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Determination of rat plasma levels of sertraline enantiomers using direct injection with achiral–chiral column switching by LC–ESI/MS/MS

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ABSTRACT

A highly sensitive and selective on-line two-dimensional reversed-phase liquid chromatography/electrospray ionization–tandem mass spectrometric (2D-LC–ESI/MS/MS) method to determine sertraline (SRT) enantiomers in rat plasma was developed and validated. The method was applied to separate and determine the diastereomers and enantiomers of SRT simultaneously. The 2D-LC–ESI/MS/MS system consisted of RAM column in first dimension for trapping proteineous part of plasma and a chiral Cyclobond column as second dimension for separation of enantiomers and diastereomers of SRT using 0.1% aqueous trifluoroacetic acid:acetonitrile (86:14, v/v) as mobile phase in an isocratic elution mode. The linear dynamic range was 0.5–200 ng/mL ($r^2 > 0.999$). Acceptable precision and accuracy were obtained over the calibration range. The assay was successfully used in the analysis of SRT enantiomers in rat plasma to support pharmacokinetic studies.

1. Introduction

Sertraline hydrochloride (+)-cis-(1S,4S)-*N*-methyl-4 (3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine hydrochloride (SRT) is a selective serotonin reuptake inhibitor (SSRI) used to treat major depression as well as obsessive-compulsive, panic and social anxiety disorders in both adults and children. It is as effective as tricyclic antidepressants (TCA) [1] with minimal side effects, such as insomnia, nervousness, nausea, diarrhea, dry mouth and dyspepsia. During its synthesis, the (−)-cis-(1R,4R)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride, trans-(1S,4R) and (1R,4S)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride are introduced in significant quantities (Fig. 1). Therefore, stereo selective separation and determination of SRT is important to assure its therapeutic efficacy and safety.

A wide variety of analytical methods including GC-ECD [2], GC–MS [3], HPLC-PDA, HPLC–UV and HPLC–MS/MS [4–13] for determination of SRT in plasma or serum were reported. These methods are suitable to determine SRT either alone or in combination of other drugs. Stereoisomers of SRT and its related enantiomeric impurities were separated (i) directly on a dimethyl β-cyclodextrin stationary phase [14] and (ii) indirectly using hydroxypropyl β-cyclodextrin as a mobile phase additive [15] by HPLC in bulk drugs and formulations. Foley and Zhou [16] separated the enantiomers of SRT by CE using highly sulphated β-cyclodextrin as a chiral selector. However these methods do not address the separation of enantiomers of SRT in biological fluids. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) in positive-ion electrospray (±ESI) and selected-ion reaction monitoring modes [17] was proved to be a valuable tool in characterization of several antidepressants including SRT and their *N*-desmethyl metabolites in raw sewage and both primary-treated wastewaters. Several LC methods to determine SRT and its main metabolite *N*-desmethylsertraline in plasma or serum suitable pharmacokinetic studies were reported [7,8,12]. All these methods follow the precipitation of proteins with organic solvents [13] and use either liquid–liquid or solid phase extraction for sample clean-up. Sample preparation is one of the most important steps in HPLC analysis of drugs and their metabolites in biological fluids. Proteins in the biological fluids can precipitate or denature and adsorb onto the packing material, leading to the build-up of back pressure. To eliminate problems such as co precipitation of analytes during extraction and avoid the adsorption of protein onto the analytical column, direct injection of the sample using column switching is becoming the method of choice [18–23]. Recently Cass and Galatti [24] reported a bidimensional achiral–chiral chromatography for determination of the plasma levels of modafinil enantiomers and its major metabolites by direct injection of human

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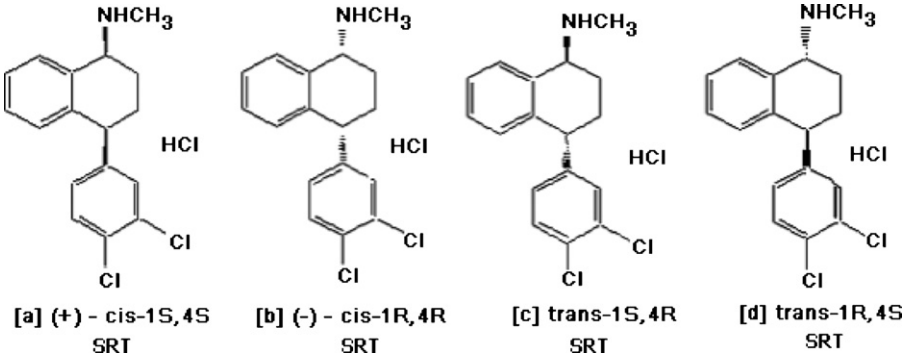

Fig. 1. Chemical structures of: (a) (+)-cis-1S,4S; (b) (−)-cis-1R,4R; (c) trans-1S,4R; (d) trans-1R,4S SRT hydrochloride.

plasma with on-line sample preparation. Two-dimensional liquid chromatography (2D-LC) and 2D-LC coupled with mass spectrometry (2D-LC/MS, 2D-LC/MS/MS) have become popular techniques in bioanalytical chemistry, pharmacology and proteomic research and these systems generate excellent resolution, enabling the comprehensive separation of complex biological matrices [19]. The specific advantages of 2D-LC over 1D-LC include (i) direct injection of plasma, (ii) on-line sample preparation, (iii) no contact with toxic solvents, (iv) no need of extraction, (v) increased column efficiency, (vi) a huge increase in peak capacity, (vii) no co precipitation of analytes with proteins, (viii) no adsorption of protein onto the analytical column and (ix) avoids extraction losses during evaporation and reconstitution steps. 2D-LC could be performed either on-line or off-line modes. The on-line approach narrows the choice of LC mode due to the mobile phase compatibility for direct transfer to the second dimension. This approach minimizes sample losses, which could be an advantage for sensitivity compared to the off-line mode.

The present paper describes the development and validation of a LC method for simultaneous determination of SRT enantiomers and diastereomers using direct injection with RAM–Cyclobond column switching by LC–ESI/MS/MS. The method was applied for the investigation of enantioselectivity in the pharmacokinetic studies of SRT administered in racemic form in a single dose to rats. The method was sensitive enough to quantify the low concentration of 0.4 ng/mL SRT in rat plasma.

2. Experimental

2.1. Chemicals and materials

Racemic mixture of SRT hydrochloride ((±)-cis-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride), (+)-cis-(1S,4S)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride, (−)-cis-(1R, 4R)-*N*-methyl-4-(3, 4-dichlorophenyl)-1, 2, 3, 4-tetrahydro-1-naphthalenemine hydrochloride, trans-(1S,4R)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride, trans-(1R,4S)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride were procured from a local pharmaceutical industry. Purified de-ionized water (Nanopure, Barnstead, USA), HPLC-grade acetonitrile, methanol, trifluoroacetic acid (Qualigens Fine Chemicals, Mumbai, India), ammonium acetate (S.D. Fine Chem., Mumbai, India) were used. The blood samples used for the development and validation of the analytical method were obtained at regular intervals of time for 24 h from male Wistar rats weighing 170 ± 10 g housed one animal per cage under standard conditions. The required environment was controlled with daily feeding of standard chow pellets and water ad libitum.

2.2. Liquid chromatography–mass spectrometry

The LC system LC-MSD Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) consisting of a binary LC pump, a vacuum degasser, a temperature-controlled microwell plate auto sampler set at 4 °C and a thermostatted column compartment set at 35 °C. The compounds were analyzed on a Astec CYCLOBOND™ I 2000 DM (25 cm × 4.6 mm, 5 μm) (Supelco, PA, USA) column, under isocratic conditions using a mobile phase containing 0.1% aqueous trifluoroacetic acid:acetonitrile (86:14, v/v) at a flow rate of 0.8 mL/min protected by a guard column Hisep-RAM (50 mm × 4.0 mm; particle size 5 μm) (Supelco, USA) under isocratic conditions using a mobile phase containing 0.02 M aqueous ammonium acetate (pH 8):acetonitrile (86:14, v/v) at a flow rate of 1 mL/min.

The analytes were monitored by mass spectrometer equipped with an electrospray ionization interface, operated in a positive mode (+ESI). Nitrogen was the nebulizer and curtain gas. Collision induced dissociation was achieved using nitrogen as collision gas. The ion source conditions were: temperature 325 °C, nebulizer gas pressure 35 psi, dry gas 8.0 L/min, ion spray voltage: 3500 V, collision energy 0.8 A, declustering potential 5.0 V (lens 1), 60.0 V (lens 2), entrance potential 40.0 V and collision exit potential 113.5 V. The data was captured using a Chemstation software. Transition of m/z 306 → 274.7 was used for detection of SRT.

2.3. Preparation of plasma standards

The calibration standards (CS) and quality control samples (QC) were prepared by spiking blank plasma with working solutions of analytes. Calibration standards were at 0.5, 1.0, 2.0, 4.0, 10.0, 20.0, 40.0, 100, 150, 200 ng/mL for all the analytes. A calibration curve was constructed using 200 μL plasma of each standard. Quadratic regression equation with peak area against concentration of SRT was used for quantification of unknown concentration of SRT enantiomers in rat's plasma. Quality controls were prepared at 0.5, 1.5, 14.0, 150, 200 ng/mL for all analytes and used for determination of accuracy and precision in determination of SRT enantiomers and diastereomers in rat plasma. The spiked plasma samples at all the levels were stored at −20 °C.

The standard stock solutions of 100 μg/mL for (±)-cis-SRT, (+)-cis-(1S,4S) SRT, (−)-cis-(1R,4R) SRT, trans-(1S,4R) SRT and trans-(1R,4S) SRT hydrochloride were prepared by dissolving requisite amounts in methanol:water (30:70, v/v). The stock solutions were further diluted with water appropriately to get an intermediate concentration of 4 μg/mL. The working solutions of all compounds for spiking calibration and quality control samples were subsequently prepared from standard and intermediate stock solutions. All the standard stock, intermediate stock and working stock solutions were prepared and stored at 4 °C until use.

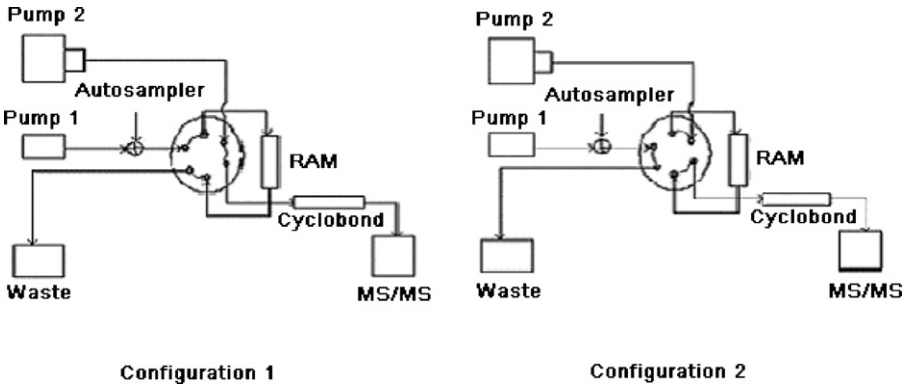

Fig. 2. Schematic diagram of the column switching system in configurations 1 and 2.

2.4. Sample preparation

Frozen plasma samples were thawed at room temperature before processing. The blood samples were centrifuged for 15 min at 4500 rpm. Supernatant was collected, filtered through 0.45μ nylon membrane filter, vortexed for 10 min and directly injected a 20 μL aliquot into 2D-LC/MS/MS containing RAM and chiral Cyclobond columns. All plasma samples including calibration curve, QC and samples for pharmacokinetic experiments were prepared as above.

2.5. Dosing and sampling

The method was applied to the investigation of enantioselectivity in the pharmacokinetics of SRT administered in the racemic form in a single dose to rats. The animals received an aqueous solution of (±)-rac-SRT hydrochloride (25 mg/kg) by oral administration. Blood samples were collected at times 0, 1, 2, 4, 6, 8, 10, 24 h after drug administration. Plasma samples were stored at −70 °C until analysis.

3. Results and discussion

3.1. Method development

Initially CHIRAL-AGP (150 mm × 4.0 mm) and CHIROBIOTEC V (150 mm × 4.6 mm) column using 0.1% aqueous acetic acid with organic modifiers, viz., methanol and acetonitrile of different composition as mobile phase were tried to separate the enantiomers and diastereomers of SRT. However, the trails did not result in good separation of the analytes. SRT and related substances are polar in nature and fairly soluble in methanol and water. The reversed-phase mode offers greatest possibilities for selectivity by taking advantage of the inclusion mechanism for which cyclodextrins are well known [15]. So reverse phase conditions were tried using a Cyclobond column which is useful for analytes having ring structures includable into the cyclodextrin cavity and form hydrogen bonds. The inclusion mechanism of cyclodextrins resolves the enantiomeric drugs which are difficult to separate on other chiral stationary phases [25]. A mobile phase containing 0.1% aqueous trifluoroacetic acid:acetonitrile (86:14, v/v) at a flow rate of 0.8 mL/min was used in an isocratic elution mode at room temperature, but all the enantiomers were not separated. However at elevated column temperatures, i.e. 35 °C it was found that all the analytes were well separated.

Initially different mobile phase conditions were tried on RAM column to separate SRT analytes. 0.05 M aqueous ammonium acetate with different proportions of acetonitrile and 0.05% aqueous acetic acid with acetonitrile of different compositions were

Table 1
Sequential steps of on-line column switching.

Step	Time interval (min)	Configuration	Analytical operation
1	0–2.5	1	Exclusion of plasma proteins by RAM column and conditioning of Cyclobond column
2	2.5–10	2	Elution of retained SRT from RAM to Cyclobond
3	10–16	1	Elution of SRT enantiomers, diastereomers from Cyclobond and conditioning of RAM column

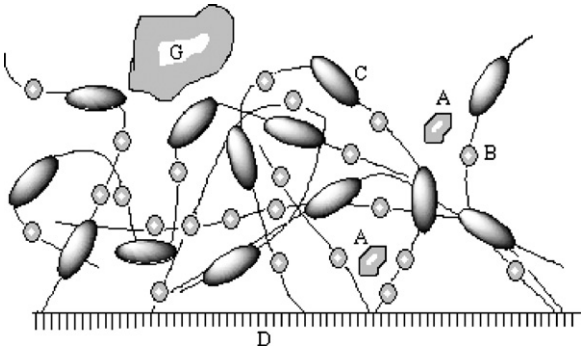

Fig. 3. Schematic representation of an embedded-network shielded hydrophobic phase, (A) small analyte molecule; (B) hydrophilic sites; (C) hydrophobic sites; (G) protein; (D) support matrix. (Reproduced from [26] with kind permission.)

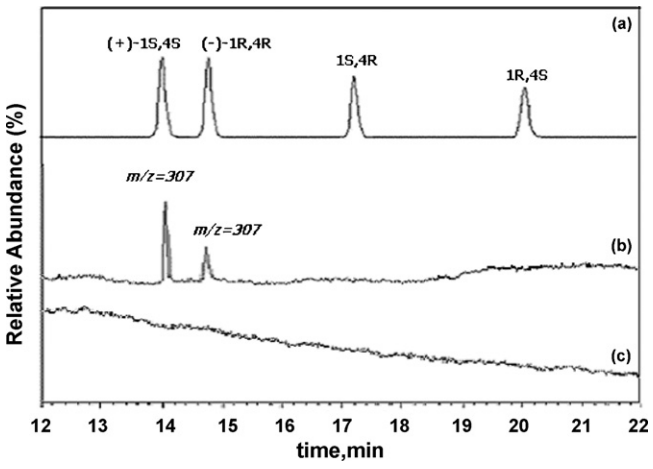

Fig. 4. Typical liquid chromatograms showing the (a) separation of 50 ng/mL of (+)-cis-1S,4S, (−)-cis-1R,4R, trans-1S,4R, trans-1R,4S SRT hydrochloride. (b) SRT enantiomers (cis-1S,4S, cis-1R,4R) in rat plasma at 6th hour after oral administration of 25 mg/kg (±)-rac-SRT to Wistar rats. (c) Blank plasma.

tried. These conditions did not result in good separation of the analytes. Isocratic conditions with a mobile phase containing aqueous ammonium acetate 0.02 M pH 8:acetonitrile (86:14, v/v) at a flow rate of 1 mL/min were successful. Depending upon the retention times of the analytes on RAM and Cyclobond columns the method was divided into three steps. The configuration of the system was changed in different steps for excluding proteins through RAM column and separation of analytes through Cyclobond column. The column switching system used for coupling of RAM and Cyclobond columns is shown schematically in Fig. 2. The sequential steps used are listed in Table 1. The rat plasma of 20 μL sample volume was applied when the system was in configuration (1) (0–2.5 min) (Fig. 2). In this configuration, the mobile phase (0.02 M aqueous NH_4OAc:ACN, 86:14, v/v) was delivered by pump 1 at a flow rate 1.0 mL/min to elute the proteins from RAM column to waste. At the same time the Cyclobond column was conditioned by the mobile phase (0.1% aqueous TFA:ACN, 86:14, v/v) delivered by pump 2. The analytes were retained on the hydrophobic phase of the RAM column. In configuration (2), (2.5–10 min) (Fig. 2) the mobile phase was delivered by pump 2 at a flow rate 0.8 mL/min and the analytes from RAM to Cyclobond column were eluted. After 10 min the system was changed to configuration (1) (Fig. 2). In this configuration the mobile phase delivered by pump 2 elutes SRT enantiomers and diastereomers from Cyclobond column while RAM column was conditioned by the mobile phase delivered by pump 1. The role of the RAM column was to remove the proteins. It could be characterized by the hydrophilic outer and hydrophobic inner phases which exclude large molecules such as proteins in the void volume while retain selectively the small hydrophobic analytes [26]. The stationary phase in RAM was a porous chromatographic support specifically designed for the removal of macromolecules, partially based on a size exclusion mechanism [27]. Only small molecules penetrate into the pores and interact with the stationary phase bound to the inner surface. During the analyses the performance of the RAM column was found to be stable with over 500 plasma injections without significant change in the back pressure. Initially the back pressure of the RAM column was 40 kgf/cm^2 and after analyses of around 500 samples

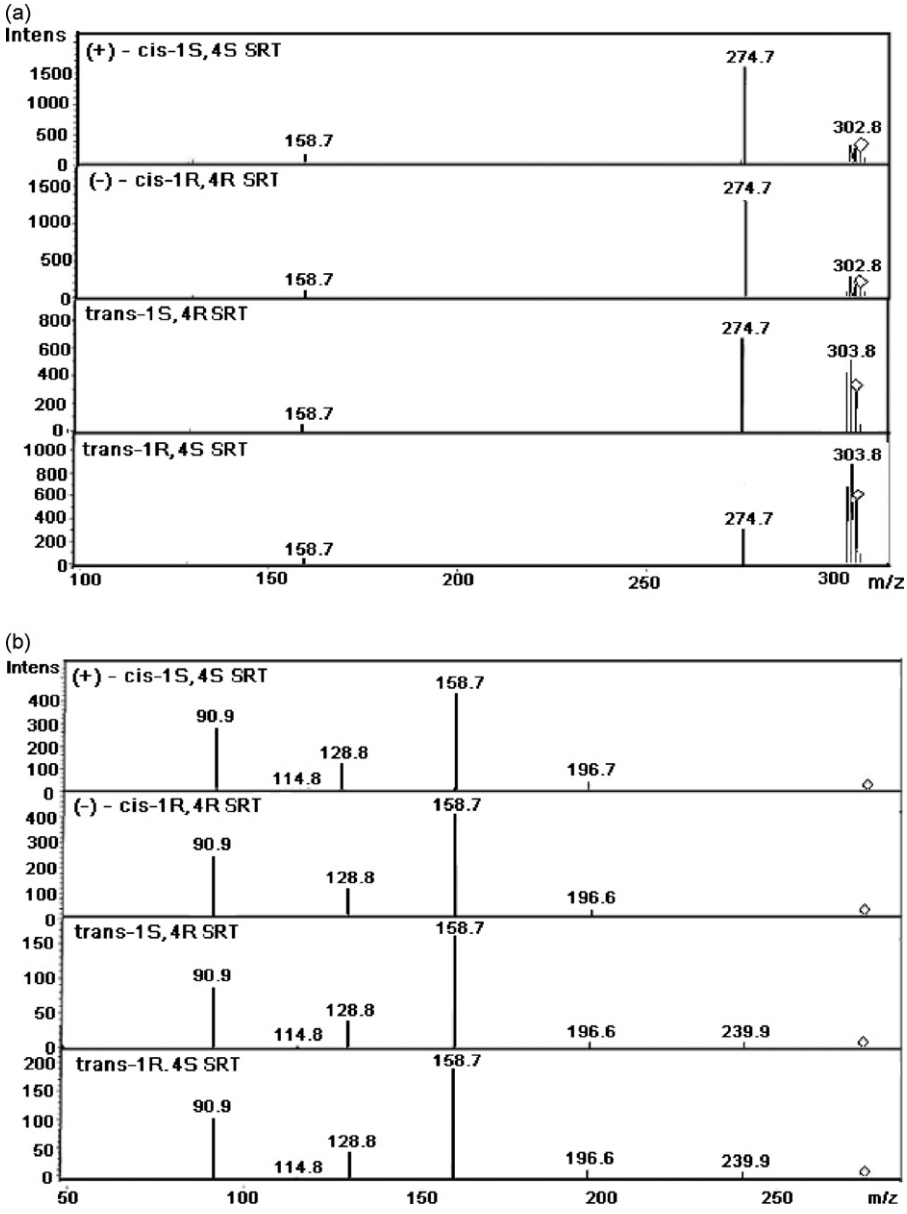

Fig. 5. (a) MS/MS1 and (b) MS/MS2 spectra of 50 ng/mL of SRT enantiomers and diastereomers.

it became 60 kgf/cm^2. Schematic representation of an embedded-network shielded hydrophobic phase is shown in Fig. 3. The elution order was (+)-cis-1S,4S (−)-cis-1R,4R, trans-1S,4R, trans-1R,4S SRT hydrochloride. Typical chromatograms of (a) standard calibration plasma spiked with (+)-cis-1S,4S (−)-cis-1R,4R, trans-1S,4R, trans-1R, 4S, (b) SRT enantiomers in rat plasma at 6th hour after oral administration of rac-SRT to Wistar rats and (c) blank plasma are shown in Fig. 4a–c. It could be seen from Fig. 4 that the trans 1S,4R and 1R,4S isomers were not found in rat plasma (Fig. 4b). It was due to the administration of rac SRT containing only the enantiomers of cis-1S,4S and -1R,4R SRT into the Wistar rats. This was studied because of the SRT formulations generally contain enantiomers of cis-1S,4S and -1R,4R selectively. Further it could be seen that there was no peak corresponding to the metabolite of SRT in the chromatogram of rat plasma (Fig. 4b). Probably it might have formed in very traces after 6th hour of injection. To detect the metabolite LC–MS/MS was studied. However there was no peak detected at 274 corresponding the *N*-desmethylsertraline. It could be due to two possibilities (i) its concentration could be very much below the detection limit or (ii) it might be not eluted from the column under the conditions used for separation. The mass spectrometric detection of SRT was investigated by ESI (+ve) SRM mode. In the full scan MS/MS1 chromatogram (Fig. 5a), all analytes formed predominately protonated molecules $[M+H]^+$ at *m*/*z* 307 and base peak at *m*/*z* 274.7. On further fragmentation all analytes gave fragments at *m*/*z* 158.7 (base peak), *m*/*z* 129 and *m*/*z* 91 in the full scan

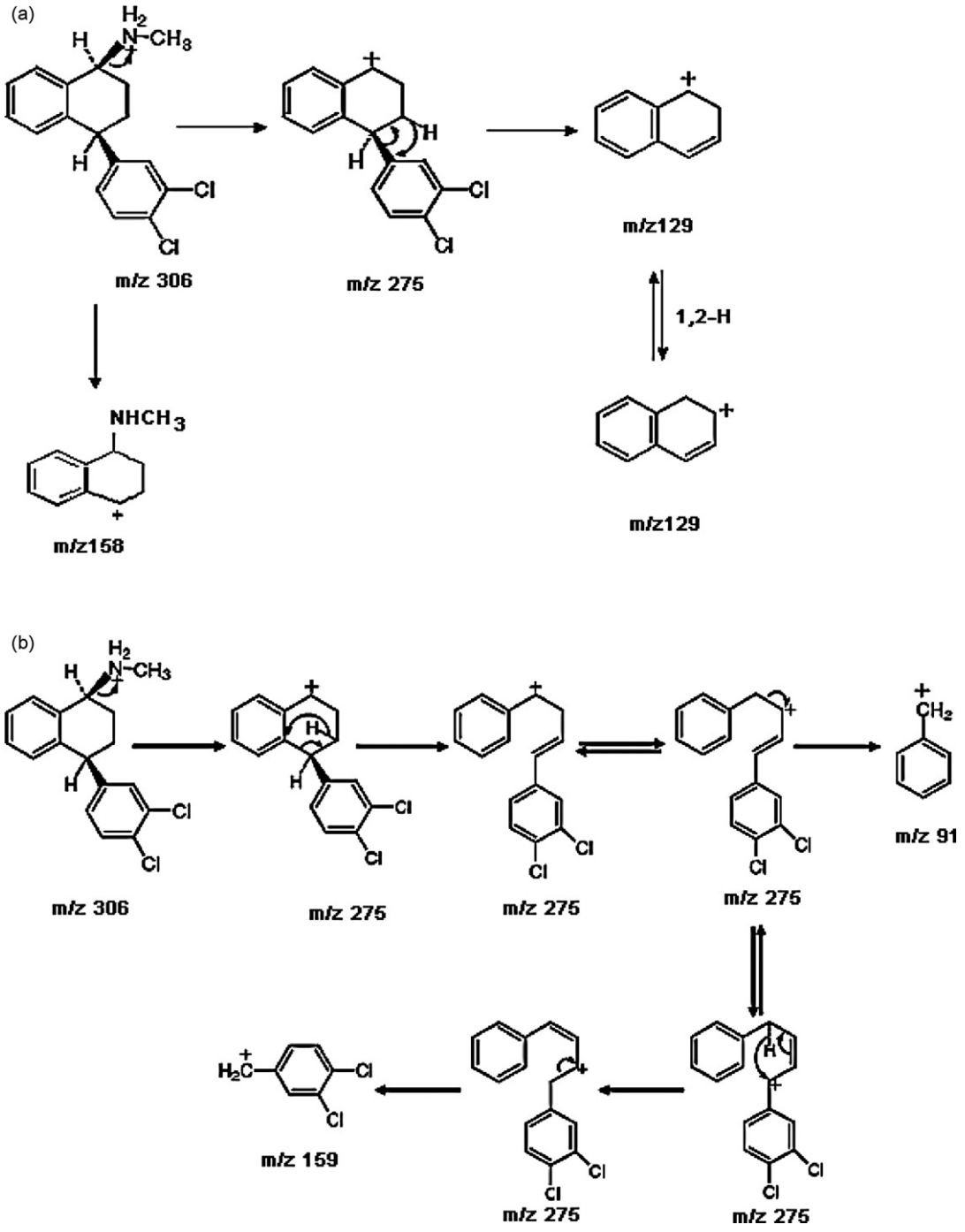

Fig. 6. (a) MS/MS1 and (b) MS/MS2. Fragmentation pathways of SRT enantiomers and diastereomers.

Table 2
Intra- and inter-batch accuracy and precision in determination of SRT enantiomers and diastereomers.

Precision and accuracy	(+)-cis-1S,4S SRT (%)	(−)-cis-1R,4R SRT (%)	trans-1S,4R SRT (%)	trans-1R,4S SRT (%)
Intra-assay precision; coefficient of variation ($n = 10$)				
0.5 ng/mL	3.02	2.45	2.17	3.01
1.5 ng/mL	4.45	3.96	2.17	2.85
14.0 ng/mL	3.53	3.13	4.38	1.05
150 ng/mL	1.06	4.62	1.92	3.94
200 ng/mL	2.05	2.97	2.84	4.47
Inter-assay precision; coefficient of variation ($n = 5$)				
0.5 ng/mL	2.62	4.45	3.22	3.44
1.5 ng/mL	3.12	2.75	3.83	2.63
14.0 ng/mL	1.44	4.75	3.26	4.87
150 ng/mL	4.17	3.48	2.99	1.90
200 ng/mL	1.45	1.4	4.94	2.05
Intra-assay accuracy; relative error ($n = 10$)				
0.5 ng/mL	4.05	−2.04	−3.07	2.03
1.5 ng/mL	2.47	−3.68	1.79	−3.88
14.0 ng/mL	3.83	4.55	4.95	2.85
150 ng/mL	2.52	3.88	−4.87	3.85
200 ng/mL	5.22	−3.02	−2.46	1.58
Inter-assay accuracy; relative error ($n = 5$)				
0.5 ng/mL	0.0	3.16	−4.64	−3.67
1.5 ng/mL	0.58	−1.99	−3.67	1.99
14.0 ng/mL	3.06	2.45	3.47	1.29
150 ng/mL	1.48	3.78	4.98	4.78
200 ng/mL	4.96	−3.88	1.58	1.38

of MS/MS2 chromatogram (Fig. 5b). The proposed fragmentation pathways during MS/MS for the $[M+H]^+$ ions of all the enantiomers and diastereomers of SRT are shown in Fig. 6.

3.2. Validation

3.2.1. Selectivity

The selectivity of the method was investigated for potential interferences of endogenous substances by using six independent batches of rat plasma (blank). Moreover, the chromatograms of the experimental samples obtained after administration of rac-SRT to Wistar rats were compared with standard chromatograms in order to detect interfering peaks. No interfering peaks were found at the retention times of SRT enantiomers and diastereomers.

3.2.2. Accuracy and precision

The quality control samples (QC) were prepared by spiking blank plasma with working solutions of analytes at 0.5, 1.0, 14.0, 150, 200 ng/mL for all 4 analytes. The accuracy, intra- and inter-day precision of the analytical method were determined by replicate processing. Precision was calculated as coefficient of variation and accuracy as % relative error. The coefficient of variation obtained in the study of intra-, inter-assay precision and accuracy were less than 5% assuring the reproducibility and repeatability of the results. Table 2 summarizes the data obtained in intra- and inter-batch accuracy and precision for SRT enantiomers and diastereomers.

3.2.3. Linearity

The calibration standards (CS) were prepared by spiking blank plasma with working solutions of analytes at 0.5, 1.0, 2.0, 4.0, 10.0, 20.0, 40.0, 100, 150, 200 ng/mL for all 4 analytes. Plots of plasma concentrations vs peak areas were constructed and the linear regression lines were used for determination of concentrations of enantiomers in plasma samples. Slopes, intercepts and correlation coefficients were calculated. The method showed linearity up to 200.0 ng/mL for all enantiomers and diastereomers of SRT and the correlation coefficients observed were in the range of 0.9995–0.9999 for all enantiomers and diastereomers. Table 3 summarizes the data obtained in recovery and linearity ranges of the SRT enantiomers and diastereomers. The recovery was calculated by using the formula given below.

$$\text{Recovery (\%)} = \left(\frac{\text{area of spiked sample}}{\text{area of standard}}\right) \times 100.$$

3.2.4. Stability

The stability of analytes was determined during blood sample collection and handling at room temperature for 2 h and after freezing plasma samples for 1st day, 2nd day, 3rd day, 1 week,15 days and 1 month. The results were compared with those obtained by freshly prepared samples. The results are summarized in Table 4.

Table 3
Recovery and linearity ranges of the SRT enantiomers and diastereomers.

	(+)-cis-1S,4S SRT	(−)-cis-1R,4R SRT	trans-1S,4R SRT	trans-1R,4S SRT
Recovery,% ($n = 3$)				
1.5 ng/mL	90.6	98.7	99.1	89.9
14 ng/mL	99.0	96.0	98.7	95.6
150 ng/mL	95.0	94.9	97.6	98.8
Linearity				
Range (ng/mL)	0.5–200	0.5–200	0.5–200	0.5–200
Regression equation	$y = 3797.9x + 1297.5$	$y = 3789x + 2747.6$	$y = 3793.9x + 2344.6$	$y = 3796.9x + 1235.8$
Correlation coefficient (r^2)	0.9999	0.9997	0.9996	0.9999

Table 4
Intra- and inter-stability data of SRT ($n = 3$).

Storage conditions	Nominal concentration (ng/mL)	RSD (%)
Freeze/thaw stability (three cycles)	0.5	1.81
	14.0	1.69
	150.0	3.22
3 days	0.5	3.32
	14.0	2.75
	150.0	4.32
1 week	0.5	6.68
	14.0	3.22
	150.0	3.35
15 days	0.5	2.78
	14.0	3.57
	150.0	3.27
1 month	0.5	4.89
	14.0	4.47
	150.0	3.78

3.2.5. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were calculated according to the ICH guidelines. The limit of detection (LOD) (lowest concentration of analyte in a sample matrix that can be detected) and the limit of quantification (LOQ) (lowest that can be quantified with acceptable accuracy and precision) were 0.17 and 0.4 ng/mL. The total ion current (TIC) chromatograms at LLOQ concentration of (+)-cis-1S,4S, (−)-cis-1R,4R, trans-1S,4R, trans-1R,4S SRT hydrochloride is shown in Fig. 7, respectively.

3.2.6. Application of the analytical method to pharmacokinetic study in rats

The developed method was applied to the study of enantioselectivity in the kinetic disposition of SRT administered in the racemic form in a single 25 mg/kg dose to rats. Fouda et al. [3] reported pharmacokinetic study on (±)-cis-1S,4S SRT. However this method does not address the enantioselectivity of SRT. The pharmacokinetics of SRT was enantioselective for the parameters $AUC_{0-\alpha}$ ($p = 0.68$), clearance ($p = 0.68$) and C_{max} ($p = 0.43$) calculated by RAMKIN software and p-values by Wilcoxon test. (Table 5). The data obtained for the Wistar male rats showed higher plasma concentrations of (+)-cis-1S,4S SRT enantiomer (Fig. 8). The higher $AUC_{0-\alpha}$ values obtained for (+)-cis-1S,4S SRT enantiomer compared to (−)-cis-1R,4R SRT enantiomer (113.45 ± 7.45 μg h/mL vs 108.69 ± 13.47 μg h/mL) could be explained by a lower apparent total clearance (222.21 ± 14.29 mL/h vs 238.16 ± 33.13 mL/h) (Table 5). The kinetic disposition of SRT is enantioselective in male Wistar rats with a (+)/(−) plasma concentration ratio (AUC) close to 1.04. C_{max} was also higher for (+)-cis-1S,4S SRT enantiomer than (−)-cis-1R,4R SRT enantiomer (Fig. 7).

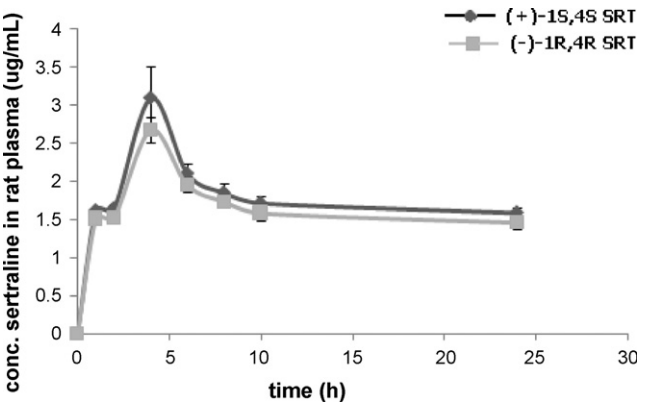

Fig. 7. Total ion current (TIC) chromatograms at LLOQ concentration of (+)-cis-1S,4S, (−)-cis-1R,4R, trans-1S,4R, trans-1R,4S SRT hydrochloride.

Table 5
Pharmacokinetic parameters of (±)-SRT enantiomers after a single oral dose of 25 mg/kg ($n = 6$) for each time point.

Parameters	(+)-cis-1S,4S SRT Mean ± S.E.M.	(−)-cis-1R,4R SRT Mean ± S.E.M.
C_{max} (μg/mL)	3.09 ± 0.40	2.67 ± 0.16
t_{max} (h)	4.00 ± 0	4.00 ± 0
AUC_{0-24} (μg h/mL)	42.90 ± 2.29	39.38 ± 1.73
$AUC_{0-\alpha}$ (μg h/mL)	113.45 ± 7.45	108.69 ± 13.47
$t_{1/2}$	31.02 ± 4.00	32.51 ± 4.37
Clearance (mL/h)	222.21 ± 14.29	238.16 ± 33.13
MRT (h)	45.57 ± 3.90	49.28 ± 6.17

C_{max}: maximum concentration of drug in rat plasma was observed at a particular time point. t_{max}: time at which maximum concentration of drug in rat plasma was observed. AUC: area under plasma concentration–time curve. $t_{1/2}$: time required for decomposition of the drug into half of the concentration. Clearance: the rate at which a substance is removed or cleared from the body. MRT (Mean Residence Time): the average time those molecules of a drug reside in the body. S.E.M.: standard deviation of standard error of the mean.

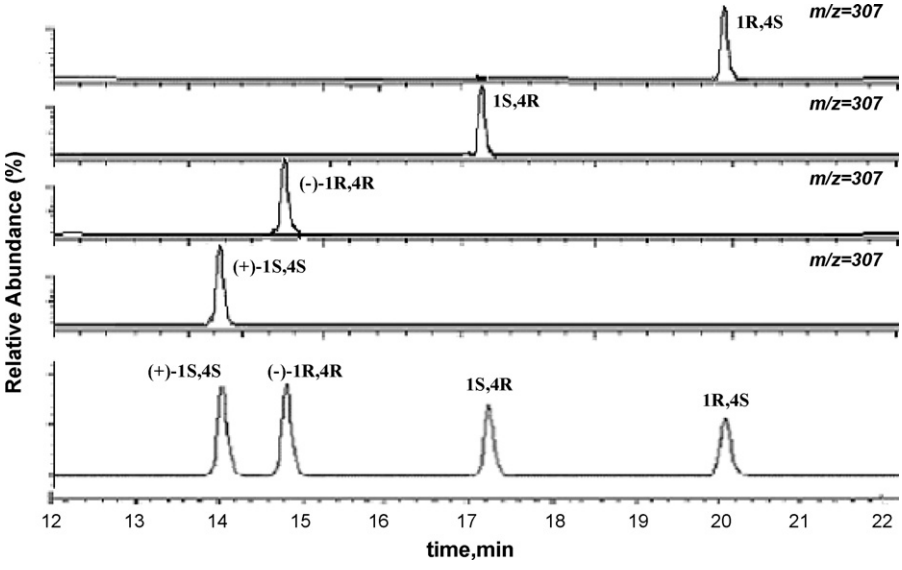

Fig. 8. Pharmacokinetic profiles for the cis enantiomers of (±)-SRT of 25 mg/kg by oral administration to rats ($n = 6$).

4. Conclusions

A LC–ESI/MS/MS column switching method for separation and determination of both enantiomers and diastereomers of SRT in rat plasma was established. LC with column switching and MS/MS detection was suitable for simple and accurate determination of SRT enantiomers and diastereomers in rat plasma. The developed method is simple and rapid, without sample pretreatment. The developed column switching technique has only three operating steps, and easy to perform. It helps in maintaining the efficiency and the lifetime of the column. The method showed adequate sensitivity, linearity, precision and accuracy and it has been successfully applied to determine enantioselectivity and the concentration–time profiles in pharmacokinetic studies.

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