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ORIGINAL RESEARCH

Determination of the lipophilic antipsychotic drug ziprasidone in rat plasma and brain tissue using liquid chromatography–tandem mass spectrometry

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ABSTRACT: A simple, sensitive and robust liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method with low matrix effects was developed and validated for the quantification of the lipophilic antipsychotic ziprasidone from rat plasma and brain tissue. Ziprasidone was extracted from rat plasma and brain homogenate using a single-step liquid–liquid extraction. Ziprasidone was separated on an Agilent Eclipse XDB C_8 column (150 × 2.1 mm i.d., 5 μm) column using a mobile phase of acetonitrile–0.02% ammonia in water (pH 7.20 adjusted with formic acid) using gradient elution. Ziprasidone was detected in the positive ion mode using multiple reaction monitoring. The method was validated and the specificity, linearity, lower limit of quantitation (LLOQ), precision, accuracy, recovery, matrix effects and stability were determined. The LLOQ was 0.2 ng/mL for plasma and 0.833 ng/g for brain tissue. The method was linear over the concentration range from 0.2 to 200.0 ng/mL for plasma and 0.833–833.3 ng/g for brain tissue. The correlation coefficient (R^2) values were more than 0.996 for both plasma and brain homogenate. The precision and accuracy intra-day and inter-day were better than 8.13%. The relative and absolute recovery was above 81.0% and matrix effects were lower than 5.2%. This validated method has been successfully used to quantify the rat plasma and brain tissue concentration of ziprasidone after chronic treatment. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: ziprasidone; plasma; brain tissue; LC-MS/MS; matrix effect

INTRODUCTION

Ziprasidone (ZIP, Fig. 1) is one of the more recently introduced second-generation antipsychotics (SGAs) to gain FDA approval (February 2001). As a benzisoxazole derivative, it is chemically different from the phenothiazine- and butyrophenone-type antipsychotic drugs. Oral ZIP (ziprasidone hydrochloride) is approved by the US FDA for the treatment of schizophrenia, and acute manic or mixed episodes associated with bipolar disorder (with or without psychotic features). ZIP intramuscular (ziprasidone mesylate) is FDA-approved for acute agitation in patients with schizophrenia. Oral ZIP appears efficacious, and has been shown to have some limited clinical advantages over chlorpromazine and haloperidol in ameliorating the negative symptoms of schizophrenia (Greenberg and Citrome, 2007). ZIP exhibits a potent and highly selective antagonistic activity on the D_2 and 5-HT_{2A} receptors. It also has a high affinity for the 5-HT_{1A}, 5-HT_{1D} and 5-HT_{2C} receptor subtypes that could contribute to the overall therapeutic effect (Seeger *et al.*, 1995; Schmidt *et al.*, 2001). ZIP is metabolized extensively, resulting in several metabolites which are far less pharmacologically active. Ziprasidone sulfoxide and ziprasidone sulfone are the major circulating metabolites in humans and are predominantly produced by the cytochrome P450 (CYP) 3A4 isozyme. Other metabolites produced include *N*-dealkylation, benzisothiazole cleavage and hydration of the C–N bond followed by oxidation and dealkylation (Prakash *et al.*, 1997, 2000; Wilner *et al.*, 2000; Miceli *et al.*, 2000).

One prerequisite for the therapeutic effects of ZIP is its ability to pass through the blood–brain barrier. Given that cognition is now recognized as a key factor that influences long-term functional outcome in schizophrenia, it is important to determine the concentration of ZIP in both plasma and its relationship to brain levels (the target organ for therapeutic action). Understanding this would allow one to discover if there were a strong correlation between the concentration of ZIP in the plasma and alterations in cognitive function when ZIP is given chronically. Such a correlation would allow for improved clinical monitoring of ZIP. Since

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Abbreviations used: CYP, cytochrome P450; SGA, second generation antipsychotic; ZIP, ziprasidone.

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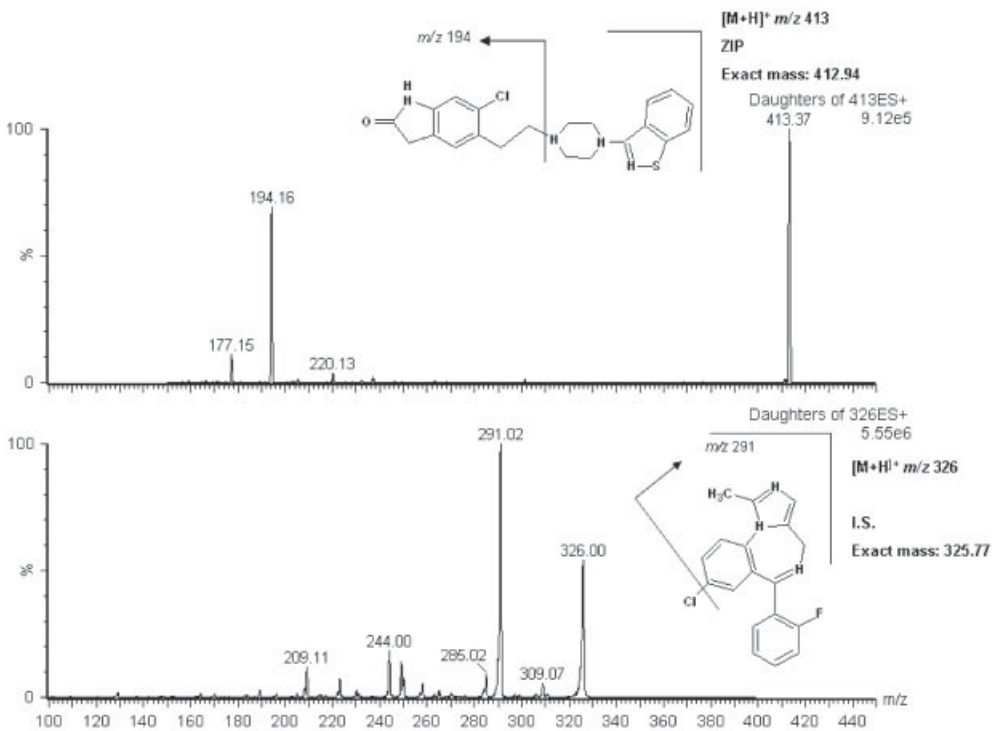

Figure 1. Product ion mass spectra of (A) ZIP, (B) IS and respective chemical structures.

ZIP is widely metabolized in the body, its concentration in plasma is low. From a bioanalytical and clinical point of view, sensitive and accurate methods are needed to determine ZIP in biological fluids for obtaining optimum therapeutic concentrations and controlling side effects. At present, the determination of ZIP in plasma has been accomplished by high-performance liquid chromatography (HPLC) with UV detection (Janiszewski *et al.*, 1995) and HPLC with fluorescence detection (Suckow *et al.*, 2004). The usefulness of liquid chromatography–electrospray–tandem mass spectrometry (LC-ESI-MS/MS) has been demonstrated for a wide range of applications in the bioanalytical, environmental and pharmaceutical fields. LC-MS methods offer several significant advantages when compared with previous methods, such as the small sample volume required, minimization of mobile phase, rapid analytical run time and improved sensitivity, selectivity and specificity (Srinivas, 2006). To date, only a few LC-MS/MS methods have been reported for the quantitation of ZIP in plasma (Janiszewski *et al.*, 1997; Al-Dirbashi *et al.*, 2006; Aravagiri *et al.*, 2007). However, these papers did not report the matrix effects for ZIP from plasma when using LC-MS methods. Recently, our lab (Zhang *et al.*, 2007a,b) described a comprehensive LC-MS/MS methods for determination of several antipsychotics including ZIP from plasma and brain tissue. However, the matix effects were high for ZIP (ion suppression: 17.1% for plasma and 16.2% for brain tissue). Matrix effects are an important issue in ESI resulting from co-eluting components, generally from the matrix, that cause variable suppression or enhancement of analyte response (King *et al.*, 2000). In these methods, liquid–liquid extraction was repeated twice to isolate ZIP from brain tissue, which was time-consuming. Owing to the lipophilic nature of ZIP, it was readily absorbed by myelin and other lipid constituents of brain homogenates. We also found that ZIP was, to some extent, irreversibly bound to the inner surface of plastic or untreated glass tubes. Hence, the recovery of ZIP from brain tissue in particular was low. Therefore, it was a challenge to improve the recovery of the hydrophobic ZIP from lipophilic brain tissue.

The purpose of this investigation was to develop and validate a simple, rapid, highly selective, sensitive and robust LC-MS/MS method for the determination of the lipophilic drug ZIP from rat plasma and brain tissue with low matrix effects.

EXPERIMENTAL

Chemicals and reagents. Ziprasidone was obtained from Pfizer Central Research (Groton, CT, USA). Midazolam (internal standard, IS) and hexamethyldisilazane were obtained from Sigma (St Louis, MO, USA). Ethyl acetate, tert-butyl methyl ether, chloroform, hexane, methyl chloride, diethyl ether, isopropyl ether, HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). The formic acid used was reagent-grade, purchased from J.T. Baker (Phillipsburg, NJ, USA). Ammonium formate, ammonium

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acetate and sodium phosphate dibasic were purchased from Sigma (St Louis, MO, USA). The deionized water used was generated from a Continental deionized water system (Natick, MA, USA).

Instrumentation. An Agilent 1100 series HPLC system, consisting of a degasser, binary pump, autosampler, and thermostatted column compartment, was used in this study (Agilent, Palo Alto, CA, USA). The mass spectrometer utilized for this work was a Quattro Micro triple-quadrupole mass spectrometer equipped with a Z-spray source and a built-in syringe pump (Waters, Manchester, UK). MS control and spectral processing were carried out using Masslynx software, version 4.0 (Waters, Beverly, MA, USA).

Liquid chromatographic and mass spectrometric conditions. The analytes were separated on an Agilent Eclipse XDB C-8 column (150 × 2.1 mm i.d., 5 μm) with a 4.0 × 2.0 mm Phenomenex Security Guard C_8 guard column. Mobile phase A consisted of 0.02% ammonia in water (adjusted to pH 7.20 using formic acid) and mobile phase B was acetonitrile. A 10 μL injection of each sample was loaded onto the column, separated and eluted using the following gradient [minutes, percentage mobile phase B, flow rate (mL/min)]: (0, 60, 0.3) (2.5, 60, 0.3) (2.6, 90, 0.6) (5.5, 90, 0.6) (5.6, 60, 0.3) (12.6, 60, 0.3). From 2.6 to 5.5 min, it was very important to use a high concentration of acetonitrile (90%) in the mobile phase to wash the column at a flow rate of 0.6 mL/min. This procedure removed the remaining brain extract residue from the column following each injection. Otherwise there was decreased response for ZIP in the next injection due to ion suppression resulting from brain extract residue from the previous injection. The column temperature was maintained at 25°C. The LC flow was introduced directly to the mass spectrometer from 1.7 to 3.5 min and diverted to waste at other times using a six-port column switching valve. The autosampler injection needle was washed with methanol to reduce carry-over after each injection. The mass spectrometer was run in positive ion ESI mode using multiple-reaction monitoring (MRM) to monitor the mass transitions. Nitrogen gas was used as the desolvation gas and was set to a flow rate of 600 L/h with a temperature of 380°C. Argon was the collision gas and the collision cell pressure was 2.4×10^{-3} mbar. For quantitation, an MRM transition from m/z 413 → 194 was performed and 413 → 177 and 413 → 220 (Fig. 1) was monitored for identification with a cone voltage of 37 V and a collision energy of 26 eV. An MRM transition from m/z 326 → 291 was performed for the IS (midazolam) with a cone voltage of 32 V and a collision energy of 30 eV. The source temperature and capillary voltage were set at 120°C and 3.20 kV, respectively.

Sample collection. ZIP chronic dosing was derived from previously published data (Terry *et al.*, 2005, 2006). Furthermore, the selected dose produced plasma concentrations that approximated therapeutic levels in humans. Male albino Wistar rats (Harlan Inc.) 2–3 months old were housed individually in a temperature-controlled room (25°C), maintained on a 12 h light–dark cycle with free access to food. Rats were treated with ZIP (12.0 mg/kg/day) orally in drinking water for periods of at least 14 days to achieve a steady-state concentration of ZIP. Rats were anesthetized with isofluorane and 3.0 mL of blood was collected via cardiac puncture to heparined tubes. The blood was centrifuged for 15 min at 2500***g*** at 8°C and the resulting plasma was frozen until analysis. The whole brains of sacrificed animals were removed and kept frozen at –70°C until analysis.

Preparation of stock, working standard and quality control solutions. Individual stock solutions of ZIP and IS (midazolam) were prepared by dissolving approximate amounts of drugs in absolute methanol to obtain final drug concentrations of 1.0 mg/mL, respectively, and were stored at –20°C. Standard solutions with concentrations of 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0, 1000.0, 2500.0 and 5000.0 ng/mL were prepared by serial dilution with acetonitrile. Precision and accuracy standards with concentrations of 5.0, 15.0, 375.0 and 3750.0 ng/mL were also prepared in the same manner. An 100.0 ng/mL IS standard solution was prepared with acetonitrile from the 1.0 mg/mL IS stock solution. The 1.0 mg/mL stock solutions were kept at –20°C when not in use and replaced every 3 months. Fresh standard solutions were prepared for each day of analysis or validation.

Preparation of calibration and QC samples. The brains were minced and homogenized in a volume of deionized water (in milliliters) equal to twice the weight (in grams) of the tissue using a Brinkmann (Westburg, NY, USA) Polytron TP 1020 homogenizer. Samples for the calibration curves and QCs were prepared by adding 10.0 μL of each standard into 250 μL of blank rat plasma or 200 μL of blank brain homogenate. This yielded calibration standard concentrations of 0.2, 0.4, 1.0, 2.0, 4.0, 10.0, 20.0, 40.0, 100.0 and 200.0 ng/mL for plasma and 0.833, 1.667, 4.165, 8.33, 16.67, 41.67, 83.33, 166.7, 416.7 and 833.3 ng/g for brain tissue. The final concentrations of QCs were 0.2, 0.6, 15.0, 150.0 ng/mL for plasma and 0.833, 2.5, 62.5, 625.0 ng/g for brain tissue. The spiked plasma and brain homogenate samples (standards and quality controls) were extracted with each analytical batch along with the unknown samples.

Sample preparation. Prior to use, all of glass extraction tubes were silanized by rinsing with 10% (v/v) hexamethyldisilazane in ethyl acetate and heated for 1 h at 150°C. To a 250 μL rat plasma or 200 μL brain homogenate sample, 10.0 μL of internal standard (100.0 ng/mL, midazolam) and 0.1 mL of 0.5 M Na_2HPO_4 (pH 10.69) for plasma or for brain homogenate were added. The samples were briefly mixed and extracted with 3 mL of isopropyl ether:methyl chloride (80:20). After centrifugation at 2000***g*** for 10 min, the upper organic layer was removed and evaporated to dryness under reduced pressure in a vacuum centrifuge. To the residue, 200 μL of acetonitrile:methanol:20 mM ammonium formate (pH 3.84 adjusted using formic acid; 48:32:20) solutions were added, ultrasonicated for 1 min, then vortexed and centrifuged at 16,000***g*** prior to LC-MS/MS analysis.

Method validation. The method was validated for linearity, recovery, matrix effect, accuracy and precision. Brain homogenate calibration curves were constructed using the peak area ratios of ZIP to that of IS, and applying a weighted ($1/x^2$) least squares linear regression analysis. Precision (expressed as percentage relative standard deviation, RSD) and

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accuracy (expressed as percentage error) were calculated for four quality control (QC) samples. Five replicates of each QC points were analyzed to determine the intra-day accuracy and precision. This process was repeated three times over three days in order to determine the inter-day accuracy and precision. Absolute and relative recoveries and matrix effects were calculated for spiked samples at 0.2, 0.6, 15.0 and 150.0 ng/mL for plasma and 0.833, 2.5, 62.5 and 625.0 ng/g for brain tissue and 4.0 ng/mL IS in plasma samples and 16.67 ng/g IS in brain tissue ($n = 5$). Absolute recovery was calculated as the peak area for ZIP and the IS in plasma or brain homogenate spiked before extraction divided by the peak area of the pure drugs in the acetonitrile:methanol:20 mM ammonium formate (pH 3.84 adjusted using formic acid; 48:32:20) solution at the same concentration. Relative recovery was calculated by dividing the peak area for ZIP and the IS spiked before extraction by the peak area for an equal concentration of the sample in the same matrix spiked after extraction. The matrix effects were calculated by dividing the response for a ZIP sample in biological matrix spiked after extraction by an equal concentration of ZIP in an acetonitrile:methanol:20 mM ammonium formate (pH 3.84 adjusted using formic acid; 48:32:20) solutions (Matuszewski *et al.*, 2003). The stability of the stock solution was determined at its storage conditions of −20°C for 3 months. ZIP was considered stable if the relative error (%RE) of the mean test responses were within 15% of appropriate controls. The bench-top stability of spiked samples stored at room temperature was evaluated for 2 h. The freeze–thaw stability was investigated by comparing the stability of samples following three freeze–thaw cycles, against freshly spiked samples. The autosampler stability was evaluated by comparing the extracted samples that were injected immediately (time 0), with the samples that were re-injected after storage in the autosampler for up to 24 h. The stability testing was performed at 0.6 and 150.0 ng/mL for plasma and 2.5 and 625.0 ng/g for brain tissue concentration levels.

RESULTS AND DISCUSSION

Method development

In order to develop and validate a highly sensitive and selective method with the designed LLOQ (0.2 ng/mL for plasma and 0.833 ng/g for brain tissue), during method development different options were evaluated to optimize the detection (MRM) parameters, chromatography and sample preparation methodology. ESI(+) MS/MS product ion mass spectra were produced by collisionally activated dissociation (CAD) of the protonated molecular ion $(M + H)^+$. The most favorable mass transition was selected and the instrument parameter settings were optimized individually for ZIP and the IS by constant infusion at 10 μL/min of a 2 μg/mL solution. The major MS/MS transitions utilized for LC-MS/MS quantitative analysis were m/z 413 → 194 for ZIP and m/z 326 → 291 for IS (midazolam).

ZIP is a highly hydrophobic drug and was found to irreversibly absorb on to the surface of plastic and untreated glass tubes. Silanized glass tubes reduced this absorption greatly. Brain samples contained substantial amount of lipid residue after evaporation of the extraction solvent to dryness. We evaluated solid phase extraction (SPE) methods using different cartridges such as the Waters Oasis HLB and Varian C_{18}. We found that the recovery of ZIP and the IS was still very low using SPE from brain tissue because of the absorption of ZIP to the SPE cartridges. Therefore liquid–liquid extraction was optimized using the extraction solvent isopropyl ether:methyl chloride (80:20), which produced the least residue and highest recovery using only a single extraction. We next optimized the gradient elution conditions. From 2.6 to 5.5 min, it was important to use a high concentration of acetonitrile (90%) in the mobile phase and to wash the column at a flow rate of 0.6 mL/min. This procedure removed the residual brain extract from the column following each run. Failure to remove this residue decreased the response of ZIP for subsequent injections due to ion suppression from brain extract residue from previous injections on the column.

Linearity and sensitivity

Table 1 shows the calibration curves for each day of validation. The curves showed good linear response ($R^2 > 0.9968$) over the range of 0.2–200.0 ng/g for plasma and 0.833–833.3 ng/g for brain tissue. Microsoft Excel or SAS JMPIN statistical software was used to generate linear regression equations for the calibration curves. A $1/x^2$-weighting scheme was used for each day of the validation and analysis for ZIP. Table 1 showed the slope, intercept and R^2 values generated from the calibration curves used in the validation study. The LLOQ, defined as the lowest concentration of analyte with accuracy within 20% and a precision <20%, was 0.2 ng/g for plasma and 0.833 ng/g for brain tissue as shown in Table 2. A signal-to-noise (S/N) > 10 at the LLOQ was observed for ZIP in biological matrices.

Table 1. Statistical data for linearity including standard deviation (SD) (linear range 0.2–200.0 ng/mL for plasma and 0.833–833.3 ng/g for brain)

Biological matrix	R^2	Slope	Intercept
Plasma	0.9984 ± 0.00090	0.28714 ± 0.01294	0.00232 ± 0.003635
Brain tissue	0.9968 ± 0.00281	0.07146 ± 0.00467	0.01065 ± 0.004806

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Table 2. The intra-day ($n = 5$) and inter-day ($n = 15$) precision (%RSD) and accuracy (%error) of the LC-MS/MS method used to quantitate ZIP in rat plasma and brain tissue

		Intra-day			Inter-day		
Matrix	Concentration added (ng/mL or ng/g)	Observed concentration (ng/mL or ng/g)	RSD (%)	Error (%)	Observed concentration (ng/mL or ng/g)	RSD (%)	Error (%)
Plasma	0.2	0.202 ± 0.009	4.68	3.70	0.204 ± 0.008	3.97	3.48
	0.6	0.606 ± 0.015	2.40	1.95	0.601 ± 0.041	6.89	5.39
	15	15.929 ± 0.398	2.50	6.19	15.789 ± 0.456	2.89	5.27
	150	144.883 ± 3.772	2.60	3.41	151.986 ± 6.056	3.98	3.60
Brain	0.833	0.833 ± 0.022	2.68	1.72	0.818 ± 0.054	6.59	5.01
	2.5	2.409 ± 0.027	1.10	3.65	2.638 ± 0.189	7.17	8.13
	62.5	63.63 ± 0.680	1.07	1.81	64.925 ± 3.835	5.91	5.31
	625	596.109 ± 27.426	4.60	4.62	596.862 ± 35.801	6.00	5.70

Precision and accuracy

Precision and accuracy measurements were acquired for the QC points. The accuracy and precision data can be seen in Table 2. The values for the intra-day precision and accuracy were better than 4.68 and 6.19%. The inter-day precision and accuracy were determined by pooling all of the validation assay ($n = 15$) QC samples. The values for the inter-day precision and accuracy were better than 7.17 and 8.13% (Table 2).

Recovery and matrix effect

ZIP and the IS are basic compounds. Therefore, extraction was influenced by the pH of the brain homogenate samples. Phosphate buffer (pH 10.69), 0.5 M, was selected because it produced the highest recovery for ZIP and the IS.

Owing to non-specific binding of lipophilic ZIP to brain constituents, it was a challenge to improve the recovery of ZIP from brain homogenate. We found that the reconstitution solution played a key role in the recovery and reduced the matrix effects from the brain homogenate residue after liquid–liquid extraction. Our laboratory (Zhang *et al.*, 2007a,b) previously described LC-MS/MS methods for the determination of several antipsychotics including ZIP from plasma and brain tissue. However, the matix effects were high (ion suppression: 17.1% for plasma and 16.2% for brain tissue) for ZIP using methanol:20 mm ammonium formate (pH 3.84 adjusted using formic acid; 70:30). Finally, we found 0.2 mL of acetonitrile: methanol:20 mM ammonium formate (pH 3.84 adjusted using formic acid; 48:32:20) as reconstitution solution and produced higher recovery (above 81.0%) and the lowest matrix effects (5.2% ion suppression). The absolute recovery, relative recovery and matrix effect data for ZIP at different concentrations in plasma or brain tissue using acetonitrile:methanol:20 mM ammonium formate (pH 3.84 adjusted using formic acid; 48:32:20) as the reconstitution solution are summarized in Table 3. The absolute recoveries ranged from 81.0 to 103.4% and the relative recoveries from 83.7 to 102.4% for ZIP and the IS from plasma or brain homogenate.

Specificity

Representative chromatograms obtained from blank biological matrices and samples spiked with the LLOQ standard (0.2 ng/mL for plasma and 0.833 ng/g for brain

Table 3. Absolute recovery, relative recovery (%), and matrix effects (mean ± SD) of ZIP and IS in rat plasma and brain tissue at different concentrations ($n = 5$) using acetonitrile:methanol:20 mM ammonium formate (pH 3.84 adjusted using formic acid; 48:32:20) as the reconstitution solution

Matrix	Concentration (ng/mL or ng/g)	Absolute recovery (%)	Relative recovery (%)	Matrix effect (%)	Type of effect
Plasma	0.2	103.4 ± 3.28	98.9 ± 3.13	102.8 ± 1.42	2.8% enhancement
	0.6	99.4 ± 2.21	97.6 ± 2.17	102.1 ± 1.95	2.1% enhancement
	15	97.3 ± 1.65	99.3 ± 1.69	98.2 ± 0.48	1.8% suppression
	150	95.8 ± 1.02	96.6 ± 1.03	98.4 ± 2.22	1.6% suppression
IS (plasma)	4.0	101.9 ± 2.48	102.4 ± 3.25	101.5 ± 2.69	1.5% enhancement
Brain	0.833	82.8 ± 4.97	87.2 ± 5.24	95.9 ± 1.75	4.1% suppression
	2.5	81.0 ± 2.58	83.7 ± 4.24	94.8 ± 0.78	5.2% suppression
	62.5	86.4 ± 2.61	90.9 ± 2.74	95.2 ± 0.92	4.8% suppression
	625.0	85.0 ± 2.29	88.5 ± 2.38	95.5 ± 2.40	4.5% suppression
IS (brain)	16.67	94.5 ± 2.30	97.3 ± 2.97	96.5 ± 1.97	3.5% suppression

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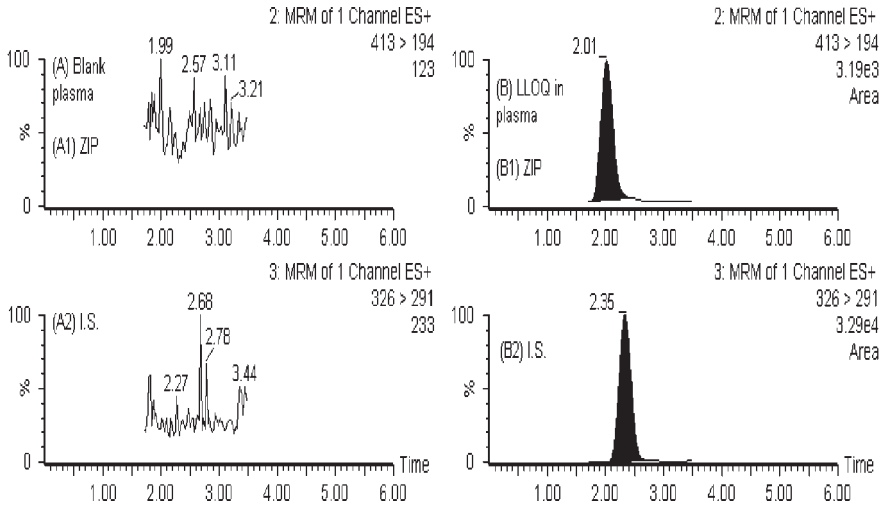

Figure 2. Representative chromatograms obtained from rat plasma: (A) blank rat plasma; (B) plasma spiked with LLOQ for ZIP at 0.2 ng/mL (B1) and IS at 4.0 ng/mL (B2).

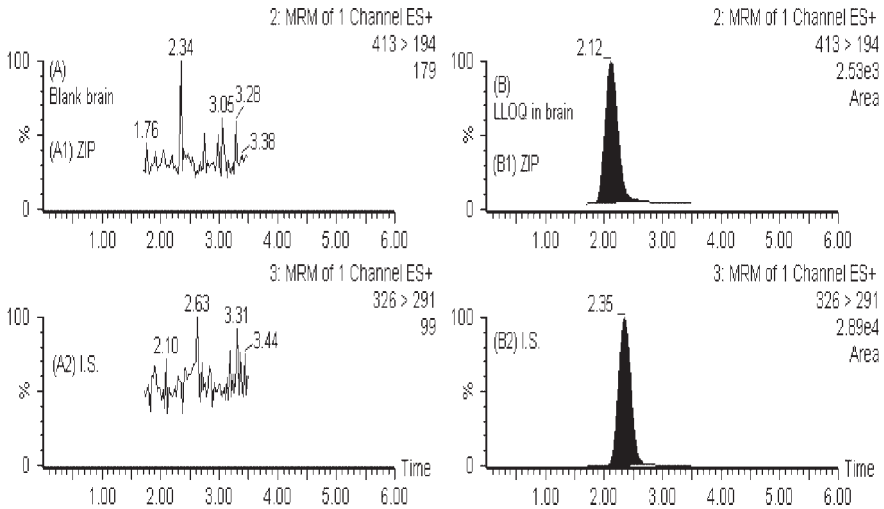

Figure 3. Representative chromatograms obtained from rat brain tissue: (A) blank rat tissue; (B) brain tissue homogenate spiked with LLOQ for ZIP at 0.833 ng/g (B1) and IS at 16.67 ng/g (B2).

homogenate) are shown in Figs 2 and 3. No interfering peaks from endogenous compounds were observed at the retention times of ZIP or IS in blank plasma or brain homogenate. Three MRM transitions have been used: one transition (413 → 194) for quantification and two transitions (413 → 177 and 413 → 220) for identification. Table 4 contains the data for the first identification MRM transition (413 → 177); the peak area ratio (413 → 194/413 → 177) in plasma or brain tissue was from 7.16 to 7.29 over the calibration curve range. The peak area ratio (413 → 194/413 → 177) from real samples was 7.18 for plasma and 7.25 for brain tissue, respectively. Also, for the second identification MRM transition (413 → 220), the peak area ratio (413 → 194/413 → 220) in plasma or brain tissue was from 23.90 to 25.03 over the calibration curve range. The peak area ratio (413 → 194/413 → 220) from real samples was 24.26 for plasma and 24.56 for brain tissue, respectively. These results showed that no significant interference existed for ZIP from plasma or brain tissue. The addition of the two MRM transitions (413 → 177 and 413 → 220) from ZIP for identification improved the specificity of the validated method.

Stability studies

Stability testing is very important for validated methods in biological samples. The stock solution was stable at the storage conditions (–20°C) for three months (data not shown). All the other stability studies were

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Table 4. Enhanced specificity of CPZ by multiple MRM transitions (413 → 194 for quantification; 413 → 177 and 413 → 220 for identification for ZIP in plasma and brain tissue)

Concentration (ng/mL in plasma/ng/g in brain tissue)	0.2/0.833	0.4/1.666	1.0/4.165	2.0/8.33	20.0/83.3	200.0/833.3	Real samples, plasma/brain
Peak area ratio in plasma (413 → 194/413 → 177)	n.d.	7.28	7.16	7.26	7.28	7.16	7.18
Peak area ratio in brain (413 → 194/413 → 177)	n.d.	7.20	7.24	7.29	7.29	7.26	7.25
Peak area ratio in plasma (413 → 194/413 → 220)	n.d.	n.d.	25.03	24.13	24.89	23.90	24.26
Peak area ratio in brain (413 → 194/413 → 220)	n.d.	n.d.	24.76	24.53	24.69	24.85	24.56

n.d., not detected with 413 → 177 or 413 → 220 MRM transition.

Table 5. Stability testing of ZIP in rat plasma and brain homogenate (n = 5)

Matrix	Stability	Spiked concentration (ng/mL or ng/g)	Observed concentration ± SD (ng/g)	RSD (%)	Relative error (%)
Plasma	Three freeze–thaw cycle	0.6	0.582 ± 0.0270	4.63	–3.00
		150.0	143.6 ± 0.649	0.45	–4.26
	Bench top (2 h)	0.6	0.583 ± 0.0522	8.95	–2.87
		150.0	143.6 ± 3.614	2.52	–4.23
	Autosampler stability (24 h)	0.6	0.586 ± 0.0078	1.34	–2.38
		150.0	153.7 ± 3.47	2.26	2.52
Brain	Three freeze–thaw cycle	2.5	2.55 ± 0.143	5.61	2.32
		625.0	665.4 ± 12.8	1.93	6.47
	Bench top (2 h)	2.5	2.38 ± 0.047	1.97	–4.52
		625.0	582.7 ± 12.49	2.14	–6.76
	Autosampler stability (24 h)	2.5	2.51 ± 0.106	4.22	3.91
		625.0	600.0 ± 17.28	2.88	–3.99

conducted at two concentration levels (0.6 and 150.0 ng/mL for plasma or 2.5 and 625.0 ng/g for brain tissue) with five determinations for each. Plasma and brain homogenate extracts were stable in the mobile phase in the HPLC autosampler for at least 24 h, indicating that samples should be processed within this period of time (Table 5). The freeze–thaw stability tests indicated that ZIP was stable in rat plasma and brain homogenate for three freeze–thaw cycles. The results of bench-top stability indicated that spiked samples were stable for 2 h. The %RE was from 2.38 to 4.26% for plasma and from 2.32 to 6.76% for brain homogenate, and RSD was from 0.45 to 8.95% for plasma and from 1.93 to 5.61% for brain homogenate (see Table 5). Finally, the storage of brain homogenate at room temperature over 8 h resulted in an increase in the viscosity of the brain homogenate samples. This increased viscosity of the brain homogenate resulted in reduced recovery of ZIP and the IS. Therefore, fresh brain homogenate should be analyzed within 2 h or frozen immediately for later analysis.

Application of the method

The validated method has been successfully used to quantify ZIP concentrations in rat plasma and brain tissue following the chronic treatment of rats with ZIP in their drinking water. The concentration data for ZIP in rat plasma and brain tissue are reported in Table 6. The representative of MRM chromatograms resulting from the analysis of real plasma and brain tissue samples following chronic treatment with ZIP are shown in Fig. 4.

Table 6. Steady-state plasma and brain tissue concentrations of ZIP after the chronic treatment of ZIP in rats (n = 3)

Matrix	Dose (mg/kg/day)	Steady-state concentrations (ng/mL or ng/g ± SD)	Concentration range (ng/mL or ng/g)
Plasma	12.0	148.2 ± 45.7	105.1–196.0
Brain tissue	12.0	238.1 ± 96.2	231.4–423.9

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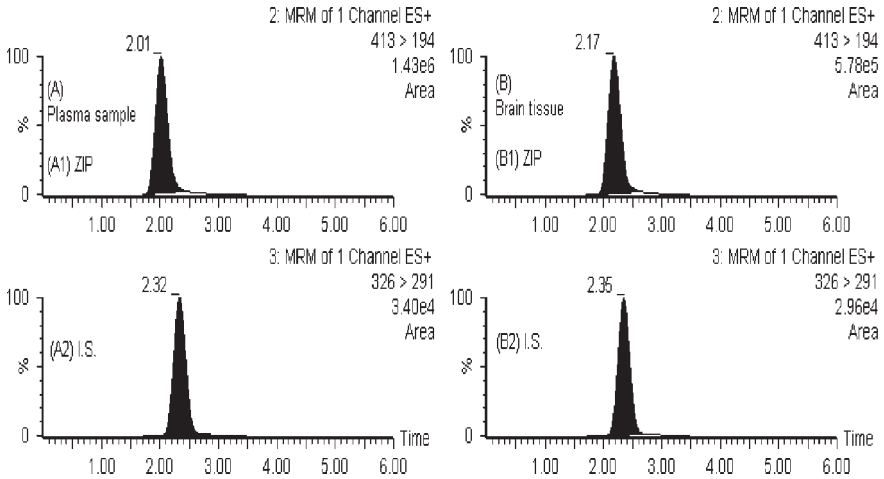

Figure 4. Representative chromatograms of plasma and brain tissue samples from rats following chronic treatment with ZIP for 15 days at 12.0 mg/kg/day: (A) the concentration of ZIP in the plasma sample was 105.08 ng/mL (A1); (B) the concentration of ZIP in brain tissue sample was 231.39 ng/g (B1).

The concentration of ZIP in brain tissue was higher than in plasma. The result was similar to the second generation antipsychotic olanzapine (Aravagiri *et al.*, 1999).

CONCLUSIONS

A simple, specific and sensitive LC-MS/MS method with low matrix effects for the determination of ZIP from rat plasma and brain tissue has been developed and validated. This method provided excellent specificity, wide linear range and an LLOQ of 0.2 ng/mL for plasma and 0.833 ng/g for brain tissue. A single step liquid–liquid extraction for sample preparation was used for 0.25 mL plasma and 0.20 mL rat brain homogenate that provided low matrix effects and high recovery for ZIP. Another advantage of this method was that only 0.25 mL for plasma and 0.2 mL for brain homogenate (small sample volume) were necessary for sample preparation. This method may be useful to determine the concentration of ZIP in different brain regions in animal experiments in the future.

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REFERENCES

Al-Dirbashi OY, Aboul-Enein HY, Al-Odaib A, Jacob M and Rashed MS. Rapid liquid chromatography–tandem mass spectrometry method for quantification of ziprasidone in human plasma. *Biomedical Chromatography* 2006; **20**: 365.

Aravagiri M, Teper Y and Marder SR. Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharmaceutics and Drug Disposition* 1999; **20**: 369.

Aravagiri M, Marder SR and Pollock B. Determination of ziprasidone in human plasma by liquid chromatography–electrospray tandem mass spectrometry and its application to plasma level determination in schizophrenia patients. *Journal of Chromatography B* 2007; **847**: 237.

Greenberg WM and Citrome L. Ziprasidone for schizophrenia and bipolar disorder: a review of the clinical trials. *CNS Drug Reviews* 2007; **13**: 137.

Janiszewski JS, Fouda HG and Cole RO. Development and validation of a high-sensitivity assay for an antipsychotic agent, CP-88,059, with solid-phase extraction and narrow-bore high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Application* 1995; **668**: 133.

Janiszewski J, Schneider P, Hoffmaster K, Swyden M, Wells D and Fouda H. Automated sample preparation using membrane microtiter extraction for bioanalytical mass spectrometry. *Rapid Communications in Mass Spectrometry* 1997; **11**: 1033.

King R, Bonfiglio R, Fernandez-Metzler C, Miller-Stein C and Olah T. Mechanistic investigation of ionization suppression in electrospray ionization. *Journal of American Society of Mass Spetrometry* 2000; **11**: 942.

Matuszewski BK, Constanzer ML and Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Analytical Chemistry* 2003; **75**: 3019.

Miceli JJ, Wilner KD, Hansen RA, Johnson AC, Apseloff G and Gerber N. Single- and multiple-dose pharmacokinetics of ziprasidone under non-fasting conditions in healthy male volunteers. *British Journal of Clinical Pharmacology* 2000; **49**(suppl. 1): 15S.

Prakash C, Kamel A, Gummerus J and Wilner K. Metabolism and excretion of a new antipsychotic drug, ziprasidone, in humans. *Drug Metabolism and Disposition* 1997; **25**: 863.

Prakash C, Kamel A, Cui D, Whalen RD, Miceli JJ and Tweedie D. Identification of the major human liver cytochrome P450 isoform(s) responsible for the formation of the primary metabolites of ziprasidone and prediction of possible drug interactions. *British Journal of Clinical Pharmacology* 2000; **49**(suppl. 1): 35S.

Schmidt AW, Lebel LA, Howard HR Jr and Zorn SH. Ziprasidone: a novel antipsychotic agent with a unique human receptor binding profile. *Europe Journal of Pharmacology* 2001; **425**: 197.

Seeger TF, Seymour PA, Schmidt AW, Zorn SH, Schulz DW, Lebel LA, McLean S, Guanowsky V, Howard HR and Lowe JA 3rd. Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. *Journal of Pharmacology and Experimental Therapeutics* 1995; **275**: 101.

Srinivas NR. Applicability of bioanalysis of multiple analytes in drug discovery and development: review of select case studies including assay development considerations. *Biomedical Chromatography* 2006; **20**: 383.

Suckow RF, Fein M, Correll CU and Cooper TB. Determination of plasma ziprasidone using liquid chromatography with fluorescence detection. *Journal of Chromatography B* 2004; **799**: 201.

Terry AV Jr, Gearhart DA, Mahadik SP, Warsi S, Davis LW and Waller JL. Chronic exposure to typical or atypical antipsychotics in rodents: temporal effects on central alpha7 nicotinic acetylcholine receptors. *Neuroscience* 2005; **136**: 519.

Terry AV Jr, Gearhart DA, Mahadik SP, Warsi S and Waller JL. Chronic treatment with first or second generation antipsychotics in rodents: effects on high affinity nicotinic and muscarinic acetylcholine receptors in the brain. *Neuroscience* 2006; **140**: 1277.

Wilner KD, Demattos SB, Anziano RJ, Apseloff G and Gerber N. Ziprasidone and the activity of cytochrome P450 2D6 in healthy extensive metabolizers. *British Journal of Clinical Pharmacology* 2000; **49**(suppl. 1): 43S.

Zhang G, Terry AV Jr and Bartlett MG. Liquid chromatography/tandem mass spectrometry method for the simultaneous determination of olanzapine, risperidone, 9-hydroxyrisperidone, clozapine, haloperidol and ziprasidone in rat plasma. *Rapid Communications in Mass Spectrometry* 2007a; **21**: 920.

Zhang G, Terry AV Jr and Bartlett MG. Sensitive liquid chromatography/tandem mass spectrometry method for the simultaneous determination of olanzapine, risperidone, 9-hydroxyrisperidone, clozapine, haloperidol and ziprasidone in rat brain tissue. *Journal of Chromatography B* 2007b; **858**: 276.

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