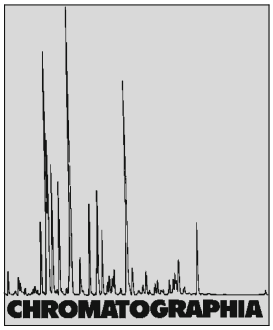
Highly Sensitive LC Method with Automated Co-Sense System and Fluorescence Detection for Determination of Sertraline in Human Plasma



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Abstract

A highly sensitive LC method with column-switching ''Co-sense'' system and fluorescence detection has been proposed for trace determination of sertraline in human plasma. A simple pre-column derivatization procedure with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole reagent was employed. Fluxetine was used as an internal standard. Under the optimum chromatographic conditions, a linear relationship with good correlation coefficient ($r = 0.9997$) was found between the peak area ratio and sertraline concentration in the range of 5–5,000 ng mL^{-1}. The limit of detection and limit of quantitation were 1.41 and 4.28 ng mL^{-1}, respectively. The intra- and inter-assay precisions were satisfactory; the relative standard deviations did not exceed 5.63%. The accuracy of the method was proved; the recovery of sertraline from the spiked human plasma was 99.76–102.62 ± 2.19–5.63%. The proposed method had high throughput as the analysis involved simple sample pre-treatment procedure and short run-time (~12 min). The results demonstrated that the method would have a great value if applied in bioavailability and pharmacokinetic studies for sertraline.

Keywords

Column Liquid chromatography
Column-switching
Fluorescence detection
Sertraline

Introduction

Sertraline (SRT); (1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine, is a potent new generation antidepressant drug. It acts as a selective serotonin reuptake inhibitor and is prescribed in the treatment of depression, obsessive–compulsive disorder, social phobia, and panic attacks [1–5]. The therapeutic index of SRT ranges from 20 to 500 ng mL^{-1} [5]. The drug is comparable to tricyclic antidepressants (TCAs) in its clinical efficacy, but it is better tolerated, and remarkably safer than TCAs in the overdose [2, 6]. Most psychiatric patients are subjected to poly-pharmacotherapy to achieve a successful control of the symptoms. Many of these antidepressants are potent inhibitors of several isozymes of cytochrome P450 enzymes, so when these drugs are given simultaneously with SRT, the risk of overdosing and adverse effects should be considered, and a determination of plasma levels becomes mandatory.

Numerous analytical methods have been developed for the quantitative determination of SRT in plasma. These methods include; gas chromatography with nitrogen-phosphorus detector [7] or tandem mass spectrometric detection [8, 9] and LC with either UV detection [10, 11] or tandem mass spectrometry [12, 13]. Only one LC with fluorescence detection using pre-column derivatisation with dansyl chloride has been reported [14]. All these methods involved a lengthy liquid–liquid or solid-phase extraction for SRT from the plasma samples prior to their analysis. Indeed, the multiple extraction procedures increase the overall analysis time (i.e. the

methods are time-consuming), and negatively affect the accuracy of the method. Therefore, these methods do not meet the needs of pharmacokinetic studies, which require an accurate and rapid feedback for the analytical information of pre-clinical and clinical specimens. Moreover, the cost and complexity of the instrumentation of LC-MS-MS limited its applications in clinical laboratories. The present work describes, for the first time, the development of a new non-extractive, highly sensitive and accurate LC method with fluorescence detection for the determination of SRT in plasma. The method involved a very simple deproteination of the plasma samples with acetonitrile followed by pre-column derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-dizole (NBD-Cl) and an automated on-line column switching using the ''Co-sense'' system. The method was successfully applied to the determination of SRT in spiked human plasma samples.

Experimental

Chromatographic System

LC apparatus consisted of a Shimadzu system (Shimadzu Corporation) equipped with two pumps (LC-10AD and LC-20AD) with FCV-10AL VP low pressure flow control valve, SCL-10A VP system controller, Rheodyne-7725 injection valve with 100 μL loop, RF-10A XL fluorescence detector. The chromatographic separations were performed on a Nucleosil C_8 analytical column (150 mm × 4.6 mm i.d., 5 μm particle diameter) manufactured by Phenomenex (USA). This chromatographic system was equipped with ''Co-sense'' system; FCV-12AH valve unit equipped with Shimpack MAY1-ODS pretreatment column of internal reversed-phase column with a hydrophilic coating (Shimadzu Corporation) which is a bio-sample analysis system capable of directly injecting the plasma samples. The column temperature was kept constant at 25 ± 2 °C. Separations were performed in isocratic mode. The mobile phase for separation (analytical mobile phase) consisted of acetonitrile—10 mmol L^{-1} sodium acetate buffer (adjusted to pH 3.5 with acetic acid)—tetrahydrofuran (40:40:20, *v*/*v*) pumped at flow rate of 1.0 mL min^{-1}. The mobile phase for sample injection (washing mobile phase) consisted of acetonitrile: 2% acetic acid (40:60) pumped for 2 min at a flow rate of 5 mL min^{-1}. The mobile phases were filtered by a Millipore vacuum filter system equipped with a 0.45 μm filter, degassed by ultrasonic bath, and by bubbling helium gas. The sample injection volume was 100 μL. The fluorescence detector was set at 470 nm as an excitation wavelength and 531 nm as an emission wavelength. The system control and data acquisition were performed by Shimadzu CLASS-VP software, version 5.032 (Shimadzu Corporation). The ratio of peak area of SRT to that of the internal standard (Fluxetine; FLX) was used for the quantitation.

Chemicals and Materials

Sertraline, as hydrochloride salt, was obtained from (Pfizer Egypt, Cairo, Egypt). Fluxetine (as HCl salt) and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) were purchased from Sigma Chemical Co., St. Louis, USA. Human plasma samples were collected from normal healthy volunteer at the King Khaled University Hospital (Riyadh, Saudi Arabia), and were stored at −20 °C until analysis. All solvents were of LC grade (Merck, Darmstadt, Germany). All other materials were of analytical grade.

Preparation of Solutions

Sertraline Standard Solution

An accurately weighed amount (25 mg) of SRT HCl was quantitatively transferred into a 25-mL calibrated flask, dissolved in 20 mL distilled water, completed to volume with the same solvent to produce a stock solution of 1 mg mL^{-1}. This stock solution was further diluted with water to obtain working standard solution of 50 μg mL^{-1}.

Fluxetine Internal Standard Solution

Accurately weighed amount of FLX HCl (25 mg) was quantitatively transferred into a 25-mL calibrated flask, dissolved in 20 mL distilled water, completed to volume with the same solvent to produce a stock solution of 1 mg mL^{-1}. This stock solution was further diluted with water to obtain working standard solution of 0.25 μg mL^{-1}.

7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole Derivatizing Reagent

Accurately weighed amount of NBD-Cl (5 mg) was quantitatively transferred into a 10-mL calibrated flask, dissolved in 5 mL acetonitrile, completed to volume with the same solvent to produce a stock solution of 0.05% (*w*/*v*). The solution was freshly prepared daily and protected from light during use.

Borate Buffer Solution

A weighed amount of 1.238 g of boric acid and 1.490 g of potassium chloride were dissolved in 100 mL distilled water. A volume of 8.0 mL of 0.2 mol L^{-1} NaOH and 80 mL of ethanol were added and the mixture was diluted to 400 mL with distilled water. The pH of the solution was adjusted to 7.9 ± 0.1 by a calibrated pH-meter (Microprocessor pH meter BT-500, Boeco, Boeckel, Hamburg, Germany).

General Derivatization Procedure

Sertraline working standard solution (400 μL) and FLX working standard solution (200 μL) were transferred into a screw-capped reaction tube using micropipettes. A volume of 4 mL borate buffer pH 7.9 and 300 μL NBD-Cl (0.05%, *w*/*v*) solutions were added. The tube was capped, swirled, and left to stand in a thermostatically controlled water bath (MLW type, Memmert, Schwabach, Germany) at 70 °C for 30 min. Then the tube was cooled rapidly, and a volume of 100 μL of HCl were added. 100 μL of the resulting solution was injected into the LC system.

Sample Preparation and Construction of Calibration Curve

The stock standard SRT solution (50 μg mL^{-1}) was serially diluted to yield concentrations of 100, 200, 500, 1,000, 5,000, 10,000, 20,000, 30,000, and 50,000 ng mL^{-1}. Aliquot (0.2 mL) of each concentration was added to a blank plasma sample (1.8 mL) to yield concentrations of 10, 20, 50, 100, 500, 1,000, 2,000, 3,000 and 5,000 ng mL^{-1}. 1.0 mL of each sample was mixed with an equal volume of acetonitrile, vortexed for 30 s, and centrifuged for 20 min at 13,000 rpm using Biofuge Pico centrifuge (Heraeus Instruments, (Germany). 400 μL portions of the supernatant layers were transferred into screw-capped tubes and manipulated as described under the general derivatization procedure. A nine-point calibration curve was constructed by plotting the peak area ratio of SRT to FLX versus SRT concentration. Analysis of calibration samples at each concentration was performed in triplicate. Slope, intercept and correlation coefficient were calculated by linear regression analysis. The calibration equation was used to calculate the concentrations of SRT in spiked plasma based on their peak-area ratios.

Operating the Co-Sense System

Figure 1 illustrates the operation of the Co-sense system at the washing and analytical phases. Before column switch; pathways of the washing and analytical mobile phases are separated. The analytical mobile phase passes from pump 1 to the analytical column without passing through the pretreatment column, and the washing mobile phase passes from another pump (pump 2) to the pretreatment column and then to the waste without passing through the analytical column. After column switch and during the analytical phase, the analytical mobile phase passes from pump 1 through the pretreatment column in the reverse direction to the analytical column.

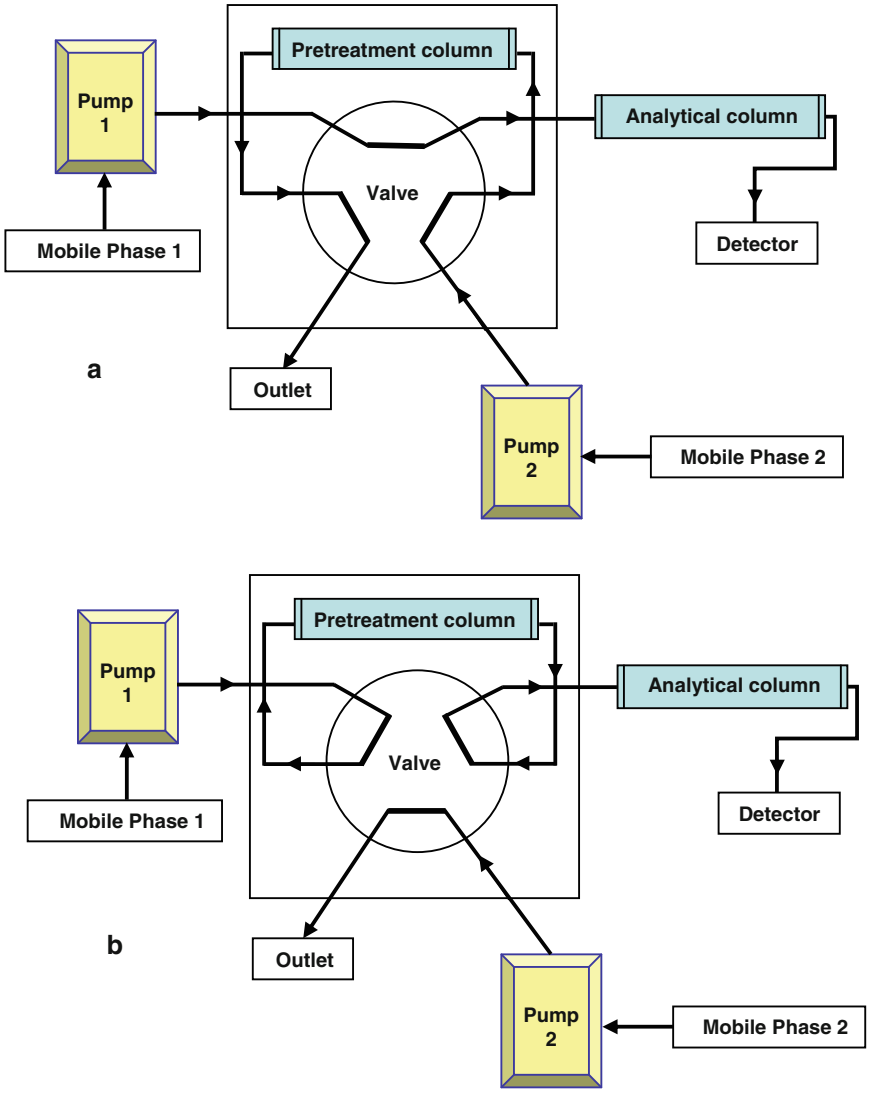

Fig. 1. **a** Flow diagram for the Co-sense system before column switching (the washing phase); pathways of the analytical and washing mobile phases are separated. **b** Flow diagram for the system after column switching (the analytical phase); the analytical mobile phase passes from pump 1 through the pretreatment column in the reverse direction to the analytical column. (Mobile phase 1 is the analytical mobile phase, while mobile phase 2 is the washing mobile phase)

Quality Control Samples

The quality control (QC) plasma samples for determination of the accuracy and precision of the method were independently prepared at low (10 ng mL^{-1}), medium (500 ng mL^{-1}), and high (5,000 ng mL^{-1}) concentrations in the same manner as the calibration standards and stored at −20 °C until use. A sample volume of 1 mL was thawed and analyzed at time intervals (0, 7 and 21 days), using FLX as internal standard (IS). The system suitability parameters were evaluated each working day.

Results and Discussion

Design and Strategy for Assay Development

Sertraline contains a weakly absorbing chromophore in its molecule, thus its LC determination in plasma using UV detection was not possible without derivatization or pretreatment of the samples. Fluorescence-based LC has been used as a sensitive alternative approach. Therefore, the present work was directed to the development of LC with fluorescence detection. Since SRT has no

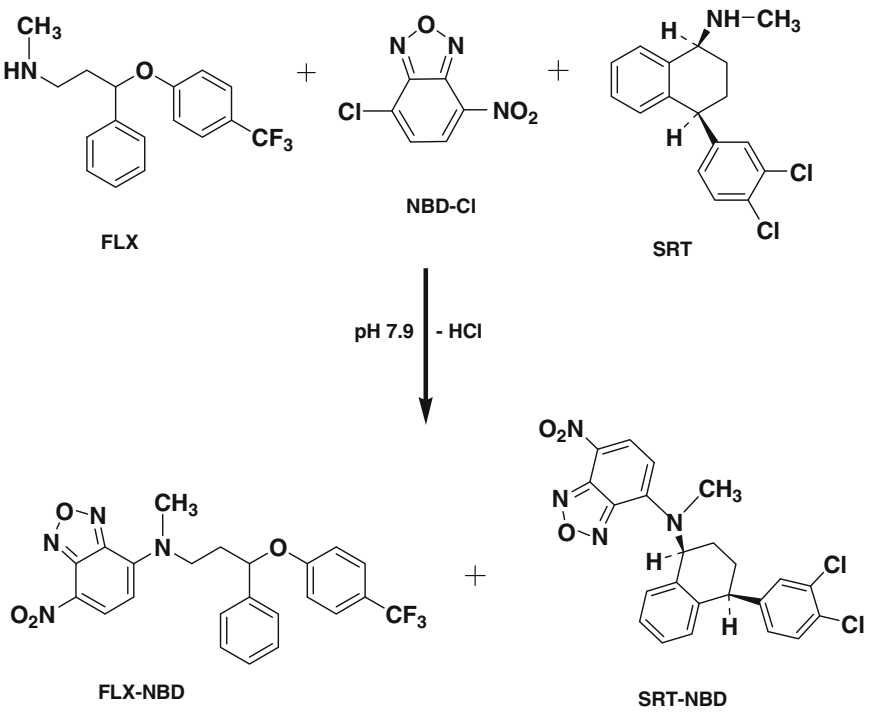

Fig. 2. Scheme for the reaction of SRT and FLX with NBD-Cl

native fluorescence, derivatization of SRT with dansyl chloride was described for its fluorescence-based LC determination [14]. However, this procedure used a lengthy liquid–liquid extraction, and it did not offer the adequate sensitivity for the pharmacokinetic study of SRT [10]. For these reasons, the present work was directed to develop a new and sensitive LC method employing non-extractive automated column switching procedure and an alternative derivatization reaction.

NBD-Cl is an activated halide derivative that has been used as a fluorogenic reagent for the determination of amino group-containing compounds [15–17]. Our preliminary experiments have showed the reactivity of SRT, via its secondary amino group, with NBD-Cl reagent. The SRT-NBD derivative was found to be fluorescent, and exhibited the highest fluorescence intensity at 531 nm after excitation at 470 nm. Therefore, the present study was devoted to adopt this reaction, for the first time, as a pre-column derivatization in the development of the method described herein. In our previous study [18], we have demonstrated the high reactivity of NBD-Cl with FLX via its secondary amino group, and the derivatized FLX has the same excitation and emission fluorescence maxima of SRT-NBD derivative. Thus, FLX was adopted as internal standard in the present study. Figure 2 shows the scheme of the reaction between SRT and FLX with NBD-Cl reagent.

Since, the proposed method was dedicated to pharmacokinetic studies, high throughput nature for the method was essential. In general, the plasma samples preparation and cleanup procedures are the limiting factor in the development of high-throughput LC method. Batch sample preparation in the 96-well format with manual or robotic liquid handling has been utilized to increase throughput [19–22]. These strategies, especially robotic liquid handling, remain problematic for plasma analyses due to the frequent formation of plasma clots or aggregates. For these reasons, this non-extraction pretreatment procedure was more efficient alternative strategy. In a previous study, Darwish et al. [23, 24] described a non-extractive pre-treatment procedure prior to LC analysis of amino compounds in plasma samples after pre-column derivatization with NBD-Cl reagent. However, the rapid elution of the massive amounts of derivatized polar biogenic amines and the remaining excess of the reagent was problematic, and required special care in establishing the optimum chromatographic conditions to achieve a good resolution of the analyte peak from the peaks of biogenic amines. Therefore, an automated on-line column-switching "Co-sense" system was employed in this study to trap the biogenic amines of the plasma and the underivatized reagents. Consequently, enhanced reliability, accuracy, and high-throughput method would be ultimately developed.

Method Development

Optimization of Derivatization Procedure

For development of a pre-column derivatization LC procedure, the optimum conditions of the derivatization reaction should be firstly established. Therefore, the effect of the NBD-Cl reagent concentration, pH of the reaction medium, type of buffer used, temperature, heating time and the diluting solvent were carefully investigated and optimized. Our experiments were initiated with studying the effect of the NBD-Cl reagent concentration. Owing to the presence of labile chloride in the chemical structure of NBD-Cl, a daily fresh solution is recommended. The maximum detector signal was obtained when the final concentration of NBD-Cl reagent in the 5-mL reaction solution was 0.003%, *w*/*v* (300 µL of 0.05%) (Fig. 3) and therefore this concentration was used in all the subsequent experiments. In order to generate the nucleophile from SRT, the reaction should be carried out in alkaline medium. The dependence of the reaction on the pH of the reaction medium was studied in the range of 7.4–9. The results indicated that maximum detector signal was obtained at pH 7.9 ± 0.1 (Fig. 3). Different buffer systems (borate, phosphate, and carbonate) of pH 7.9 were tested. The highest signals were obtained when the reaction was carried out using

borate buffer. Studies for optimization of ionic strength of borate buffer revealed that the optimum concentration was 100 mmol L^{-1}, and the complete reaction was achieved after 30 min at 70 °C. Under these conditions, significantly high fluorescence background was also observed. This was attributed to the hydrolysis of NBD-Cl to its corresponding hydroxy derivative [25]. The fluorescence of NBD-OH was found to be quenched in acid medium [26]. Therefore acidification of the reaction mixture with HCl prior to its injection into the LC system was necessary to remarkably decrease the background signal. Meanwhile, the reaction product was not affected, thus the sensitivity was ultimately increased. The amount of hydrochloric acid required for acidification was found to be 0.1 mL per 5-mL reaction volume.

Optimization of Chromatographic Conditions

Experiments were performed using Nucleosil C_8 analytical column (150 mm × 4.6 mm i.d., 5 µm particle diameter) manufactured by Phenomenex (USA). The separation of SRT and FLX (IS) was achieved adopting isocratic mode trying different compositions of the mobile phase. Several experiments have shown that the employment of an appropriate ratio of sodium acetate buffer solution in the mobile phase may improve the chromatographic peaks [27]. Different ratios of sodium acetate and acetonitrile contents in the mobile phase were tested. The results indicated that the best resolution was achieved when the content of aqueous sodium acetate: acetonitrile was 40:60 (*v*/*v*). It was found that addition of tetrahydrofuran in the mobile phase in a ratio of 20% (*v*/*v*) on the expence of acetonitrile gave better resolution and symmetric peaks. Moreover, different concentrations of sodium acetate buffer in the mobile phase; 10, 20, 30, 40, and 50 mmol L^{-1} were also tested. The results showed that the lowest concentration of 10 mmol L^{-1} sodium acetate buffer was able to improve the shape of the chromatographic peaks of both SRT and FLX and resulting in more symmetric peaks. The acidic pH of the mobile phase was beneficial to reduce the significantly high fluorescence background as mentioned above. Therefore, the sodium acetate buffer was adjusted to pH 3.5 by acetic acid. FLX was found to be quantitatively derivatized by NBD-Cl under the same conditions required for SRT, and it has shown good resolution and good chromatographic profile with the applied chromatographic conditions. Under these chromatographic conditions, the run time of the sample was 12 min, and the retention times were 9.93 ± 0.06 and 8.13 ± 0.04 min ($n = 5$) for SRT and FLX, respectively (Fig. 4).

Determination of SRT in Plasma by "Co-Sense System"

Co-sense is an automated pre-treatment column switching system for analysis of biological samples such as blood plasma and serum. This system enables the direct injection of bio-samples. The system was used as an effective tool in satisfying the requirements for high-throughput analysis in pharmacokinetic testing. The role of the pretreatment column in the method described herein was to selectively exclude the undesired derivatized products of other plasma constituents and the underivatized reagent. It is obvious that the pretreatment process is accompanied by a small increase in the overall analysis time. However, increasing the washing mobile phase flow rate to 5 mL min^{-1} in this investigation shortened the pretreatment time to 2 min. Therefore, an interval time of 2 min was selected as pretreatment time before the column switching. Use of the Co-sense system enabled us to obtain highly reliable, sensitive, and accurate results.

Method Validation

Selectivity, Linearity, Limit of Detection and Limit of Quantitation

The selectivity of the method was evaluated by carrying out blank experiments in the mobile phase and different batches of blank human plasma to identify the reagent peaks and the peaks due to the derivatized biogenic amines in the plasma samples. Typical chromatograms

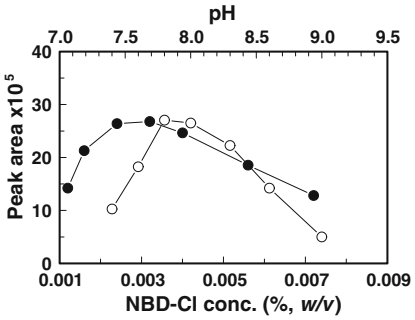

Fig. 3. Effect of NBD-Cl concentration (•) and pH (○) on the derivatization of SRT with NBD-Cl

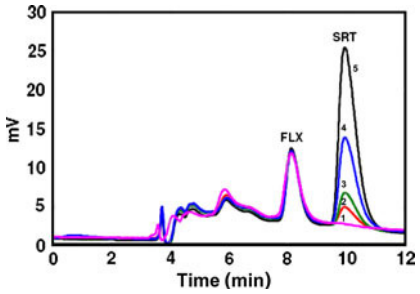

Fig. 4. Representative chromatograms. **5** = plasma sample spiked with SRT (1,000 ng mL^{-1}); **4** = plasma sample spiked with SRT (500 ng mL^{-1}); **3** = plasma sample spiked with SRT (100 ng mL^{-1}); **2** = plasma sample spiked with SRT (50 ng mL^{-1}); **1** = blank plasma. All plasma samples, including the blank, were spiked with FLX (10 ng mL^{-1}) as internal standard. mV is the detector response in millivolts

obtained from blank plasma containing 10 ng mL^{-1} of FLX as internal standard and plasma samples spiked with the same concentration of FLX and varying concentrations of SRT are shown in Fig. 4. The chromatograms showed complete separation of SRT and FLX from the reagent and endogenous plasma constituents. The chromatographic performance parameters of the SRT and FLX are presented in Table 1.

Under the above optimum conditions, linear relationship with good correlation coefficient ($r = 0.9997$, $n = 3$) was found between the peak area ratio of SRT to that of FLX (Y) versus SRT concentration (X) in the range of 5–5,000 ng mL^{-1}. The mean regression equation (mean ± SD, $n = 3$) obtained from the nine-points calibration curve was: $Y = 1.97 \times 10^{-3}$ ($\pm 3.79 \times 10^{-4}$) + 8.84×10^{-4} ($\pm 3.43 \times 10^{-5}$) X (ng mL^{-1}). The percentage rela-

Table 1. Chromatographic parameters of the proposed method

Parameter	Value
Retention time of SRT (min)	9.93 ± 0.06
Retention time of IS; FLX (min)	8.13 ± 0.04
Retention factor of SRT, k (min)	1.69
Resolution, SRT and IS peaks	1.79
Peak asymmetry at 10% peak height	2.05
Number of theoretical plates	1299
Height equivalent to theoretical plate (μm)	116
Correlation coefficient (r)	0.9997
Slope (b) ± SD	0.000884 ± 0.0000343
Intercept (a) ± SD	0.00197 ± 0.000379
LOD (ng mL^{-1})	1.41
LOQ (ng mL^{-1})	4.28
Linear range (ng mL^{-1})	5.0–5000

Table 2. Recovery of SRT from spiked human plasma

Nominal conc. (ng mL^{-1})	Measured conc. (ng mL^{-1})	Recovery (% ± RSD)
10	10.14 ± 0.34	101.40 ± 3.43
50	50.81 ± 2.89	101.61 ± 5.63
100	99.95 ± 2.19	99.95 ± 2.19
500	510.59 ± 11.81	102.12 ± 2.36
1,000	1,023.81 ± 28.60	102.38 ± 2.86
3,000	3,078.60 ± 79.22	102.62 ± 2.64
5,000	4,988.00 ± 120.03	99.76 ± 2.40

tive standard deviation (RSD%) value for the slopes of the calibration curves was 3.88% ($n = 3$). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the ICH guidelines for validation of the analytical procedures based on the standard deviation of the response and the slope of the calibration curve [28] using the formula: LOD or LOQ = $\kappa \times$ SDa/b, where $\kappa = 3.3$ for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope of the calibration curve. Calculations based on five replicate experimental injections revealed the LOD and LOQ of 1.41 and 4.28 ng mL^{-1} respectively. It is worth mentioning that the high sensitivity of the proposed method and its wide dynamic range allows the analysis of the clinical specimens without pre-concentration or dilution as the clinical therapeutic range of SRT in plasma is 20–112 ng mL^{-1} [10].

Precision

Intra-assay precision was studied at three concentration levels (10, 500, and 5,000 ng mL^{-1}) of SRT and 10 ng mL^{-1} level for FLX (IS). Six aliquots were prepared from each concentration level, and injected into the LC system. The RSD% calculated for the values of the peak areas corresponding to the IS during the study was 3.17% ($n = 18$). The RSD% calculated for the values of the retention time corresponding to the chromatographic peaks was 0.61 and 0.49% for SRT and IS, respectively. The RSD% of the determinations were 3.12, 1.41, and 1.86% for 10, 500, and 5,000 ng mL^{-1}, respectively. Inter-assay precision was carried out on five different days at the same concentration levels for spiked plasma samples freshly prepared daily. The RSD% of the determinations were 3.43, 2.36, and 2.40% for 10, 500, and 5,000 ng mL^{-1}, respectively.

Accuracy

The accuracy of the proposed method was determined by recovery studies. Plasma samples spiked with different concentrations (10, 50, 100, 500, 1,000 and 5,000 ng mL^{-1}) of SRT and 10 ng mL^{-1} for IS were subjected to the analysis by the proposed method. The values of the ratios between SRT and IS were then interpolated in the linear regression equation computed in the linearity study, to calculate the experimental concentration values. The recovery values ranged from 99.76 to 102.62 (± 2.19–5.63%) indicating accuracy of the method (Table 2).

Robustness and Ruggedness

In order to measure the extent of the method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged. The chromatographic parameters were interchanged within the range of 1–10% of the optimum recommended conditions. The studied parameters were: the pH and the composition of the mobile phase, percentage of tetrahydrofuran, concentration and pH of sodium acetate buffer, pH of the derivatization reaction mixture, and column temperature. The chromatographic profile included: retention factor (k), retention time (R_t), peak asymmetry, resolution, column efficiency, etc.) were calculated and compared to those of the system suitability (Table 1). The results revealed that the method was robust for these small changes with tetrahydrofuran content, concentration of sodium acetate in the mobile phase, and its pH in the range of 3.3–3.6. With respect to the pH of the derivatization reaction, the results did not significantly change in the range of 7.9–8.1. However, increasing the pH value above 8.3 resulted in dramatic decrease in the detector signal. The increase in the column temperature generally decreased the k values, and the column temperature has to be maintained at 25 ± 2 °C.

The ruggedness of the method was evaluated by applying the recommended analytical procedures on the same LC system (independently on different days) on the analysis of a series of SRT samples. The RSD% values of the k, R_t, and peak areas obtained from the three operators were not more than 4%.

Stability of SRT in Plasma

The stability of SRT in plasma was studied under a variety of storage and handling conditions at low, medium, and high concentration levels (10, 500, and 5,000 ng mL^{-1}, respectively). The short-

term temperature stability was assessed by analyzing three aliquots of each concentration level that were thawed at room temperature (25 ± 5 °C) and kept at this temperature for 6 h. Freeze–thaw stability (−20 °C in plasma) was checked through three cycles. Three aliquots at each concentration level were stored at −20 °C for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycles were repeated three times, and then analyzed on the third cycle. The long-term stability was determined by analyzing three aliquots of each concentration level stored at −20 °C for 21 days. The results revealed the stability of SRT under the tested conditions as the RSD% of the recovered SRT were 2.97–4.22%.

Post-preparative stability of SRT was tested by processing stock samples obtained from 100 ng mL^{-1} SRT spiked plasma samples and stored at room temperature on the bench top. The samples were assayed immediately and after 1, 3, 6, 12, and 24 from processing. RSD% calculated for recovered SRT concentration were 0.74, 1.28, 0.84, 1.11, and 2.39%, respectively. These results indicated the stability of derivatized SRT, and suitability of the system for processing a large number of samples without negative effect on the analytical results.

Conclusions

The present study described, for the first time, the development of a highly sensitive, accurate, non-extractive LC method with automated online column switching and fluorescence detection for the determination of SRT in plasma after pre-column derivatization with NBD-Cl reagent. The proposed method, being based on non-extractive sample preparation and simple derivatization reaction, is easy to perform and yielded highly reliable and accurate analytical results. The derivatized sample was directly injected into the LC system using isocratic elution mode for only 2 min as pretreatment time before online column switching and less than 12 min as analysis time after column switching. These qualities added the property of high throughput to the method. The analytical results demonstrated that the proposed method is suitable for accurate quantification of SRT in human plasma. The performance of the method makes it valuable for combined pharmacokinetic studies and bioavailability evaluation of SRT in human subjects after oral administration of therapeutic doses.

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