ELSEVIER

Journal of Chromatography B, 714 (1998) 299–308

JOURNAL OF CHROMATOGRAPHY B

Determination of clozapine, desmethylclozapine and clozapine *N*-oxide in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection

Angela Avenoso[a], Gabriella Facciolà[a], Giuseppe Maurizio Campo[a], Antonio Fazio[b], Edoardo Spina[a,*]

[a] *Institute of Pharmacology, School of Medicine, University of Messina, P.zza XX Settembre 4, I-98122 Messina, Italy*
[b] *Institute of Neurological and Neurological Sciences, University of Messina, I-98122 Messina, Italy*

Received 13 January 1998; received in revised form 22 April 1998; accepted 27 April 1998

Abstract

An isocratic high-performance liquid chromatography (HPLC) method with ultraviolet detection for the simultaneous determination of clozapine and its two major metabolites in human plasma is described. Analytes are concentrated from alkaline plasma by liquid–liquid extraction with *n*-hexane–isoamyl alcohol (75:25, v/v). The organic phase is back-extracted with 150 μl of 0.1 *M* dibasic phosphate (pH 2.2 with 25% H_3PO_4). Triprolidine is used as internal standard. For the chromatographic separation the mobile phase consisted of acetonitrile–0.06 *M* phosphate buffer, pH 2.7 with 25% phosphoric acid (48:52, v/v). Analytes are eluted at a flow-rate of 1.0 ml/min, separated on a 250×4.60 mm I.D. analytical column packed with 5 μm C_6 silica particles, and measured by UV absorbance detection at 254 nm. The separation requires 7 min. Calibration curves for the three analytes are linear within the clinical concentration range. Mean recoveries were 92.7% for clozapine, 82.0% for desmethylclozapine and 70.4% for clozapine *N*-oxide. C.V. values for intra- and inter-day variabilities were ≤13.8% at concentrations between 50 and 1000 ng/ml. Accuracy, expressed as percentage error, ranged from −19.8 to 2.8%. The method was specific and sensitive with quantitation limits of 2 ng/ml for both clozapine and desmethylclozapine and 5 ng/ml for clozapine *N*-oxide. Among various psychotropic drugs and their metabolites, only 2-hydroxydesipramine caused significant interference. The method is applicable to pharmacokinetic studies and therapeutic drug monitoring.

Keywords: Clozapine; Desmethylclozapine; Clozapine *N*-oxide

1. Introduction

Clozapine, a dibenzodiazepine derivative, is an atypical antipsychotic agent with proven efficacy in the management of treatment-resistant schizophrenia and other psychotic disorders [1,2]. Unlike traditional antipsychotics, clozapine has a low propensity for extrapyramidal side effects and minimal effect on prolactin secretion. Despite its therapeutic potential, wider use of clozapine has been limited by the high risk of agranulocytosis occurring in 1–2% of the patients [3], which makes frequent haematological monitoring necessary. Clozapine is extensively metabolized in the liver by cytochrome P450 iso-

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00202-3

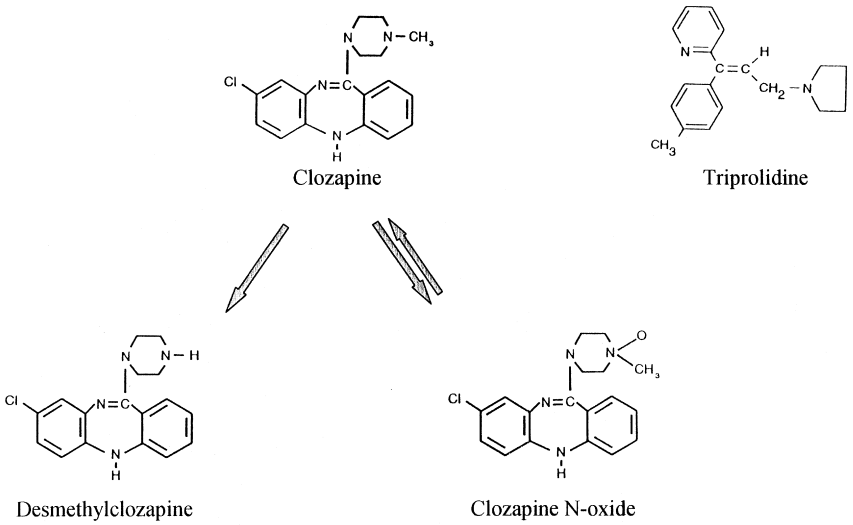

Fig. 1. Structures of triprolidine (I.S), clozapine and its two major metabolites desmethylclozapine and clozapine *N*-oxide.

enzymes yielding several derivatives, mainly desmethylclozapine, which is pharmacologically active, and clozapine *N*-oxide (Fig. 1) [4]. Although a well defined relationship between plasma clozapine concentrations and effects has not yet been documented, the large inter-individual variability in bioavailability and elimination, the potential for clinically relevant pharmacokinetic interactions with other drugs and the high probability of patient noncompliance suggest that therapeutic drug monitoring may be useful in the clinical management of patients treated with clozapine [5].

Several analytical methods have been described for the quantification of clozapine and its metabolites in human plasma or serum, based on a variety of detection and separation techniques. The first published methods include gas chromatography with nitrogen-specific [6,7] or mass spectrometric [8] detection. These techniques do not determine clozapine metabolites and require long analysis time or expensive equipment and therefore are not suitable for routine analysis. On the other hand, high-performance liquid chromatography (HPLC) assays have been published [9–21], all of which use a reversed-phase separation and both liquid–liquid [9,10,12,17,18,21] or solid-phase [11,13,15,19,20] extraction. Only a few methods for the simultaneous determination of clozapine and its two main metabolites have been reported [11,15,18,20,21] and some require time consuming chromatographic analysis. In addition, clozapine *N*-oxide is not always detected. Since the pathway between clozapine and clozapine *N*-oxide is reversible [22–24], even if clozapine *N*-oxide is inactive, it may contribute to a longer duration of action of clozapine than predicted by its blood levels. Therefore, the determination of this metabolite may be important for a correct monitoring of clozapine plasma concentrations.

A simple, fast and sensitive HPLC assay with UV detection has been developed for the separation and measurement of clozapine and its two main metabolites in human plasma. The method was validated by recovery, linearity, accuracy and precision according to Good Laboratory Practice Guidelines [25].

2. Experimental

2.1. Chemicals

Clozapine, 8-chloro-11-(4′-methyl)piperazinyl-5H-dibenzo[(*b,e*)]-[1–4]diazepine, was kindly donated by Sandoz (Basle, Switzerland). *N*-Desmethylclozapine and clozapine *N*-oxide were purchased from Research Biochemicals (Natick, MA, USA) and

the internal standard triprolidine from Sigma (Munich, Germany).

Potassium phosphate monobasic was obtained from Carlo Erba (Milan, Italy), 1-heptanesulfonic acid sodium salt from Acros Organics (NJ, USA), sodium hydroxide and isoamyl alcohol from Janssen Chimica (Beerse, Belgium), *n*-hexane, acetonitrile and water from J.T. Baker (Deventer, The Netherlands). Water was deionized and purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

Stock standard solutions were prepared by dissolving pure substances in methanol. Clozapine, *N*-desmethylclozapine, clozapine *N*-oxide and triprolidine were prepared at a concentration of 5 mg of free base per ml. Each solution was stable for up to two months when stored at −25°C.

Working standard solutions containing 10 μg/ml of each analyte were prepared by making appropriate dilutions of the stock solutions with 0.1 *M* KH_2PO_4 (pH 2.2 with 25% H_3PO_4). Triprolidine standard solution was prepared in a similar manner at the concentration of 20 μg/ml. All standards were stored at 4°C for one month.

Drug-free plasma used for the validation of the method was obtained from healthy volunteers. Blood collected in EDTA-sodium was centrifuged and the plasma was pooled and stored at −30°C. Calibrators were prepared by adding known amounts of working standard solution to 1 ml blank plasma.

An unextracted solution of 0.1 *M* KH_2PO_4 (pH 2.2 with 25% H_3PO_4) containing the three drugs at a concentration of 0.5 μg/ml and the internal standard at the concentration of 1 μg/ml, was injected daily to check the resolution of the chromatographic system.

2.3. Extraction procedure

To 1.0 ml plasma in a 13-ml tube were added 25 μl of the working internal standard solution (0.5 μg/ml), and 1 ml of NaOH (0.5 *M*). The plasma was briefly mixed with a vortex mixer and 4 ml of *n*-hexane–isoamyl alcohol (75:25, v/v) was then added. The solution was shaken for 10 min at 230 cycles/min and centrifuged at 3000 *g* for 5 min. The organic phase was transferred to another 13-ml tube containing 150 μl of 0.1 *M* KH_2PO_4 (pH 2.2 with 25% H_3PO_4) and mixed for 5 min at 300 cycles/min. After centrifugation at 3000 *g* for 2 min, the organic layer was discarded by aspiration and 1 ml of diethyl ether was added to the remaining acid layer. The ethereal phase was eliminated after gentle mixing and 50 μl of the acid phase was injected into the chromatographic system.

2.4. Apparatus

The chromatographic equipment was purchased from Shimadzu (Kyoto, Japan) and consisted of a Model LC-10ADvp dual reciprocating pump coupled to a manual injector (Rheodyne 7725i) with a 50-μl fixed loop, a Model SPD-10AV UV–Vis detector with variable wavelength set at 254 nm and a Model SCL-10A system controller. The apparatus was connected to a personal computer (Digital Venturis FX 5166s) with a CLASS-VP 4.1 Chromatography Data System software (Shimadzu).

2.5. Chromatographic conditions

The chromatographic separation was performed using a Waters Spherisorb S5 C_6 analytical column (250 mm×4.6 mm I.D.). The mobile phase consisted of acetonitrile–water (48:52, v/v) containing 0.009 *M* eptansulphonic acid sodium salt, and 0.06 *M* dibasic phosphate, adjusted to pH 2.7 with orthophosphoric acid (25%). Acetonitrile and the phosphate buffer (0.06 *M*, pH 2.7) were filtered using 0.45 μm filters (Alltech, Milan, Italy) in a Millipore solvent filtration apparatus (Millipore, Molsheim, France). Prior to use, the HPLC eluent was degassed by sonication. Flow-rate was 1 ml/min at ambient temperature with an operating pressure of 10.8 MPa.

2.6. Calculations

Concentration values were obtained by using the CLASS-VP chromatography data system software. Instrument calibration was performed by using plasma calibrators spiked with clozapine (0.025, 0.05, 0.1, 0.25, 0.5, 1 and 2 μg/ml) and its two metabolites (0.025, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 μg/ml). Quantitative determination was performed by inter-

polation from linear calibration areas constructed by plotting peak height ratios (analyte to internal standard) as a function of the concentration of the calibrators.

3. Results

3.1. Chromatography

A chromatogram of pure standard of clozapine, desmethylclozapine, clozapine *N*-oxide and triprolidine in 0.1 *M* KH_2PO_4 (pH 2.2 with 25% H_3PO_4) injected without extraction is shown in Fig. 2A. The drugs are well resolved with retention times of 4.9, 3.9, 5.6 and 6.6 min, respectively at a flow-rate of 1 ml/min and a pH value of the mobile phase of 2.7. Although this pH is lower than that used by others, there was no deterioration in peaks resolution after injection of approximately 500 plasma samples.

When the pH of the mobile phase was increased from 2.7 to 3.7, retention time increased for each substance (5.9, 4.7, 7.2 and 7.7 min, respectively). Clozapine and desmethylclozapine peaks retained a good resolution while the peak of clozapine *N*-oxide was broadened and poorly separated from the internal standard. At pH values below 2.7 the retention times decreased and the peak of clozapine *N*-oxide was poorly separated from that of clozapine.

A chromatogram of a drug-free plasma sample is shown in Fig. 2B, while a chromatogram of extracted plasma sample spiked with 0.4 μg/ml clozapine, 0.3 μg/ml desmethylclozapine and 0.2 μg/ml clozapine *N*-oxide is illustrated in Fig. 2C. All peaks were well resolved and symmetrical.

The chromatogram of an extracted sample of plasma collected from a patients taking 400 mg/day of clozapine is shown in Fig. 2D. There were no interference peaks which could impair the quantitation of the analytes.

3.2. Recovery and linearity

The recovery of clozapine and its metabolites was determined by comparing the peaks height (analytes and internal standard) of extracted samples containing known amounts of the four compounds with the peaks height obtained after direct injection of the compounds dissolved in 0.1 *M* KH_2PO_4 (pH 2.2 with 25% H_3PO_4).

The recovery of clozapine (0.025–2 μg/ml) ranged from 88.2 to 96.0% (92.7±2.7%, mean±S.D.; $n=5$). The recovery of *N*-desmethylclozapine and clozapine *N*-oxide (both tested at 0.025–0.5 μg/ml) ranged from 76.3 to 86.8% (81.7±4.8%, mean±S.D.; $n=5$) and from 64.0 to 77.6% (70.4±5.0%, mean±S.D.; $n=5$), respectively (Table 1).

The analysis of calibrators gave linear curves over the range tested (Fig. 3). Linear regression equations (mean values) of peak-height ratios of drug to internal standard (y) vs. drug concentrations (x) were $y=0.0008x-0.001$ ($r=0.9995$) for clozapine, $y=0.0008x-0.004$ ($r=0.9991$) for *N*-desmethylclozapine and $y=0.0004x+0.003$ ($r=0.9924$) for clozapine *N*-oxide.

3.3. Precision and accuracy

Intra-day and the inter-day precision and accuracy were determined by assaying blank plasma spiked with five different concentrations of clozapine, *N*-desmethylclozapine and clozapine *N*-oxide.

Intra-day precision was assessed by assaying five samples at three drug concentrations (0.05, 0.25 and 1 μg/ml). Inter-day precision was evaluated by assaying (seven days) ten samples at two concentrations (0.1 and 0.5 μg/ml).

In the range investigated intra-day and inter-day coefficients of variation (C.V.s) were less than 6.6%, 7.5% and 13.8% for clozapine, *N*-desmethylclozapine and clozapine *N*-oxide, respectively (Table 2).

Fig. 2. (A) Chromatogram of clozapine, desmethylclozapine, clozapine *N*-oxide and triprolidine, dissolved in 0.1 *M* KH_2PO_4 and injected without extraction. (B) Chromatogram obtained from the analysis of a drug-free plasma sample after extraction (C) chromatogram of an extracted plasma sample spiked with 400 ng/ml clozapine, 300 ng/ml desmethylclozapine, 200 ng/ml clozapine *N*-oxide and 500 ng/ml triprolidine. (D) Chromatogram from a patient receiving 400 mg of clozapine/day. The concentration of I.S. in this analysis was 400 ng/ml.

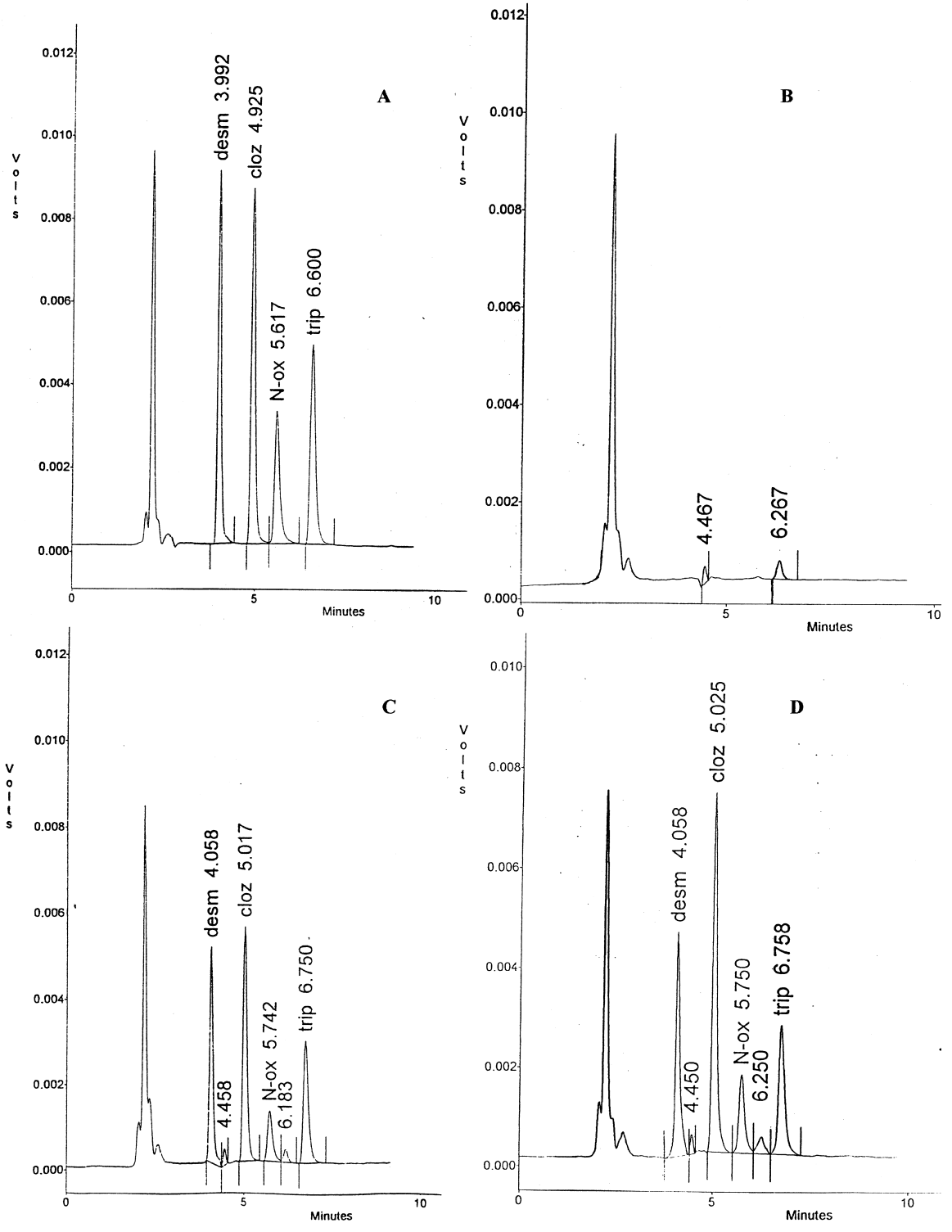
A
desm 3.992
cloz 4.925
N-ox 5.617
trip 6.600
B
4.467
6.267
C
desm 4.058
cloz 5.017
4.458
N-ox 5.742
6.183
trip 6.750
D
desm 4.058
cloz 5.025
4.450
N-ox 5.750
6.250
trip 6.758
Volts
Minutes

Table 1
Recoveries of clozapine, desmethylclozapine and clozapine *N*-oxide

Concentration (ng/ml)	Recovery (%) (mean±S.D., $n=5$)		
	Clozapine	Desmethylclozapine	Clozapine *N*-oxide
25	88.2±3.9	78.9±5.9	64.0±11.2
50	90.4±3.4	76.3±3.0	68.0±5.5
100	93.5±5.0	86.8±4.9	72.2±5.7
250	92.2±4.8	86.8±6.5	70.2±2.2
500	96.0±6.0	79.7±5.4	77.6±6.8
1000	93.3±7.7	–	–
2000	95.3±5.2	–	–

The accuracy was expressed as percentage error [(found concentration − spiked concentration)/spiked concentration]×100 (%) (Table 2).

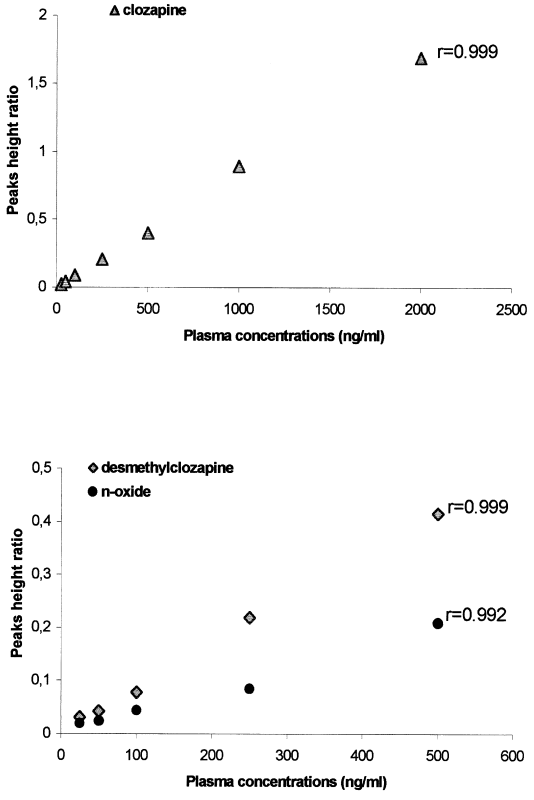

Fig. 3. Linear regression curves of clozapine (A) and its two metabolites (B) determined by plotting peak height ratio (analyte/internal standard) in the *y* axis versus the concentration of calibrators in the *x* axis ($n=5$ for each concentration).

3.4. Limit of quantitation and limit of detection

The lower limit of quantitation, based on an upper limit of 20% for precision and a range of 80–120% for accuracy, was 2 ng/ml for clozapine and *N*-desmethylclozapine and 5 ng/ml for clozapine *N*-oxide with a signal-to-noise ratio>10. Detection limits (signal-to-noise ratio>3) were 1 ng/ml for clozapine and desmethylclozapine and 3 ng/ml for clozapine *N*-oxide.

3.5. Specificity

Potential interferences were assessed by assaying solutions of a number of psychoactive drugs and their metabolites, as well as plasma from patients receiving antipsychotic drugs commonly used in combination with clozapine (Table 3). Fig. 4 shows the chromatogram of a sample spiked with some of the tested substances. Some of the analysed drugs were not well extracted with our method and some of them were not extracted at all. Plasma was spiked with 1 μg/ml of each tested drug, 0.25 μg/ml of clozapine and metabolites and 0.5 μg/ml of triprolidine.

4. Discussion

The present study describes a HPLC method for the simultaneous determination of clozapine and its two major metabolites in human plasma. The assay is fast, easy to perform, and accurate, and per-

Table 2
Intra-day and inter-day precision and accuracy of the method for clozapine, desmethylclozapine and clozapine *N*-oxide

Spiked value (ng/ml)	*n*	Measured value (ng/ml) (mean±S.D.)	Intra-day ($n=5$) C.V. (%)	Inter-day ($n=10$) C.V. (%)	Relative error (%)
Clozapine					
50	5	51.3±3.4	6.6		2.6
100	10	96.8±3.1		3.2	3.2
250	5	240.1±7.8	3.3		3.9
500	10	513.0±17.4		3.4	2.6
1000	5	1000.4±29.9	3.0		0.04
Desmethylclozapine					
50	5	42.1±2.9	6.9		15.8
100	10	102.8±7.7		7.5	2.8
250	5	252.5±8.9	3.6		1.0
500	10	471.1±25.1		5.3	5.8
1000	5	992.0±31.7	3.2		0.8
N-oxide					
50	5	40.1±3.9	9.7		19.8
100	10	84.6±7.9		13.8	15.4
250	5	219.1±2.8	1.2		12.4
500	10	409.0±22.8		5.6	18.2
1000	5	815.0±27.7	3.4		18.5

Table 3
Compounds assayed for possible interference and their relative retention times

Extracted drugs	Unextracted drugs	Retention time (min)
Theophylline		2.9
Carbamazepine-10,11-epoxide		3.7
Desmethylclozapine		**4.0**
Chlordiazepoxide		4.4
	Oxazepam	5.0
Clozapine		**5.0**
2-Hydroxy-desipramine		5.0
	Carbamazepine	5.0
	Clonazepam	5.3
Clozapine *N*-oxide		**5.8**
Triprolidine		**6.8**
Mianserin		7.9
	Diazepam	9.1
Desipramine		9.3
Protriptyline		9.0
Haloperidol		10.4
Nortriptyline		10.6
Imipramine		10.7
Amitriptyline		11.9
Clomipramine		14.9
Thioridazine		19.1

Oxazepam, carbamazepine, clonazepam and diazepam were not extracted by the extraction procedure.

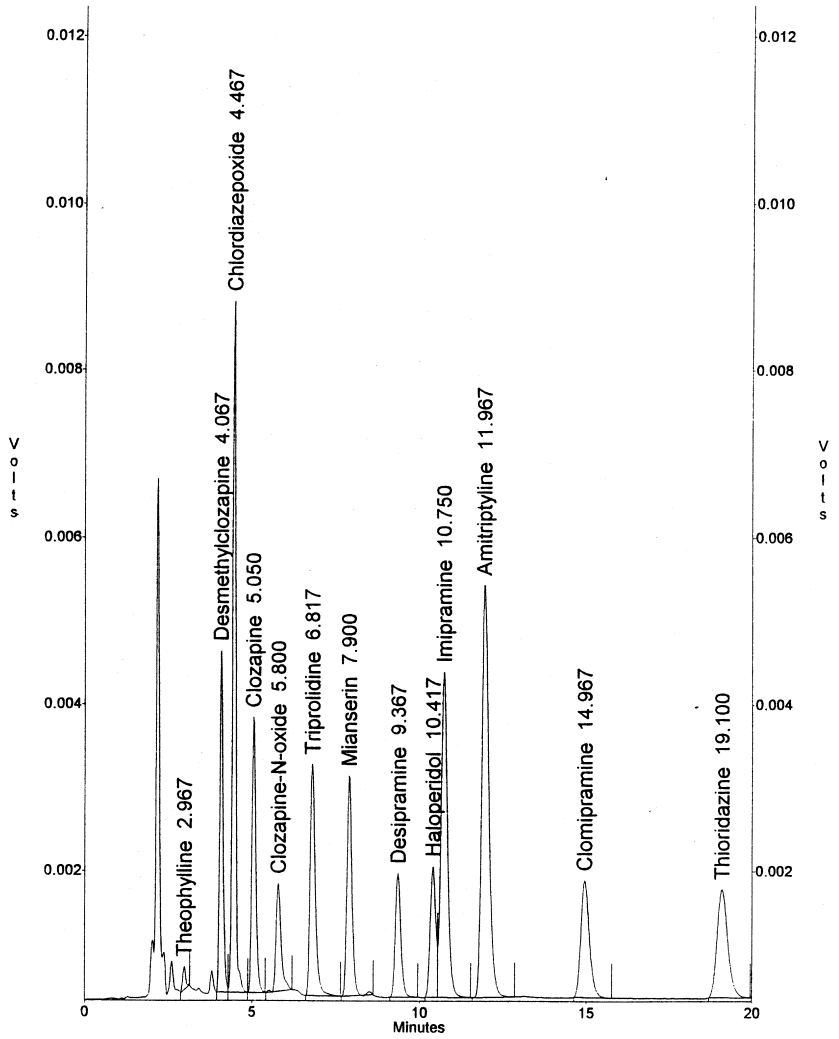

Fig. 4. Chromatogram obtained after the extraction of a plasma sample spiked with 1 μg/ml for potential interferents, 250 ng/ml of clozapine and its two metabolites and 500 ng/ml of triprolidine. Carbamazepine-10,11-epoxide, 2-hydroxydesipramine, protriptyline and nortriptyline were not included in the chromatogram.

formance characteristics are adequate for therapeutic drug monitoring.

The antisthaminic triprolidine [18], was selected as internal standard because it is stable, well extracted (recovery 89.3±4.5%, $n=5$) and elutes after a short time (6.6 min). Since triprolidine is not used in psychiatric practice, it is a more appropriate internal standard than imipramine [15,16], protriptyline [14,17], amoxapine [19], fluphenazine [10], clonazepam [13] and loxapine [21], all of which may be coadministered with clozapine.

The time required for the chromatographic separation (7 min), is shorter than that reported for other methods [11,15,20,21]. In the robust HPLC method described by Guitton et al. [21], complete separation requires 20 min. Only Volpicelli et al. [18] described a method in which separation takes 6 min.

Mean recovery values were all greater than 70% and were higher than those described for other methods allowing the simultaneous determination of the three analytes [11,15,18,20,21]. In particular, the recoveries described by Weigmann and Heimke [15]

Table 4
Recoveries of clozapine, desmethylclozapine and clozapine *N*-oxide after extraction of a sample containing the three drugs at concentrations of 400 ng/ml, 300 ng/ml and 200 ng/ml, respectively

n-Hexane–isoamyl alcohol (%)	Recovery (%) (mean±S.D, $n=5$)		
	Clozapine	Desmethylclozapine	Clozapine *N*-oxide
95:5	98.4±4.6	44.2±5.2	6.7±1.2
90:10	95.8±3.8	66.4±3.3	15.5±2.8
80:20	94.7±5.1	80.3±4.3	60.2±4.4

Three different extractive mixtures were used.

were higher than ours for clozapine *N*-oxide (77%), but lower for clozapine (85%) and desmethylclozapine (78%). Fadiran et al. [20] reported a higher recovery for clozapine (95%), but lower values for desmetyhylclozapine (70%) and clozapine *N*-oxide (65%).

The improved recoveries in our method are due to a novel extraction procedure in which the alkalinized sample is mixed with *n*-hexane–isoamyl alcohol in a 75:25 (v/v) ratio. Other methods [14,17] which used a *n*-hexane–isoamyl alcohol (98.5:1.5) mixture allowed the detection of clozapine and desmethylclozapine only, the latter with a poor recovery. Table 4 shows that by increasing the percentage of isoamyl alcohol the recovery of both desmethylclozapine and clozapine *N*-oxide improved, while that of clozapine worsened slightly. Based on these results, we selected the mixture which ensures the highest recovery of the two metabolites (82.0%, 70.4%) while retaining a good recovery of clozapine (92.7%). The recovery of triprolidine (I.S.) was similar to that of clozapine, decreased slightly with increasing proportion of isoamyl alcohol. The treatment of the acid extract with diethyl ether during the last step of the extraction procedure provided a cleaner sample with less interfering peaks, resulting in improved sensitivity.

Another advantage of the present method is the low limit of quantitation for the three analytes, which ranged from 2 ng/ml for both clozapine and *N*-desmethylclozapine to 5 ng/ml for clozapine *N*-oxide. These values are the lowest described for the simultaneous determination of the three compounds [11,15,18,20,21] and for clozapine *N*-oxide in particular. This is important because the plasma concentrations of clozapine *N*-oxide are frequently low.

The use of a C_6 column, which is more polar than those employed in other methods, allows a better resolution and a faster elution. A C_8 column was also tested, but it was associated with longer retention times, lower resolution and broadened peaks.

Several commonly coadministered psychotropic drugs were tested to rule out possible interferences (Table 3). Among these only 2-hydroxydesipramine, the metabolite of desipramine, interferes with the determination of clozapine. Two other drugs which coelute with clozapine, oxazepam and carbamazepine, are not extracted with our procedure.

In conclusion, this method ensured fast analysis time, a good reproducibility and accuracy and a low limit of quantitation. The assay has been already used to study the pharmacokinetic interaction between fluoxetine and clozapine [26], and it could be usefully applied to further pharmacokinetic studies and therapeutic drug monitoring.

Acknowledgements

This study was supported by EU Biomed 2 (BMH4-CT96-0291).

References

[1] A. Fitton, R.C. Heel, Drugs 40 (1990) 722.
[2] R.J. Baldessarini, F.R. Frankenburg, New Engl. J. Med. 324 (1991) 746.
[3] J.M. Alvir, J.A. Lieberman, A.Z. Safferman, J.L. Schwimmer, J.A. Schaff, New Engl. J. Med. 329 (1993) 162.
[4] M.W. Jann, S.R. Grimsley, E.C. Gray, W.H. Chang, Clin. Pharmacokinet. 24 (1993) 161.
[5] D.J. Freeman, L.K. Oyewumi, Clin. Pharmacokinet. 32 (1997) 93.
[6] K. Richtner, J. Chromatogr. 434 (1988) 465.

[7] R. Heipertz, H. Pilz, W. Beckers, Arch. Toxicol. 37 (1977) 313.
[8] V. Bondesson, L.H. Lindstrom, Psychopharmacology 95 (1988) 472.
[9] W. Zeren, L. Minglian, X. Peipei, Z. Yanlin, Biomed. Chromatogr. 1 (1986) 53.
[10] C. Haring, C.C. Humpel, B. Auer, A. Saria, C. Barnas, W. Fleischhlacker, H. Hinterhuber, J. Chromatogr. 428 (1988) 160.
[11] J.P. Chovan, J.D. Vermeulen, J. Chromatogr. 494 (1989) 413.
[12] C. Humpel, C. Haring, A. Saria, J. Chromatogr. 491 (1989) 235.
[13] D. Wilhelm, A. Kemper, J. Chromatogr. 525 (1990) 218.
[14] M.J. Lovdahl, P.J. Perry, D.D. Miller, Ther. Drug Monit. 13 (1991) 69S.
[15] H. Weigmann, C. Hiemke, J. Chromatogr. 583 (1992) 209.
[16] O.V. Olesen, B. Poulsen, J. Chromatogr. 622 (1993) 39.
[17] M.C. Chung, S.K. Lin, W.H. Chang, J. Chromatogr. 613 (1993) 168.
[18] R. Volpicelli, F. Centorrino, P.R. Puopolo, J. Kando, F.R. Frankenburg, R.J. Baldessarini, J.G. Flood, Clin. Chem. 39 (1993) 1656.
[19] R.N. Gupta, J. Chromatogr. B 673 (1995) 311.
[20] O. Fadiran, J. Leslie, M. Fossler, D. Young, J. Pharm. Biomed. Anal. 13 (1995) 185.
[21] C. Guitton, J.M. Kinowski, R. Aznar, F. Bressolle, J. Chromatogr. B 690 (1997) 211.
[22] M.W. Jann, Y.W.F. Lam, W.-H. Chang, Arch. Int. Pharmacodin. Ther. 328 (1994) 243.
[23] M. Pirmohamed, D. Williams, S. Madden, E. Templeton, B.K. Park, J. Pharmacol. Exp. Ther. 272 (1995) 984.
[24] G. Lin, G. McKay, K.K. Midha, J. Pharm. Biomed. Anal. 14 (1996) 1561.
[25] F. Bressolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[26] E. Spina, A. Avenoso, G. Facciolà, M. Fabrazzo, P. Monteleone, M. Maj, E. Perucca, A.P. Caputi, Int. Clin. Psychopharmacol. 13 (1998) 141.