×100.

% bias = [(measured concentration-mean theoretical concentration)/measured concentration]×100.

Table 2. Precision and accuracy of the simultaneous assay method for R- warfarin, S-warfarin and etravirine in rat plasma.

3.3 Extraction recovery

The plasma concentration range of 2.5-500 ng/mL displayed a mean recovery rate (n=3) of R-warfarin, S-warfarin, etravirine 121%± 0.06, 125.5% ± 0.08, 108.6%± 0.40, respectively. The mean recovery was calculated from the ratio of the slopes of the analytes calibration curves in plasma to that in mobile phase.

3.4 Accuracy and precision of the assay

To investigate the intra-day and inter-day precision and accuracy in rat plasma; low, medium, and high quality control spiked plasma samples were used. Table 2 illustrates the intra-day (n=6) and inter-day (n=3) quality control samples for racemic warfarin and etravirine.

3.5 Stability

Stability results in plasma were summarized in Table 3. The racemic warfarin and etravirine quality control samples were not affected by the three freeze-thaw cycles. Racemic warfarin and etravirine were stable in plasma under refrigeration conditions and room temperature for up to 12 hours.

Storage condition	Nominal concentration	R-Warfarin Mean (\pm SD) percent of nominal concentration remaining (n=3)	S-Warfarin Mean(\pm SD) percent of nominal concentration remaining (n=3)	Etravirine Mean(\pm SD) percent of nominal concentration remaining (n=3)
4°C temperature for 12 hours in rat plasma	10 ng/mL	97.0 \pm 0.46	103.0 \pm 0.38	108.5 \pm 0.89
	100 ng/mL	96.0 \pm 2.47	99.0 \pm 3.27	95.2 \pm 0.17
	400ng/mL	99.5 \pm 11.79	105.5 \pm 18.16	98.8 \pm 11.69
Room temperature in the autosampler for 12 hours in rat plasma	10 ng/mL	96.3 \pm 0.37	102.2 \pm 0.16	102 \pm 1.24
	100 ng/mL	97.2 \pm 12.29	96.6 \pm 10.17	114 \pm 22.1
	400ng/mL	103 \pm 63.4	103.5 \pm 66.6	83 \pm 110.8
Freeze-thaw Cycle 1	10 ng/mL	74.29 \pm 0.37	81.88 \pm 0.73	107.43 \pm 0.31
	100ng/mL	69.30 \pm 0.63	77.71 \pm 1.52	92.93 \pm 1.41
	400 ng/mL	92.58 \pm 8.38	82.64 \pm 3.59	91.90 \pm 25.63
Cycle 2	10 ng/mL	78.8 \pm 0.03	89.6 \pm 0.31	121.88 \pm 0.18
	100ng/mL	71.58 \pm 0.43	81.5 \pm 2.38	102.45 \pm 5.22
	400 ng/mL	100.31 \pm 4.23	90.39 \pm 24.33	107.63 \pm 10.73
Cycle 3	10 ng/mL	76.78 \pm 0.21	85.63 \pm 0.14	133.4 \pm 2.7
	100ng/mL	82.28 \pm 3.62	91.46 \pm 4.67	150.97 \pm 19.24
	400 ng/mL	112.82 \pm 68.23	111.17 \pm 76.69	94.55 \pm 19.47

Table 3. Stability of warfarin and etravirine in rat plasma.

3.6 Matrix effect

The matrix factor percentages of 118.2 ± 10.9 , 114 ± 5.8 , 143.6 ± 17.57 for warfarin-R, warfarin-S, and etravirine were obtained. These results are in agreement with international guidelines (FDA, 2001).

3.7 Application of the assay method

The analytical method was applied to study the pharmacokinetics of concomitant administration of racemic warfarin and etravirine using the rat as an animal model (Figure 3 and 4).

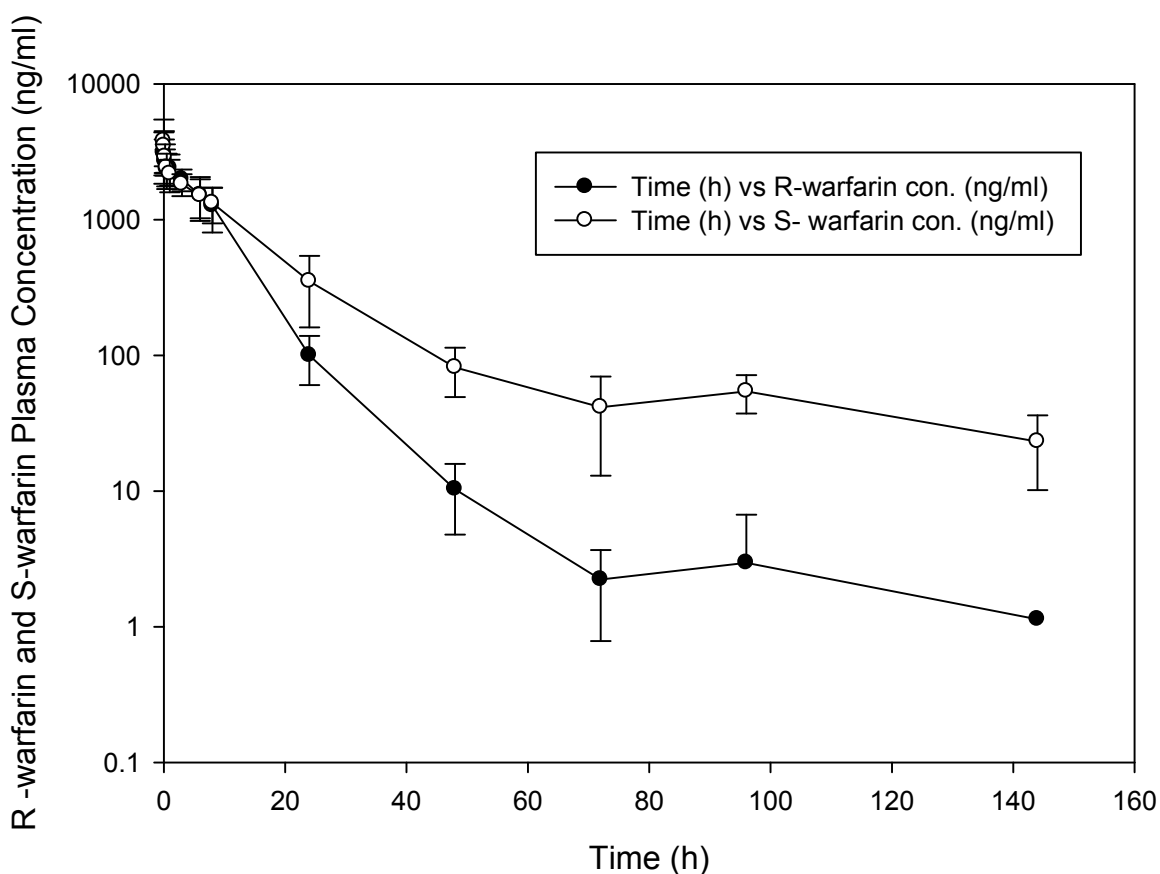


Fig. 3. Mean (\pm SD; n=6) plasma concentration – time profile of R-warfarin and S- Warfarin after an intravenous 1mg/kg dose of racemic warfarin to male Sprague Dawley rats.

The mean (\pm SD) areas under the plasma concentration curve for R-warfarin, S-warfarin, and etravirine were 30000 ± 5584.4 , 32009 ± 10231 , and 5203.5 ± 500 ng x hr/mL, respectively. The half-lives for these analytes were 7.5 ± 10.7 , 16 ± 7 , and 22 ± 11 hrs, respectively. Furthermore, these analytes exhibited clearances of 39.5 ± 12.8 , 31.6 ± 12 and 4.6 ± 0.0002 mL/hr, respectively. The volumes of distribution for R-warfarin, S-warfarin, and etravirine were calculated to be 414 ± 162.4 , 637.5 ± 554 , and 144 ± 72 mL, respectively. Based upon these findings it can be suggested that R-warfarin is metabolized and eliminated quickly. S-warfarin has a longer half-life and is not cleared as quickly as R-warfarin. Therefore, this demonstrates that S-warfarin is the more potent enantiomer, circulating longer throughout

the body, and has the potential for eliciting more therapeutic efficacy. Etravirine demonstrated a decrease in clearance, suggesting the saturation of the metabolic pathways responsible for its clearance upon concomitant administration with warfarin. Further pharmacokinetic studies are underway to examine the steady state drug interactions upon concomitant administration of racemic warfarin and etravirine.

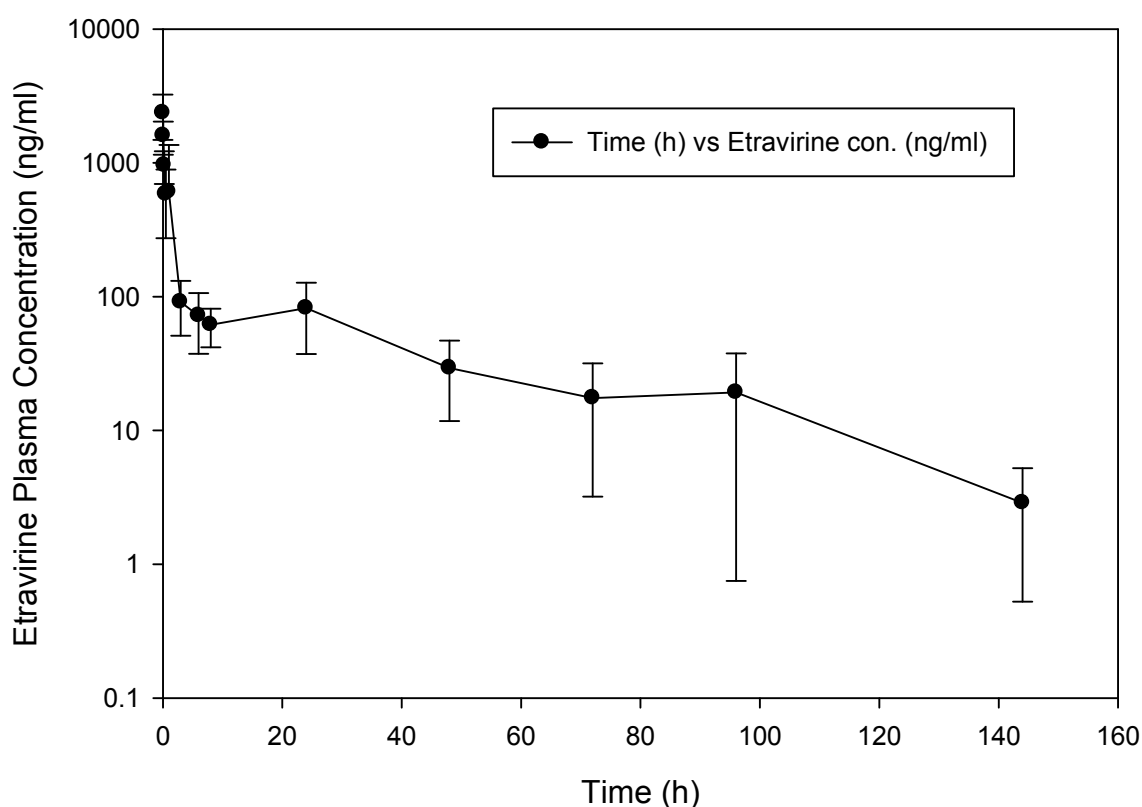


Fig. 4. Mean (\pm SD; n=6) plasma concentration - time profile of etravirine after an intravenous 25mg/kg dose of etravirine to male Sprague Dawley rats.

4. Conclusion

In conclusion, we have developed a rapid, simple and sensitive LC-MS/MS assay using a chiral column, for the simultaneous detection and quantification of R-warfarin, S-warfarin and etravirine in small volumes (100 μ l) of rat plasma. This method enabled us to do the simultaneous pharmacokinetic evaluation of intravenously administered racemic warfarin and etravirine using Sprague-Dawley rat as an animal model. Among the two enantiomers of warfarin, the S-warfarin is more potent and stays in the body for a longer period of time as observed from its longer half life. This observation is in agreement with the previous studies on the pharmacokinetics of racemic warfarin reported by Eli Chan et al. 2009 and others. Furthermore, upon concomitant administration of etravirine and racemic warfarin, there is a statistically significant increase in the half life of etravirine which is a clear indication of a drug-drug interaction between these two commonly administered pharmaceuticals. Hence the concomitant administration of warfarin and etravirine could

increase the efficacy of this NNRTI due to the above mentioned reasons. Due to the limited pharmacokinetic studies for etravirine in the literature, we need to proceed further with similar studies to fully understand the therapeutic impacts of this combination drug regimen. Hence this study can be used for preclinical analysis which can be applied for further pharmacokinetic characterization of racemic warfarin with etravirine.

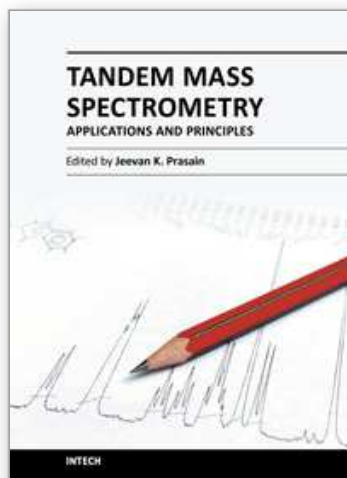
5. Acknowledgements

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6. References

- Abobo, C.V., & Lei, W., & John, J., & Joseph, M., & Bates, T., & Liang, D. (2010). LC-MS/MS determination of etravirine in rat plasma and its application in pharmacokinetic studies. *Journal of Chromatography B*, 878: 3181-3186.
- Alexander P, Areg H., 2009. Pharmacokinetic and Pharmacodynamic Study of Interaction of Rhodiola Rosea SHR-5 Extract with warfarin and theophylline in Rats. *Phytotherapy research* 23: 351-357.
- Barclay, V.K., & Tyrefors, N.L., & Johansson, I.M., & Pettersson, C.E. (2011). Trace analysis of fluoxetine and its metabolite norfluoxetine. Part I: Development of a chiral liquid chromatography-tandem mass spectrometry method for wastewater samples. *Journal of Chromatography A*, 1218: 5587-5596.
- Breckenridge, A., & Orme, M., & Wesseling, H., & Lewis, R.J., & Gibbons, R. (1974). Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. *Clinical Pharmacology and Therapeutics*, 15: 424-430.
- Boffito, M., & Jackson, A., & Lamorde, M., & Back, D., & Watson, V., & Taylor, J., & Waters, L., & Asboe, D., & Gazzard, B., & Pozniak, A. (2008). Pharmacokinetics and safety of etravirine administered once or twice daily after 2 weeks treatment with efavirenz in healthy volunteers. *Journal of acquired immune deficiency syndromes* (1999), 52(2): 222-227.
- Chan, E., & Hegde, A., & Chen, X. (2009). Effect of rutin on warfarin anticoagulation and pharmacokinetics of warfarin enantiomers in rats. *The Journal of Pharmacy and Pharmacology*, 61:451-458.
- Else, L. & Watson, V., & Tji, J., & Hughes, J., & Siccardi, M., & Khoo, S., & Back, D. (2010). Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. *Journal of chromatography B, analytical technologies in the biomedical and life sciences*, 878:1455-1465.
- Fayet, A., & Béguin, A., & Zanolari, B., & Cruchon, S., & Guignard, N., & Telenti, A., Cavassini, M., & Günthard, H.F., & Buclin, T., & Biollaz, J., & Rochat, B., & Decosterd, L.A. (2009). A LC-tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, maraviroc, darunavir, and etravirine. *Journal of chromatography B, analytical technologies in the biomedical and life sciences*, 877:1057-1069

- Kakuda, T.N., & Schöller-Gyüre, M., & Hoetelmans, R.M. (2011). Pharmacokinetic interactions between warfarin and non-antiretroviral drugs. *Clinical Pharmacokinetics*, 50: 25-39.
- Kaminsky, L.S., & Zhang, Z.Y. (1997). Human p450 metabolism of warfarin. *Pharmacology & Therapeutics*, 73: 67-74.
- Quaranta, S., & Woloch, C., & Paccou, A., & Giocanti, M., & Solas, C., & Lacarelle, B. (2009). *Therapeutic Drug Monitoring*, 31(6):695-702.
- Rezk, N. L., & White, N.R., & Jennings, S.H., & Kashuba, A.D. (2009). A novel LC-ESI-MS method for the simultaneous determination of etravirine, darunavir and ritonavir in human blood plasma. *Talanta*, 79(5): 1372-1378.
- Radwan, M.A., & AlQuadeib, B.T., & Aloudah, N.M., & Aboul Enein, H.Y. (2010). Pharmacokinetics of ketorolac loaded to polyethylcyanoacrylate nanoparticles using UPLC MS/MS for its determination in rats. *International Journal of Pharmaceutics*, 397(1-2):173-178.
- Schöller-Gyüre, M., & Kankuda, T.N., & De Smedt, G., & Vanaken, H., & Bouche, M.P., & Peeters, M., & Woodfall, B., & Hoetelmans, R.M. (2008). A pharmacokinetic study of etravirine (TMC125) co-administered with ranitidine and omeprazole in HIV-negative volunteers. *British Journal of Clinical Pharmacology*, 66(4): 508.
- Sharma, K., & Kandaswamy, M., & Mithra, C., & Meena, A.K., & Giri, S., & Rajagopal, S., & Mullangi, R. (2011). Highly sensitive LC-MS/MS-ESI method for simultaneous quantitation of albendazole and ricobendazole in rat plasma and its application to a rat pharmacokinetic study. *Biomedical Chromatography: BMC*, doi:10.1002/bmc:1654.
- Ter Heine, R., & Rosing, H., & van Gorp, E.C., & Mulder, J.W., & Beijnen, J.H., & Huitema, A.D. (2009). Quantification of etravirine (TMC125) in plasma, dried blood spots and peripheral blood mononuclear cell lysate by liquid chromatography tandem mass spectrometry. *Journal of pharmaceutical and biomedical analysis*, 49: 393-400.
- U.S. Food and Drug Administration. (2001). Guidance for industry, bioanalytical method validation, Available from: < <http://www.fda.gov/cder/guidance> >
- Wei, B., & Dong, L., & Bates, T.R. (2008). Development and validation of an HPLC method to determine griseofulvin in rat plasma: Application to pharmacokinetic studies. *Analytical Chemistry Insights*, 3: 103-109.
- Welzen, M.E., & van den Berk, G.E., & Hamers, R.L., & Burger, D.M. (2011). Interaction between antiretroviral drugs and acenocoumarol. *Antiviral Therapy*, 16(2):249-52.



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