ELSEVIER

Journal of Chromatography B, 668 (1995) 85–90

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Analysis of olanzapine in human plasma utilizing reversed-phase high-performance liquid chromatography with electrochemical detection

John T. Catlow, Richard D. Barton, Matt Clemens, Todd A. Gillespie, Michael Goodwin, Steven P. Swanson*

Lilly Research Laboratories, Department of Drug Metabolism and Disposition, Indianapolis, IN 46285, USA

First received 11 October 1994; revised manuscript received 30 January 1995; accepted 31 January 1995

Abstract

A sensitive reversed-phase HPLC method for the analysis of olanzapine in human plasma is described. Isolation of olanzapine from plasma was accomplished by solid-phase extraction utilizing an ion-exchange/reversed-phase cartridge designed for basic drug extraction. The drug was subsequently separated by reversed-phase HPLC and monitored by electrochemical detection (ED). Electrochemical analysis was used to detect olanzapine due to its uniquely low oxidative potential. Ascorbic acid was added to prevent oxidation during extraction. The limit of quantitation for the assay was established at 0.25 ng/ml utilizing a 1-ml human plasma sample. The average inter-day accuracy was 96.6% with a average precision (%C.V.) of 3.22% over the concentration range of 0.25 to 100 ng/ml. This method was applied to human plasma samples from human clinical trials with olanzapine. The HPLC–ED method compared favorably with a negative chemical ionization GC–MS method previously utilized for analysis of olanzapine in human plasma.

1. Introduction

Schizophrenia is a debilitating disorder of the central nervous system whose symptoms have been divided into two classes: positive symptoms, including hallucinations, delusions and conceptual disorganization; and negative symptoms, including social withdrawal, blunting affect and poverty of speech. Traditionally, classical antipsychotics like haloperidol have been used to treat the positive symptoms of schizophrenia. However, classical antipsychotics have a much lower efficacy in treating negative symptoms of schizophrenia and can cause extrapyramidal motor side-effects (EPS), such as tremor and rigidity. Clozapine has been described as an atypical antipsychotic because it is effective in the treatment of both positive and negative symptoms of schizophrenia and has low EPS. However, the use of clozapine has been severely limited by the occurrence of agranulocytosis in a small percentage of the patient population [1].

Olanzapine (LY170053, 2-methyl-4(4-methyl-1-piperazinyl)-10H-thieno[2,3-*b*][1,5]benzodiazepine) is an atypical antipsychotic agent that is structurally similar and has a binding profile similar to clozapine; each displays high affinity for D1, D2, and D4 dopamine, serotonin (5-

* Corresponding author.

0378-4347/95/$09.50
SSDI 0378-4347(95)00061-5

HT)2a, 5-HT2c, and 5-HT3, α1-adrenergic, histamine1, and muscarinic receptors [2]. In vitro and in vivo studies suggest that olanzapine and clozapine have similar pharmacological profiles, and might therefore have similar atypical antipsychotic profiles in man [3]. Indeed, in clinical studies of schizophrenic and schizophreniform patients, olanzapine was effective in the treatment of both positive and negative symptoms of schizophrenia with a low incidence of EPS and no agranulocytosis [4].

A sensitive analytical method which can detect low ng/ml and sub ng/ml concentrations was needed for analysis of olanzapine in plasma samples at therapeutic dosages. Previously, sub ng/ml analysis of olanzapine in human plasma samples was only accomplished by the gas chromatography–mass spectrometry (GC–MS) method utilizing a liquid–liquid extraction and negative chemical ionization mass spectrometry with selected ion monitoring [5]. The GC–MS method is complex, labor-intensive, and expensive. In this report we describe the development of a sensitive method for the analysis of olanzapine in human plasma which utilizes solid-phase extraction, reversed-phase HPLC, and electrochemical detection (ED).

2. Experimental

2.1. Chemicals and reagents

High-purity-grade methanol, acetonitrile, and ethyl acetate were purchased from Burdick and Jackson (Muskegon, MI, USA). Water used in the assay was purified with a Milli-Q Plus Analytical Water System (Millipore, Bedford, MA, USA). Ammonium hydroxide and acetic acid were high-purity grade (Suprapur; EM Science, Cherry Hill, NJ, USA). Potassium carbonate–potassium borate certified buffer (pH 10, 0.05 *M*) and potassium phosphate certified buffer (pH 6, 0.05 *M*) were obtained from Fischer Scientific (Pittsburg, PA, USA). All other chemicals were reagent grade. Human plasma was purchased from Biological Specialty Corporation (Lansdale, PA, USA). Olanzapine (LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-*b*][1,5]benzodiazepine) and internal standard (LY170222, 2-ethyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-*b*][1,5]benzodiazepine) (Fig. 1) were synthesized at Eli Lilly and Company (Indianapolis, IN, USA).

2.2. Apparatus and instrumentation

Solid-phase extraction was performed using a Supelco vacuum manifold (Bellefonte, PA, USA), Varian LRC Certify I cartridges (Harbor City, CA, USA) with cation-exchange and hydrophobic functional groups [6]. All glassware utilized in the extraction and HPLC analysis was treated by gas-phase silylation with hexamethyldisilane [7].

2.3. Solid-phase extraction

Solid-phase extraction (SPE) cartridges were conditioned with 10 ml of methanol followed by 10 ml of SPE buffer (pH 6 phosphate buffer containing 17.6 g ascorbic acid/l). Plasma samples (1 ml) were combined with 0.5 ml of SPE buffer and 50 μl internal standard (100 ng/ml) in 12 × 100 mm test tubes. The samples were mixed gently and added to conditioned SPE cartridges. The cartridges were washed in order with 5 ml of SPE buffer, 1 ml of 30% methanol in carbonate–borate buffer (pH 10), and 1 ml of 1 *M* acetic acid. The cartridges were then dried by applying

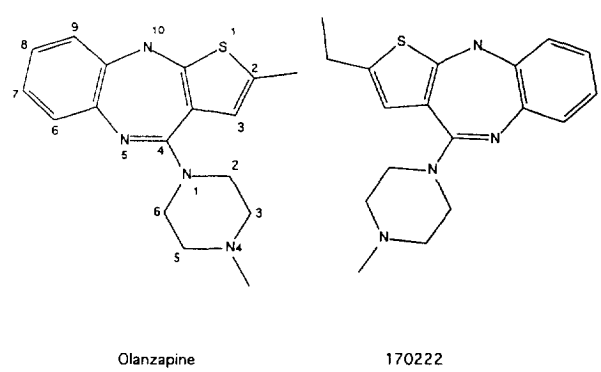

Fig. 1. Structure of olanzapine and internal standard.

full vacuum (ca. 40 cmHg; 1cmHg = $1.3 \cdot 10^3$ Pa) for 5 min. A final cartridge wash was conducted with 6 ml of methanol followed by 2 min of full vacuum in order to remove residual solvent. The drug was eluted with three 1-ml aliquots of 2% ammonium hydroxide in ethyl acetate. The eluates were dried under nitrogen at approximately 50°C and the residue was redissolved in 100 μl of mobile phase for HPLC analysis.

2.4. HPLC conditions

The chromatographic system consisted of a Hewlett-Packard HP1050 pump and autosampler (Kennett Square, PA, USA), an ESA Coulochem Model 5100A electrochemical detector with a Model 5011 electrode cell (Bedford, MA, USA), and a 150 mm × 4.6 mm I.D. (5 μm) YMC basic column by YMC (Wilmington, NC, USA). The electrochemical detector guard cell was set at 0.3 V and the analytical cell was utilized in an oxidation (+0.2 V)/reduction (−0.2 V) mode. Aliquots of the plasma extracts (15–40 μl) were injected into the HPLC system utilizing a mobile phase of 75 m*M* sodium phosphate (pH 7)–methanol–acetonitrile (48:26:26), a flow-rate of 1.2 ml/min, and a column temperature of 40°C. Peak-height ratios of olanzapine and internal standard were determined with a Perkin Elmer-Nelson Access*Chrom chromatography data system (Cupertino, CA, USA) using an eight point standard curve ranging from 0.25 to 100 ng/ml.

2.5. Method validation

The precision and accuracy of the method was assessed by analyzing replicate human blank plasma samples spiked at concentrations of 0.25, 0.5, 5, 25 and 100 ng/ml. The extraction efficiency, limits of quantitation, linearity of the standard curves, and the precision and accuracy of the data were then determined on each of three days. In addition, spiked plasma samples and plasma samples from patients orally administered olanzapine were analyzed by both the GC–MS and HPLC–ED method described in this report. The human samples were from a Phase II clinical study. During this study patients were given 2.5 to 17.5 mg/day for a period from two weeks to a maximum of one year. The patients included 250 randomized schizophrenic patients, and allowed women of childbearing potential. The two analytical methods were compared by plotting the concentrations obtained from the GC–MS assay versus the HPLC–ED assay and determining the correlation coefficient.

2.6. GC–MS method

Selected plasma samples were analyzed by a negative chemical ionization GC–MS method as previously reported [5]. Briefly, to 1 ml plasma samples were added 5 ng of internal standard, 200 μl of 1 *M* HCl, and 10 ml of *n*-butanol–hexane–ethyl acetate (0.15:50:50), and the contents were mixed gently for 10 min. After centrifugation the organic layer was discarded. The aqueous phase was adjusted to approximately pH 10 with 1 *M* sodium hydroxide and then extracted with 4 ml of ethyl acetate. The ethyl acetate layer was transferred to a clean tube, washed with 2 ml of 0.1 *M* HCl and discarded. An aliquot of 1 ml of 1 *M* sodium hydroxide was added to the aqueous layer followed by 4 ml of methylene chloride. The contents were mixed and the methylene chloride layer was removed and concentrated to dryness using a Speed Vac. After reacting the extracted drug with heptafluoroacetic acid anhydride, the derivatized drug was separated by capillary gas chromatography on a 30-m DB-1701 fused-silica column (J&W Scientific, 0.25 μm film, 0.25 mm I.D.) and detected by mass spectrometry with a Finnigan 4500 quadrapole mass spectrometer operated in the methane negative chemical ionization mode (NCI) and selected ion monitoring. The ratio of ions $m/z = 370$ (derivatized olanzapine) and $m/z = 384$ (derivatized internal standard) were used for quantitation. The average intra-day accuracy was 97.6% with a average precision (% C.V.) of 7.44% over the concentration range of 0.1 to 25.6 ng/ml utilizing the NCI GC–MS analytical method.

3. Results

3.1. Oxidation potential evaluation

Olanzapine displayed a reversible biphasic oxidation potential with an initial signal plateau observed at an applied potential of 0.2 V and a second oxidation potential starting at 0.6 V. Although the higher oxidation potentials yielded greater absolute sensitivity, a potential of 0.2 V was selected as this provided sufficient sensitivity for the assay and yielded greater electrode stability. In addition, greater specificity was observed by utilizing the reduction/oxidation (REDOX) detector mode where the compounds in detector one are initially subjected to an oxidation at a potential of 0.2 V followed by reduction at detector two to −0.2 V. Monitoring the reduction reaction resulted in greater selectivity since fewer endogenous compounds present in the plasma are reversibly oxidized at this potential. Stability of the detector in the REDOX mode was acceptable under the conditions utilized. No noticeable loss in sensitivity or stability was observed after hundreds of injections.

3.2. Extraction efficiency

The average solid-phase extraction recovery for olanzapine and the internal standard over the concentration range of 0.25 to 50 ng/ml was 78 and 72%, respectively (see Table 1). Slightly higher recoveries were obtained without the 30% methanol buffer wash step, however this step removed an endogenous compound which otherwise prevented quantitation of olanzapine at subnanogram per milliliter levels.

Table 1
Extraction efficiency of olanzapine and internal standard from human plasma

	Olanzapine % recovery[a]	170222 % recovery[a]
Plasma spike 0.25 ng/ml	71	76
Plasma spike 1 ng/ml	80	69
Plasma spike 5 ng/ml	80	77
Plasma spike 50 ng/ml	82	68
Overall average	78	72

[a] $n = 3$ and C.V. was less than 25% for all concentration levels.

3.3. HPLC chromatography

Representative HPLC–ED chromatograms of control plasma and plasma spiked with olanzapine are shown in Fig. 2. Olanzapine and the internal standard eluted with retention times of 7.0 and 10.5 min, respectively. No interferences were noted at the retention times of olanzapine or the internal standard and no late eluting peaks were observed. The limit of detection for olanzapine by this method was established at 0.25 ng/ml. At this concentration olanzapine gave a signal-to-noise ratio of greater than 5:1. A representative chromatogram of plasma from a patient orally administered olanzapine is shown in Fig. 3.

3.4. Precision and accuracy

The precision and accuracy of the HPLC–ED method were determined by measuring plasma

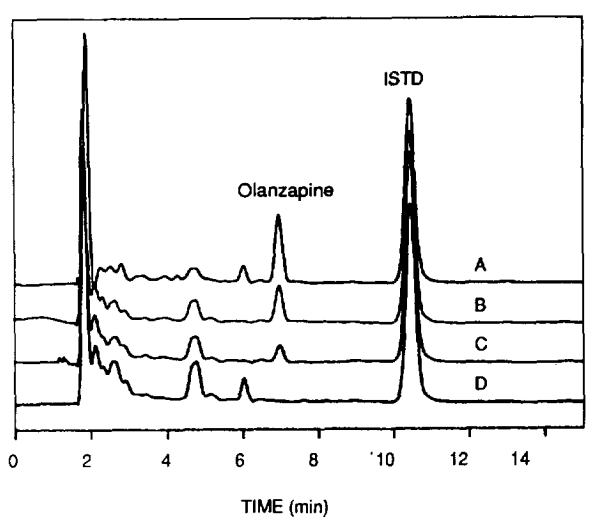

Fig. 2. Human plasma spiked with olanzapine at 1.0 ng/ml (A), 0.5 ng/ml (B), 0.25 ng/ml (C), and control plasma (D). ISTD = internal standard.

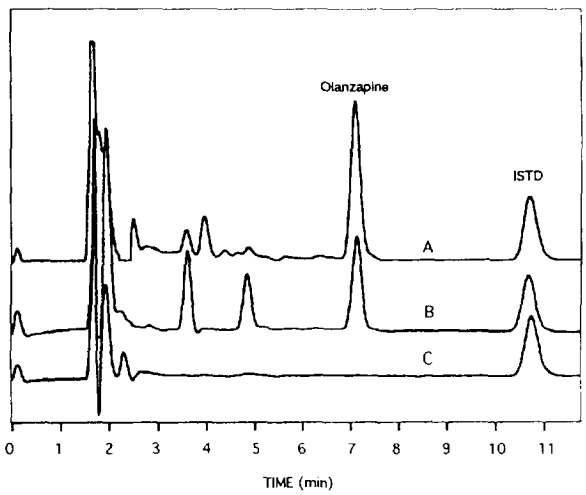

Fig. 3. Human plasma sample from Phase II study F1D-MC-HGAD (A), human plasma spiked with olanzapine at 5 ng/ml (B) and a control human plasma (C).

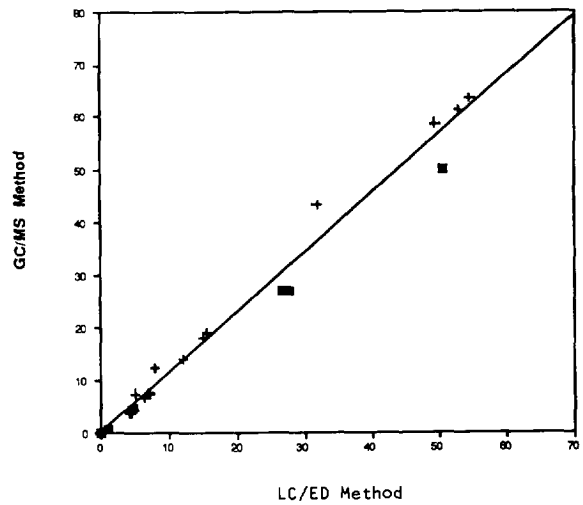

Fig. 4. Concentrations of human samples from Phase II study F1D-MC-HGAD and human plasma spiked with olanzapine as determined by both the HPLC–ED and GC–MS methods. $y = 1.2361 \cdot 10^{-2} + 1.1303x$; $r^2 = 0.985$; + = Human samples; ■ = Spiked human plasma. Units for both axes are ng/ml.

samples prepared at olanzapine concentrations of 0.25, 0.5, 1, 5, 25 and 100 ng/ml on three separate days. The inter-assay accuracy from 94.4 to 99.7% over the three days (Table 2). The inter-assay precision (% C.V.) ranged from 1.29 to 6.49%. Standard curves were highly reproducible with correlation coefficient typically greater than 0.997 and y-intercepts close to zero. The GC–MS analytical method was validated from 0.1 to 25.6 ng/ml over 1 day. The intra-assay accuracy and precision for the GC–MS analytical method ranged from 89.8 to 112.5% and 2.13 to 13.64%, respectively.

Samples of plasma from several patients receiving olanzapine orally at daily dosages of 2.5 to 17.5 mg/day, in addition to plasma samples spiked with olanzapine were analyzed by both HPLC–ED and GC–MS. The correlation between the methods was determined by least squares linear regression obtained after plotting the concentrations obtained by GC–MS against those obtained by HPLC–ED. Two points were removed statistically in accordance with Cook's D [8] for each point since they exert undue influence on the linear regression (Fig. 4). The formula for the original regression line and after removing two points were y(GC–MS) = 1.15 + 1.03x(HPLC–ED) and y(GC–MS) = 1.24 + 1.13x(HPLC–ED), respectively. The coefficient of determination (r^2) for the original regression line and after removing two points were 0.912 and 0.985, respectively.

Table 2
Statistics on three-day validation of HPLC method for analysis of olanzapine in human plasma

Inter-assay statistics	0.25 ng/ml	0.5 ng/ml	5 ng/ml	25 ng/ml	100 ng/ml	Overall average
Mean (ng/ml)	0.248	0.472	4.73	23.7	99.7	–
Accuracy (%)	99.2	94.4	94.6	94.9	99.7	96.6
Precision (% CV)	6.49	1.29	2.97	3.65	1.71	3.22
Days	3	3	3	3	3	3
n (total)	15	15	15	15	15	75

4. Discussion

The HPLC–ED method presented provides subnanogram per milliliter analysis of human plasma for olanzapine. The LC–ED method has similar detection limits, accuracy and precision, but is less labor-intensive and less expensive than the earlier GC–MS method.

The maximum current response of olanzapine was obtained at a potential of +0.85 V (the highest potential tested), but a secondary plateau was observed at +0.2 V. This secondary plateau is particularly suited for coulometric electrochemical analysis due to its uniquely low oxidative potential. Advantages of a lower oxidation potential include a decrease in the noise level, greater specificity due to a reduced incidence of endogenous interfering peaks, and longer electrode performance.

Initial investigations with olanzapine at low plasma concentrations revealed reduced and variable recovery, particularly at concentrations below 10 ng/ml. Further examination suggested that oxidation of olanzapine was occurring during the extraction process and prior to HPLC. The addition of the anti-oxidant ascorbic acid decreased the variability and increased the extraction efficiency.

Samples of plasma from several patients receiving olanzapine orally at daily dosages of 2.5 to 17.5 mg/day, in addition to plasma samples spiked with olanzapine, were analyzed by both HPLC–ED and GC–MS. The least-squares linear regression obtained after plotting the concentrations obtained by GC–MS against those obtained by HPLC–ED is shown in Fig. 4. Two points were removed statistically in accordance with Cook's D [8] for each point since they exert undue influence on the linear regression. The GC–MS analytical method tended to give slightly higher results for the same human samples analyzed by the LC–ED analytical method, but the spiked human plasma gave similar results by either methods. This discrepancy may be due to the human samples being stored at −20°C for over 8 months between the analysis by GC–MS and LC–ED, whereas the spiked human plasma was stored at −20°C for less than two weeks between the analysis by GC–MS and LC–ED.

In summary, a sensitive HPLC–ED method was developed for the analysis of olanzapine in human plasma. The method has a high degree of precision and accuracy and has a detection limit of 0.25 ng/ml. The assay is sufficiently sensitive and easy to use for the analysis of plasma samples in human clinical trials.

References

[1] U. Juul Povlsen, U. Noring, R. Fog and J. Gerlach, *Acta Pschiatr. Scand.*, 71 (1985) 176–185.
[2] R.W. Fuller and H.D. Snoddy, *Res. Comm. Chem. Path. Pharmacol.*, 77 (1992) 87–93.
[3] N.A. Moore, N.C. Tye, M.S. Axton and F.C. Risus, *J. Pharmacol. Exp. Ther.*, 262 (1992) 545–551.
[4] C.M. Beasley, S. Montgomery and N.C. Tye, *Second International Conference on Schizophrenia, Vancouver, BC, July 19–22, 1992*, abstract 332.
[5] R.M. Goodwin, R.D. Barton and T.A. Gillespie, in A.G. Marshall (Editor), *Proceedings of the 41st ASMA Conference on Mass Spectrometry*, San Francisco, CA, May 31–June 4, 1993, Professional Association Management.
[6] X. Chen, J. Franke, J. Wijsbeek and R. Zeeuw, *J. Anal. Toxicol.*, 16 (1992) 351–355
[7] D.C. Fenimorfe, C.M. Davis, J.H. Whitford and C.A. Harrington, *Anal. Chem.*, 48 (1976) 2289–2290.
[8] R.D. Cook, *Technometrics*, 19 (1977) 15–18.