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Determination of plasma ziprasidone using liquid chromatography with fluorescence detection

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Abstract

A liquid chromatographic procedure was developed for the determination of a new antipsychotic agent ziprasidone in plasma using fluorescence detection. A one-step liquid–liquid extraction from 1 ml of alkalinized plasma containing an internal standard α-ergocryptine using methyl-*t*-butyl ether afforded a greater than 84% recovery of ziprasidone. Chromatography was performed using a reversed-phase trimethylsilyl bonded silica column with a mobile phase of 72:28 phosphate buffer:acetonitrile at a flow rate of 1.5 ml/min. Detection of the eluted peaks was observed using excitation and emission wavelengths of 320 and 410 nm, respectively. Chromatographic run time did not exceed 14 min with no interference from endogenous material. The calibration curve was linear over the concentration range of 0.5 to 200 ng/ml and the inter- and intra-assay imprecision (CV) was less than 10%. The lower limit of quantitation was assessed at 0.5 ng/ml. Specificity of the method is demonstrated by the lack of interference from a large number of commonly used drugs and their metabolites in clinical use. The utility of the method is exemplified with the presentation of clinical data from patients receiving ziprasidone.

Keyword: Ziprasidone

1. Introduction

Ziprasidone HCl is a novel antipsychotic with a unique pharmacologic profile. It is chemically unrelated to the phenothiazines and the atypical antipsychotics currently available (Fig. 1). Ziprasidone exhibits a potent and highly selective antagonistic activity on the D_2 and 5-HT_{2A} receptors [1]. 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 [2].

The metabolic fate of ziprasidone has been studied in both rats and humans and found to be extensively metabolized in both species [3–5]. The principle routes of ziprasidone biotransformation in humans involve *N*-dealkylation, oxidation to form the sulfone and sulfoxide metabolites, reductive cleavage of the benzisothiazole moiety, and the hydration of the C–N double bond followed by sulfur oxidation or N-dealkylation. Subsequent in vitro studies indicated that ziprasidone is predominantly metabolized in human liver microsomes by the CYP isoform 3A4 [6].

There are several published procedures that describe the quantitation of ziprasidone in plasma. Liquid chromatography with UV detection at 215 nm, preceded with a solid-phase extraction with a structurally similar analog as the internal standard, achieved a lower limit of quantitation (LLOQ) of 1 ng/ml with acceptable precision and accuracy [7]. A method for serum ziprasidone using LC–APCI–MS was reported with an emphasis on automated sample preparation [8]. This procedure resulted in a LLOQ of 0.5 ng/ml with good precision and accuracy. Serum ziprasidone was also quantitated using LC–MS–MS but full details of the method were not presented [4].

We present an LC procedure that offers an alternative to the existing methods. A highly selective assay with a LLOQ equal to the LC–MS technique is achieved using the native fluorescence of ziprasidone. Application of the assay to clinical samples is demonstrated.

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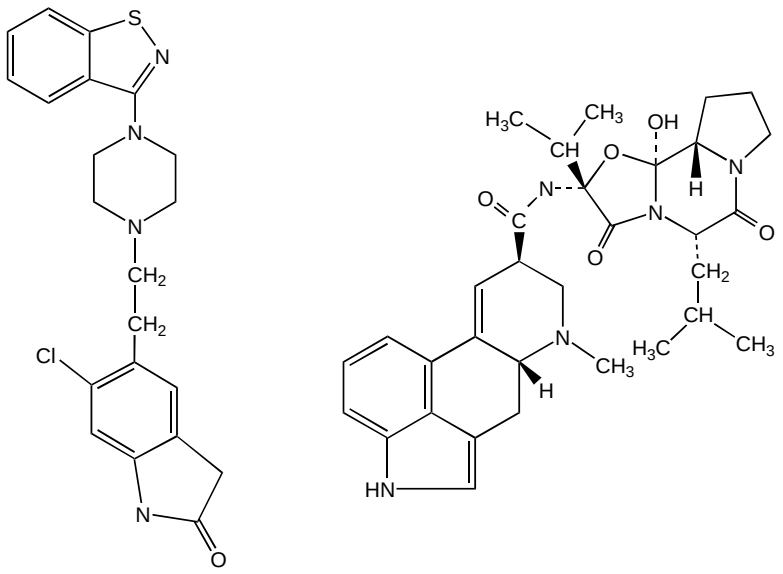

Fig. 1. Chemical structures of ziprasidone and α-ergocryptine (internal standard).

2. Experimental

2.1. Chemicals and reagents

Ziprasidone hydrochloride reference standard (88.3% pure) was obtained from Pfizer Central Research (Groton, CT, USA) and the internal standard α-ergocryptine (99% pure) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other drugs and metabolites used for specificity tests were obtained either from their respective manufacturers or from Sigma Chemical Co. Chemicals used in the extraction process and chromatography were of reagent or HPLC grade from Fisher Chemical Co. (Fairlawn, NJ, USA). Acetonitrile was HPLC grade from J.T. Baker Co. (Phillipsburg, NJ, USA). Water used in the preparation of buffers and standards was generated using a Milli-RO 10 Plus and a Milli-Q purification system. The standard stock solution of ziprasidone was prepared at a concentration of 1.0 mg/ml in methanol/chloroform (1:1) and diluted to 1 and 0.05 μg/ml in methanol/water (1:1) for the working solutions. The internal standard α-ergocryptine was prepared as 1 mg/ml in methanol and diluted to 0.5 μg/ml in 0.01 M HCl for the working solution. Stock solutions were stored at −20 °C and working solutions were maintained at 4 °C.

2.2. Sample collection and processing

Generally, ziprasidone blood samples were collected from fasting subjects 10–14 h post-evening dose using EDTA blood collection tubes. The plasma was separated from cells by centrifugation, transferred to polypropylene tubes and stored at −20 °C until the day of analysis.

To extract the drug from samples, 1.0 ml of plasma was transferred to a 16 mm × 100 mm disposable screw-capped borosilicate tube to which 30 μl (15 ng) of the internal standard α-ergocryptine, 1.0 ml of 0.6 M carbonate buffer (pH ∼ 9.8) and 4.5 ml of methyl-*t*-butyl ether were added. The contents were mixed for 15 min on a rocking platform at 'moderate' speed, followed by centrifugation at 1500 × *g* for 10 min. The supernatant (organic) phase was transferred to a 5 ml screw-thread tapered disposable borosilicate centrifuge tube and evaporated to dryness under reduced pressure at room temperature in a vacuum centrifuge for ∼45 min. To the residue, 100 μl of mobile phase was added, vortexed, and transferred to inserts for injection. The injection volume of the calibration extracts ranged from 20 to 80 μl while sample extracts were routinely injected at 30 μl.

A nine-point calibration curve was constructed in drug-free plasma in the range of 0.5–200 ng/ml and processed similarly with each batch of samples. Three levels of quality control samples (run in duplicate) were included with each day's analyses.

2.3. Instrumentation

Chromatographic separation was achieved using a Model 590 Programmable Solvent Delivery Module and a Model 717 plus Autosampler with the sample compartment cooled to 10 °C (Waters Corp., Milford, MA, USA). A Series 1100 Model G1321A fluorescence detector equipped with an 8 μl standard flow cell and a xenon flash lamp, and operated with a Model G1323A Control Module (Agilent Technologies, Palo Alto, CA, USA) was used to detect the analytes. Chromatographic signals were processed with a PC-based chromatography data system using ChromPerfect

for Windows v.3.5 (Justice Laboratory Software, Palo Alto, CA, USA) for data collection, construction of the calibration curves, sample analysis and storage of quality control data. Chromatographic tracings were also recorded on a Recordall strip chart recorder (Fisher Scientific, Springfield, NJ, USA).

A Speed-Vac Sample Concentrator System consisting of a Model SC110A centrifuge, a Model RT4104 refrigerated solvent trap and a Model VP190 oil-vacuum pump (ThermoSavant Instruments, Holbrook, NY, USA) were used to evaporate sample extracts during sample preparation.

2.4. Chromatographic conditions

The mobile phase consisted of 720 ml of 0.05 M monobasic potassium phosphate, 280 ml of acetonitrile and 1.0 ml of *o*-phosphoric acid, with 1.2 ml of *n*-butylamine added to enhance peak symmetry. Following filtration and degassing, the mobile phase was pumped at 1.5 ml/min through a 250 mm × 4.6 mm column containing 5 μm particles of trimethylsilyl-bonded silca (Supelcosil LC-1, Supelco, Bellefonte, PA, USA) resulting in a backpressure of about 12.1 MPa. The column temperature was maintained at 32 °C with a Model CH-30 column heater and a Model TC-50 Temperature Controller (Eppendorf, Westbury, NY, USA). The excitation wavelength of the fluorescence detector was set at 320 nm (bandwidth: 20 nm). The emission wavelength was optimized at 410 nm (bandwidth: 20 nm), with a gain set to 11, the analog output to the data system set to 25 and the signal filter (RC time constant) tuned to 4 s. The detector signal to the data system was 1 V full-scale.

Quantitation was achieved by calculating the peak-height ratios of ziprasidone to the internal standard α-ergocryptine. The data system performed the least-square linear regression analysis for each calibration curve with equal weighting for each point.

3. Results and discussion

3.1. Chromatography

Ziprasidone in plasma was quantitated using LC coupled with fluorescence detection. While a previous report indicated that ziprasidone is non-fluorescent [7], our studies indicate that it exhibits natural fluorescence under the

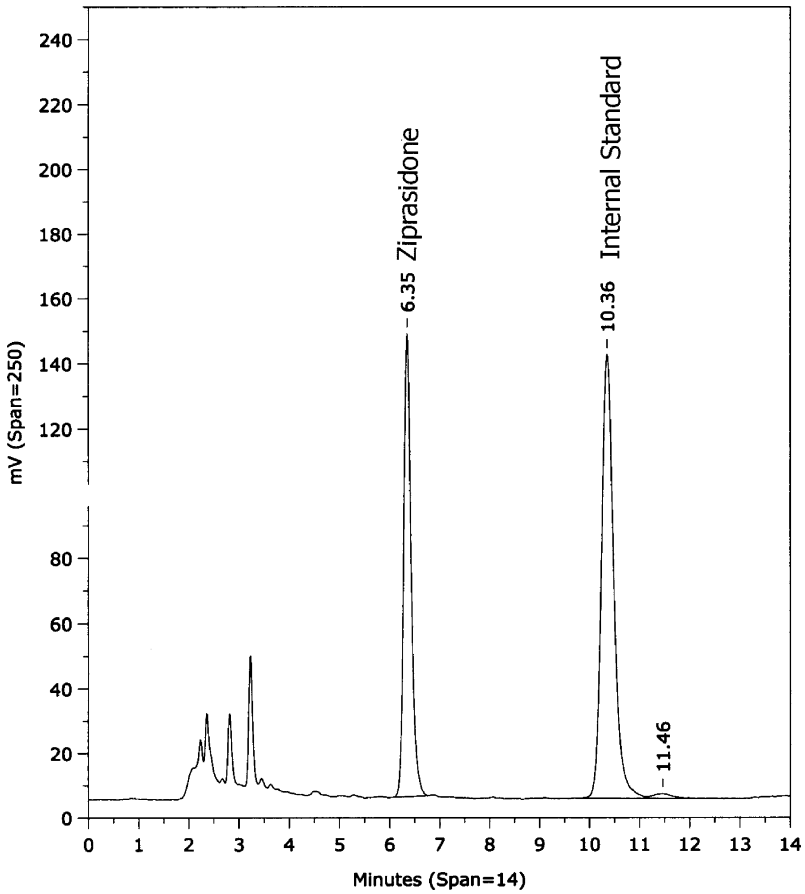

Fig. 2. Chromatogram of a 40 μl injection of a supplemented 1 ml plasma extract containing 20 ng/ml of ziprasidone and 15 ng/ml of α-ergocryptine (internal standard).

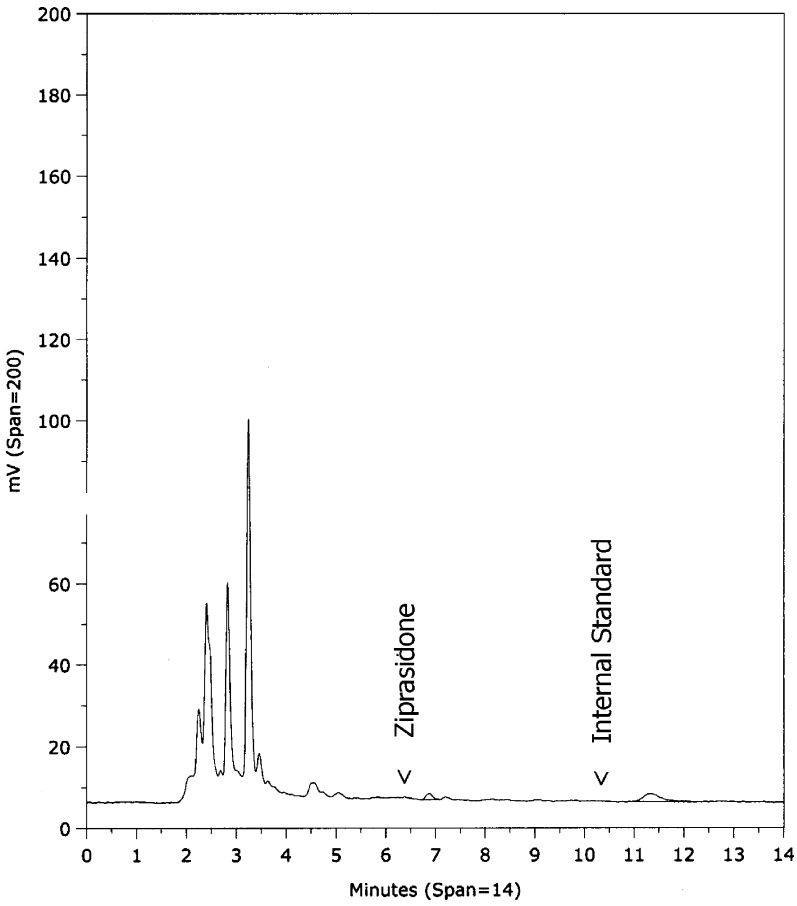

Fig. 3. Chromatogram of an 80 μl injection of a 1 ml drug-free plasma extract.

conditions described herein. Peak scanning revealed the excitation and emission maxima to be 320 and 410 nm, respectively. In the absence of the dimethyl-fluoro analog of ziprasidone, α-ergocryptine was selected as an internal standard, which displayed similar optimum fluorescent excitation and emission spectra, a suitable retention time (∼10.3 min) and a similar extraction recovery (∼85%). Fig. 2 shows a chromatogram of a 1 ml drug-free plasma sample extract supplemented with 20 ng of a ziprasidone reference standard. A chromatogram of a drug-free plasma extract illustrates a clean, stable baseline with no interfering endogenous peaks (Fig. 3). Run-time was set at 14 min, and no carry-over peaks were detected in subsequent chromatograms of patient samples. Varying the injection volumes of the calibration and sample extracts had no apparent effect on the robustness of the method since the calibration curves were linear over the concentration range ($r > 0.999$), and no peak distortions were visible.

3.2. Precision, accuracy and recovery

The intra-day precision of the method was determined by supplementing twelve 1 ml aliquots of drug-free plasma with various amounts of ziprasidone. After the addition of the internal standard, the samples were processed as described, together with the standard calibration curve. The average result for each added concentration and the respective CVs are listed in Table 1. Inter-day variability was assessed by analyzing quality control samples with each batch of samples. The quality control samples were prepared at 5, 50 and 150 ng/ml, and stored frozen at −20 °C. The respective day-to-day variations (CVs) are listed in Table 1. The accuracy of the method, measured by the intra- or inter-day percentage difference between the mean concentration found and the amount added, did not exceed 10%. Inspection of the daily calibration curve parameters, i.e. slope, intercept, and correlation coefficient, was another measure of day-to-day consistency.

The absolute recovery of three concentrations of ziprasidone, and the defined concentration (15 ng/ml) of internal standard, from plasma was determined by comparing peak heights of the quantitatively extracted compounds to the peak heights of the equivalent concentrations of the un-extracted, directly injected reference standards. The ratios of the peak heights of the extracted and the un-extracted ziprasidone and internal standard × 100 reflects the overall extraction efficiency. The percent recovery of ziprasidone from plasma at 200, 50 and 2 ng/ml was found to be 84.9 (±3.0), 85.2 (±2.0)

Table 1
Plasma ziprasidone intra- and inter-assay variation

Intra-assay variation[a]		
Ziprasidone added (ng/ml)	Ziprasidone recovered (ng/ml)	Coefficient of variation (%)
200	205	0.83
100	103	1.16
50.0	48.2	1.78
20.0	19.4	2.66
10.0	9.6	2.07
5.0	4.9	1.90
2.0	2.1	6.60
1.0	1.1	9.26
0.50	0.55	5.79
Inter-assay variation[b]		
Ziprasidone quality controls (ng/ml)	Ziprasidone recovered (ng/ml)	Coefficient of variation (%)
150	148	3.84
50.0	47.6	6.11
5.0	4.7	7.56

[a] $N = 12$ at each concentration.
[b] $N = 18$ consecutive days.

and 86.0 (±3.2), respectively ($n = 12$ for each concentration). The recovery of α-ergocryptine (%) from plasma at 15 ng/ml was 85.1 (±2.3) ($n = 12$).

3.3. Selectivity, sensitivity and stability

Table 2 lists a number of drugs and their respective metabolites that were processed by this method with their retention times and a comparison between fluorescence and UV detection at 210 nm. Of the 45 compounds tested, only protriptyline and imipramine would interfere on the fluorescence detector at or near the retention time of the internal standard. Clearly, more compounds would directly interfere with ziprasidone if UV detection were employed. At this low concentration, the internal standard α-ergocryptine did not appreciably absorb with UV detection
at 210 nm.

The LLOQ was determined to be 0.5 ng/ml (intra-assay variation: 5.79%, $n = 12$, Table 1). Fig. 4 represents a chromatogram of 0.5 ng/ml plasma extract of a ziprasidone standard. This level compares favorably with the existing LC–MS method also having the same LLOQ [8]. The upper limit of quantitation (ULOQ) was determined at 200 ng/ml (intra-assay variation: 0.83%, $n = 12$, Table 1).

The stability of ziprasidone in plasma was assessed by the 'freeze–thaw' procedure. A set of nine tubes each of ziprasidone quality controls (5, 50 and 150 ng/ml) supplemented in drug-free human plasma were allowed to thaw at room temperature (in darkness) for 2 h three times during a 1 week period. Each set was then processed, with a calibration curve, and nine tubes of equivalent concentration that were kept frozen during the same period. There was a slight but statistically significant decline in the thawed samples (−2 to −3%; $P < 0.05$, Student's t-test, $n = 9$) at the 5 and 50 ng/ml concentrations, but no difference at 150 ng/ml. Stability of ziprasidone plasma samples at room temperature was determined by preparing 22 samples, each containing 100 ng/ml in human drug-free plasma. Eleven samples were stored frozen at −20 °C and 11 were left unfrozen at room temperature (22–24 °C) in darkness for 24 h. Both sets were

Table 2
Retention times of some commonly used drugs and their metabolites

Drug/metabolite	Retention time (min) (UV at 210 nm)	Retention time (min) (fluorescence)
O-Desmethylvenlafaxine	<4.0	ND
N-Desmethylvenlafaxine	<4.0	ND
O,*N*-Didesmethylvenlafaxine	<4.0	ND
Atenolol	<4.0	ND
Nadolol	<4.0	ND
7-Hydroxy quetiapine	<4.0	ND
Quetiapine sulfone	<4.0	ND
7-Hydroxy dealkylquetiapine	<4.0	ND
9-Hydroxy risperidone	4.2	ND
Venlafaxine	4.4	ND
Norclozapine	5.3	ND
Propranolol	5.5	5.5
Risperidone	5.8	ND
Thioridazine	6.1	6.1
Clozapine	6.3	ND
Quetiapine	6.4	ND
Mesoridazine	6.5	ND
Ziprasidone	**6.6**	**6.6**
Didesmethylcitalopram	6.6	ND
Reduced Haloperidol	6.7	ND
Desmethylcitalopram	7.0	ND
Citalopram	7.7	ND
Haloperidol	8.8	ND
Desipramine	10.1	10.1
Paroxetine	10.1	10.1
α-Ergocryptine (internal standard)	**ND**	**10.8**
Protriptyline	10.8	10.8
Imipramine	10.9	10.9
Fluvoxamine	11.1	ND
Nortriptyline	11.6	ND
Amitriptyline	12.4	ND
Maprotiline	12.6	ND
Perphenazine	13.5	ND
Methadone	13.5	ND
Chlorpromazine	15.2	ND
Desmethylsertraline	15.4	ND
Norfluoxetine	15.7	ND
Sertraline	16.0	ND
Fluoxetine	17.0	ND
Desmethylclomipramine	17.2	ND
Clomipramine	18.3	ND
Nefazodone	18.5	ND
Fluphenazine	20.7	ND
Sertindole	29.5	29.5
Olanzapine	ND	ND

ND: not detected.

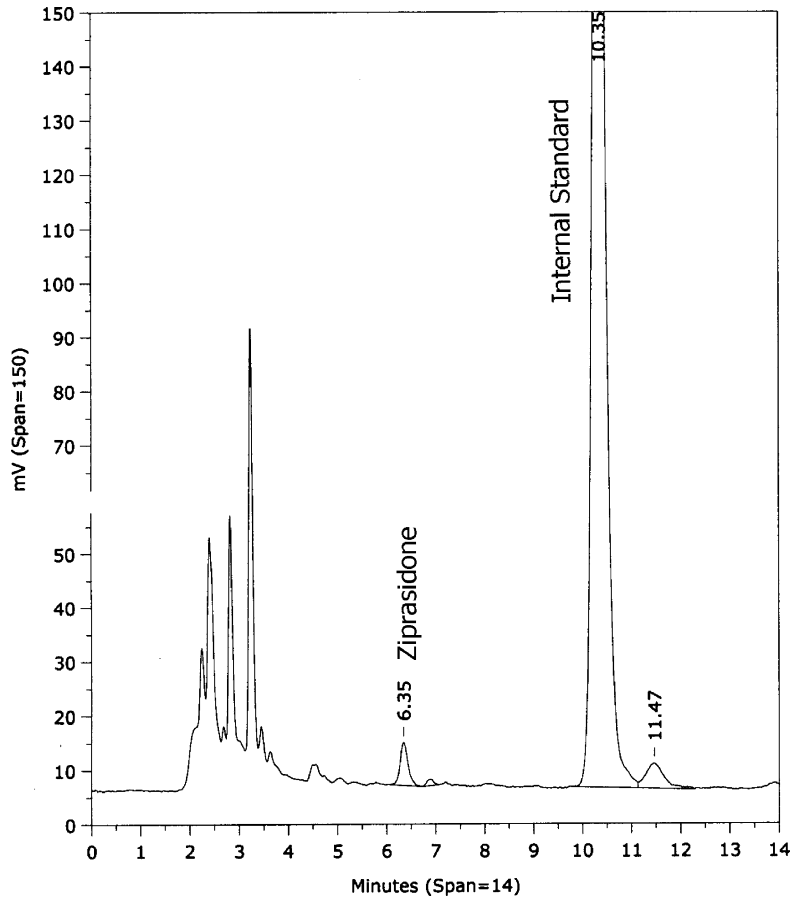

Fig. 4. Chromatogram of a 80 μl injection of a 1 ml supplemented plasma extract containing 0.5 ng/ml (the LLOQ) of ziprasidone and 15 ng/ml internal standard.

processed identically the following day. Once again, a small but statistically significant decline (−2%) in ziprasidone levels was observed in the unfrozen set ($P < 0.05$, Student's *t*-test) (Fig. 5). The magnitude of this difference would not normally be clinically significant. However, a repeat of the above test, which extended the temperature exposure time from 24 to 72 h, revealed a more significant decline (−16.5%, $P < 0.01$, Student's *t*-test) in plasma concentration. No significant difference between the 24 and 72 h frozen ziprasidone samples in human plasma was observed. This would preclude shipping unfrozen plasma ziprasidone samples.

Commercially available bovine serum is widely used in many laboratories as a substitute for human drug-free plasma in the preparation of quality controls, calibration standards and other validation tests. Caution must be exercised, however, as some drugs are not stable in this medium. A recent report demonstrates that olanzapine, another widely used neuroleptic, rapidly degrades in calf serum at room temperature, and over a longer period of time while frozen [9]. To determine the stability of ziprasidone in bovine serum, the above experiments were repeated using Adult Bovine Serum, (Cocalico Biologicals Inc., Reamstown, PA, USA). It was observed that ziprasidone deteriorates more rapidly at room temperature in bovine serum than in human plasma in both the 24 and 72 h tests. While there was no significant ziprasidone deterioration between frozen bovine serum and human plasma at 24 h, a 5.7% loss occurred in bovine at 72 h ($P < 0.01$, Student's *t*-test) (Fig. 5). The addition of ascorbic acid (2.5 mg to each ml of sample) as an antioxidant to the bovine samples did not completely inhibit ziprasidone degradation at room temperature. It was previously reported that ziprasidone is stable at −20 °C for at least 6 months [6]. Several clinical samples were re-analyzed following storage at −20 °C. A slight decrease in plasma ziprasidone was observed in most samples over a 3–9-month period (Table 3). This trend, however, was not corrected for inter-assay variability, and could not be statistically evaluated. A larger number of samples repeated over time will determine if a significant deterioration of ziprasidone occurs in frozen plasma samples.

Finally, stability of the processed sample extracts was routinely assessed by the inclusion of at least two processed calibration standards injected at the end of every run. Following an average total run time of 8.7 h (range = 5.9–10.8 h.), a 200 and 50 ng/ml standard injected indicated no

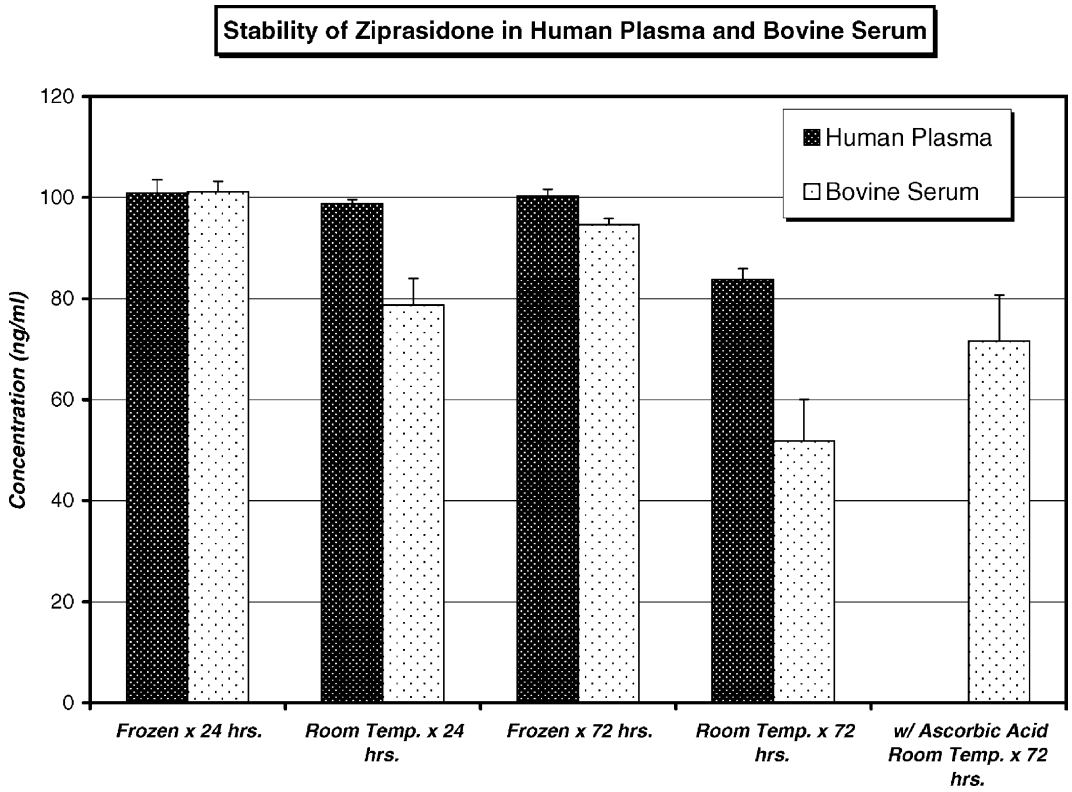

Fig. 5. The stability of ziprasidone in human plasma and bovine serum stored frozen and at room temperature for 24 and 72 h.

Table 3
Steady-state plasma concentrations and storage stability

Subject number	Dose (mg per day)	Original plasma concentration (ng/ml)	Re-analyzed plasma concentration (ng/ml)
1	80	48	43[b]
1[a]	80	22	21[b]
1[a]	80	38	37[b]
1[a]	80	24	[c]
2	160	49	44[d]
2[a]	160	42	40[d]
2[a]	160	58	[c]
3	80	38	32[d]
3[a]	80	75	68[d]
3[a]	80	32	28[d]
4	120	68	70[b]
5	120	94	[c]
6	80	62	57[b]
7	160	81	73[b]
8	160	158	149[b]
9	40	40	35[b]
10	40	20	19[b]
11	80	36	[c]

[a] Same subject: different sample date.
[b] Re-analyzed 3 months from original.
[c] Insufficient sample for re-analysis.
[d] Re-analyzed 9 months from original.

deterioration in the sample extract concentration (202.9 ng/ml, CV = 5.3%; 50.6 ng/ml, CV = 5.4%, $n = 10$ days each).

3.4. Application to biological samples

This method was developed for the purpose of monitoring individuals receiving ziprasidone in several multi-center studies that occasionally included the co-administration of a number of other psychotropic medications. Fig. 6 shows a chromatogram of an actual plasma sample extract from a subject in steady-state receiving 160 mg per day of ziprasidone. No evidence of any ziprasidone metabolites appears in the chromatogram. Table 3 lists several subjects with the dose and the corresponding steady-state plasma levels of ziprasidone. The lack of a dose—plasma concentration correlation is due, in part, to the uncontrolled nature of the samples (i.e., the subjects were not matched for age, gender, co-morbidity, concurrent medications, etc.). The well-documented genetically controlled inter-individual differences in drug metabolizing enzymes are a major contribution to these variations in the plasma levels of ziprasidone. Studies involving plasma levels of other neuroleptics revealed similar inter-individual variations [10]. Steady-state concentrations in normal volunteers have been estimated in single and multiple-dose pharmacokinetic studies [11,12].

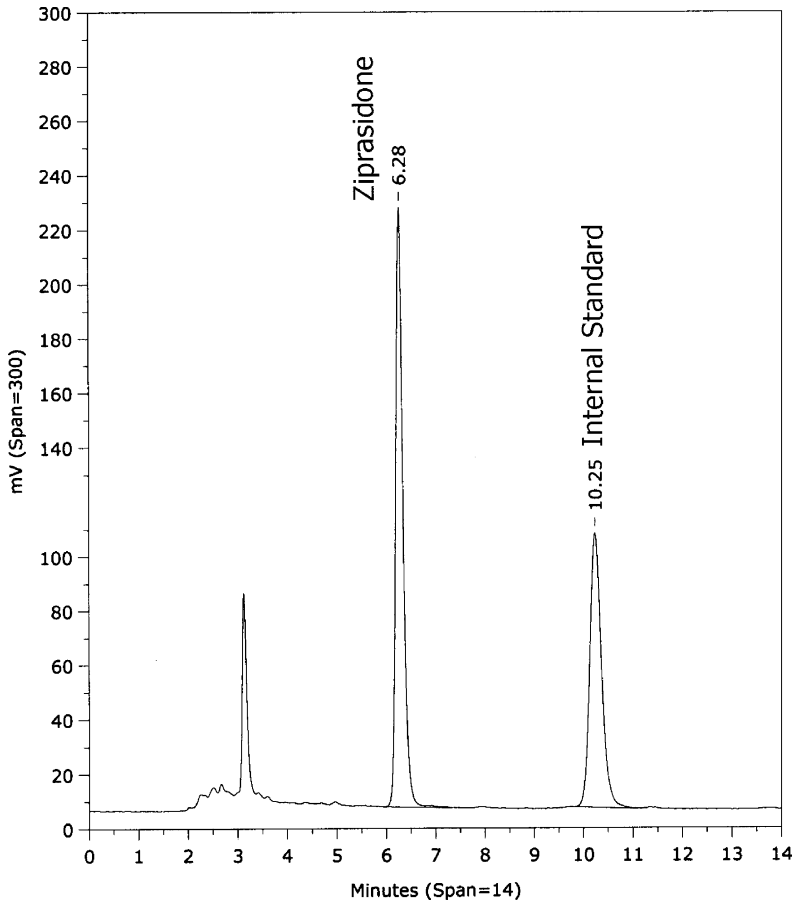

Fig. 6. Chromatogram of a 30 μl injection of a 1 ml plasma extract from a subject in steady-state receiving 160 mg per day of ziprasidone. The plasma concentration of ziprasidone was determined to be 42 ng/ml.

4. Conclusions

A liquid chromatographic assay using fluorescence detection was developed as an alternate to the LC–UV and LC–MS procedures for detection of plasma ziprasidone. In our laboratory, the method has demonstrated reliability and selectivity while offering the capability of detection limits equivalent to LC–MS. It would be potentially useful in most bioanalytical studies.

Acknowledgement

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