CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 42, Issue 12, December 2014
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2014, 42(12), 1717–1722.

RESEARCH PAPER

Determination of Pharmacokinetics Differences of Ammuxetine Isomers in Rat Plasma Using On-Line Solid Phase Extraction Coupled with Liquid Chromatography-Tandem Mass Spectrometry

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Abstract: An on-line solid phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to determine the novel chiral antidepressant, *S*/*R*-ammuxetine in rat plasma. The plasma sample pretreatment consisted of the following steps: protein precipitation using methanol and acetonitrile (50:50, *V*/*V*), an on-line SPE process to remove proteins and most matrices in plasma, and a separation step using a C_{18} analytical column after elution of ammuxetine isomers enriched on SPE column. Then *S*/*R*-ammuxetine were determined by tandem mass spectrometry. The SPE column was a Retain PEP Javelin column (10 mm × 2.1 mm, 5 μm), while the chromatographic separation was achieved on a ZORBAX SB-C_{18} (50 mm × 2.1 mm, 3.5 μm) analytical column. The multiple reaction monitoring mode of the positive ion was adopted in MS detection, and the precursors to the product ion transitions of *m/z* 292.1/154.0 and *m/z* 260.4/116.2 were used to measure *S*/*R*-ammuxetine and internal standard (propranolol). The method was linear over *S*/*R*-ammuxetine concentration range from 0.2 μg L^{-1} to 1000 μg L^{-1} with the correlation coefficients (*R*) of 0.9903 and 0.9951, respectively. The average intra-day precision values (RSD) were 1.2%–12% for *S*-ammuxetine and 0.4%–11.2% for *R*-ammuxetine, respectively. The average recovery values were 94.2%–101.6% for *S*-ammuxetine and 94.3%–109.4% for *R*-ammuxetine. This method exhibited a dramatically increased sensitivity compared to previous reports, thus could be used in the pharmacokinetic study of ammuxetine isomers in rat after intragastric administration.

Key Words: On-line solid phase extraction; Liquid chromatography coupled with tandem mass spectrometry; Chiral isomers; Ammuxetine; Pharmacokinetics

1 Introduction

Depression is a common and severe psychiatric disorder with high rate of morbidity, recurrence, disability and suicide, and is becoming one of the most prevalent public health problems worldwide, producing a serious burden to the patients and society[1]. Pharmacotherapy is the most effective strategy to treat depression currently. At present, the main antidepressants include tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), and serotonin and norepinephrine reuptake inhibitors (SNRIs), etc[2]. SNRIs were reported to have higher efficacy and/or faster acting rate than other commonly used antidepressants. SNRIs, the first-line antidepressants having great economic revenue, are attractive to pharmaceutical companies[3–5].

Ammuxetine, a novel SNRIs synthesized by our institute, exhibits better anti-depression effect compared with the same

Received 31 August 2014; accepted 14 October 2014
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This work was supported by the National Natural Science Foundation of China (Nos. 81102498, 81202466).

DOI: 10.1016/S1872-2040(14)60787-8

type antidepressant duloxetine[6], together with low acting dosage, good effect after oral administration and stronger effect under the same dosage. Ammuxetine and duloxetine are all chiral drugs. Chiral drugs usually have chiral discriminative properties, which display the stereoselectivity in targeting specific molecules and in the process of absorption, distribution, metabolism and excretion, and even exhibit the opposite pharmacological, toxicological and pharmacokinetic characteristics[7–9]. Therefore, the research and development of new chiral antidepressant drugs have caused more and more attention. The first-line antidepressants such as paroxetine, escitalopram and duloxetine, etc were developed in recent years. It is very important to discriminate the pharmacokinetic differences of chiral isomers of ammuxetine. However, due to the low blood concentration of *R*-ammuxetine, sensitive and specific detection methods are required in the pharmacokinetic study. Recently, an assay for the quantification of ammuxetine using the reversed-phase liquid chromatography coupled with tandem mass spectroscopy (RPLC-MS/MS) was reported[10]. However, the limit of quantification (LOQ) was about 2 μg L^{-1}, which could not meet the *in vivo* research requirements of *R*-ammuxetine. In addition, more sensitive detection methods are urgently needed for the upcoming clinical research of ammuxetine.

Recently, on-line solid-phase extraction (SPE) is a new sample pretreatment technique with simple operation, high recovery and excellent reproducibility[11]. The purification or preconcentration of the sample could be fulfilled with on-line SPE, and thus automatic, high-throughput extraction of target compounds from complex matrixs could be accomplished. In this paper, a rapid, sensitive and selective on-line SPE-MS/MS method was established to quantify the *in vivo* concentrations of *S*/*R*-ammuxetine isomers. The present method was successfully applied to the pharmacokinetic research of ammuxetine isomers in rat after intragastric administration with satisfactory results.

2 Experimental

2.1 Chemicals and Instruments

Ultimate 3000 liquid chromatography system (Thermo, USA) consisted of a DGP-3600SD dual-gradient pumps (left pump and right pump), a SRD-3600 solvent rack with integrated vacuum degasser, a WPS-3000 (RS) autosampler equipped with a 100 μL loop, a TCC-3x00 (RS) thermostatted column compartment with a two-port, six-port (2P-6P) valve, a VWD detector with Chromeleon® 7.1 software. The mass spectra were obtained using an Agilent 6410B triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA), equipped with an ESI ion source, a MSD detector and Agilent workstation. Aglient MassHunter Optimizer® was used for optimizing MS parameters like characteristic ion pair, collision energy and fragmentor voltage. Vortex mixers were purchased from Genie® (Vortex-Genie 2, Scientific Industries, USA). CentriVap centrifuge vacuum concentrator was supplied by Labconco® (USA).

S-ammuxetine (purity > 98%) and *R*-ammuxetine (purity > 98%) were synthesized by Beijing Institute of Pharmacology and Toxicology. Propranolol (purity ≥ 99%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). Ammonium formate and formic acid were supplied by Sinopharm (Shanghai, China). Deionised water, purified with a MilliQ system (Millipore, Billerica, MA, USA), was used throughout the study. Acetonitrile and methanol were of HPLC grade and purchased from Sigma-Aldrich (Milwaukee, WI, USA).

2.2 Experimental animal

Male Sprague-Dawley rats (0.18–0.22 kg) were supplied by Beijing Vital River Laboratory Animal Technology Company, Beijing, China. The animals were acclimatized under standard condition: room temperature of (25 ± 2) °C and relative humidity of 40%–60% under natural light/dark conditions for one week, and were given food and water ad libitum prior to the experiment.

2.3 HPLC-MS/MS conditions

The chromatographic separations were performed on a Thermo Retain PEP Javelin SPE column (10 mm × 2.1 mm, on-line SPE) and an Agilent ZORBAX SB-C18 column (50 mm × 2.1 mm, 3.5 μm, analysis column). The mobile phase was acetonitrile-water (containing 5 mM ammonium formate) (20:80, *V*/*V*) for the sample loading and flushing of SPE column, acetonitrile-water (5:95, *V*/*V*) for the regeneration of SPE column. ZORBAX SB-C_{18} column was used as analytical column, and the mobile phase was acetonitrile-water (containing 0.1% formic acid) (40:60, *V*/*V*). The column temperature was 35 °C, and the sample cell was kept at 4 °C. The flow diagram of on-line SPE is shown Fig.1 and the gradient elution procedure is depicted in Table 1.

Equipped with an ESI ion source, Agilent Agilent 6410B triple quadrupole mass spectrometer was operated in positive ion mode under the following conditions: ion spray voltage at 4000 V; nebulizer gas pressure at 40 psi, nitrogen as drying gas (9 L min^{-1}, 350 °C), under multiple reaction monitoring (MRM) mode. The detected ion pairs of ammuxetine and propranolol respectively were *m/z* 292.1/154.0 (collision energy of 5 eV, fragmentation voltage of 80 V) and m/z 260.4/116.2 (collision energy of 16 eV, fragmentation voltage of 112 V). Scan time was set at 200 ms.

2.4 Pretreatment of the plasma sample