ELSEVIER

Journal of Chromatography B, 714 (1998) 309–315

JOURNAL OF CHROMATOGRAPHY B

Determination of olanzapine in serum by high-performance liquid chromatography using ultraviolet detection considering the easy oxidability of the compound and the presence of other psychotropic drugs

Ole Vendelin Olesen*, Kristian Linnet

Institute for Basic Research in Psychiatry, Department of Biological Psychiatry, Clinical Biochemical Laboratory, Psychiatric Hospital, Aarhus University Hospital, Skovagervej 2 DK-8240 Risskov, Denmark

Received 15 December 1997; received in revised form 8 April 1998; accepted 28 April 1998

Abstract

A method for determination of the atypical neuroleptic drug olanzapine in serum was developed. After a single-step liquid–liquid extraction, the compound was separated from other constituents on a normal-phase silica gel column using a buffer–methanol mobile phase and measured by UV absorption at 270 nm. Addition of 0.25% ascorbic acid to serum protects olanzapine against oxidation during extraction and stabilizes the easily oxidised compound during storage. Inter-day variation was <8% at serum levels found in olanzapine treated patients. Analytical interference from coadministered psychoactive drugs and their metabolites were studied. Only risperidone, also a relatively newly developed antipsychotic drug, interfered, but the most commonly used antidepressants and traditional antipsychotics and their metabolites did not interfere.

Keywords: Olanzapine

1. Introduction

Olanzapine belongs to the new generation of neuroleptic drugs introduced in the treatment of schizophrenia. The new drugs are atypical because compared with the older neuroleptics there is a better possibility of obtaining a sufficient clinical response without extrapyramidale side-effects, and additionally, they are considered more useful than the conventional drugs for treating the negative symptoms of schizophrenia [1,2]. Olanzapine, which is structurally related to clozapine (Fig. 1), is extensively metabolized in the liver via N-glucuronidation, hydroxylation, N-oxidation and N-demethylation but none of the different metabolites are clinically active [3]. The recommended daily dose of olanzapine in the treatment of schizophrenia is in the range 5–20 mg, but no therapeutic serum concentration level has been established [4]. Nevertheless, therapeutic drug monitoring (TDM) can be used to check for compliance and disclose drug–drug interactions. Furthermore, when more serum concentration data from patients treated with olanzapine have accumulated, it may be

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00205-9

possible to explain an insufficient therapeutic response by an unusually low concentration to dose ratio (C/D) in some cases, and if a correlation between some side-effects of olanzapine treatment and the serum concentration of the drug is found, serum monitoring might be useful in patients with disturbing side-effects.

For these reasons, we wanted to include serum olanzapine determinations in our TDM service, but the previously published two HPLC methods used electrochemical detection, which in our experience, is more demanding than UV detection [5,6]. Furthermore, at a psychiatric hospital most patients are given more than one psychotropic drug, and the possible interference of commonly used comedication was either not reported or insufficiently described, because only pure compounds and no metabolites were included [5,6]. During our preliminary studies we also became aware of the fact that the concentration of olanzapine in serum decreased significantly during the extraction procedure. Aravagiri et al. [6] used liquid–liquid extraction, and although the tubes containing the plasma samples and organic fluid were shaken for 10 min, the authors did not mention problems due to oxidation of olanzapine. Catlow et al. [5] used solid-phase extraction and found that addition of the antioxidant ascorbic acid to the buffer used to condition the cartridges increased extraction efficiency and decreased the variability of the recovery. The authors could use redox potential setting mode (+0.2 V for oxidation and −0.2 V for reduction) of the electrochemical detector for the quantitation of olanzapine, which shows that the first step in the oxidation of olanzapine is reversible.

The aim of the present study was to develop a simple, accurate method for the quantitative determination of olanzapine in serum and to investigate the most commonly comedicated psychoactive drugs and their metabolites for analytical interference in order to make the method suitable for routine TDM. We also wanted to investigate whether addition of ascorbic acid to serum was able to protect olanzapine against oxidation during the extraction procedure, and whether ascorbic acid was able to reduce the oxidised compound, which might have been formed during storage.

2. Experimental

2.1. Chemicals

Olanzapine, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-*b*] [1,5] benzodiazepine)(Fig. 1) was donated by Eli Lilly (Indianapolis, IN, USA) and trifluoperazine, used as internal standard (I.S.), was a gift from Rhône-Poulenc Rorer (Copenhagen, Denmark). HPLC grade heptane, isoamylalcohol, methanol and acetonitrile were from Fisons (Loughborough, UK). Ammonium acetate, sodium hydroxide and ammonium water, all analytical grade, were obtained from Merck (Darmstadt, Germany). Water was deionized and purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

Stock solutions were prepared by dissolving pure substances in ethanol: olanzapine at a concentration of 5 m*M* (1.562 mg/ml) and trifluoperazine in a concentration of 10 m*M* (4.804 mg/ml). The stock solutions were stored at −20°C. Further dilution was performed with ethanol–water (50:50, v/v) for olanzapine and with methanol for trifluoperazine.

Serum standards and controls containing known amounts of olanzapine were prepared by spiking serum from healthy drug-free donors. Ascorbic acid (0.25% (w/v)) was added to all standard and control sera as antioxidant (10 μl of 25% aqueous ascorbic acid/ml) before olanzapine was added, and the sera were stored at −20°C.

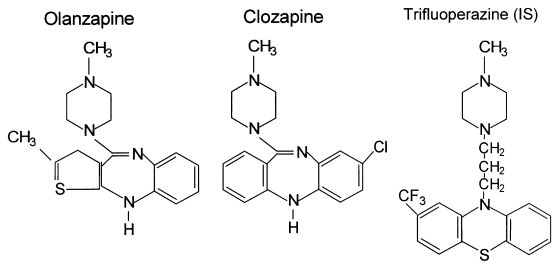

Fig. 1. Molecular structures of olanzapine, clozapine and trifluoperazine used as internal standard.

2.3. Extraction

In 12-ml centrifuge tubes, 1.0 ml of serum was mixed with 0.5 ml 0.5 *M* NaOH and 50 μl I.S., 0.77 μg/ml percloroperazin. A 5-ml volume of heptane–isoamylalcohol (97.5:1.5, v/v) was added, and the mixture was shaken for 5 min in horizontal position at 250 shakings/min on a HS 500 (Janke & Kunkel, Staufen, Germany) shaking apparatus. After centrifugation at 1500 *g* for 10 min, the aqueous layer was frozen by immersing the tubes into a cooling bath consisting of dry ice and ethanol. The heptane layer was decanted into centrifuge tubes and evaporated to dryness at 60°C in a gentle stream of nitrogen. The residue was dissolved in 75 μl of mobile phase of which 65 μl was injected into the chromatograph.

2.4. Chromatography and calculations

The chromatographic analysis was performed on a Perkin–Elmer (Norwalk, CT, USA) system consisting of a LC Model 250 pump, ISS-200 autosampler, LC 90 UV photometer set at 270 nm, and chromatograms were recorded by TURBOCHROM Navigator software (PE Nelson). The analytical column and mobile phase were as previously described for determination of clozapine [7]. A 150×4.6 mm Sperisorb S5W (Phase Separation, Queensferry, UK) was used, and the mobile phase was 50 m*M* ammonium acetate buffer adjusted to pH 9.9 with ammonia water–methanol (15:85, v/v). The mobile phase was degassed with helium before use. The flow-rate was 1.1 ml/min.

From recorded peak heights, the ratios of drug to I.S. were calculated. The results obtained from serum standards spiked with different known amounts of olanzapine were used to calculate the factor for multiplying the ratios between heights of unknown and I.S. peaks.

2.5. Protection of olanzapine by ascorbic acid

Ascorbic acid 0.25% (10 μl of an aqueous solution of 25% ascorbic acid/ml serum) was routinely added to serum samples from patients treated with olanzapine immediately after separation of serum from red blood cells by centrifugation, and the samples were stored at −18°C until analysis. The protective role of ascorbic acid against oxidation during extraction was studied by comparing the recovery of olanzapine from freshly prepared standards with and without addition of antioxidant. Other samples with and without ascorbic acid were stored at 4°C for 2 weeks or at room temperature for 24 h before analysis. Furthermore, the ability of ascorbic acid to reduce the oxidised olanzapine formed during storage was studied using samples from olanzapine treated patients. Half of the serum volume from patients was treated as described above and the other half kept at ambient temperature for 24 h, before ascorbic acid was added and the samples analyzed.

3. Results

3.1. Chromatography and recovery

A chromatogram of a serum blank from a healthy drug-free blood donor is shown in Fig. 2A. After the solvent peaks, no interfering peaks were detected, and Fig. 2B shows that olanzapine and the I.S. prochloroperazine were eluted within 5 min. A chromatogram of serum from a patient treated with olanzapine is shown in Fig. 2C. Fig. 2D shows that for routine analysis of unknown sera it was necessary to use 20 min for each chromatogram in order to avoid late eluting peaks from comedicated drugs appearing in the next chromatogram. The latter chromatogram also shows that the high-dose neuroleptic drug methotrimeprazine often used in combination with other psychotropic drugs as a sedative, did not disturb the quantitation of olanzapine.The recovery was calculated by comparing the peak heights after injection of olanzapine and the I.S. dissolved in mobile phase with the peak heights obtained after extraction of the same amount of the compounds from serum. The average recoveries of olanzapine and trifluoperazine were 81 and 87%, respectively.

3.2. Serum concentrations in patients, linearity

The serum concentrations of olanzapine were analyzed by the present method in 45 patients

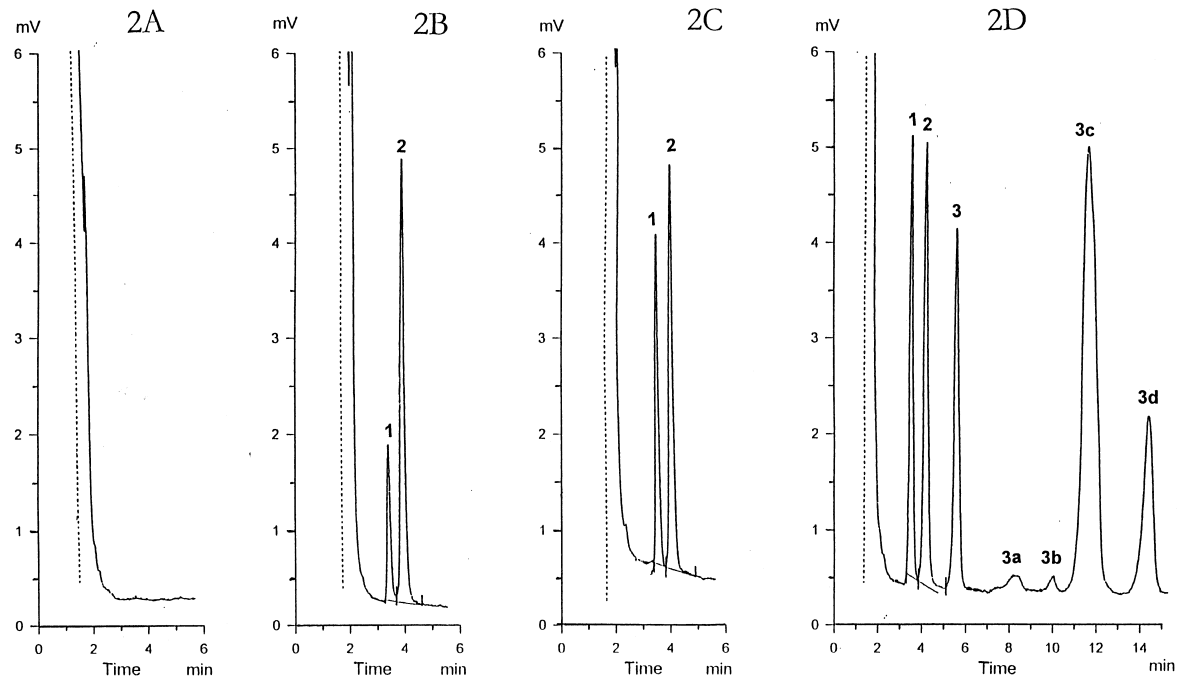

Fig. 2. Chromatograms of (A) 1-ml blank serum sample; (B) serum blank spiked with 15.6 ng/ml olanzapine and the internal standard trifluoperazine; (C) serum from a patient treated with olanzapine 10 mg/day. The measured concentrations of olanzapine was 35 ng/ml; (D) serum from a patient treated with 15 mg/day of olanzapine and 50 mg/day of methotrimeprazine. The measured concentration of olanzapine was 43 ng/ml. Peaks: 1=olanzapine, 2=trifluoperazine, 3=methotrimeprazine, 3a to d=methotrimeprazine metabolites.

referred to our TDM service for the first time. The 24-h dose (median and range) was 15 mg (5–22.5 mg), and the serum olanzapine concentration was 24.7 ng/ml (1.72–78.1 ng/ml). Ninety percent of the patients displayed serum olanzapine concentrations within the range 3–55 ng/ml. There was a great inter-individual variation in the olanzapine concentration obtained per mg daily dose (C/D). The median C/D was 1.84 (ng/l)/(mg/24 h), but the total range extended from 0.17 to 4.19 (ng/ml)/(mg/24 h), i.e. the ratio varied by a factor of 24.

3.3. Precision, and accuracy and linearity

The within-day and day-to-day precision and accuracy were evaluated by analyzing blank serum spiked with different amounts of olanzapine. The results are given in Table 1 which shows that the day-to-day variation was less than 8% within the concentration range found in 95% of the patients treated with olanzapine. The inter-day variability of the response factor was 5.97%. The lower level of quantitation was 1.56 ng/ml, and at this level the C.V. was about 20%.

Blank serum was spiked with olanzapine in the range 0–469 ng/ml and analyzed according to the method described. There was a linear correlation ($r=1.000$, $n=14$) between added and found olanzapine, and the equation of the regression line was $y=0.997x-2.4$.

3.4. Analysis for interference from other drugs

Sera from patients receiving drugs which may be used in combination with olanzapine, were analyzed in order to study interference with regard to olanzapine determination. The retention times are given in Table 2. It appears that none of the commonly used antidepressant drugs and sedatives are supposed to interfere, but the method does not allow determination of olanzapine in the presence of risperidone and its active metabolite 9-OH-risperidone.

Table 1
Precision and accuracy of the determination of olanzapine in spiked serum

Olanzapine (ng/ml)	*n*	Intra-day		Inter-day	
		C.V. (%)	Accuracy (%)	C.V. (%)	Accuracy (%)
1.56	5			19.8	107
4.69	12			4.69	110
4.69	9	4.05	113		
16.4	12			7.67	101
16.4	9	5.18	99		
46.9	10			5.17	98
46.9	9			4.52	103

3.5. Protective effects of ascorbic acid

The amount of ascorbic acid (0.25%) was chosen arbitrarily. A comparison of the recovery obtained when 0.125, 2.5 or 0.5% of ascorbic acid was added to serum spiked with olanzapine showed no difference. At three different serum levels (4.69, 15.62 and 46.9 ng/ml) the mean accuracy at the three different ascorbic acid concentrations was 99.3, 105 and 100%, respectively. Drug free serum with or without addition of 0.25% ascorbic acid was spiked with olanzapine and immediately extracted. Extraction reduced recovery by about 16% in the samples without antioxidant. Storage at 4°C for 1 or 2 weeks

Table 2
Retention time (t_R) of olanzapine, trifluoperazine (internal standard) and other drugs and metabolites

Compound	t_R (min)	Compound	t_R (min)
Olanzapine	3.58	*Tricyclic anti depressants*	
Trifluoperazine	4.20	Amitriptyline	7.58
Other neuroleptics		Clomipramine	7.73
Clozapine	2.90	Desmethyl-clomipramine	>15
Clozapine N-demethyl	6.19	Imipramine	9.51
Clozapine N-oxide	2.36	Desipramine	>15
Flupentixol	2.33	Nortriptyline	16.2
Haloperidol	3.20	10-OH-Nortriptyline	18.5
Perphenazine	2.82	*Selective serotonin reuptake inhibitors*	
Risperidone	4.26[a]	Citalopram	9.90
9-OH-Risperidone	3.41[b]	Desmethyl citalopram	16.8
Zuclopenthixol	2.45	Didesmethylcitalopram	10.8
Neuroleptics used as sedatives		Fluvoxamine	5.89
Chlorpromazine	6.53	Fluoxetine	11.1
Chlorprothixen	4.33	Norfluoxetine	12.0
Chlorprothixene metabolite	9.86	Paroxetine	15.6
Methotrimeprazine	5.26	*Other drugs*	
Demethylmethotrimeprazine	13.5	Hydroxyzine	2.43
Methotrimeprazine sulfoxide	14.2	Diltiazem	2.84
Thioridazine	12.3	Mianserine	2.77
Thioridazine metobolites 1, 2 and 3	>13	Venlafaxine	9.28
		Benzodiazepines	N.D

[a] Interference internal standard.
[b] Interference olanzapine.
N.D., not detected

Table 3
Serum olanzapine determinations in patients

A (ng/ml)	B (ng/ml)	Δ (%)
14.4	13.4	−6.5
17.8	17.2	−3.5
11.3	11.3	0
28.4	20.9	−26.4
12.5	12.2	−2.5
41.6	43.1	+3.8
45.9	46.6	+1.4
25.3	25.3	0
21.2	19.7	−7.4
45.6	45.6	0

A: According to Section 2.
B: Serum kept at room temp. for 24 h before addition of ascorbic acid.

diminished recovery in samples without antioxidant further to 45% and 13%, respectively. Storage at ambient temperature for 24 h led to about 40% decrease of recovery. Thus, both extraction and storage contributed to the loss of recovery. Half of the serum volume from ten different patients was treated routinely with ascorbic acid and the other half kept at ambient temperature for 24 h before ascorbic acid was added. The samples were analyzed and the results shown in Table 3. It appears that apart from one sample almost identical results were obtained, indicating that ascorbic acid was able to reduce the oxidised olanzapine formed during storage to the parent compound.

4. Discussion

The two methods previously described for determination of olanzapine in human plasma by HPLC were based on electrochemical detection and both gave 0.25 ng/ml as the lower level of quantitation when 1 ml serum was extracted. Catlow et al. [5] found serum olanzapine concentrations in patients treated with the drug (2.5 to 17.5 mg/day) in the range 4–55 ng/ml, and Aravagiri et al. [6] found serum concentrations in almost the same range in patients given daily doses of 10–20 mg. Ninety percent of our patients treated with doses of 5–22.5 mg/day had serum olanzapine concentrations within the range 3–55 ng/ml. Thus there is a good agreement between the three methods with respect to the serum concentration levels found in patients, and it is also apparent that our lower limit of quantitation of 1.56 ng/ml is sufficient for routine TDM.

Addition of an antioxidant to serum was necessary in order to prevent a loss of recovery of olanzapine during extraction and storage. Such a loss can easily be overlooked when standards, samples and controls are subjected to the same procedure. The easy oxidability of the compound is disclosed, when the absolute peak heights of freshly prepared standards are compared with standards kept in the refrigerator or at ambient temperature. Catlow et al. [5] found reduced and variable recovery, particularly at concentrations below 10 ng/ml and solved this problem by adding ascorbic acid to the phosphate buffer used to condition the cartridge before extraction. Furthermore, these authors used the reduction potential of −0.2 V for quantitation, and if olanzapine and a possible amount of the unknown oxidised compound are poorly separated on the column, this may also contribute to the reproducible results. Aravagiri et al. [6] solely used the second oxidation step of olanzapine for quantitation, i.e. a setting of 0.3 V for the screening electrode and 0.93 V for the analytical cell. The authors did not mention problems due to oxidation during extraction, but the mean inter-individual variability of the serum olanzapine concentration in seven patients on constant doses was about 40% or twice as high as the inter-individual variation of the C/D ratio in the same patients. This is quite unexpected considering the mean half-life of 30 h for olanzapine [8].

The use of unmodified silica as a column material and a mixture of methanol and aqueous buffer as the mobile phase was originally suggested by Jane in 1975 [9] for the separation of basic drugs. The chromatographic conditions for determination of olanzapine were almost identical to those we used for the determination of the structurally related clozapine in serum [7]. The method has turned out to be very robust and reproducible during 5 years of routine TDM of clozapine. Furthermore, only risperidone interfered with the determination, and since this drug also belongs to the new generation of neuroleptic drugs, comedication with olanzapine is unlikely. Other psychotropic drugs and sedatives commonly used in combination therapy did not

interfere, but due to the frequent use of comedication it was necessary routinely to use an elution time of 20 min for each chromatogram in order to avoid late eluting peaks appearing in the next chromatogram.

Acknowledgements

The authors are grateful for the excellent technical assistance of Mrs. Birgit Poulsen. This work was supported by Geert-Jørgensen's, Krista and Viggo Petersens' and Albert Nielsen's Foundations.

References

[1] E. Richelson, J. Clin. Psychiatry 57(Suppl. 11) (1996) 4.
[2] S.R. Marder, J. Clin. Psychiatry 57(Suppl. 11) (1996) 26.
[3] K. Kassahun, E. Mattiuz, E. Nyhart Jr, B. Obermeyer, T. Gillespie, A. Murphy, R.M. Goodwin, D. Tupper, J.T. Callaghan, L. Lemberger, Drug Metab. Dispos. 25 (1997) 81.
[4] C.B. Nemeroff, J. Clin. Psychiatry 58 (1997) 45.
[5] J.T. Catlow, R.D. Barton, M. Clemens, T.A. Gillespie, M. Goodwin, S.P. Swanson, J. Chromatogr. B 668 (1995) 85.
[6] M. Aravagiri, D. Ames, W.C. Wirshing, S.R. Marder, Ther. Drug Monit. 19 (1997) 307.
[7] O.V. Olesen, B. Poulsen, J. Chromatogr. 622 (1993) 39.
[8] L. Ereshefsky, J. Clin. Psychiatry 57(Suppl. 11) (1996) 12.
[9] I. Jane, J. Chromatogr. 111 (1975) 227.