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Selective microemulsion liquid chromatography analysis of dopamine receptor antagonist LE300 and its N-methyl metabolite in mouse sera by using a monolithic silica column

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ABSTRACT

A highly selective, sensitive, and rapid microemulsion liquid chromatography (MELC) method was developed and validated for the simultaneous determination of a novel type of dopamine receptor antagonist LE300 and its N-methyl metabolite in mouse sera. LE300, its N-methyl metabolite, and pindolol (an internal standard) were detected using excitation and emission wavelengths of 275 and 340 nm, respectively. HPLC analysis by using a monolithic column was performed by directly injecting the sample after appropriate dilution with the microemulsion mobile phase. The chromatographic behaviour of these compounds was studied to demonstrate their chromatographic efficiency, retention, and peak symmetry. The MELC method was validated for its specificity, linearity, accuracy, precision, robustness and stability. An experimental design was used during validation to evaluate method robustness. The calibration curves in serum showed excellent linearity (r = 0.997) over concentrations ranging from 10 to 400 ng mL^{-1} for LE300 and 15 to 500 ng mL^{-1} for its N-methyl metabolite. The mean relative standard deviation (RSD) of the results of inter- and intra-day precision and accuracy of LE300 and its N-methyl metabolite were ≤5%. The overall recoveries of LE300 and its N-methyl metabolite from mouse sera were in the range 97.9–101.5% with %RSD ranging from 0.98% to 3.63%, which were in line with ICH guidelines. The assay was successfully applied in a pharmacokinetic study.

1. Introduction

The compound 7-methyl-6,7,8,9,14,15-hexahydro-5 H-benz[d]indolo[2,3-g]azecine (LE300; Fig. 1) is an azecine-type dopamine (D) receptor antagonist and represents a novel class of dopamine receptor ligands [1,2]. Azecine-type antagonists have high affinities for dopamine receptors and display unique selectivity profiles against the D_1-like receptors [3]. Dopamine is a key neurotransmitter in the brain, where it controls numerous physiological functions, including locomotion, behaviour, emotion, cognition, learning, and motivation. Dysfunctions in the dopaminergic system have been linked with several neuropsychiatric disorders [4]. Method validation is a necessary process to demonstrate that an analytical method is suitable for its intended use, thus, that it can offer accurate, precise and reproducible results. These reliable results are essential for bioavailability, bioequivalence, pharmacokinetic, pharmacodynamics or toxicological studies where analytes must be quantified in biological matrices such as urine or plasma [5–7]. When a method is implemented for the first time or a new drug or metabolite is included a full validation is mandatory. According to FDA, a full validation should study all the fundamental parameters including accuracy, precision, selectivity, calibration curve, sensitivity, reproducibility and stability [8]. Microemulsion liquid chromatography (MELC) is a type of reversed-phase liquid chromatography by using microemulsions as the mobile phase, which has been successfully applied in the separation of pharmaceutical compounds [9]. Microemulsions possess a unique mobile phase property in that they can solubilise both polar and non-polar substances, because of emulsions formed between oils and the aqueous phase [10]. A search of the scientific literature indicates that no analytical method has been published concerning the analysis of LE300 and/or its N-methyl metabolite. Our microemulsion mobile phase approach enabled the determination of LE300 and its N-methyl

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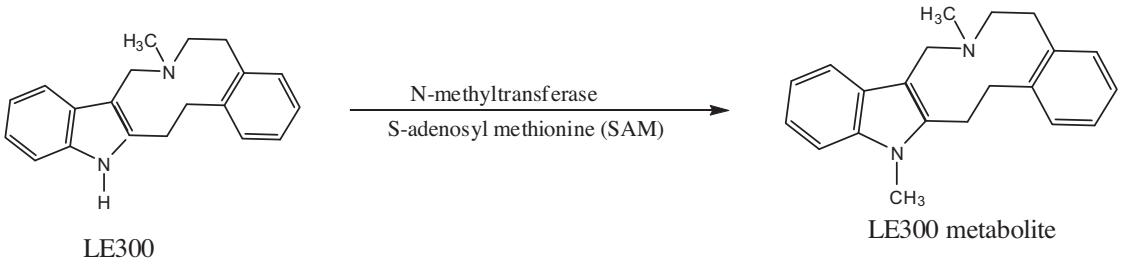

Fig. 1. Metabolic pathway of LE300.

metabolite in spiked and biological mouse serum samples. No initial extraction step is needed because the microemulsion dissolves amino acids present in mouse sera, which thereby decreases the analysis time and reduces hazards associated with the use of organic solvents. This study, for the first time, describes the development and validation of an MELC method for the simultaneous determination of potential metabolic LE300 products in mouse sera, by using a monolithic silica column. The analytical method was developed in accordance with criteria recommended in the ICH guidelines.

2. Experimental

2.1. Materials

Standard LE300 and pindolol (internal standard) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The LE300 N-methyl metabolite was kindly provided by Dr. J. Lehmann (Institut fur Pharmazie, Universitat Jena, Jena, Germany). Sodium dodecyl sulphate was obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade diisopropyl ether and 2-propanol were obtained from Thermo Fisher Scientific (Fairlawn, NJ, USA). Potassium dihydrogen phosphate was obtained from Probus (Badalona, Barcelona, Spain). Phosphoric acid (85.0%) was purchased from BDH Chemicals (Poole, UK). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Adult male Swiss albino mice were provided by the Experimental Animal Care Centre at the college of Pharmacy, KSU.

2.2. Instrumentation and chromatographic conditions

Experiments were performed on a Waters Corporation System (Milford, MA, USA), equipped with a 1500 series HPLC pump, a dual wavelength fluorescence detector (2475), and an autosampler (2707). Data integration was performed using Empower Pro Chromatography Manager Software (Waters Corporation). All solutions were filtered through a 0.22-μm Millex filter (EMD Millipore, Milford, MA, USA) and degassed by ultrasonication (Tecnal, São Paulo, Brazil). Chromatographic separation was achieved on a reversed-phase RP-18e Chromolith Performance column (100 mm × 4.6 mm; Merck, Darmstadt, Germany). Samples were chromatographed using liquid phase flow rates of 2.0 mL min^{-1} and injection volumes of 20 μL. Chromatograms were monitored by fluorescence detection, using 275 and 340 nm as excitation and emission wavelengths, respectively. All chromatographic experiments were conducted at ambient temperature. The microemulsion mobile phase was prepared by mixing diisopropyl ether (1.0%, w/v), 2-propanol (13.0%, w/v), and sodium dodecyl sulphate (4.0%, w/v) with potassium dihydrogen phosphate (50 mM). This mixture was sonicated for 15 min to aid dissolution. The pH of the resulting optically transparent microemulsion was adjusted to 3.5 with 85% orthophosphoric acid and was filtered through a 0.45-μm Millex filter (Milford, MA, USA). The microemulsion mobile phase solution was stable for 3 months.

2.3. Preparation of stock and standard solutions

Primary stock solutions of LE300 and its N-methyl metabolite were prepared for use as standards and quality controls (QC) by dissolving reference standards in methanol at concentrations of 1.0 mg mL^{-1}. An internal standard stock solution was prepared by dissolving pindolol in methanol at a concentration of 1.0 mg mL^{-1}. Working standard solutions of LE300 and its N-methyl metabolite were prepared in deionized water to get the working solution of 4.0 μg mL^{-1}. All solutions were found to be stable for 1 month when stored at −4 °C. Appropriate dilutions of the individual working solutions of LE300 and its N-methyl metabolite were made and used for constructing the calibration curves and spiking serum. All solutions contained the internal standard at a concentration of 10 μg mL^{-1}.

2.4. Preparation of serum quality control samples

The quality control (QC) samples at five concentration levels, i.e. 25, 45, 100, 250, 350 ng mL^{-1} for LE300 and 30, 60, 120, 250, 400 ng mL^{-1} for its N-methyl metabolite were prepared by spiking the drug-free serum with appropriate volumes of individual LE300 and its N-methyl metabolite and were stored frozen until analysis. Before spiking, the drug free serum was tested to make sure that there was no endogenous interference at retention time of LE300 and its N-methyl metabolite and the internal standard. The QC samples were extracted with the calibration standards and the percentage biological content of the stored QC samples was found in the accepted range (98–101.5%).

2.5. Assay method

A mouse sera sample (50 μL) was placed in 1.5 mL Eppendrof tubes, and accurately measured aliquots of the individual working standard LE300 and its N-methyl metabolite solutions (4.0 μg mL^{-1}) were added. Then 10 μL of the internal standard solution (1.0 mg mL^{-1}) was added to each tube and diluted to 500 μL with deionized water and mixed well to give final concentrations of 25, 45, 100, 250, 350 ng mL^{-1} for LE300 and 30, 60, 120, 250, 400 ng mL^{-1} for its N-methyl metabolite, respectively. The mixture was treated with 500 μL microemulsion mobile phase and vortexed vigorously for 60 s and 20 μL was injected into HPLC system in triplicate runs. Blank mice sera samples were processed in the same manner using deionized water instead of LE300 and its N-methyl metabolite.

2.6. Selectivity

The selectivity of the assay was checked by analyzing six independent blank mouse sera samples. The chromatograms of