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Ultra-performance liquid chromatography/tandem mass spectrometric determination of diastereomers of SCH 503034 in monkey plasma

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Abstract

This paper describes the development and qualification of a fast, sensitive and specific ultra-performance liquid chromatography/tandem mass spectrometric (UPLC/MS/MS) method for the determination of diastereomers of SCH 503034 in monkey plasma. The analytical method involves direct protein precipitation with a mixture of methanol/acetonitrile (10/90) containing an internal standard, followed by separation of the stereoisomers on an Acquity UPLC™ C_{18} column and detected by selected reaction monitoring (SRM) in positive ionization mode using atmospheric pressure chemical ionization (APCI). The effects of ion-pairing agents on separation and ionization efficiency were investigated. The two diastereomers were well separated ($R = 1.3$) with a runtime of 5 min under an isocratic condition. The method was qualified. The linear concentration range was 1–2500 ng/ml for the both stereoisomers. Inter-assay mean bias and relative standard deviation (R.S.D.) were in the range of −1.2% to 3.6% and 2.8–10%, respectively. Intra-assay mean bias and R.S.D. were in the range of −1.3% to 5.5% and 2.3–7.8%, respectively. Recoveries of the stereoisomers at concentration levels of 2.5, 50 and 1000 ng/ml were 87.2–90.0%, 89.1–90.4% and 92.3–94.3%, respectively. The LLOQ for this assay was 1 ng/ml. No matrix interferences were observed in six different sources of blank monkey plasma.

Keywords: UPLC; LC/MS/MS; SCH 503034; Diastereomers; Plasma

1. Introduction

It is important to explore the biological responses of new chemical entities with respect to stereochemistry as part of lead characterization. According to the US Food and Drug Administration's (FDA) policy statement for the development of new stereoisomeric drugs to evaluate the pharmacokinetics of a single enantiomer or mixture of enantiomers, manufacturers should develop quantitative assays for individual enantiomers in in vivo samples early in drug development [1]. Rapid assessment of the drug-like properties of potential compounds is always desirable to enhance the success rate of drug candidates entering into drug development. The increased speed with which scientists can profile new chemical entities for various drug metabolism and pharmacokinetic characteristics will certainly shorten the iteration times for lead selection and optimization [2,3].

SCH 503034 (Fig. 1) discovered at Schering-Plough Research Institute is a potent, orally active, novel HCV serine protease inhibitor. This drug candidate is a diastereomeric compound and is manufactured as a 1:1 mixture of SCH 534129 ((*R*)-isomer) and SCH 534128 ((*S*)-isomer). The topic of stereoselectivity in bioanalytical methods has received much attention [4–6]. Chromatographic techniques using normal phase and supercritical fluid [7–9] for the separation of stereomers have been used as a useful tool to enhance sample throughputs when hyphenating to a mass spectrometer. The ultra-performance liquid chromatography (UPLC) system utilizing high linear velocities with columns packed with porous 1.7 μm particles, coupled to a tandem mass spectrometer is another powerful approach to dramatically improve peak resolution, sensitivity and speed of analysis [10–13].

In order to support preclinical pharmacokinetic studies of SCH 503034, a quantitative method for the determina-

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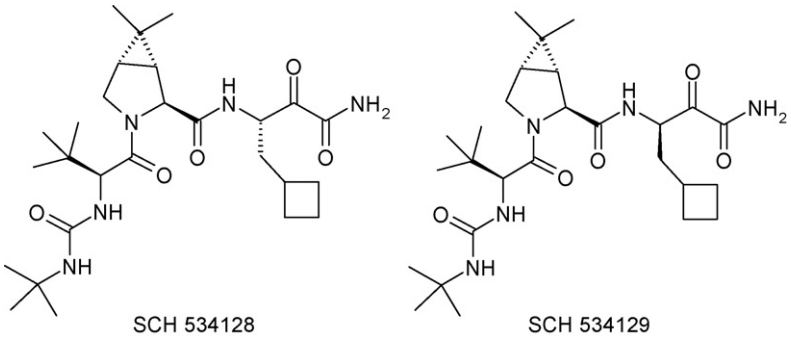

Fig. 1. Structures of the stereoisomers of SCH 503034.

tion of SCH 503034 diastereomers in monkey plasma by ultra-performance liquid chromatography/tandem mass spectrometry was developed and qualified. We also investigated the differences in LC–MS performance by conducting a comparison of UPLC with other methods previously optimized for HPLC-based separation and quantification of SCH 503034 diastereoisomers.

2. Experimental

2.1. Chemicals and reagents

Single reference standards of SCH 534129 and SCH 534128 and $^{2}H_{9}$-SCH 503034 (Internal standard, diastereomeric ratio ≈1) were synthesized at Schering-Plough Research Institute. Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Fisher Scientific (Pittsburg, PA, USA). Ammonium acetate (99.99%) was from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Perfluopentanoic acid (PFPA) was purchased from Fluka Chemie Gmbh (CH-9471 Buchs/Schweiz). De-ionized water was generated from a Milli-Q water purifying system purchased from Millipore Corporation (Bedford, MA, USA). Drug free monkey plasma was purchased from Bioreclamation Inc. (Hicksville, NY, USA).

Concentrations of stock solutions of each isomer of SCH 503034 and $^{2}H_{9}$-SCH 503034 were 1 mg/ml in acetonitrile. Concentrations of working solutions for each isomer were 0.05, 0.5, 5 and 50 μg/ml in 50% acetonitrile. Internal standard working solution was 0.1 μg/ml of $^{2}H_{9}$-SCH 503034 in methanol/acetonitrile (10:90).

Mobile phase A-1 and A-2 were methanol/water (5:95) with 40 mM PFPA and methanol/water (95:5) with 40 mM PFPA, respectively. Mobile phase B-1 and B-2 were methanol/water (5/95) with 4 mM of ammonium acetate and methanol/water (95/5) with 4 mM of ammonium acetate, respectively.

2.2. Instrumentation and conditions

An API 4000 LC–MS/MS triple quadrupole mass spectrometer equipped with a Heated Nebulizer^{TM} ionization source (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada) was used for tandem mass spectrometry. MS/MS analysis was performed in the selected reaction monitoring (SRM) positive ionization mode, using mass transitions m/z 520.4 → 421.4 for the diastereoisomers and m/z 529.4 → 421.4 for internal standard. Sensitivity was optimized for each compound by varying the declustering potential (DP), collision energy (CE) and collision exit potential (CXP) in SRM mode and maximizing ion intensity. For these experiments, using a collision activated dissociation (CAD) gas setting of 3, the DP, CE and CXP voltages were 50 V, 25 eV, 10 V for the analyte and IS. Dwell time for the compound of interest and IS was set at 400 ms. The nebulization temperature was 450 °C. Peak areas were integrated using Analyst software (version 1.3). Linear regression with weighting of $1/x^2$ was used for constructing the standard curves.

The chromatographic system A consisted of a Waters Acquity UPLC^{TM} system and an Acquity UPLC^{TM} BEH C_{18} column (1.7 μm particle size, 50 mm × 2.1 mm i.d.) operated at 40 °C. The mobile phase flow rate was set at 0.7 ml/min. An isocratic elution was run over 5 min with 50:50 mobile phase A-1 and A-2.

The chromatographic system B consisted of Shimadzu LC-10AD VP liquid chromatography pumps, Shimadzu SCL-10A VP System controller, Shimadzu CTO-10A VP column oven, DGU-14A Degasser (Columbia, MD, USA), CTC Analytics PAL autosampler with a refrigerated sample compartment (set at 8 °C) from Leap Technologies (Carrboro, NC, USA), and MonoChrom C_{18} column (3 μm particle size, 75 mm × 3 mm i.d., operated at 40 °C) from Varian, Inc. (Lake Forest, CA). The flow rate was 1 ml/min. Gradient program 1: a linear gradient from 45% mobile phase B-2 to 68% B-2 was run over 18 min, held for 2 min, returned to 45% B-2 immediately, and then equilibrated for 0.5 min. Gradient program 2: a linear gradient from 45% mobile phase A-2 to 64% A-2 was run over 12 min, held for 2 min, returned to 45% A-2 immediately, and then equilibrated for 0.5 min.

2.3. Sample preparation

Calibration standards were prepared by spiking standard working solutions in blank monkey plasma to give final concentrations of 0, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000 and 2500 ng/ml of each isomer. QC samples were prepared on three levels at the concentrations of 2.5, 50, 1000 ng/ml of each isomer in the same manner as the calibration standards. Study samples were thawed in a refrigerator and placed on crushed ice. Then,

50 μl of each standard, QC sample or study sample were loaded into designated wells of a 96-well plate. All the plasma procedures were performed on crushed ice due to the instability of the diastereomers at room temperature in plasma. Next, 200 μl of 10/90 methanol/acetonitrile containing 0.1 μg/ml of internal standard was added into each well using a Tomtec Quadra 96. The samples were slightly mixed for 30 s (at this point, samples no longer need to be maintained on ice). The 96-well plate was centrifuged for 10 min at 4000 rpm. A 200 μl of the supernatant were transferred into a clean 96-well plate using a Tomtec Quadra 96. A 10–20 μl of the supernatant were injected onto the HPLC column for LC/MS/MS analysis.

2.4. Extraction recoveries

Extraction recovery of the compounds of interest was measured by adding known amounts of the isomers to monkey plasma and extracting with methanol/acetonitrile (10/90) as described above except with the addition of internal standard. After transferring to a clean 96-well plate, 25 μl of internal standard (1 μg/ml) were added into each well and mixed. An aliquot was injected onto the column. The peak area ratios of the drug to internal standard were compared to those obtained from blank plasma samples (extracted with 10:90 methanol/acetonitrile and followed by spiking same amounts of the isomers and internal standard as above into the supernatant). The concentrations used for evaluation of extraction efficiency were 2.5, 50 and 1000 ng/ml of the each isomer, respectively, with each concentration being carried out in triplicate.

2.5. Accuracy and precision

The method was qualified by analysis of monkey plasma quality control samples prepared as above. For the inter-assay reproducibility, one standard curve and two sets of low, medium and high QCs were prepared and assayed within one run. Six replicate runs were conducted. Intra-assay reproducibility was performed by preparing one standard curve and six sets of QC samples and assaying within one run. Precision was expressed as the relative standard deviation (R.S.D.) of the determined concentrations of inter- or intra-assay. Accuracy was measured according to the following equation: percent difference (bias %) from nominal value = $(X - C_N)/C_N \times 100$, where X is the measured mean value of intra- or inter-assay quality control samples and C_N is the nominal concentration.

2.6. Stability studies

For bench-top stability, four duplicate plasma samples were spiked with SCH 534128 or SCH 534129. The samples retained at room temperature for following time intervals: 0, 0.5, 1, 2, 4 and 6 h.

For refrigerator stability (4 °C), four duplicate plasma samples were spiked with SCH 534128 or SCH 534129. The samples were retained at refrigerator for the following time intervals: 0, 0.5, 1, 2, 4 and 6 h.

For processed sample stability in auto-sampler (8 °C), two sets of QC samples spiked with the SCH 534128 or SCH 534129 were injected after the first standard curve and injected again at the end of the run (36 h interval).

2.7. Matrix interferences

The method was checked for potential matrix interferences by analysis of plasma (spike samples after extraction) from six different sources in duplicate at the low QC concentration for the drugs and at the method concentration for the internal standard.

2.8. Monkey PK study

Three male cynomolgous monkeys were orally dosed at 10 mg/kg with SCH 503034 and blood samples were collected at designated time points (0, 0.25, 0.5, 1, 2, 4, 6, 8, 24 and 48 h).

3. Results and discussion

Full scan mass spectra and product ion mass spectra of SCH 503034 and $^2H_9$-SCH 503034 (IS) in the mobile phase obtained in APCI positive ion mode using flow injection (0.3 μg/ml of the analyte or 1 μg/ml of IS) are presented in Figs. 2 and 3, respectively. SCH 503034 and $^2H_9$-SCH 503034 were evidenced by their protonated molecule $[M+H]^+$ at m/z 520.4 and m/z 529.4 which were chosen as the precursor ions for analyte and IS, respectively. The compounds were fragmented almost exclusively by collision induced dissociation to produce product ions at m/z 421.4 and 308.5.

The topic of stereo-selectivity in bioanalytical methods has received much attention [4–7]. The policy statement of the FDA for the development of new stereoisomeric drugs states that "when stereoisomers are biologically distinguishable, they might seem to be different drugs" [1]. It is also important to understand the biological responses of new drugs with respect to stereochemistry as part of lead characterization. Therefore, a chiral or diastereomeric chromatographic separation has become a key step in developing HPLC-MS/MS methods for enantiomers, which are generally not distinguishable by the mass spectrometry. In our initial efforts for the determination of the diastereomers, methanol/water with 4 mM of ammonium acetate was used as a mobile phase for the separation of the diastereomers with a runtime of 20 min as shown in Fig. 4A. While this method was acceptable, it clearly needed to be improved due to incomplete isomer separation and a long runtime. Although baseline separation could be achieved, it would take about 40 min for each run and was therefore not amenable as a high throughput assay for a large number of samples. Over the past decade, trifluoroacetic acid (TFA) has been used as a common ion-pairing agent for reversed phase HPLC. Unfortunately, TFA can be a problem in terms of ionization suppression in atmospheric pressure ionization (API). Recently, other perfluorinated carboxylic acids [14–16], such as perfluoropentanoic acid (PFPA), have become more commonly used ion-pairing agents in reversed phase chromatography/mass spectrometry since they have the same advantages as TFA, but are less likely to result in

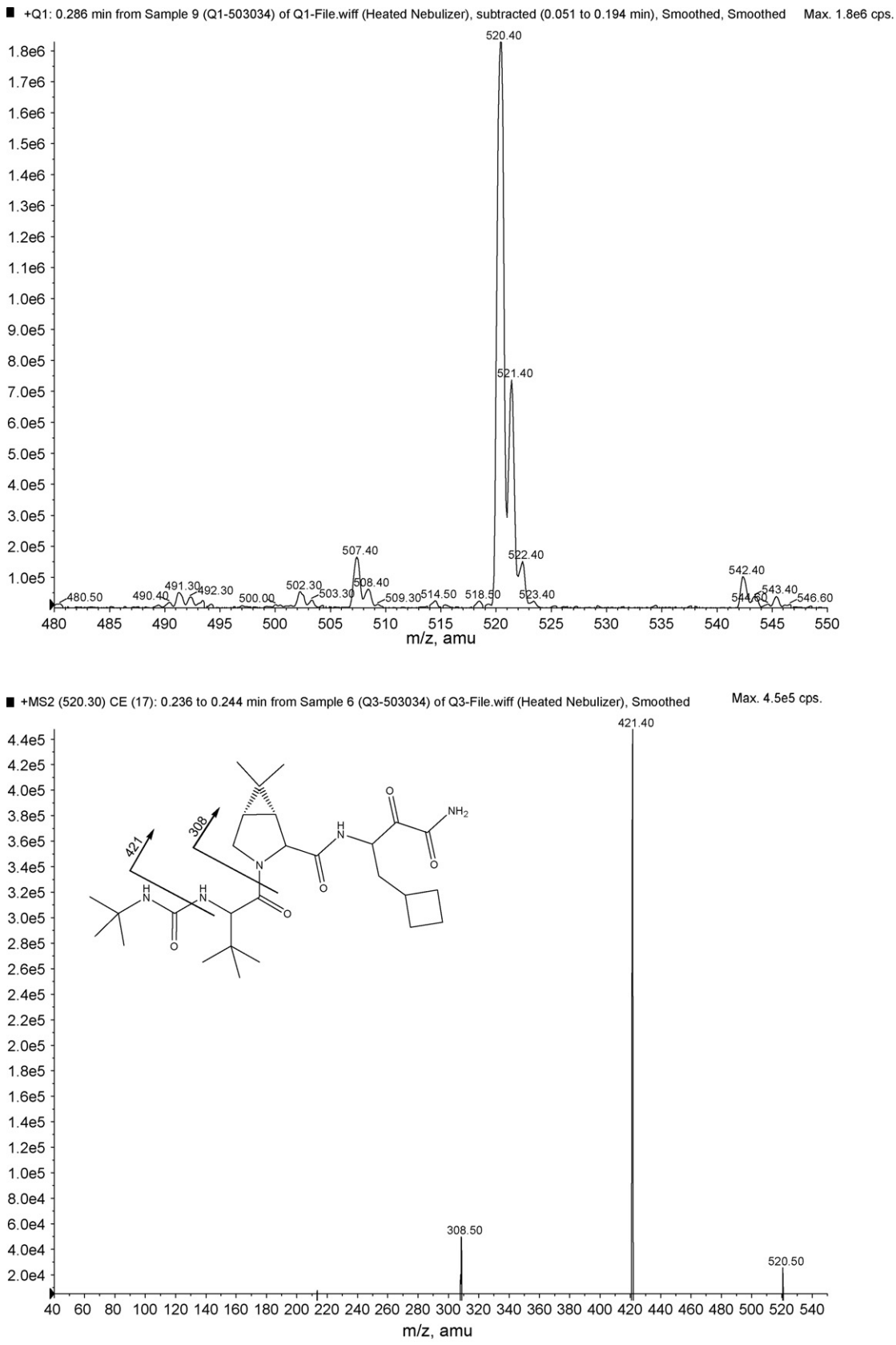

Fig. 2. Full scan spectrum of a 0.3 μg/ml SCH 503034 standard solution in mobile phase, along with product ion scan spectra of its protonated molecule $[M+H]^+$ at *m/z* 520.4. See Section 2.2 for MS conditions. Parent ion (top) and product ion (bottom) mass spectra of SCH 503034.

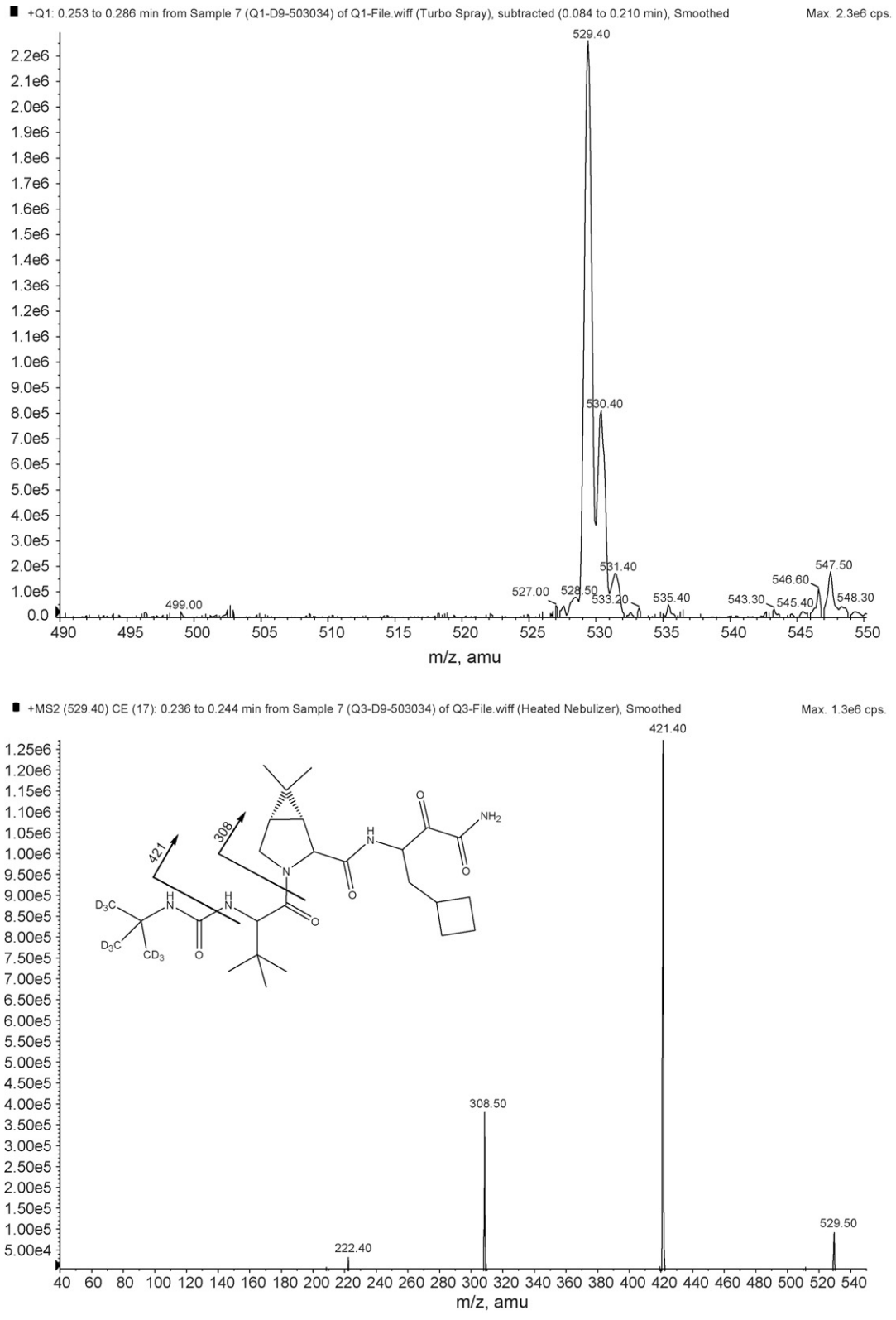

Fig. 3. Full scan spectrum of a 1 μg/ml $^{2}H_{9}$-SCH 503034 standard solution in mobile phase, along with product ion scan spectra of its protonated molecule $[M+H]^{+}$ at *m/z* 529.4. See Section 2.2 for MS conditions. Parent ion (top) and product ion (bottom) mass spectra of $^{2}H_{9}$-SCH 503034.

ion suppression in API-MS than TFA. We tested TFA and PFPA as ion-pairing agents for the separation of the compounds of interest. TFA gave a very poor selectivity and lower sensitivity for the isomers. Fig. 4B shows the separation of the diastereomers on a Varian MonoChrom C_{18} column using the mobile phase with PFPA ion-pairing agent. It can be seen that both separation and runtime were improved compared to the same column using the mobile phase with ammonium acetate.

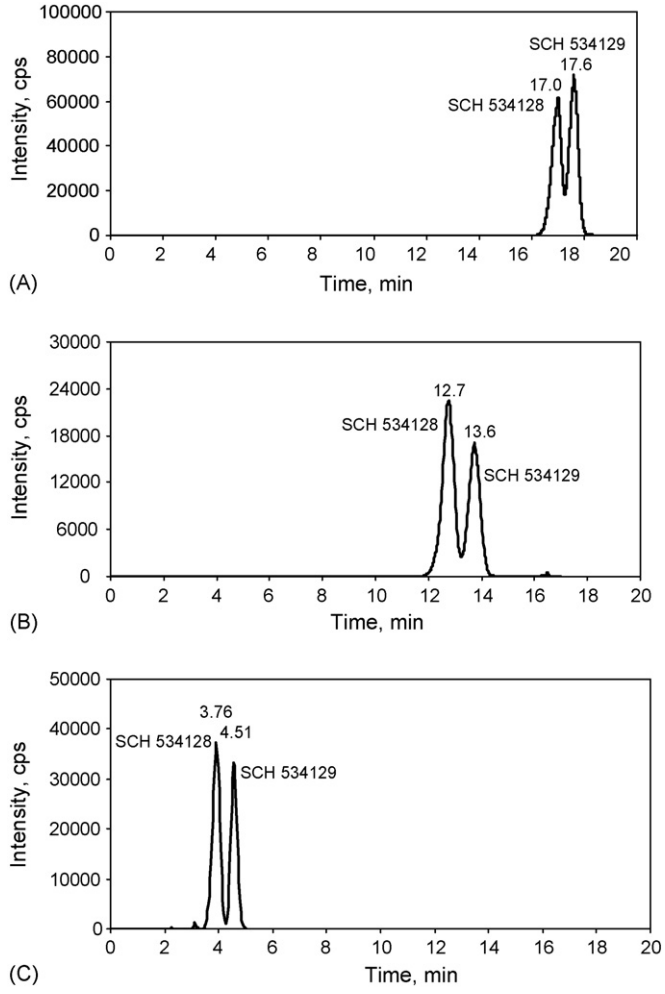

Fig. 4. Reconstructed chromatograms of SCH 534128 and SCH 534129 reference standards obtained on: (A) MonoChrom C_{18} column/mobile phase B-1 and B-2; (B) MonoChrom C_{18} column/mobile phase A-1 and A-2; (C) Acquity BEH C_{18} column/mobile phase A-1 and A-2.

Fig. 5 shows that the effect of PFPA concentrations in mobile phase on the resolution of the diastereomers. The resolution increased with elevation of PFPA concentrations in mobile phase. The best resolution ($R = 1.3$) was obtained at 40 mM of PFPA.

According to liquid chromatographic theory, one effective way to improve the column efficiency is to reduce the particle size of the stationary phase as follows:

$$H = A + \frac{B}{\mu} + C \cdot \mu = 2\lambda d_p + 2G D_m/\mu + \omega(d_p \text{ or } d_c)^2 \mu/D_m + R d_f^2 \mu/D_s$$

Where: H, λ, d_p, D_m, d_c, d_f, and D_s, represent height equivalent of a theoretical plate, particle shape, particle diameter, the diffusion coefficient of the mobile phase, the capillary diameter, the film thickness and the diffusion coefficient of the stationary phase, respectively. G, ω, and R are constants. In general, as the particle size is lowered by a factor of three, for example, from 5 μm (HPLC scale) to 1.7 μm (UPLC scale), column efficiency (N) is increased by three and resolution (R) is increased by the

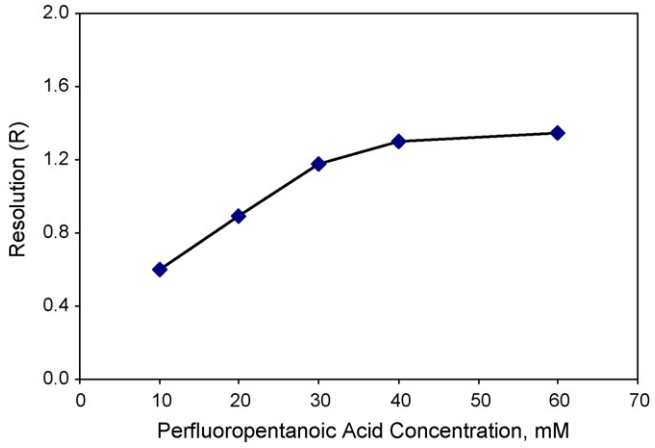

Fig. 5. Peak resolution of the diastereomers as a function of perfluoropentanoic acid concentration in mobile phase.

square root of three or 1.7. The column backpressure would also increase by 27-fold.

Recent technological advances have made available reversed phase chromatographic media with a 1.7 μm particle size along with a liquid handling system that can operate such columns at much higher pressures (max. 16,000 psi) [10–13]. This technology, termed ultra-performance liquid chromatography (UPLC), offers significant theoretical advantages in resolution, speed and sensitivity for analytical determinations due to the use of smaller particle size and smaller column diameter, particularly when coupled with mass spectrometers capable of high-speed acquisitions. From Fig. 4C, we can see that the UPLC using the mobile phase with PFPA ion-pairing agent proved to be the best one among the three approaches. Runtime was shortened significantly from 17 to 5 min compared to the conventional HPLC (Fig. 4B), furthermore, separation and sensitivity were improved as well.

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. For this method, no significant endogenous interfering peaks for the diastereomers, or the internal standard, were observed in blank monkey plasma. Representative chromatograms of the diastereomers in monkey plasma samples are shown in Fig. 6.

The back-calculated concentrations of SCH 534128 and SCH 534129 calibration standards assayed in five separate runs are presented in Tables 1 and 2. Calibration curves for the each diastereoisomers were linear over the concentration range of 1–2500 ng/ml in monkey plasma with the correlation coefficients (r^2) being >0.99.

The mean recoveries of the two diastereoisomers were 87.2% and 90.0% for low level samples (2.5 ng/ml), 90.4% and 89.1% for medium level samples (50 ng/ml) and 92.3% and 94.3% for high level samples (1000 ng/ml), respectively, as shown in Table 3.

The results of intra- and inter-assay precision and accuracy were presented in Table 4. Inter-assay mean biases ($n = 12$) were in the range of −1.2% to 3.6% for SCH 534128 with precision (R.S.D.) 4.3–7.7%, respectively, and 1.3–2.4% for SCH 534129

Table 1
Back-calculated concentrations of SCH 534128 calibration standards assayed in five separate runs

Nominal conc. (ng/ml)	Back-calculated standard concentration											Slope	*Y*-intercept	r^2
	1	2.5	5	10	25	50	100	250	500	1000	2500			
Run 1	1.14	2.57	4.58	10.5	21.9	57.2	90.2	235	529	1110	2450	0.00303	−0.00418	0.995
Run 2	0.89	2.21	4.69	11.2	25.9	56.6	89.3	241	544	956	2380	0.00287	−0.00404	0.999
Run 3	1.05	2.69	5.25	9.82	27.2	48.9	108	279	489	988	2650	0.00356	−0.00336	0.995
Run 4	0.91	2.25	5.12	9.55	27.5	53.2	101	224	447	1080	2440	0.00297	0.00452	0.996
Run 5	1.10	2.38	4.75	9.60	24.5	54.2	105	251	540	1030	2330	0.00332	−0.00384	0.997
Mean	1.02	2.42	4.88	10.1	25.4	54.0	98.7	246	510	1033	2450	0.00315	−0.00218	0.996
S.D.	0.11	0.21	0.29	0.71	2.3	3.3	8.5	21	41	63	122	0.00028	0.00376	0.0017
%CV	11	8.5	6.0	7.0	9.0	6.1	8.6	8.5	8.1	6.1	5.0	9.0	NA	0.17
%Dev	1.8	−3.2	−2.4	1.3	1.6	8.0	−1.3	−1.6	2.0	3.3	−2.0			
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 2
Back-calculated concentrations of SCH 534129 calibration standards assayed in five separate runs

Nominal conc. (ng/ml)	Back-calculated standard concentration											Slope	*Y*-intercept	r^2
	1	2.5	5	10	25	50	100	250	500	1000	2500			
Run 1	1.15	2.67	4.60	9.98	22.6	56.6	88.6	240	524	1120	2390	0.00177	−0.00530	0.996
Run 2	0.90	2.29	4.55	11.3	27.0	52.3	91.4	238	539	944	2410	0.00231	−0.00405	0.998
Run 3	1.09	2.71	5.50	9.65	25.9	45.2	106	266	477	998	2590	0.00198	−0.00498	0.997
Run 4	0.89	2.38	5.38	9.45	28.6	54.2	96.2	231	465	1020	2410	0.00223	0.00542	0.995
Run 5	1.14	2.35	4.66	9.89	25.2	51.9	108	259	537	1070	2240	0.00218	−0.00479	0.994
Mean	1.03	2.48	4.94	10.1	25.9	52.0	98.0	247	508	1030	2408	0.00209	−0.00274	0.996
S.D.	0.13	0.19	0.46	0.73	2.2	4.3	8.6	15	35	67	124	0.00022	0.00458	0.0016
%CV	12	7.9	9.4	7.2	8.6	8.2	8.8	6.0	6.9	6.5	5.2	10	NA	0.16
%Dev	3.4	−0.8	−1.2	0.5	3.4	4.1	−2.0	−1.3	1.7	3.0	−3.7			
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 3
Extraction recoveries of the stereoisomers of SCH 503034

Analyte	Nominal conc. (ng/ml)	Recovery (%) (mean ± S.D., $n = 3$)	R.S.D. (%)
SCH 534128	2.5	87.2 ± 7.1	8.2
	50	90.4 ± 5.8	6.4
	1000	92.3 ± 4.7	5.1
SCH 534129	2.5	90.0 ± 6.6	7.3
	50	89.1 ± 7.8	8.8
	1000	94.3 ± 4.2	4.5

with corresponding R.S.D. 2.8–10%, respectively. Intra-assay mean biases ($n = 6$) were in the range of −1.3% to 5.2% for SCH 534128 with R.S.D. 2.3–6.8%, respectively, and 0.7–5.5% for SCH 534129 with corresponding R.S.D. 3.4–7.8%, respectively. The lower limit of quantitation (LLOQ) for this assay is equivalent to the lowest point of the standard curves for the test articles, or nominally 1 ng/ml. The mean biases and R.S.D. ($n = 6$) for the LLOQ assay were −1.8% and −1.1%, and 11% and 13%, respectively.

The aim of the stability study was to obtain information on the stability of the analyte in real study samples in the matrix of interest, and to establish storage conditions and lengths of

Table 4
Intra- and inter-assay precision and accuracy

Analyte	Nominal conc. (ng/ml)	Inter-assay precision (mean ± S.D., $n = 12$)	R.S.D. (%)	Bias (%)	Intra-assay precision (mean ± S.D., $n = 6$)	R.S.D. (%)	Bias (%)
SCH 534128	2.5	2.59 ± 0.11	4.3	3.6	2.63 ± 0.18	6.8	5.2
	50	50.6 ± 3.9	7.7	1.2	50.5 ± 1.2	2.3	1.0
	1000	988 ± 60	6.1	−1.2	987 ± 29	3.0	−1.3
SCH 534128	1 (LLOQ)	0.982 ± 0.10	11	−1.8	985 ± .023	2.3	−1.5
SCH 534129	2.5	2.56 ± 0.26	10	2.4	2.64 ± 0.21	7.8	5.5
	50	50.7 ± 3.1	6.1	1.4	50.5 ± 1.8	3.6	1.0
	1000	1013 ± 28	2.8	1.3	1007 ± 34	3.4	0.7
SCH 534129	1 (LLOQ)	0.989 ± 0.129	13	−1.1	0.971 ± 0.025	2.5	−2.9

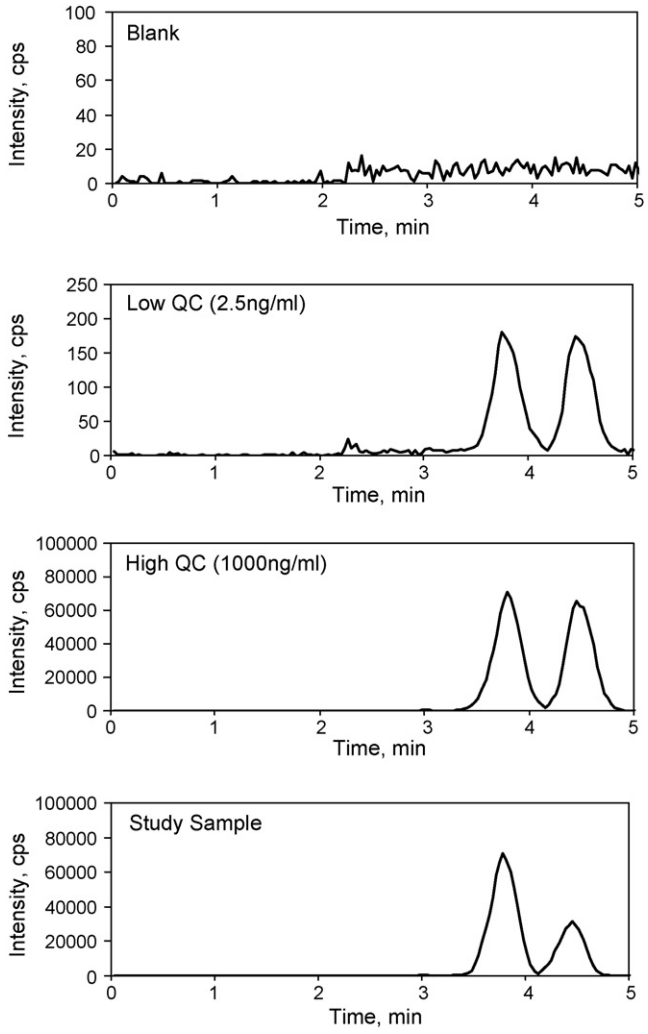

Fig. 6. Reconstructed chromatograms of SCH 534128 and SCH 534129 in monkey plasma.

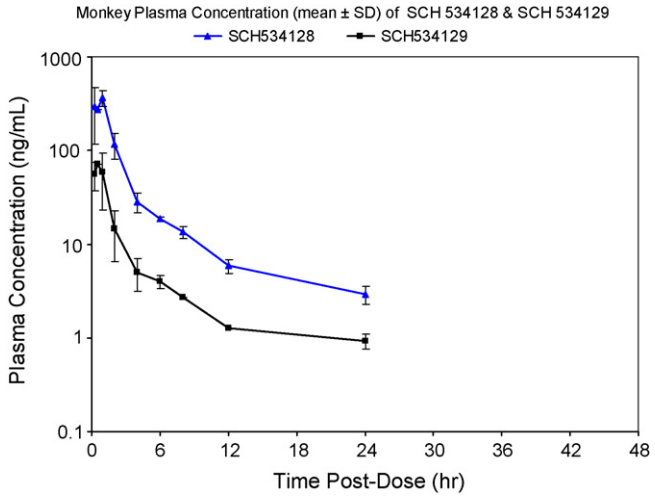

Fig. 7. Representative plasma concentration-time profiles for SCH 534128 and SCH 534129 after an oral administration of 10 mg/kg of SCH 503034 to monkeys.

storage as well as sample processing conditions. SCH 503034 is a compound containing a ketoamide functional group, which is reactive in biological matrices on certain conditions. The bench-top stability study showed that the test articles were not stable at room temperature in monkey plasma (data not shown). Therefore, sample preparation steps before protein precipitation should be performed on ice. However, no significant changes were observed for the concentrations of the stereoisomers after standing for 6 h at 4 °C. For processed samples, the stereoisomers of the test articles could be stable at least 36 h at 8 °C.

A general concern on the reliability of any new HPLC-MS/MS methods is the ionization suppression or enhancement caused by co-eluting endogenous compounds in biological matrices [17–20]. The matrix interferences study demonstrated that there were no significant differences in both peak areas of the diastereoisomers or the internal standard and peak area ratios

Table 5
Peak area variations of the test articles in 6 different sources of blank monkey plasma

Matrix no.	Peak area	Individual mean ($n = 2$)	Total mean ($n = 12$)	Deviation[a] (%)	Peak area ratio[b]	Individual mean ($n = 2$)	Total mean ($n = 12$)	Deviation[a] (%)
1	2090 2200	2145	2041	5	0.00992 0.01100	0.0105	0.0103	2
2	2390 2300	2345		15	0.01131 0.01098	0.0111		8
3	1790 1880	1835		−10	0.00904 0.00961	0.0093		−9
4	1890 2020	1955		−4	0.00923 0.01080	0.0100		−3
5	2330 2100	2215		9	0.01286 0.00998	0.0114		11
6	1780 1720	1750		−14	0.00919 0.00941	0.0093		−10

[a] Deviation = (individual mean − total mean)/total mean × 100.
[b] Ratio = drug peak area/IS peak area.

of the diastereoisomers over the internal standard in six different sources of blank monkey plasma spiked with the same amount of the test articles and internal standard since deviations of the individual mean ($n = 2$) form the total mean ($n = 12$) were less than 20% (Table 5).

The method presented here has been successfully applied to pharmacokinetic studies of SCH 503034 in monkeys. Fig. 7 shows representative plasma concentration–time profiles for SCH 534128 and SCH 534129 after oral administration of 10 mg/kg of SCH 503034 to the monkeys.

4. Conclusions

A fast, sensitive and specific UPLC-MS/MS method for the determination of diastereoisomers of SCH 503034 in monkey plasma has been developed and qualified. In this paper, we have demonstrated that the use of the ion-pair reversed phase UPLC method resulted in improvements over the initial HPLC methods in terms of runtime, separation and sensitivity for the diastereoisomers of interest than commonly used HPLC. The UPLC/MS/MS method has been applied to the pharmacokinetic studies of SCH 503034 in monkeys and proved to be reproducible and reliable for the high throughput PK studies. This method has not only been used for the determination of SCH 503034 in monkey plasma, but also used in rat, dog and human plasma samples.

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