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Determination of ziprasidone in human plasma by liquid chromatography–electrospray tandem mass spectrometry and its application to plasma level determination in schizophrenia patients

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Abstract

An accurate, rapid and simple liquid chromatography–tandem mass spectrometry (LC–MS–MS) assay method was developed for the determination of ziprasidone (ZIP) in the plasma of schizophrenia patients. A simple one step liquid–liquid extraction with 20% methylene dichloride in pentane was used to isolate ZIP and the internal standard from the plasma matrix. The compounds were separated on a C-18 column by an isocratic elution and the eluted compounds were analyzed by a triple quadrupole mass spectrometer with a TurboIon spray interface using the positive ion atmospheric pressure electrospray ionization method and detected using multiple reaction monitoring mode. The ZIP standard calibration curve was linear over the range of 0.25–500 ng/ml when 0.5 ml of plasma was used for the analysis ($r^2 > 0.998$). The intra-assay (within-day) and inter-assay (between-day) variations were less than 12% for the spiked standard curve and quality control samples. The absolute extraction efficiency was 82% for ZIP and 68% for INS-RSP. The analysis time for each sample was less than 3 min and useful for high turnaround plasma level determinations. This LC–MS–MS assay method for ZIP is highly specific, sensitive, accurate and rapid and is currently being used for the plasma level determination of ZIP in schizophrenia patients treated with various daily oral doses of ZIP. The data showed large inter-individual variations.

Keywords: Ziprasidone; LC–MS–MS; Schizophrenia patients; Plasma analysis; Ziprasidone levels

1. Introduction

Ziprasidone (ZIP), a benzisothiazolylpiperazine derivative, is a second-generation newer atypical antipsychotic drug and it is chemically different from the phenothiazine and butyrophenone type antipsychotic drugs (Fig. 1). ZIP is found to have approximately eight-fold greater affinity for 5-HT_{2A} receptors than for D_2 receptors [1], apart from exhibiting high affinity for 5-HT_{2C}, 5-HT_{1A}, 5HT_{1B} and 5HT_{1D} receptors. The receptor binding profile of ZIP indicates that it may be associated with a decreased incidence of inducing extra pyramidal symptoms (EPS) [1–5] as compared to first-generation antipsychotic drugs. Unlike most other second-generation antipsychotics, ZIP is not generally associated with weight gain or hyperlipidemia [6–10].

Orally administered ZIP is absorbed easily and absorption increased two-fold in the presence of food. It is >99% bound to plasma proteins primarily to albumin and alpha-1-acid glycoprotein [11,12]. ZIP is metabolized extensively by phases I and II metabolic pathways resulting in several metabolites which are far less pharmacologically active. The ZIP-sulfoxide and ZIP-sulfone metabolites are major circulating metabolites in human and predominantly metabolized by cytochrome P450 (CYP) 3A4 isozyme. Other metabolites are produced by pathways including N-dealkylation, benzisothiazole cleavage and hydration of C–N bond followed by oxidation and dealkylation [11,13–15].

To facilitate plasma level monitoring and to conduct pharmacokinetic evaluations of ZIP in schizophrenia patients, a highly specific, sensitive and rapid method of analysis was developed. There are number of reported quantitative methods using high performance liquid chromatography (HPLC)–thin layer chromatography (TLC) [17], HPLC–ultra violet [18], HPLC–mass

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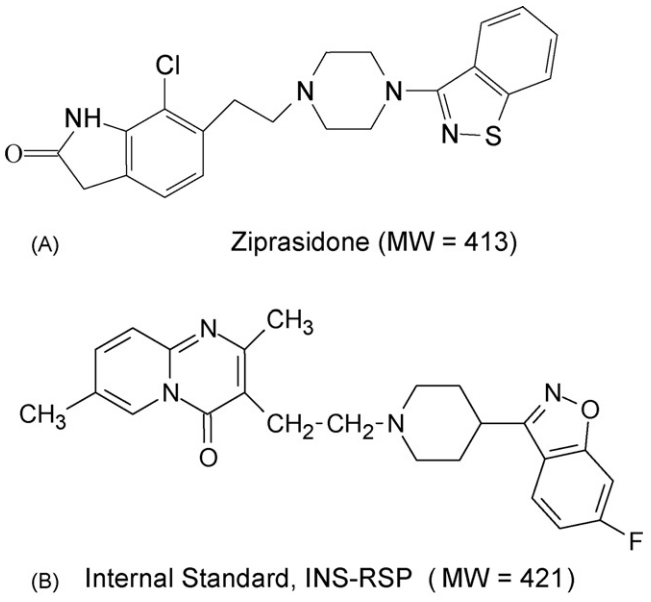

Fig. 1. Chemical structures of Ziprasidone (MW = 413, A), and Internal Standard INS-RSP (MW = 421, B).

spectrometry [19] and HPLC–fluorescence [20] for the determination of ZIP. Previously reported LC–MS–MS [16,19] methods did not provide sufficient details of the method to reproduce it in our laboratory. A recently published article describes a LC–MS–MS method with a range of 0.5–200 ng/ml when 1 ml of plasma was used for analysis [21]. We developed and describe in this article a simple, rapid and sensitive LC–MS–MS method for the analysis of ZIP in human plasma with a range of 0.25–500 ng/ml when 0.5 ml was use for analysis. This method is being used in the determination of ZIP plasma levels in samples from an ongoing study.

2. Experimental

2.1. Chemicals and materials

Ziprasidone was generously donated by Pfizer Inc., Groton, CT, USA, and the internal standard (INS-RSP), an analog of risperidone (R 68808) (Fig. 1) was donated by Janssen Research Foundation (Beerse, Belgium). All solvents and reagent chemicals were HPLC grade, procured from Fisher Scientific (Tustin, CA, USA) and used without further purification. De-ionized pure water was produced in the laboratory by reverse osmosis using a ROpure Nanopure water purification system (Barnstead, MA, USA). Centrifugations were carried out using a refrigerated centrifuge (Centra GP 8R, IEC, Fisher Scientific) at 1725 × *g*.

2.2. Analytical instrumentations

Chromatographic separations were performed using a HPLC system consisted of constant pressure solvent delivery system (Perkin-Elmer Miro200 pumps), and an autosampler with 50 μl sample loop (PE Micro 200). Both were interfaced to a triple quadrupole tandem mass spectrometer (API 2000, Applied BioSystems, Foster City, CA, USA). The HPLC system, mass spectrometer, data acquisition and data analysis were controlled with a personal computer and Windows 2000 (Microsoft) based integrated suite of software system (Analyst 1.3, Applied BioSystems).

2.3. Chromatographic conditions

The compounds were separated on a silica Luna-C-18 column (4.6 mm × 50 mm, 5 μm particle size, Phenomenex, Torrance, CA, USA). The mobile phase consisted of 10% aqueous ammonium acetate (0.65 mM, pH not adjusted) in 45% methanol and 45% acetonitrile, degassed by filtering through a 0.25 μm nylon membrane filter under suction. The column was kept at 37 °C using a column heater and compounds were eluted by isocratic elution with a flow rate of 0.6 ml/min (pressure ∼ 300 psi).

Stock solutions of ZIP and INS-RSP were prepared in methanol–water (1:1) mixture by dissolving accurately weighed amounts of compounds (Cahn Ultramicro Balance, Thomas Sc, Los Angeles, CA, USA). Calibration standard curve samples were prepared from stock solution by a serial dilution in drug free blank plasma. The calibration standard curve consisted of at least nine points ranging from 0.25 to 500 ng/ml. Three quality control samples (QC) were prepared at each time the standard curve was made and used to check the reliability of standard curve samples. Three spiked samples were prepared in bulk and used as long-term quality control samples (Lt-QC) to validate the reliability and accuracy of standard curve samples made at different times. All plasma samples were stored at −70 °C until analysis.

2.4. LC–MS–MS conditions

The compounds were separated by isocratic elution from the C-18 column and introduced into the triple quadrupole tandem mass spectrometer for detection and quantitative determination. The mass spectrometer was operated in the positive ion atmospheric pressure electrospray ionization mode using the TurboIonSpray ion source interface. The source temperature was kept at 300 °C. Nitrogen was used as the nebulizer gas (Gas 1), auxiliary gas (Gas 2), curtain gas and the gas for collision activated dissociation (CAD) in the collision cell. The gas flows were kept at the API2000 instrumental settings of 30, 30, 30 and 4, for Gas1, Gas2, curtain gas and CAD gas, respectively. The ZIP and INS-RSP were detected for quantitative determination by a multiple reaction monitoring (MRM) mode. The ion transitions monitored were *m/z* 413 → 194 for ZIP and *m/z* 421 → 201 for INS-RSP (Fig. 2). These transition ions were selected based on predominant fragmentation pathways of ZIP and INS-RSP and their intensity as observed in their product ion spectra (Fig. 2). The dwell time for each transition was set at 200 ms with an inter-channel pause time of 5 ms to provide optimal sampling of each peak of interest. The total scan time was 410 ms. The CAD was ensured with nitrogen gas with a thickness in the collision cell at approximately 177×10^{13} atoms $(cm^2)^{-1}$. The optimum collision energies were 38 and 35 for ZIP and INS-RSP, respectively. Ions are detected and counted by a Channel Electron Multiplier kept at 2100 V. The mass spectrometer, LC

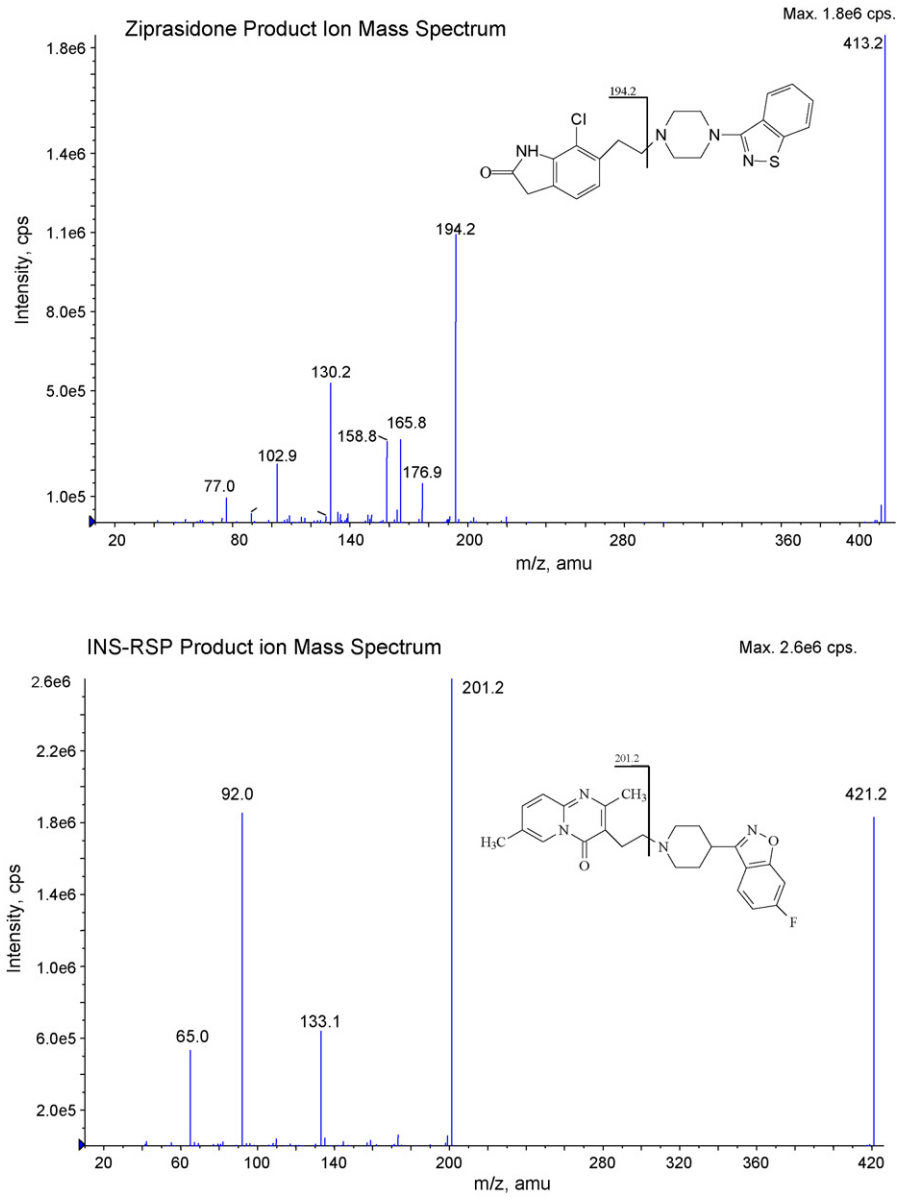

Fig. 2. Product ion mass spectrum of Ziprasidone and Internal Standard (INS-RSP).

system, mass calibration, data acquisition, data representation and post-acquisition quantitative analyses were carried out using a suite of software applications (Analyst 1.3.2, Applied Biosystems).

2.5. Plasma samples

Patients who met the DSM-IV criteria for schizophrenia were admitted into the study, Clinical Atypical Trials of Intervention Effectiveness (CATIE) [NIMH #N01MH90001]. The patient population consisted of 93 males and 41 females of various ethnic origin (Caucasian, Hispanic, African American and others). The age of patients varied from 20 to 67 years with an average age of 42 ± 10 years. All patients gave informed consent for venipuncture and plasma concentration determination. These schizophrenic patients were out patients and treated with daily oral doses ranging from 40 to 200 mg of ZIP. Drugs for other clinical conditions were co-administered when prescribed by physicians. Blood samples were collected by venipuncture in Vacutainer blood collection tubes containing EDTA as anticoagulant (Becton and Dickinson, Rutherford, NJ, USA). The blood samples were immediately centrifuged for 15 min at 4 °C at $1725 \times g$, and plasmas were separated and stored frozen at −70 °C until analysis.

2.6. Plasma concentration by LC–MS–MS

2.6.1. Sample preparation

Plasma samples were analyzed for ZIP after the extraction of ZIP from plasma matrix by a simple one step extraction procedure. Briefly, to 0.5 ml aliquots of patient's plasma taken in borosilicate glass tube (125 mm × 16 mm), 0.5 ml of a saturated aqueous solution of sodium carbonate and 20 ng of (100 μl of 200 ng/ml solution) INS-RSP were added and mixed. The com-

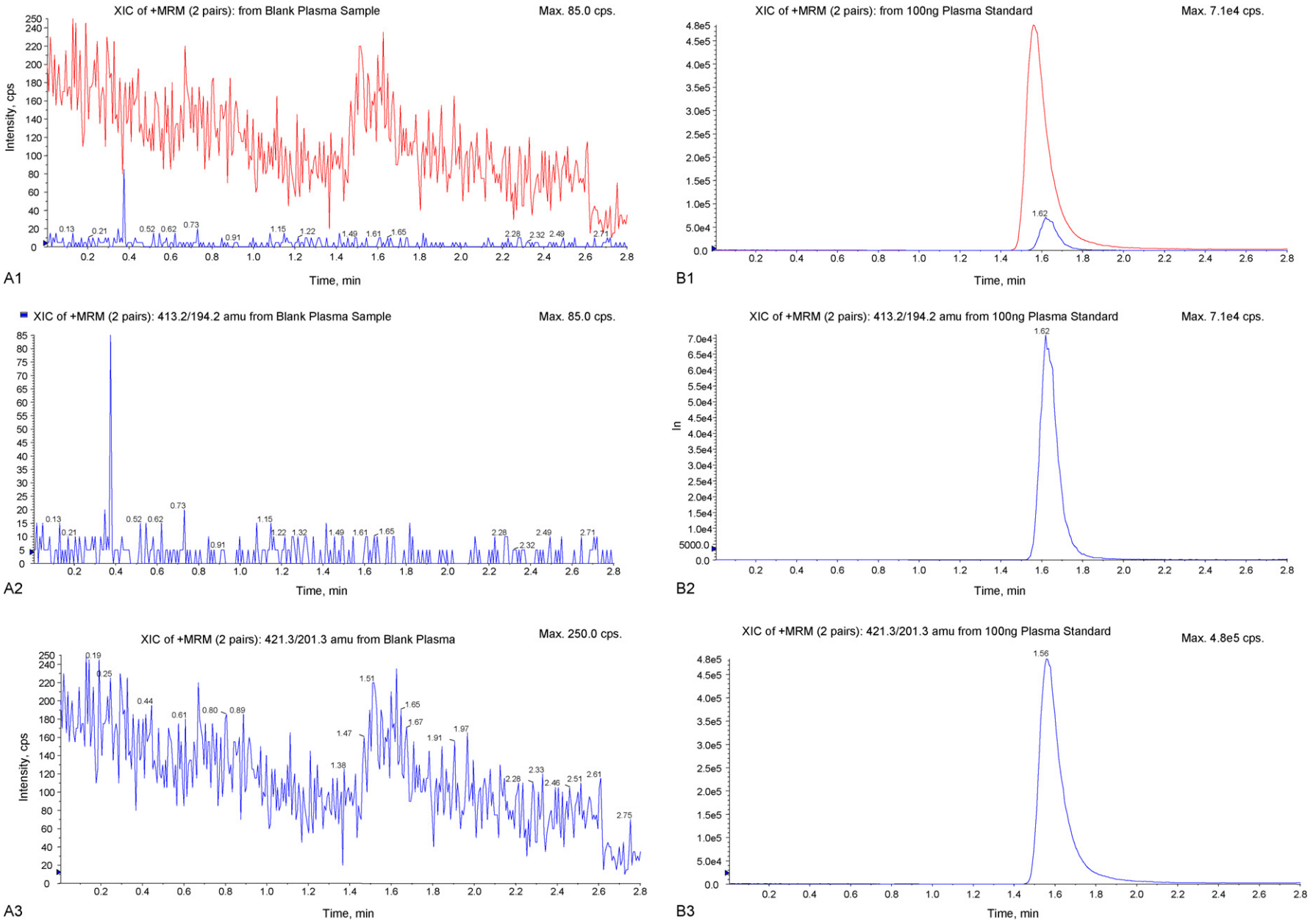

Fig. 3. LC/MS/MS MRM total ion chromatograms (TIC) of blank plasma (A1) and plasma standard (B1) containing ZIP and INS-RSP (100 and 20 ng). The extracted ion chromatograms (XIC) of ZIP and INS-RSP from TIC of blank plasma (A2 & A3) and the plasma standard (B2 & B3), respectively.

pounds were extracted by a simple one step extraction with 7 ml of a mixture of 20% methylene dichloride in pentane. After shaking for 10 min, the upper organic layer was transferred to a borosilicate glass tube (100 mm × 16 mm) and dried at 60 °C in a dry-bath under a slow flow of nitrogen. The residue was dissolved in 250 μl of mobile phase and an aliquot of this reconstituted solution was injected into LC–MS–MS system for analysis. Quantitative analysis was carried out by multiple reaction monitoring mode using ion transitions *m/z* 413 → 194 for ZIP and *m/z* 421 → 201 for INS-RSP. The assay was linear for ZIP over the range of 0.25–500 ng/ml when 0.5 ml of plasma was used in the extraction ($r^2 > 0.998$). The overall intra-assay (within-day) and inter-assay (between-day) variations were less than 12%. The variations in the concentrations of long-term quality control samples analyzed over a period of 6 months were less than 12% (Tables 1 and 2).

2.7. Data analysis

Statistical and graphical analyses of plasma concentration data were performed using commercially available microcomputer software programs (Analyst 1.3, Applied BioSystems; Prism, GraphPad Software Inc., San Diego, CA, USA; Microsoft Office, Microsoft Corp.; WinNonlin, Pharsight Corp., Mountain View, CA, USA).

3. Results and discussion

3.1. LC–MS–MS

The fragmentation of ZIP and INS-RSP (Fig. 1) to their product ions under optimal MS conditions is given in (Fig. 2). The mass spectrometer parameters including ion source temperature, collision energy and nebulizer, auxiliary, curtain and CAD gas settings were optimized to produce maximum intensity of selected product ions formed in the ion transition of 413 → 194 for ZIP and *m/z* 421 → 201 for INS-RSP.

The LC–MS–MS total ion chromatogram of blanks plasma did not show (Fig. 3, A1–A3) any interfering endogenous substances in the plasma matrix. The chromatograms of spiked plasma standard (Fig. 3, B1–B3), and plasma from a patient receiving 200 mg/day of ZIP are shown in Fig. 4 (C1–C3). Both ZIP and INS-RSP were eluted with in 3 min after injection. Both

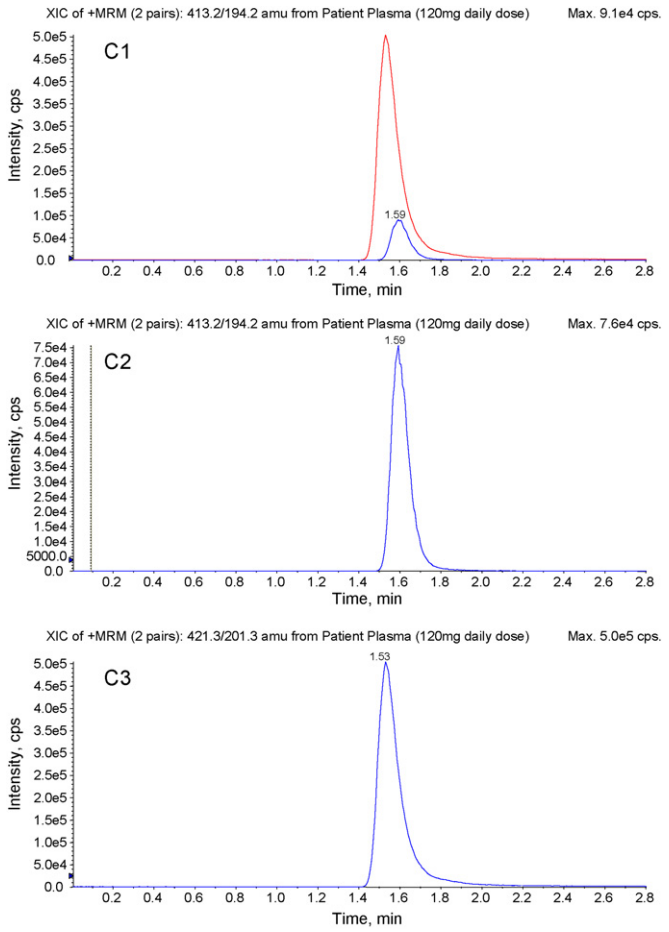

Fig. 4. LC/MS/MS MRM total ion chromatogram (TIC) of plasma from a patient (C1) treated with a daily dose of 120 mg of ZIP extracted with 20 ng of INS-RSP. The extracted ion chromatograms (XIC) of ZIP and INS-RSP (C2 & C3) respectively, from TIC (C1) of plasma extract.

Table 1
Accuracy and precision of ziprasidone standard calibration curve

	Spiked concentrations of ziprasidone calibration standard curve (ng/ml)										Correlation coefficient
	0.25	0.5	1	2.5	10	25	50	100	250	500	
Intra-assay variability[a] ($n = 4$)											
Mean	0.26	0.48	0.94	2.57	10.27	25.82	51.1	102.9	237.8	447.2	0.9984
S.D.	0.01	0.08	0.10	0.13	0.33	1.65	2.6	4.02	9.00	43.2	0.002
CV%	3	10	11	5	3	6	5	4	4	10	0.2
Precision (%)	104	97	94	103	103	103	102	103	95	89	
Inter-assay variability[b] ($n = 18$)											
Mean	0.25	0.49	0.98	2.43	10.11	26.62	53.1	103.6	238.8	446.7	0.9986
S.D.	0.02	0.06	0.11	0.25	1.13	2.15	3.03	4.90	9.65	30.5	0.002
CV%	6	11	11	10	11	8	6	5	4	7	0.2
Precision (%)	99	99	98	97	101	106	106	104	96	89	

S.D.: standard deviation; CV%: coefficient of variation (S.D./mean); precision (%): determined concentration/actual spiked concentration.

[a] Intra-assay (within-day) variations were calculated as CV% from the determined concentrations of four aliquots of each spiked plasma standard curve samples in a single day.

[b] Inter-assay (between-day) variations were calculated as CV% from the determined concentration of plasma standard curve samples assayed on 18 different days.

Table 2
Accuracy and precision of ziprasidone quality control samples

	Spiked concentrations of ziprasidone quality control and long-term quality control samples (ng/ml)					
	QC i 1.2	QC ii 30	QC iii 180	Lt-QC i 4	Lt-QC ii 40	Lt-QC iii 200
Intra-assay variability[a] ($n=4$)						
Mean	1.2	30.3	165.6	4.43	44.8	199.4
S.D.	0.08	1.17	8.68	0.20	3.25	12.42
CV%	7	4	5	4	7	6
Precision (%)	100	101	92	111	112	100
Inter-assay variability[b] ($n=18$)						
Mean	1.3	30.2	170.6	4.5	43.8	206.2
S.D.	0.12	1.69	15.74	0.51	3.79	16.99
CV%	9	6	9	11	9	8
Precision (%)	107	101	95	113	109	103

S.D.: standard deviation; CV%: coefficient of variation (S.D./mean); QC: quality control samples made at the time of each standard curve; Lt-QC: long-term QCs to verify the validity of standard curve and QCs made at different times; precision (%): determined concentration/actual spiked concentration.

[a] Intra-assay (within-day) variations were calculated as CV% from the determined concentrations of four aliquots of each spiked QC plasma samples assayed in a single day.

[b] Inter-assay (between-day) variations were calculated as CV% from the determined concentration of QC samples assayed on 18 different days.

ZIP and INS-RSP eluted simultaneously. However, there was no interference in the quantitation of both compounds as one of the advantages of using the LC–MS–MS and MRM method is that the complete chromatographic resolution of the analytes peaks is not essential for accurate quantitation. The absolute extraction recovery was determined by comparing peak areas of ZIP and INS-RSP in the extracted spiked plasma samples and that obtained after direct on column injection of known amounts of ZIP and INS-RSP. The overall mean extraction recovery was 82 and 68%, respectively, for ZIP and INS-RSP ($n=4$).

3.2. Assay linearity, precision, accuracy and stability

The assay was linear over the range of 0.25–500 ng/ml of ZIP when 0.5 ml of the plasma samples was used in the analysis. The calibration standard curve was constructed by plotting the peak area ratio of ZIP to INS-RSP versus the spiked ZIP concentration in the plasma.

The precision and accuracy of the LC–MS–MS assay method for ZIP were evaluated from the intra-assay (within-day) and inter-assay (between-day) variations. Precision was determined by the percentage ratio of spiked concentration versus determined concentration. The accuracy was determined by the coefficient of variation (CV%) of concentrations determined for the quality control samples and the concentrations of standard curve samples (Tables 1 and 2). The intra-assay variations were determined by analyzing four sets of standard calibration curve samples and quality control samples and long-term quality control samples. The inter-assay variations were determined from the concentration data of calibration and quality control samples analyzed on 18 different days. The data showed that the calibration curve is linear over the range of 0.25–500 ng/ml of ZIP and highly reproducible with correlation coefficients >0.998, slope ~0.001 and intercept ~0.0001. The precision and accuracy for both inter- and intra-assay determinations were ~113 and ~111%, respectively (Tables 1 and 2).

Lower limit of quantitation (LLQ) is the lowest concentration determined with precision of ±20% (assayed concentration/spiked concentration) with a CV% lower than 15%. The LLQ for the ZIP when analyzed using this method was 0.25 ng/ml and the upper limit of quantitation was 500 ng/ml. None of the analyzed plasma samples had ZIP concentrations above upper limit of quantitation. All concentrations below the LLQ were extrapolated values and concentrations below 0.1 ng/ml were reported as non-detectable.

Table 3
Typical concentrations of plasma ziprasidone in a few individual schizophrenic patients receiving various daily doses of ziprasidone determined on different days during the treatment

Patient (I.D.)	Dose (mg/day)	Time after last dose (h)	ZIP concentration (ng/ml)
1	40	13.6	13.03
1	40	14.5	8.96
1	40	15.0	13.10
1	40	15.0	15.04
1	40	15.3	13.27
1	40	15.4	31.05
2	80	4.3	71.30
2	80	4.5	67.01
2	80	7.0	49.71
2	80	16.5	23.33
2	80	17.8	20.51
3	120	1.5	26.63
3	120	2.0	63.38
3	120	2.0	76.37
3	120	10.5	26.21
3	120	13.5	114.41
3	120	14.0	46.39
4	160	4.3	50.81
4	160	5.3	136.10
4	160	3.0	180.72
4	160	2.0	253.91
4	160	1.2	175.33

Table 4
Mean ± S.D. plasma ziprasidone concentrations in patients receiving different daily doses at various time intervals after the administration of last dose

Dose (mg/day)	Patients (*n*)	Time after last dose (h)					
		0–1	2–4	5–8	9–12	13–18	19–24
40	10	28.3 ± 24.3					
80	55	126.6 ± 74.1	66.4 ± 45.3	59.5 ± 42.3	47.8 ± 41.9	46.4 ± 48.0	
120	54	44.1 ± 42.7	75.2 ± 56.5	89.0 ± 73.4	55.6 ± 43.4	47.2 ± 38.0	
160	75	75.4 ± 58.5	95.1 ± 72.0	80.4 ± 49.9	54.1.9 ± 52.3	54.1 ± 52.3	17.8 ± 20

Mean ± S.D. values were calculated for the time periods when available plasma sample numbers were greater than 3.

The long-term QC samples were made in bulk quantity and used for over a period of 1 year to verify the accuracy and stability within and between spiked calibration plasma standards and stock solutions. Each time a new calibration standard curve samples were made, the bulk long-term QCs were thawed and required numbers of aliquots were made. The remaining bulk long-term QCs were stored frozen for future use. The QC samples were incorporated into each batch analysis enabling to continuously monitor the stability of ZIP and accuracy of the assay within and between stock solutions and spiked calibration plasma standards. The data (Table 2) showed that there is no significant variation in ZIP concentration indicating that ZIP is stable under the experimental conditions. Thus the long-term QCs are useful to monitor stability of analytes during many freeze and thaw cycles, and variations due to human errors which may occur during various steps in making calibration standards, including weighing analytes, preparation of stock solutions, dilutions from stock solutions and making spiked plasma standards at different times.

3.3. Application to plasma level determination

ZIP plasma levels were determined for more than 500 samples collected for an ongoing multi-center study to establish population pharmacokinetic characteristics of a ZIP and other antipsychotic drugs (CATIE study). The plasma level data of a typical patient each receiving a daily dose of 40, 80, 120 and 160 mg of ZIP are given in Table 3. The mean plasma concentrations found in patients receiving different dose level at different time intervals after the last dose are given in Table 4 and showed wide variations. There was no strong relationship between the daily administered dose of ZIP and plasma concentrations. These variations and the apparent lack of correlation between plasma concentration and daily dose may be, in part, due to the fact that the sample collection was not strictly time controlled and samples were collected at different time intervals after the last dose but before the administration of the next dose. Pharmacokinetic results of this study will be discussed elsewhere after the completion of the study and the final data analysis.

The recently published article [21] described a LC–MS–MS method with a range of 0.5–200 ng/ml when 1 ml of plasma was used for the analysis. Due the absence of quality control and patient plasma level data in that article, it is not easy to compare its applicability with the current method described in this article. The current method has similar sensitivity and broader range when a smaller amount of sample (0.5 ml) is used for analysis. The method has been used to determine plasma level of ZIP in large number of samples from schizophrenia patients.

4. Summary and conclusion

A sensitive LC–MS–MS method has been developed and validated for the determination of ZIP in human plasma. The method is simple, accurate and sensitive with an LLQ of 0.25 ng/ml when 0.5 ml of plasma was used for the assay. The chromatographic analysis time/sample is less than 3 min as compared to HPLC methods, generally several times longer (>15 min) and therefore the method is useful for high turnaround sample analysis and has sufficient sensitivity for pharmacokinetic studies. The method is currently being used for the plasma level determination of ZIP in schizophrenia patients treated with various daily oral doses of ZIP.

Acknowledgments

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