BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **20**: 365–368 (2006)
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.571

ORIGINAL RESEARCH

Rapid liquid chromatography–tandem mass spectrometry method for quantification of ziprasidone in human plasma

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Received 23 June 2005; accepted 20 July 2005

ABSTRACT: A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the determination of ziprasidone (ZIP) in human plasma was developed. ZIP and *N*-methyl ziprasidone as internal standard (IS) were extracted from alkalinized plasma using *tert*- butyl methyl ether. Separation was performed isocratically on a C8 column with 90% acetonitrile containing 2 mmol/L ammonium acetate as a mobile phase with a total run time of 2.5 min. MS/MS transitions of m/z 413 → 194 and m/z 427 → 177 of the analyte and internal standard were used for quantification. Confirmatory ions of m/z 413 → 177 and m/z 427 → 180 were collected as well. The calibration curve based on peak-area ratio was linear up to at least 200 ng/mL with a detection limit of 0.1 ng/mL. The method showed satisfactory reproducibility with a coefficient of variation of less than 5%. The method was successfully applied to the analysis of ZIP in spiked human plasma. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: ziprasidone; therapeutic drug monitoring; LC-MS/MS

INTRODUCTION

Ziprasidone (ZIP, Fig. 1) is an antipsychotic agent for the treatment of schzophrenia owing to its combined antagonistic activity on dopamine and serotonin receptors (Seeger *et al.*, 1995; Zorn *et al.*, 1995; Gunasekara *et al.*, 2000a,b; Clay and Cooper, 2002). Pharmacokinetic studies demonstrated that this drug has a 59% oral bioavailability in humans with an elimination half-life of 4 h (Miceli *et al.*, 1994). This compound is metabolized in the human liver microsomes by the CYP isoform 3A4, as indicated by *in vitro* studies (Prakash *et al.*, 2000).

Determination of ZIP in biological samples has received limited attention. Methods used include high-performance liquid chromatography (HPLC) with ultraviolet detection (Janiszewski *et al.*, 1995; Sachse *et al.*, 2005), HPLC with fluorescence detection (Suckow *et al.*, 2004), and by mass spectrometry (MS; Janiszewski *et al.*, 1998). Recently, we reported a stability-indicating HPLC method for ZIP and applied it to the determination of this compound in bulk powder and in pharmaceutical formulations (El-Sherif *et al.*, 2004).

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Abbreviations used: MRM, multiple reaction monitoring; ZIP, ziprasidone.

Published online 15 September 2005

In this paper, we describe a validated method for the determination of ZIP in human plasma based on liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). This method represents an attractive alternative for existing methods owing to its simplicity, sensitivity, selectivity and short turn-around time. The method was applied for analysis of ZIP in spiked human plasma samples.

EXPERIMENTAL

Materials and chemicals. ZIP and *N*-methyl-ziprasidone as an internal standard (IS) were kindly supplied by Pfizer Egypt. HPLC-grade acetonitrile, HPLC-grade methanol and ammonium acetate were purchased from Fisher Scientific (Fairlawn, NJ, USA), whereas *tert*-butyl methyl ether was supplied by Fluka Chemie AG, Switzerland. Boric acid was purchased from BDH Chemicals Ltd (Poole, England). Sodium hydroxide was obtained from Sigma (St Louis, MO, USA). Control human plasma was supplied by the blood bank of King Fang Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. Water was prepared by reverse osmosis and further purified by passing through a Milli-Q System (Millipore, Bedford, MA, USA).

Stock solutions of 100 µg/mL ZIP and IS were prepared in methanol. These solutions were kept at 4°C in the dark and were stable for at least one month.

Sample pretreatment. To 1 mL of plasma in a 13 × 100 mm screw-capped borosilicate glass test tube (Fisher Scientific), 10 µL of IS were added to give a concentration of 25 ng/mL

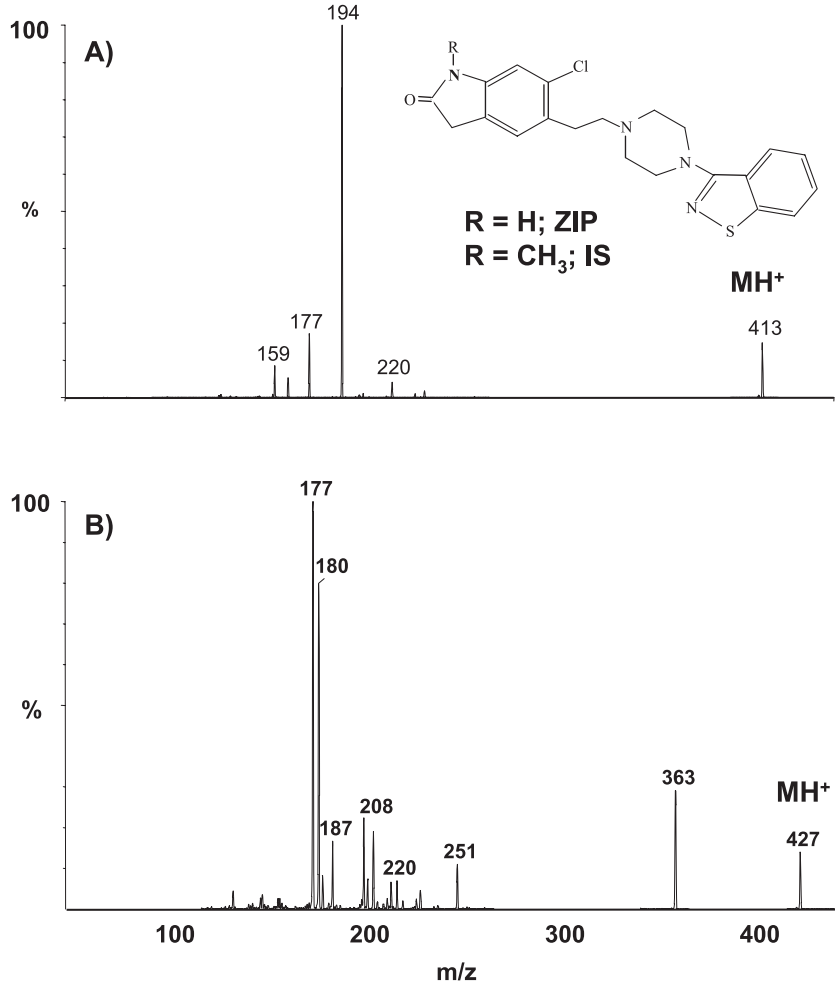

Figure 1. The product ion spectra obtained by ESI-MS/MS analysis for the MH^+ ions for ZIP at m/z 413 (A) and for IS at m/z 427 (B).

followed by 100 µL of sodium borate buffer (0.4 mol/L, pH 10.2) and 2 mL of *tert*-butyl methyl ether. The mixture was vortex-mixed for 2 min and centrifuged at 3000 rpm for 5 min. After collecting and evaporating the organic layer under a gentle stream of nitrogen gas at 45°C, the residue was reconstituted in 200 µL of 90% acetonitrile containing 0.02% formic acid. Ten microliters were injected onto the LC-MS/MS system.

Chromatographic and tandem mass spectrometric systems. The LC system used consisted of a Waters Alliance 2795 separations module (Waters, Milford, MA, USA) for solvent delivery and sample introduction. A Micromass Quattro micro API bench-top triple quadrupole mass spectrometer (Micromass, Manchester, UK), interfaced with a Z-spray electrospray ionization (ESI) source was used as a detector. Separations were performed on a 2.1 × 150 mm C_8 column packed with 5 µm particles (Symmetry, Waters) connected to the outlet of a 2.1 × 10 mm, 3.5 µm, guard column (Symmetry). The mobile phase consisted of 2 mmol/L ammonium acetate in acetonitrile:water (9:1 v/v) and delivered at a flow rate of 0.3 mL/min at room temperature. MassLynx software (version 4.0, Micromass) running under Microsoft Windows XP professional environment was used to control the instruments and data acquisition.

The ESI source was operated in the positive-ion mode at a capillary voltage of 3.3 kV, a cone voltage of 30 V and a collision energy of 26 eV using argon as collision gas. Nitrogen was used as the nebulizing and desolvation gas. The ion source and the desolvation temperature were maintained at 125 and 350°C, respectively. ZIP and the IS were detected by multiple reaction monitoring (MRM) at the following transitions of mass to charge (m/z): 413 → 194 and 413 → 177 for ZIP and 427 → 177 and 427 → 180 for the IS. The resolution of both MS1 and MS2 was maintained at a level that yielded a 10% valley between adjacent ions and 0.75 atomic mass unit (amu) peak widths at half heights.

Method validation. To establish the linear range, control plasma spiked with ZIP at the following concentrations was used: 0, 0.5, 1, 2, 5, 20, 50, 100, 150 and 200 ng/mL. These samples were kept at 4°C except during use. The calibration curve was constructed by plotting the peak area of ZIP to that of IS against the concentration in ng/mL.

The within- and between-day variations of ZIP determination were evaluated by repeatedly analyzing plasma samples

spiked with ZIP at concentrations of 5 and 100 ng/mL over a period of one week.

RESULTS AND DISCUSSION

For therapeutic drug monitoring purposes of ZIP, the analytical method should be sensitive and selective with a rapid turn-around time between samples. UV, fluorescence and mass spectrometric detection that have been employed previously are inferior to LC-MS/MS in terms of the three parameters mentioned above. The use of a precursor to product ion transitions in MRM renders the latter a highly selective technique that typically requires a minimal or even no chromatographic separation. To the best of our knowledge, LC-MS/MS has never been reported for the determination of ZIP in human plasma; therefore, we investigated its utilization and applied the developed method to spiked human plasma samples.

MS and MS/MS studies

The positive-ion ESI-MS and ESI-MS/MS were similar to what we reported previously (El-Sherif *et al.*, 2004). In brief, ZIP and IS showed protonated molecular ions at *m*/*z* of 413 and 427, respectively, with a characteristic envelope for the presence of a single chlorine atom on the molecule. Upon collision-induced dissociation (CID), the *m*/*z* of 413 gave a major fragment at *m*/*z* 194, corresponding to the neutral loss of benzisothiazole-piperazine moiety together with the following less abundant ions: 159, 177 and 220. On the other hand, CID of the *m*/*z* of 427 gave a major fragment at 177 that is common with ZIP. Moreover, it showed a fragment at *m*/*z* 208 that is 14 amu higher than the major fragment of ZIP at *m*/*z* 194 and agrees with the difference of one methyl group. Figure 1 shows the product ion spectra of ZIP and IS.

Method development

The MS/MS results described above were used to develop an LC-MS/MS method. One MRM scan function included the following transitions: *m*/*z* 413 → 194 and 413 → 177 for ZIP and *m*/*z* 427 → 177 and 427 → 180 for the IS. Several stationary and mobile phases were tested and among them a combination of a C8 column and a mobile phase of 90% acetonitrile containing 2 mmol/L ammonium acetate gave the optimum separation of the target analytes from other interfering compounds co-extracted from plasma. ZIP and IS co-eluted as a single peak with a retention time of 2 min. A complete run time of 2.5 min was employed to ensure the elution of any late eluting peaks. Figure 2 shows typical MRM chromatograms for control plasma and ZIP spiked plasma at 2 ng/mL. The use of MRM for detection resulted in a great sensitivity with confidence regarding the compound identity. The hydrophobic properties of the target compounds resulted in satisfactory retention at high percentage of organic modifier and increased signal-to-noise ratio (S/N).

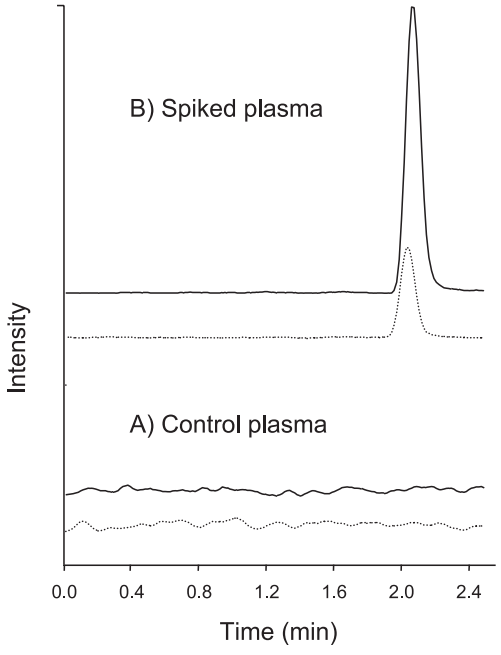

Figure 2. Typical chromatograms with multiple reaction monitoring for: (A) control plasma (lower); (B) plasma spiked with ZIP at 2 ng/mL. The solid line represents the 413 → 194 transition of ZIP and dotted line represents the 427 → 177 transition of the IS.

Method validation

Using an *N*-methyl analog of ZIP as IS, calibration curves constructed using spiked plasma were linear between 0.5 and 200 ng/mL, a range that covers the concentrations usually found in those receiving ZIP as a part of their therapy (Suckow *et al.*, 2004; Sachse *et al.*, 2005). The curve was constructed by plotting the peak-area ratio of ZIP to the IS against the concentration in plasma in ng/mL. A linear relationship was obtained up to 200 ng/mL. The average (standard deviation) of the slope and *y*-axis intercept of three regression curves obtained on three different days were 1.32 (0.08) and 2.1 (0.5), respectively, with a correlation coefficient (*r*) of ≥0.997. The limit of detection was 0.1 ng/mL (S/N > 3).

Precision expressed as coefficient of variation (CV%) was evaluated using pooled plasma spiked with ZIP at 5 and 100 ng/mL representing low and high concentrations, respectively. Within-day precision was assessed by processing five 1 mL aliquots of each concentration within the same day through the whole analytical

Table 1. Recovery and within- and between-day reproducibility of ZIP in human plasma

		Within-day (n = 5)			Between-day (n = 3)		
Compound	Concentration added (ng/mL)	Mean (ng/mL)	CV[a] (%)	Recovery[b] (%)	Mean (ng/mL)	CV (%)	Recovery (%)
ZIP	5	4.2	1.1	84.0	4.2	2.8	84.0
	100	120.9	4.9	120.9	118.8	0.3	118.8

[a] CV = coefficient of variation.
[b] Recovery (%) = 100 × found concentration/added concentration.

procedure. Alternatively, between-day precision was assessed by analyzing samples at the previously mentioned concentrations on three different days. As shown in Table 1, the CV% was less than 5%, an acceptable level taking into consideration the multiple step pretreatment and the nature of the biological matrix. The overall analytical recovery of ZIP from plasma was calculated from these samples and was in the range 84–121%.

The proposed method is an attractive alternative to the existing ones. Owing to its high throughput and short turn-around time of 2.5 min and the trend towards LC-MS/MS technology in modern clinical chemistry laboratories, the method should be useful for therapeutic drug monitoring purposes. The current method is also superior in terms of sensitivity (limit of detection of 0.1 ng/mL), and this will enable the use of smaller plasma volumes due to the fact that ZIP is usually present at high concentrations (serum median steady-state concentration 76 ng/mL; Sachse *et al.*, 2005).

In summary, we developed and validated an LC-MS/MS method for the analysis of ZIP. The method was linear over a wide dynamic range and satisfactorily sensitive and selective. The method fulfils the criteria in terms of high throughput and reproducibility to be used for therapeutic drug monitoring purposes.

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