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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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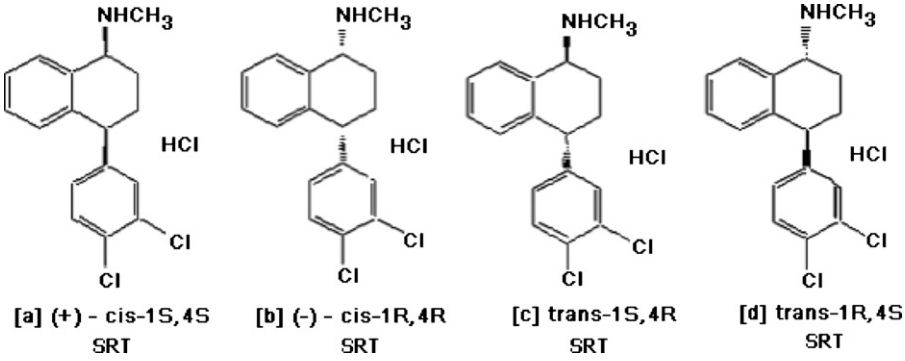

Fig. 1. Chemical structures of: (a) (+)-cis-1S,4S; (b) (−)-cis-1R,4R; (c) trans-1S,4R; (d) trans-1R,4S SRT hydrochloride.

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.