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A sensitive high-performance liquid chromatographic method using electrochemical detection for the analysis of olanzapine and desmethylolanzapine in plasma of schizophrenic patients using a new solid-phase extraction procedure

Maria Augusta Raggi[a,*], G. Casamenti[a], R. Mandrioli[a], V. Volterra[b]

[a]*Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b]*Psychiatric Clinic, University of Bologna, Viale Pepoli 5, 40123 Bologna, Italy*

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Abstract

A high-performance liquid chromatographic method with amperometric detection for the analysis of the novel antipsychotic drug olanzapine and its metabolite desmethylolanzapine in human plasma has been developed. The analysis was carried out on a reversed-phase column (C_8, 150×4.6 mm I.D., 5 μm) using acetonitrile–phosphate buffer, pH 3.8, as the mobile phase. The detection voltage was +800 mV and the cell and column temperature was 30°C. The flow-rate was 1.2 ml min^{-1}. Linear responses were obtained between 5 and 150 ng ml^{-1}, with repeatability <3.3%. A careful pretreatment of the biological samples was implemented by means of solid-phase extraction (SPE) on C_8 cartridges. The method requires 500 μl of plasma for one complete analysis. Absolute recovery exceeded 97% for both olanzapine and desmethylolanzapine, and the detection limit was 1 ng ml^{-1} for both analytes. Repeatability, intermediate precision and accuracy were satisfactory. This sensitive and selective method has been successfully applied to therapeutic drug monitoring in schizophrenic patients treated with Zyprexa® tablets.

Keywords: Olanzapine; Desmethylolanzapine; Liquid chromatography; Amperometric detection; Solid-phase extraction; Human plasma

1. Introduction

Olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3*b*][1,5]benzodiazepine, Fig. 1) is an antipsychotic drug recently introduced in therapy. It has a chemical structure and therapeutic properties very similar to those of clozapine [1]. In fact, it appears to be effective against both positive (hallucinations, delusions) and negative (poverty of speech, social withdrawal) symptoms of schizophrenia [2,3], whereas 'traditional' antipsychotic drugs (e.g., phenothiazines, butyrophenones) are only effective in the treatment of the positive symptoms of this illness. The different therapeutic profiles can be explained by the different pharmacodynamic properties of the molecules. In fact, early theories explained the onset of schizophrenia as a dysfunction of dopaminergic transmission, and thus, the tradition-

*Corresponding author. Tel.: +39-51-209-9700; fax: +39-51-209-9734.

E-mail address: raggima@alma.unibo.it (M.A. Raggi).

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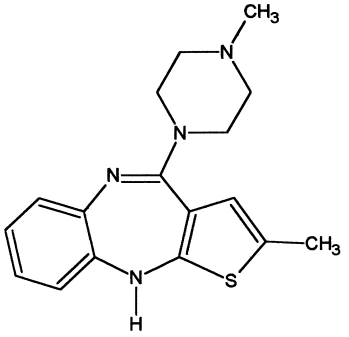
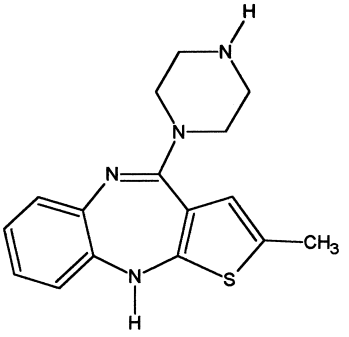
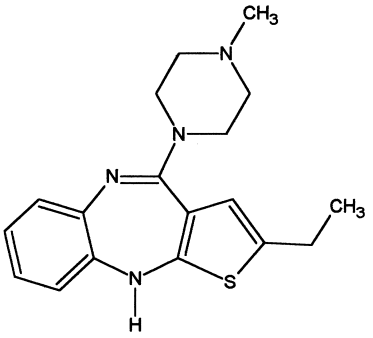


Olanzapine



N-desmethylolanzapine



2-methylolanzapine

Fig. 1. Chemical structures of olanzapine, desmethylolanzapine and 2-methylolanzapine.

al antipsychotics were typically antagonists of the central D_2 dopaminergic receptors. On the contrary, today schizophrenia is believed to be a multifactorial disease [4,5] which can be treated most effectively using drugs which interact with multiple neurotransmitter systems [6]. Olanzapine is one of these drugs and shows high affinity for dopamine D_1, D_2, D_4, serotonin 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, α_1-adrenergic, histamine H_1 and muscarinic receptors [7–10]. Olanzapine, like clozapine, does not cause extrapyramidal motor side effects like traditional antipsychotic drugs (e.g., haloperidol) [11]; unlike clozapine [6,12], however, olanzapine seems not to cause agranulocytosis [1].

This is not to say that treatment with olanzapine does not cause adverse effects. The most common of these are somnolence, constipation, weight gain and dry mouth [6,13], which rarely require discontinuation of the treatment.

Olanzapine is extensively metabolised by the cytochromes P450 (CYP), and in particular by the isozymes CYP1A2 and CYP2D6 [14]; one of the main metabolites thus formed is *N*-desmethylolanzapine [15,16].

The levels of olanzapine in the plasma of treated schizophrenic patients should be monitored. In fact, the therapeutic response seems to be correlated to the plasma olanzapine concentration [16] which in turn tends to increase with the daily dosage [17]; reliable data on the concentration of olanzapine in plasma, however, are still insufficient.

Due to the high efficacy of the drug, olanzapine is

administered to patients at very low dosages (generally from 2 to 20 mg daily [1]); this means that the plasma levels of the drug and its metabolites are very low. Therefore, very sensitive and reliable methods are necessary in order to determine the plasma concentrations of olanzapine and its metabolites.

Several techniques have been reported in the literature for the determination of olanzapine levels in biological fluids: gas chromatography [18,19], HPLC with electrochemical [12,19] or UV detection [21], liquid chromatography–tandem mass spectrometry [22], liquid chromatography–atmospheric pressure chemical ionization mass spectrometry [23].

The GC techniques with nitrogen detection and the HPLC techniques with UV detection generally lack the necessary sensitivity to perform a therapeutic monitoring when low daily dosages are administered, unless complicated and time-consuming concentration procedures are used (i.e., the sample has to be dried and then redissolved; high-volume injection loops have to be used, leading to a dramatic decrease in the column lifespan). On the other hand, the HPLC procedures with electrochemical (coulometric) detection show good sensitivity, but are often laborious generally due to the sample pretreatments by means of either liquid–liquid extraction [22] or solid-phase extraction (SPE) [12].

Most papers determine the olanzapine levels in plasma; only one of them, to our knowledge, quantifies desmethylolanzapine (in rat plasma) as well [24] using an HPLC method with coulometric detection and an SPE procedure, which however does not give a high enough extraction yield.

Following our previous study on the analysis of only olanzapine levels in human plasma [25], we describe here a new procedure for the simultaneous determination of olanzapine and its metabolite *N*-desmethylolanzapine in human plasma using high efficiency SPE. The proposed method was successfully applied to the analysis of the plasma of some patients treated with Zyprexa® tablets. This work is part of a wider study which is aimed at finding a correlation between the olanzapine dosages administered to patients and the levels of olanzapine and desmethylolanzapine in plasma. These results can lead to an improvement in the therapy of patients and a reduction in side effects.

2. Experimental

2.1. Chemicals

Eli Lilly (Indianapolis, IN, USA), kindly donated olanzapine, *N*-desmethylolanzapine and 2-methylolanzapine (used as the internal standard (I.S.)). Novartis Italia (Origgio, Milan, Italy) kindly donated clozapine and clotiapine. Carbamazepine, lorazepam, triprolidine hydrochloride, protriptiline hydrochloride, haloperidol and imipramine hydrochloride were purchased from Sigma. Clonazepam and flurazepam were kindly donated from Roche Pharmaceuticals (Basel, Switzerland). Paroxetine hydrochloride hemihydrate, lorazepam, gabapentin, amoxapine, risperidone and phenobarbital were gifts of SmithKline Beecham Pharmaceuticals (Melbourne, Victoria, Australia), Wyeth Laboratories (Taplow, Maidenhead, Berkshire, UK), Parke Davis Pharmaceuticals (Ann Arbor, MI, USA), Lederle Laboratories (Gosport, Hampshire, UK), Janssen-Cilag Italia (Borgo S. Michele, Latina, Italy) and Rhône-Poulenc Rorer Italia (Milan, Italy), respectively.

Acetonitrile and methanol were HPLC grade from Carlo Erba (Milan, Italy). Phosphoric acid (85%, m/m), potassium dihydrogen phosphate (KH_2PO_4), triethylamine were analytical grade from Carlo Erba. Ultrapure water (18.2 MΩ cm) was obtained by means of a Millipore (Milford, MA, USA) Milli-Q apparatus.

2.2. Apparatus and chromatographic conditions

For the voltammetric assays an AMEL (Milan, Italy) Model 433 voltammeter was used.

The chromatographic apparatus consisted of a Varian (Harbor City, CA, USA) Model 9002 chromatographic pump and an Antec (DB Leiden, The Netherlands) Decade amperometric detector (working electrode, glassy carbon; reference electrode, Ag/AgCl; auxiliary electrode, stainless steel 316) set at +800 mV.

A Jasco (Tokyo, Japan) Model PU-980 chromatographic pump and a Jasco UV-975 detector (λ=260 nm) were also used.

Separation was achieved on a Varian ResElut

reversed-phase column (C_8, 150×4.6 mm I.D., 5 μm), with a Varian ResElut precolumn (C_8, 30×4.6 mm I.D., 5 μm). Column, precolumn and detector cell were thermostatted at 30°C. The mobile phase was a mixture of acetonitrile (20%) and pH 3.8 phosphate buffer (15.4 mmol l^{-1}) containing 19.7 m*M* triethylamine (80%). This mixture has an apparent pH of 4.3. It was filtered (Millipore membrane filters: nylon, 47 mm diameter, 0.2 μm pore size) and degassed by ultrasonication (Transsonic T-310 apparatus from Elma, Singen, Germany) before use. The flow-rate was 1.2 ml min^{-1} and the injection loop was 20 μl. Data were handled by means of Varian Star Chromatography software running on an 80486 processor.

2.3. Patients and sample collection

The blood samples were drawn from six schizophrenic patients of the Psychiatric Clinic of the University of Bologna, subjected to treatment with Zyprexa® tablets at a constant daily dose for at least 4 weeks. Blood samples were obtained immediately before a first daily dose of olanzapine and 12 h after the last dose.

Blood samples were drawn into test tubes, containing EDTA, and centrifuged at 1400 *g* for 20 min. The supernatant plasma was transferred into test tubes and frozen at −20°C until analysis, usually within 1 week.

This procedure was also used to separate the plasma from the blood of healthy volunteers ('blank' plasma).

2.4. Method selectivity

Standard solutions of several drugs active on the Central Nervous System and commonly used in the Psychiatric Clinic were injected into the HPLC. The drugs tested were: amoxapine, clotiapine, clozapine, haloperidol, risperidone, imipramine, paroxetine, protriptiline, triprolidine, clonazepam, flurazepam, lorazepam, carbamazepine, gabapentin and phenobarbital.

When clozapine (t_R=60.3 min) was injected, the column was flushed for 15 min at a flow-rate of 2 ml min^{-1} after the third run.

2.5. Solutions and SPE procedure

Stock solutions of olanzapine, desmethylolanzapine and 2-methylolanzapine (I.S.) were 1 mg ml^{-1} in methanol. All subsequent dilutions were in the mobile phase, brought to pH* 2.0 with phosphoric acid.

The solid-phase extraction procedure was carried out on IST (Hengoed, Mid Glamorgan, South Wales, UK) StepBio C_8 cartridges (100 mg, 1 ml). The cartridges were activated with 1 ml of methanol two times and conditioned with 1 ml of water five times. After loading the cartridge with 500 μl of patient plasma (or blank plasma) diluted with 500 μl of water and spiked with 25 ng ml^{-1} of the I.S., the cartridge was washed with 1 ml of water two times and dried by applying full vacuum (ca. 40 kPa) for 30 s. The analytes were eluted from the cartridge with 500 μl of the mobile phase brought to pH* 2.0 with phosphoric acid, applying full vacuum again for 30 s.

2.6. HPLC method

2.6.1. Absolute recovery and precision

Known amounts of olanzapine and desmethylolanzapine standard solution were added to 500 μl of blank plasma, to obtain concentrations of 5, 25 and 100 ng ml^{-1}, then subjected to the above described analytical procedure. The analyte peak areas were then compared to the areas obtained injecting standard solutions at the same nominal concentration, obtaining the absolute recovery or extraction yield. The procedure was repeated six times, and values of percentage relative standard deviations (RSD%) were obtained for repeatability (intra-day precision) and intermediate precision (inter-day precision).

2.6.2. Calibration curves

Standard solutions of the two analytes having different concentrations in the 5–150-ng ml^{-1} range were added to known amounts of blank plasma, then subjected to the above described analytical procedure. Plotting the analyte peak area value against the

analyte concentration, the 10-point calibration curves were set up.

2.6.3. Sample analysis

The area values of the peaks obtained injecting the eluates from the SPE procedure into the HPLC were interpolated on the respective calibration curves.

2.6.4. Accuracy assays

Known amounts of olanzapine, desmethylolanzapine and I.S. solutions were added to known amounts of plasma samples taken from patients. The method accuracy was evaluated by calculating the difference between the spiked sample peak areas and the original sample peak areas, then comparing these differences with the peak areas obtained by injecting standard solutions having the same concentration as the sample spiking.

3. Results and discussion

3.1. Preliminary studies

Our previous paper on the quantitative determination of olanzapine alone in human plasma [27] prompted us to develop a new HPLC method with amperometric detection, in order to also monitor the plasma levels of the main metabolite desmethylolanzapine.

N-Desmethylolanzapine, like olanzapine, is an electroactive compound which can easily be oxidised, as we found by means of electrochemical experiments. The same is true of 2-methylolanzapine which was used in this study as the I.S. [12,26] for the control of retention times only. The linear scan voltammograms (LSV) of olanzapine (a), desmethylolanzapine (b) and 2-methylolanzapine (c) standard solutions are shown in Fig. 2; the voltammogram of the supporting electrolyte (a pH 3.8, 15.4 mmol l^{-1}

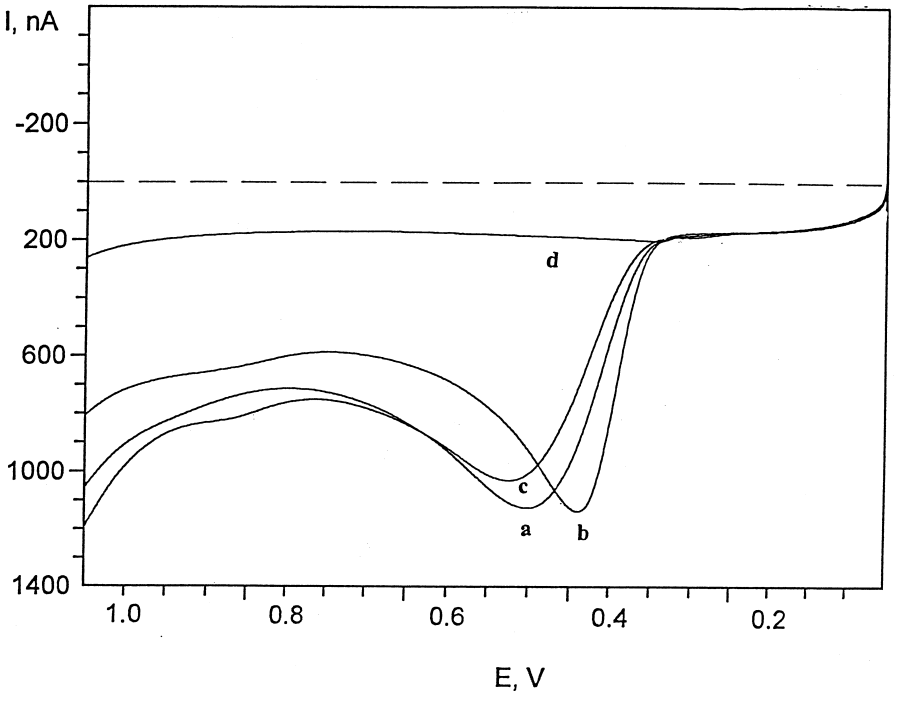


Fig. 2. Voltammograms of (a) a 25 μg ml^{-1} olanzapine standard solution; (b) a 25 μg ml^{-1} desmethylolanzapine standard solution; (c) a 25 μg ml^{-1} I.S.; (d) the supporting electrolyte.

phosphate buffer (d)) is also included. As can be seen, each compound gives rise to an anodic wave which reaches a maximum intensity value in the 400–500 mV potential range; the compounds are thus suitable for electrochemical detection coupled with liquid chromatography.

3.2. HPLC conditions

Based on the above-mentioned results, we used an HPLC procedure with amperometric detection and the potential of the electrochemical cell was set at +800 mV in order to be certain that the three analytes were oxidised even under different conditions; some experiments were also carried out at lower potential values (250 mV) according to literature [12], the results were however not satisfactory, as could be expected given the reported voltammograms (Fig. 2). This fact can be explained by the different pH value of the buffer used by the other author [12] (pH 7.0, instead of pH 4.3) for the HPLC experiments. In fact, it has been recently found [26] that the pH of the solution heavily influences the oxidation of olanzapine which is oxidised at lower potential values if the pH is increased.

The mobile phase used in our previous paper for the determination of olanzapine [27] did not allow for the determination of desmethylolanzapine levels because under those experimental conditions the analyte was not detected, probably because it has a very short retention time and its peak is masked by the injection peak. Thus, it was necessary to select a mobile phase with a different composition.

Peak retention times were enhanced both by raising the pH (from 2.0 to 3.8) and by lowering the organic modifier percentage. The influence of the organic modifier on the retention times of the analyte peaks at pH 3.8 can be seen from Fig. 3. It is apparent that all retention times decrease, at different rates, when the percentage of the organic modifier is increased from 15 to 30%. For this reason, we chose to work at an acetonitrile percentage of 20% in order to not have very long analysis times. For the same reason, the flow-rate was raised from 1.0 to 1.2 ml min^{-1} which led to a further decrease in the chromatographic run times. Furthermore, triethylamine was added to lessen peak asymmetry.

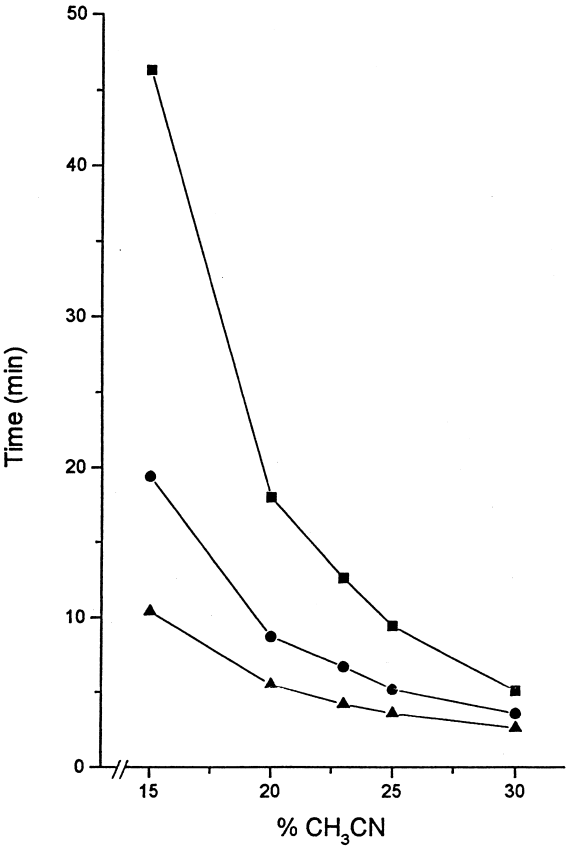


Fig. 3. Effect of the percent volume of organic modifier in the mobile phase on analyte separation. (▲) Desmethylolanzapine (●) olanzapine; (■) I.S.

The temperature previously used for the column and the detector [27] was decreased from 50 to 30°C to increase the column lifespan and prevent electrode fouling.

Therefore, the optimal leading conditions were obtained using a C_8 column (150×4.6 mm I.D., 5 μm) and a mobile phase composed of acetonitrile and 15.4 mmol l^{-1} phosphate buffer, pH 3.8, containing 19.7 m*M* triethylamine (20:80, v/v), flowing at 1.2 ml min^{-1}, and carrying out the detection at +800 mV and at 30°C. Under these conditions, olanzapine, desmethylolanzapine and the I.S. are chromatographically well separated and have retention times (t_R) of 6.1, 3.9 and 12.6 min, respectively.

3.3. Stability studies

At the acidic pH value selected for the mobile phase, the standard solutions of the analytes were found to be sufficiently stable for the analysis. Thus, it was not deemed necessary to add ascorbic acid to the solutions in order to block the oxidation process of olanzapine during the extraction procedure as reported by other authors [12,23] who worked at higher pH values (pH≥7).

3.4. Implementation of a new SPE procedure

The implementation of a careful procedure for the extraction of olanzapine from biological matrices is a very important step in the method development.

One paper [23] proposes a liquid–liquid extraction method which has satisfactory absolute recovery but is very laborious. Other papers propose SPE procedures [12,26]. Both procedures are rather complicated and time-consuming (involving several washing steps with buffers and other solutions) and do not give satisfactory extraction yields (72–78% [12], 57–78% [26]). The SPE method we previously used [27], based on the use of BondElut C_8 cartridges, gave a good extraction yield for olanzapine; it did however introduce a 4-fold dilution of the sample which therefore decreases the method sensitivity. In order to increase the sensitivity of the method, we used a different kind of cartridge, namely Isolute C_8 cartridges (100 mg, 1 ml) instead of BondElut C_8 cartridges (50 mg, 1 ml), and tested various eluents to minimise elution volumes: methanol, the mobile phase, a mobile phase–acetonitrile mixture (80/20, v/v) and the mobile phase brought to a lower pH value (pH* 2.0). Best results were obtained using the last solution: only 500 μl of this modified mobile phase were needed to elute the analytes with a good extraction yield, after loading the cartridge with 500 μl of plasma. In this way, no dilution of the sample occurs, and the sensitivity of the assay is increased 4-fold with respect to the previous paper.

3.5. Method validation

The chromatogram of a blank plasma sample spiked with 25 ng ml^{-1} of the I.S. is shown in Fig. 4a, while the chromatogram of the same blank plasma sample spiked with 25 ng ml^{-1} of olanzapine, desmethylolanzapine and the I.S., is shown in Fig. 4b. The latter chromatogram is almost identical to that of a standard solution and no interference is present in either chromatogram.

This new SPE procedure gave good results with respect to selectivity and reproducibility (Table 1). The absolute recovery data were obtained by injecting blank plasma samples to which 5, 25 or 100 ng ml^{-1} of olanzapine and desmethylolanzapine were added.

The extraction yield was very satisfactory: mean values of 99.3 and 100.0% (for olanzapine and desmethylolanzapine, respectively) were obtained, while the mean extraction yield for the I.S. was

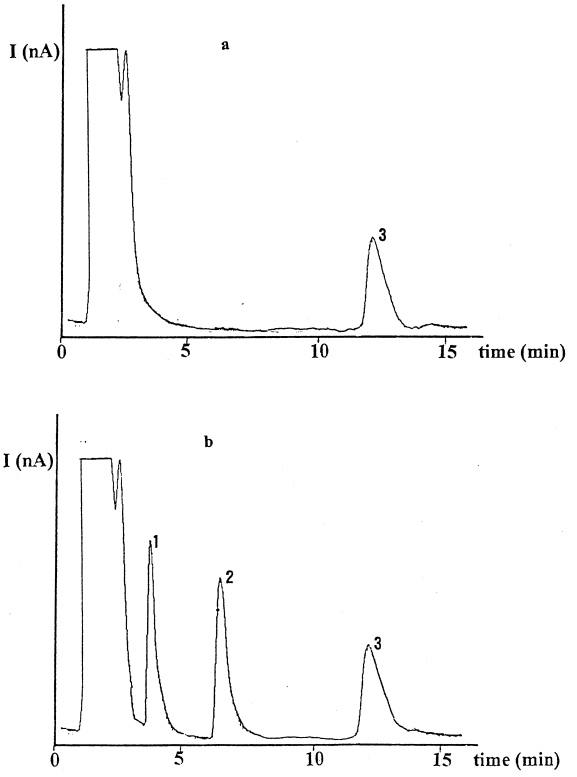


Fig. 4. Chromatograms of a blank plasma sample after the SPE procedure, spiked with 25 ng ml^{-1} of the I.S. (a) and the same sample spiked with 25 ng ml^{-1} of the analytes and the I.S. (b). Peaks: (1) desmethylolanzapine, (2) olanzapine; (3) I.S.

Table 1
Assay characteristics

Compound	Linearity range (ng ml^{-1})	Amount added to blank plasma (ng ml^{-1})	Repeatability (RSD%)	Intermediate precision (RSD%)	Absolute recovery (%)
Olanzapine	5–150	5	3.5	3.8	101.0
		25	2.3	2.8	98.1
		100	1.6	2.4	98.8
Desmethylolanzapine	5–150	5	3.2	3.9	102.9
		25	2.1	2.3	97.4
		100	2.2	2.2	99.8
Internal standard	–	25	2.0	2.2	95.1

95.1%. The mean RSD% value for repeatability was 2.5 (for both olanzapine and desmethylolanzapine, $n=6$), while the mean RSD% values for intermediate precision were 3.0 (for olanzapine) and 2.8 (for desmethylolanzapine) ($n=6$). The overall results of these assays are summarised in Table 1.

Linearity was found between 5 and 150 ng ml^{-1} for both analytes, and the least-square regression equations were $y=6485x+2364$ ($r=0.9996$) for olanzapine and $y=5058x+5716$ ($r=0.9990$) for desmethylolanzapine, where x is the analyte concentration expressed as ng ml^{-1}, and y is the peak area value of the analyte expressed as arbitrary area units. The limit of quantitation (LOQ), calculated according to the USP XXIV guidelines [27], was 3 ng ml^{-1} for both olanzapine and desmethylolanzapine; the limit of detection (LOD) was 1 ng ml^{-1} for both analytes.

3.6. Method selectivity

Selectivity studies were carried out because psychiatric patients are often subjected to treatment with multiple CNS drugs which could potentially interfere with the analytical determination of olanzapine and desmethylolanzapine. For methodological reasons the results obtained by means of the HPLC-ED apparatus were compared to those obtained by means of an HPLC-UV apparatus set at 260 nm. The drugs tested for interference were other antipsychotics, antidepressants, benzodiazepines and antiepileptics (used as mood stabilizers). The results are reported in Table 2 and clearly demonstrate the superior selectivity of the electrochemical detection over traditional UV detection; in fact, none of the tested drugs was detected by means of the HPLC-ED method within 60 min of run.

3.7. Application to plasma samples of patients

Having thus validated the method, it was then

Table 2
Retention times (t_R) of olanzapine, desmethylolanzapine, 2-methylolanzapine (I.S.) and other drugs tested for interference

Compound	HPLC-ED t_R (min)	HPLC-UV t_R (min)
Desmethylolanzapine	3.9	3.9
Olanzapine	6.1	6.0
2-Methylolanzapine	12.6	12.5
Other antipsychotics		
Amoxapine	n.d.	n.d.
Clotiapine	n.d.	n.d.
Clozapine	60.7	53.6
Haloperidol	n.d.	n.d.
Risperidone	n.d.	18.5
Antidepressants		
Imipramine	n.d.	n.d.
Paroxetine	n.d.	n.d.
Protriptiline	n.d.	41.3
Triprolidine	n.d.	34.4
Benzodiazepines		
Clonazepam	n.d.	n.d.
Flurazepam	n.d.	49.6
Lorazepam	n.d.	20.3
Antiepileptics used as mood stabilizers		
Carbamazepine	n.d.	55.6
Gabapentin	n.d.	n.d.
Phenobarbital	n.d.	15.2

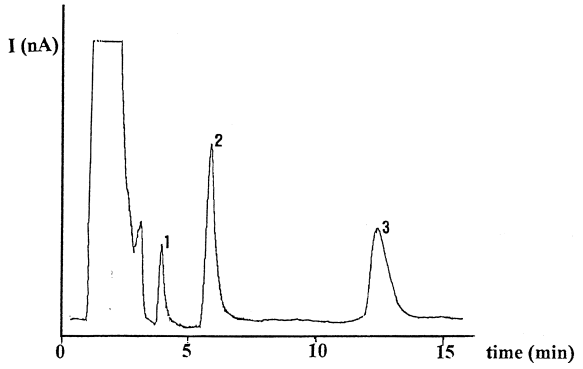


Fig. 5. Chromatogram after the SPE procedure of a plasma sample from a patient who received 20 mg of olanzapine daily. The analyte concentrations found were: 32.6 ng ml^{-1} of olanzapine and 12.3 ng ml^{-1} of desmethylolanzapine. Peaks: (1) desmethylolanzapine; (2) olanzapine; (3) I.S.

applied to the plasma of patients undergoing therapy with olanzapine. The chromatogram of a plasma sample from a patient receiving 20 mg/day of olanzapine as Zyprexa® tablets, after the SPE procedure, is shown in Fig. 5; again, no interference is present, thus highlighting the good selectivity of the method. In this chromatogram olanzapine is detected at t_R=6.1 min, desmethylolanzapine at t_R=3.9 min and the I.S. at t_R=12.6 min. The analyte concentrations in plasma, obtained by interpolation on the appropriate calibration curves, were found to be: 32.6 ng ml^{-1} of olanzapine and 12.3 ng ml^{-1} of desmethylolanzapine.

Accuracy was assessed from patient plasma spiked with three different concentrations of each analyte. The results are reported in Table 3 and are very near to 100% in all cases, demonstrating the good accuracy of the method.

Some plasma samples were analysed by means of both the HPLC-ED apparatus and the HPLC-UV apparatus. The results obtained for olanzapine are in good agreement between them, the HPLC-UV method, however, allows only for a reliable determination of high plasma levels of olanzapine, namely concentrations higher than 25 ng/ml. With regard to desmethylolanzapine, it was not quantifiable by means of the HPLC-UV method because all samples had desmethylolanzapine levels lower than the LOQ.

Table 3
Recovery assays

Concentration added (ng ml^{-1})	Mean recovery values (%)±SD[a]	
	Olanzapine	Desmethylolanzapine
5	95.2±2.2	93.3±3.6
25	100.3±0.4	96.3±1.0
50	99.5±0.2	97.7±0.7

[a] n=3.

The patients whose blood was analysed received daily olanzapine doses between 10 and 20 mg (average, 15.8 mg) and the mean plasma concentrations of the analytes were 43.2 ng ml^{-1} for olanzapine (ranging from 12.6 to 86.4 ng ml^{-1}) and 10.1 ng ml^{-1} for desmethylolanzapine (ranging from 5.2 to 15.0 ng ml^{-1}). These results are in satisfactory agreement with those found by other authors, who report daily doses between 2.5 and 17.5 mg of olanzapine which result in olanzapine plasma levels between 4 and 55 ng ml^{-1} [12] and daily doses between 5 and 22.5 mg of olanzapine with resulting plasma olanzapine levels in the 1.7–78 ng ml^{-1} range [23].

4. Conclusion

The method proposed herein, based on HPLC-ED, can reliably determine olanzapine and desmethylolanzapine in human plasma, with good accuracy and precision. Furthermore, it is more sensitive with respect to the previously published method [27] thanks to the improved SPE procedure.

In addition, this new SPE procedure is faster and more feasible than the liquid–liquid extraction procedure proposed by other authors [23], and gives better extraction yields than other SPE procedures [12,26]. Thanks to the electrochemical detection, the analytical method is very sensitive (LOD=1 ng/ml) and selective, in fact 15 CNS drugs were tested and none of them caused any interference on the determination of olanzapine and desmethylolanzapine.

This method has the advantage of being rapid and allows for the simultaneous determination of low levels of olanzapine and its metabolite desmethylolanzapine in human plasma, hence it seems to be suitable for therapeutic drug monitoring. This study

will thus continue with the analysis of a larger number of blood samples, the quantitation of olanzapine, desmethylolanzapine and possibly other metabolites and the study of olanzapine pharmacokinetic changes due to the simultaneous administration of other drugs commonly used in the psychiatric clinic.

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References

[1] B. Fulton, K.L. Goa, Drugs 53 (1997) 281.
[2] C.M. Beasley Jr., G. Tollefson, P. Tran, W. Satterlee, T. Sanger, S. Hamilton, Neuropsychopharmacology 14 (1996) 111.
[3] J. Gerlach, L. Peacock, Int. Clin. Psychopharmacol. 10 (1995) 39.
[4] N.C. Andreasen, Lancet 346 (1995) 477.
[5] A. Carllson, Int. Clin. Psychopharmacol. 10 (1995) 21.
[6] G.D. Tollefson, C.M. Beasley, P.V. Tran, J.S. Street, J.A. Krueder, R.N. Tamura, K.A. Graffeo, M.E. Thieme, Am. J. Psychiatry 154 (1997) 457.
[7] N.A. Moore, D.O. Calligaro, D.T. Wong, F. Bymaster, N.C. Tye, Curr. Opin. Invest. Drugs 2 (1993) 281.
[8] F.P. Bymaster, D.O. Calligaro, J.F. Falcone, Neuropsychopharmacol. 14 (1996) 87.
[9] F.P. Bymaster, S.K. Hemrick-Luecke, K.W. Perry, R.W. Fuller, Psychopharmacol. 124 (1996) 87.
[10] J.T. Catlow, R.D. Barton, M. Clemens, T.A. Gillespie, M. Goodwin, S.P. Swanson, J. Chromatogr. B 668 (1995) 85.
[11] C.M. Beasley Jr., G. Tollefson, P. Tran, W. Satterlee, T. Sanger, S. Hamilton, Neuropsychopharmacol. 14 (1996) 111.
[12] R.J. Baldessarini, F.R. Frankenburg, New Engl. J. Med. 324 (1991) 746.
[13] C.M. Beasley, T. Sanger, W. Satterlee, Psychopharmacology 124 (1996) 159.
[14] B.J. Ring, J. Catlow, T.J. Lindsay, T. Gillespie, L.K. Roskos, B.J. Cerimele, S.P. Swanson, M.A. Hamman, S.A. Wrighton, J. Pharmacol. Exp. Ther. 276 (1996) 658.
[15] 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.
[16] P.J. Perry, T. Sanger, C. Beasley, J. Clin. Psychopharmacol. 17 (1997) 472.
[17] M. Aravagiri, D. Ames, W.C. Wirshing, S.R. Marder, Ther. Drug Monit. 19 (1997) 307.
[18] L. Xue, S.B. Crookham, F.X. Diamond, D.J. Davis, T.M. Reiber, Clin. Chem. 44 (1998) 103.
[19] A.J. Jenkins, K.M. Sarconi, H.N. Raaf, J. Anal. Toxicol. 22 (1998) 605.
[20] S.C. Kasper, E.L. Mattiuz, S.P. Swanson, J.A. Chiu, J.T. Johnson, C.O. Garner, J. Chromatogr. B 726 (1999) 203.
[21] O.V. Olesen, K. Linnett, J. Chromatogr. B 714 (1998) 309.
[22] M. Berna, R. Shugert, J. Mullen, J. Mass Spectrom. 33 (1998) 1003.
[23] M.J. Bogusz, K.D. Kruger, R.D. Maier, R. Erkwoh, F. Tuchtenhagen, J. Chromatogr. B 732 (1999) 257.
[24] J.A. Chiu, R.B. Franklin, J. Pharm. Biomed. Anal. 14 (1996) 609.
[25] M.A. Raggi, G. Casamenti, R. Mandrioli, S. Fanali, D. De Ronchi, V. Volterra, Chromatographia 51 (2000) 562.
[26] M.A. Raggi, G. Casamenti, R. Mandrioli, G. Izzo, E. Kenndler, J. Pharm. Biomed. Anal., in press.
[27] United States Pharmacopeia XXIV – National Formulary XIX, United States Pharmacopeial Convention, Rockville, MD, 2000, pp. 2000–2002.