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A simple and accurate liquid chromatography–tandem mass spectrometry method for quantification of zonisamide in plasma and its application to a pharmacokinetic study

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ABSTRACT

Zonisamide (ZNM) is an antiepileptic drug that is used as an adjunctive therapy in the treatment of adults with partial seizures. An LC–MS/MS method for quantification of ZNM in human and rabbit plasma using $^{2}H_{4}$,^{15}N-Zonisamide as an internal standard (IS) has been developed and validated. The drug and IS were extracted by ether and analyzed on Symmetry® C_{18} column. Quantitation was achieved using ESI-interface employing MRM mode. The method was validated over the concentration range of 0.5–50 μg/mL and 0.5–30 μg/mL ($r^2 > 0.99$) in human and rabbit plasma samples, respectively. Intra- and inter-run precision of ZNM assay in human and rabbit plasma samples ranged from 0.8 to 8.5% with accuracy (bias) varied from −11.3 to 14.4% indicating good precision and accuracy. Stability of ZNM in human and rabbit plasma samples at various conditions showed that the drug was stable under the studied conditions. Analytical recoveries of ZNM and IS from spiked human and rabbit plasma samples were in the range of 70.8–77.3% and 85.6–110.4%, respectively. Matrix effect study showed a lack of matrix effect on mass ions of ZNM and IS. The developed method was successfully applied for a pharmacokinetic study by measuring ZNM in rabbit plasma samples. Moreover, the method is routinely utilized for TDM of ZNM.

1. Introduction

Zonisamide (ZNM) is a 1,2-benzisoxazole-3-methanesulfonamide, structurally unrelated to other antiepileptic drugs (AEDs) in clinical practice. It is used as an adjunctive therapy in the treatment of adults with refractory partial seizures [1]. Following oral administration, ZNM is almost completely absorbed reaching maximal plasma concentrations within 2–5 h [2]. It is extensively metabolized by acetylation and further conjugation with glucuronic acid with only 15–30% of the parent drug is excreted unchanged in the urine [3–5]. Although ZNM is not more than 60% plasma protein bound, it is extensively bound to erythrocytes [6,7].

Several chromatographic methods have been reported for quantification of ZNM in biological fluids. These involve gas chromatographic (GC) assays [8,9], liquid chromatographic (HPLC) assays [10–14] and LC–MS assays [15–18]. The GC methods however, are tedious and time-consuming. The reported HPLC assays suffer from long analytical run times [11], large sample volumes [11,13] or inadequate lower limit of quantification (LLOQ) [10,11,13,14]. On the other hand, LC–MS is considered a gold standard technique for drug analysis in biological fluids. However, the reported methods encountered several disadvantages such as employing external standard methods (no IS was used) [16], the use of inappropriate internal standards [15,17], laborious sample pre-treatment procedures such as solid phase extraction (SPE) [17], inappropriate validation procedures, employing only one quality control (QC) sample [16] or describing incomplete validation parameters [18], lack of specificity due to utilization of selected ion monitoring (SIM) rather than multiple reaction monitoring (MRM) modes [17] or inadequate LLOQ [15]. In addition, some of the reported methods could presumably be appropriate for therapeutic drug monitoring (TDM) and not for pharmacokinetic applications [16,18].

The objective of the present study was to develop and validate a rapid, selective, accurate and reproducible electrospray LC–MS/MS method for quantification of ZNM in human and rabbit plasma samples. In addition, the developed method was aimed to be employed in the analysis of ZNM plasma samples for a pharmacokinetic study

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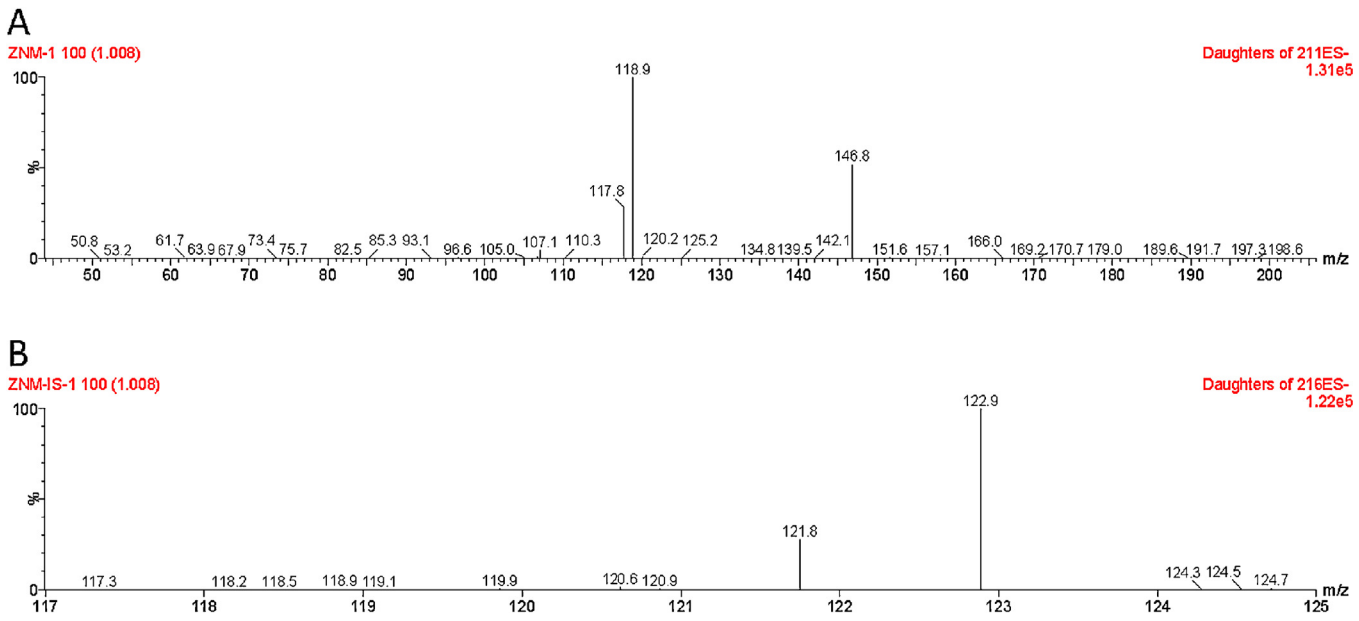

Fig. 1. Full scan mass spectra of precursor ions of (A) ZNM and (B) ZNM–IS (IS).

involving an oral administration of ZNM at a dose of 10 mg/kg to rabbits as well as routine TDM of ZNM in plasma samples of epileptic patients on the drug therapy.

2. Materials and methods

2.1. Chemicals and reagents

Zonisamide was purchased from Sigma–Aldrich (St. Louis, MO, USA) and the internal standard (IS), [$^2H_4$,^{15}N]-Zonisamide, was purchased from Alsachim Co. (Strasbourg, France). Water was purified using a Milli-Q water device (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade and solvents were of HPLC grade.

2.2. Instrumentation

A liquid chromatographic system, Alliance 2695, consisted of a solvent delivery system, and an autosampler (Waters Assoc., Milford, MA, USA) was used. Chromatographic separation of the analytes was achieved on Symmetry® C_{18} column (5 μm, 3.9 mm × 50 mm) equipped with a pre-column filter of the same packing material. The mobile phase consisted of acetonitrile-0.1% triethylamine (80:20, v/v; pH = 9.9) and delivered at a flow rate of 0.2 mL/min to a negative electrospray ionization interface (ESI-) of triple quadrupole mass spectrometer (Quattro LC, Micromass, Manchester, UK). Tuning parameters of MS were optimized by direct infusion of solutions of ZNM and IS in the mobile phase into the ionization probe at a flow rate of 10 μL/min using Hamilton syringe. The ion source and desolvation temperatures were set at 150 °C and 350 °C, respectively. The capillary voltage was adjusted at 3.18 kV, cone voltage at 20 V, collision energy at 12 eV and collision gas pressure at <1.0e^{-4} mbar. The MRM transitions at *m/z* 211 > 118.8 and 216 > 122.8 were selected for quantification of ZNM and IS, respectively. Data acquisition, handling, and system control were performed by MassLynx Software (Version 4.1, Micromass, Manchester, UK).

2.3. Preparation of calibration standards and quality control samples

Stock solutions of ZNM and the internal standard ([$^2H_4$,^{15}N]-Zonisamide) were prepared by dissolving the compounds in methanol to yield 1.0 mg/mL solutions. Aliquots of ZNM and the IS stock solutions were further diluted with methanol to yield the working standard solutions of 500 μg/mL and 100 μg/mL, respectively. The calibration standards of ZNM at concentrations of 0.5, 1.0, 5, 10, 15, 20, 30, and 50 μg/mL and 0.5, 1.0, 5, 10, 15, 20, 30 μg/mL were prepared by spiking drug-free human or rabbit plasma with ZNM standard solution, respectively. Similarly, quality control (QC) samples were prepared by spiking drug-free human plasma, at concentrations of 1.5, 12, 25, and 40 μg/mL or drug-free rabbit plasma at concentrations of 1.5, 12, and 25 μg/mL, with ZNM. The spiked plasma samples were aliquoted (200 μL) into Eppendorf polypropylene tubes and kept frozen at −80 °C pending analysis.

2.4. Sample preparation

Prior to assay, frozen human or rabbit plasma samples, including calibrators or QC samples, were thawed at ambient temperature. A 100 μL aliquot of each plasma sample was transferred to a 1.5 mL Eppendorf tube and then 20 μL of IS (100 μg/mL) was added and vortex-mixed for 30 sec. To each tube, 20 μL of ammonium acetate (1.0 mM) and 1.0 mL of diethylether were added and vortex-mixed for 30 sec. The tube was centrifuged at 9000 × *g* for 10 min, the organic layer was separated and evaporated under stream of purified N_2 gas and then reconstituted with 150 μL of mobile phase. A 10 μL of this sample was then injected into the LC–MS system for analysis.

2.5. Method validation

The proposed assay method was validated for linearity, accuracy, precision, selectivity, stability, and matrix effect according to the standard guidelines [19,20].