ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 441–449

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

A capillary gas–liquid chromatographic method for the assay of the neuroleptic drug zotepine in human serum or plasma

S. Ulrich[a,*], F.P. Meyer[a], B. Bogerts[b]

[a] *Institute of Clinical Pharmacology, University Hospital, Leipziger Strasse 44, D-39120 Magdeburg, Germany*
[b] *Psychiatric Clinic, University Hospital, Leipziger Strasse 44, D-39120 Magdeburg, Germany*

Received for review 17 May 1995; revised manuscript received 10 July 1995

Abstract

A capillary gas–liquid chromatographic method suitable for the assay of the atypical neuroleptic drug zotepine in human serum or plasma was developed. A liquid–liquid extraction with three subsequent extraction steps was applied for sample preparation. The minimum detectable concentration was 1.0 ng ml^{-1}. The within-day relative standard deviation (RSD) ($n = 6$) was 5.3% at 5 ng ml^{-1}, 3.6% at 10 ng ml^{-1} and 6.1% at 100 ng ml^{-1}. The day-to-day RSD ($n = 6$) was 9.3% at 10 ng ml^{-1} and 5.1% at 100 ng ml^{-1}. Steady-state serum levels of four schizophrenic patients were measured.

Keywords: Gas–liquid chromatography; Neuroleptics; Psychotherapeutics; Therapeutic drug monitoring; Zotepine

1. Introduction

Zotepine (Fig. 1) is an antipsychotic drug developed in Japan from 1970 to about 1979 [1–3]. The clinical efficiency in schizophrenia has been shown in several studies [4,5]. In Germany, 2 million defined daily doses (DDD) were prescribed in 1993, which was about 0.7% of all neuroleptics. The increased use of zotepine can be associated with a favourable therapeutic spectrum. It is effective in acute exacerbation of schizophrenia, in reducing anxiety–depression and both negative and positive symptoms. It has fewer extrapyramidal adverse effects (EPS) than conventional antipsychotics, e.g. haloperidol. As for the newer antipsychotic drug resperidone, these decreased EPS are considered to be caused by the simultaneous dopamine-D_2 and serotonin-$5HT_2$ receptor blocking properties. For comparison, haloperidol has no antiserotonergic effect.

For the assay of zotepine in human and dog serum, a gas–liquid chromatographic method using a 0.8 m × 2.5 mm i.d. column packed with 3% OV-17 on 80–100 mesh Chromosorb W HP and electron-capture detection has been described [2]. A liquid–liquid extraction procedure with *n*-hexane–isoamyl alcohol (98.5:1.5, v/v) with (three subsequent extraction steps) was applied for sam-

*Corresponding author.

SSDI 0731-7085(95)01637-6

ple preparation. This method has also been used in more recent investigations [6–9]. For the assay of [^{14}C]zotepine in serum of rats and mice, thin-layer chromatography with radioactivity detection has been used [2].

After a single dose of 100 mg of zotepine, maximum plasma levels $c_{max} = 30–240$ ng ml^{-1} (mean 129 ng ml^{-1}) at $t_{max} = 1–4$ h (mean 2.2 h) have been found in five Asian schizophrenics [2]. Much lower c_{max} after a 100 mg single dose were reported for 15 Caucasian volunteers (mean $c_{max} = 19.6$ ng ml^{-1} after $t_{max} = 2–4$ h) [10]. Steady-state plasma levels after several weeks of treatment of 14 Asian schizophrenic patients were 39.6 ± 25.0 ng ml^{-1} (mean $\pm$ SD) for 150 mg day^{-1}, 62.9 ± 32.4 ng ml^{-1} for 200 mg day^{-1} and 71.8 ± 30.6 ng ml^{-1} for 250 mg day^{-1} [11]. In a study of 21 Asian patients at a mean dose of 298.6 mg day^{-1} the serum levels were 71.6 ± 54.2 ng ml^{-1} after 6 weeks of treatment. The serum levels were not dependent on anticholinergic comedication (biperiden or piroheptine) [6].

A 26-fold variation in the concentration–dose ratio of zotepine was found in 59 Asian patients: 2.4–62.2 ng ml^{-1} per mg kg^{-1}, mean 13.2 (=0.21 ng ml^{-1} mg^{-1}). Smokers had significantly lower concentration–dose ratios. The serum levels were increased by benzodiazepine co-medication [7]. No definite relationship between clinical outcome and serum levels could be detected. However, only few data at high serum concentrations (>60 ng ml^{-1}) were included [8]. Using the 21-item UKU Side Effect Rating Scale, only the item akathisia revealed a significantly positive correlation with the zotepine serum concentration [9].

The purpose of the present investigation was to develop a gas–liquid chromatographic method with a bonded-phase fused-silica capillary for the assay of zotepine in human serum. In contrast to Ref. [2], nitrogen–phosphorus selective detection, which is usually more often encountered in assays of pharmaceuticals, was applied.

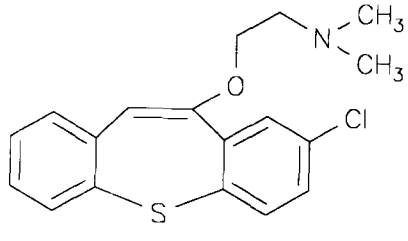

Fig. 1. Structure of zotepine.

2. Experimental

2.1. Chemicals

Zotepine (Z) was purchased from Klinge Pharma (Munich, Germany and 2-chloramitriptyline·HCl (Lu 8-112C) from H. Lundbeck (Copenhagen, Denmark). The organic solvents *n*-hexane, *n*-heptane, methanol, isopropyl alcohol, isoamyl alcohol and toluene (all of analytical-reagent grade) were from Merck (Darmstadt, Germany). For the preparation of aqueous NaOH and HCl, deionized, distilled water was used. NaOH and concentrated HCl (analytical-reagent grade) were obtained from Merck and NaCl was obtained from Laborchemie (Apolda, Germany). Dichlorodimethylsilane was purchased from Chemiewerk Nünchritz (Nünchritz, Germany).

2.2. Solutions

A 1 N aqueous solution of NaOH containing 6% NaCl was prepared by dissolution of 20 g of NaOH and 30 g of NaCl in 500 ml of water and 0.1 N HCl was prepared by diluting 4.2 ml of concentrated HCl in 500 ml water. The extraction solvent was prepared by mixing nine volumes of *n*-heptane with one volume of isopropyl alcohol. For the silanization of glassware, a solution of 5% dichlordimethylsilane in toluene was used.

2.3. Reference and standard solutions

The reference solution (1 μg ml^{-1}) was prepared daily by diluting 1:100 with 0.001 N HCl a stock solution of zotepine in methanol (100 μg ml^{-1}). The stock solution was stored at −20°C. A standard solution (4 μg ml^{-1}) was prepared by diluting 1:25 a stock solution of 100 μg ml^{-1} chloramitriptyline base (=111.6 μg ml^{-1} chloramitriptyline·HCl) in water. The standard solution was stored at 4°C.

2.4. Glassware

Glassware was silanized with 5% dichlordimethylsilane in toluene monthly. A special cleaning procedure including 30 min of sonification in 0.001 N HCl was applied.

2.5. Sample preparation

Serum (2 ml) was mixed in a 10 ml glass tube with 100 μl of standard solution, then 0.5 ml of aqueous NaOH containing 6% NaCl and 4 ml of *n*-heptane–isopropyl alcohol (9:1, v/v) were added. The first extraction step was carried out by shaking for 30 min. After 5 min of centrifugation at 1500*g*, as much as possible (ca. 3.0 ml) of the organic layer was transferred into 1.25 ml of 0.1 N HCl in a 10 ml glass tube and shaken for 30 min. After 2 min of centrifugation at 1500*g*, the organic layer was discarded, 1 ml of *n*-heptane–isopropyl alcohol (9:1, v/v) was added, the two phases were vortex mixed for 30 s and separated again by centrifugation for 2–5 min. From the lower phase (0.1 N HCl), 1.0 ml was removed carefully and placed in a smaller 4 ml glass tube, then 150 μl of the aqueous NaOH and 100 μl of *n*-heptane–isopropyl alcohol (9:1, v/v) were added and vortex mixed for 30 s. After 5 min of centrifugation at 1500*g*, as much as possible of the organic layer (ca. 80 μl) was separated into a tapered 4 ml glass tube. The solution was evaporated to dryness for 5 min in a vacuum evaporator and the residue was reconstituted in 10 μl of *n*-hexane–isoamyl alcohol (98.5:1.5, v/v). Volumes of 2 μl were injected into the GC system.

2.6. Apparatus

A Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a nitrogen–phosphorus detector (NPD) and a split–splitless injector port was used for the analysis. Separation was obtained with a 25 m × 0.2 mm i.d. HP-5 capillary (0.33 μm film thickness) and nitrogen (0.7 ml min^{-1}, automatic pressure control) as the carrier gas. Flow rates of the detector gases were air 100 ml min^{-1} (275 kPa), hydrogen 2.5 ml min^{-1} (100 kPa) and the auxiliary gas nitrogen 27 ml min^{-1} (220 kPa). The injector was operated at 290°C in the split–splitless mode. The split (30 ml min^{-1}) was opened 0.05 min after injection. A temperature programme was used for the oven ($T_1 = 260$°C, $T_2 = 280$°C, rate = 2°C min^{-1}). The detector port was maintained at 300°C. The NPD was operated at a baseline of 25 to 30 pA. An HP3398 Series II integrator was used for the calculation of retention times, peak widths and peak areas.

2.7. Quantitation

Calibration curves were constructed by plotting the peak-area ratios ($A_Z/A_{I.S.}$) obtained from blank serum spiked with the above-mentioned reference solution of zotepine. Eight equidistant concentrations between 10 and 80 ng ml^{-1} were used.

2.8. Accuracy and precision

Accuracy was assessed by determining the concentrations of drug measured in samples (5, 10 and 100 ng ml^{-1}, $n = 6$ each) relative to the known concentration added. Precision was determined utilizing the within-day and between-day ($n = 6$) relative standard deviations (RSDs).

2.9. Patients

Serum levels of zotepine were measured for four patients (two male, two female, 26.2 ± 3.9 years) weekly over different times of therapy: 1, 3, 4 and 8 weeks. The patients received 25–175 mg day^{-1} of Nipolept in divided doses. Doses >100 mg day^{-1} were divided into four doses with 50 mg in the evening. Doses of 75 mg day^{-1} resulted from three divided doses, 50 mg day^{-1} from two divided doses and 25 mg day^{-1} from one dose, with each 25 mg in the evening. Co-medication was with biperidene (three patients) and flupenthixol decanoate (two patients). Blood samples were drawn into vacutainers once a week between 7.00 and 8.00 a.m. before the morning dose.

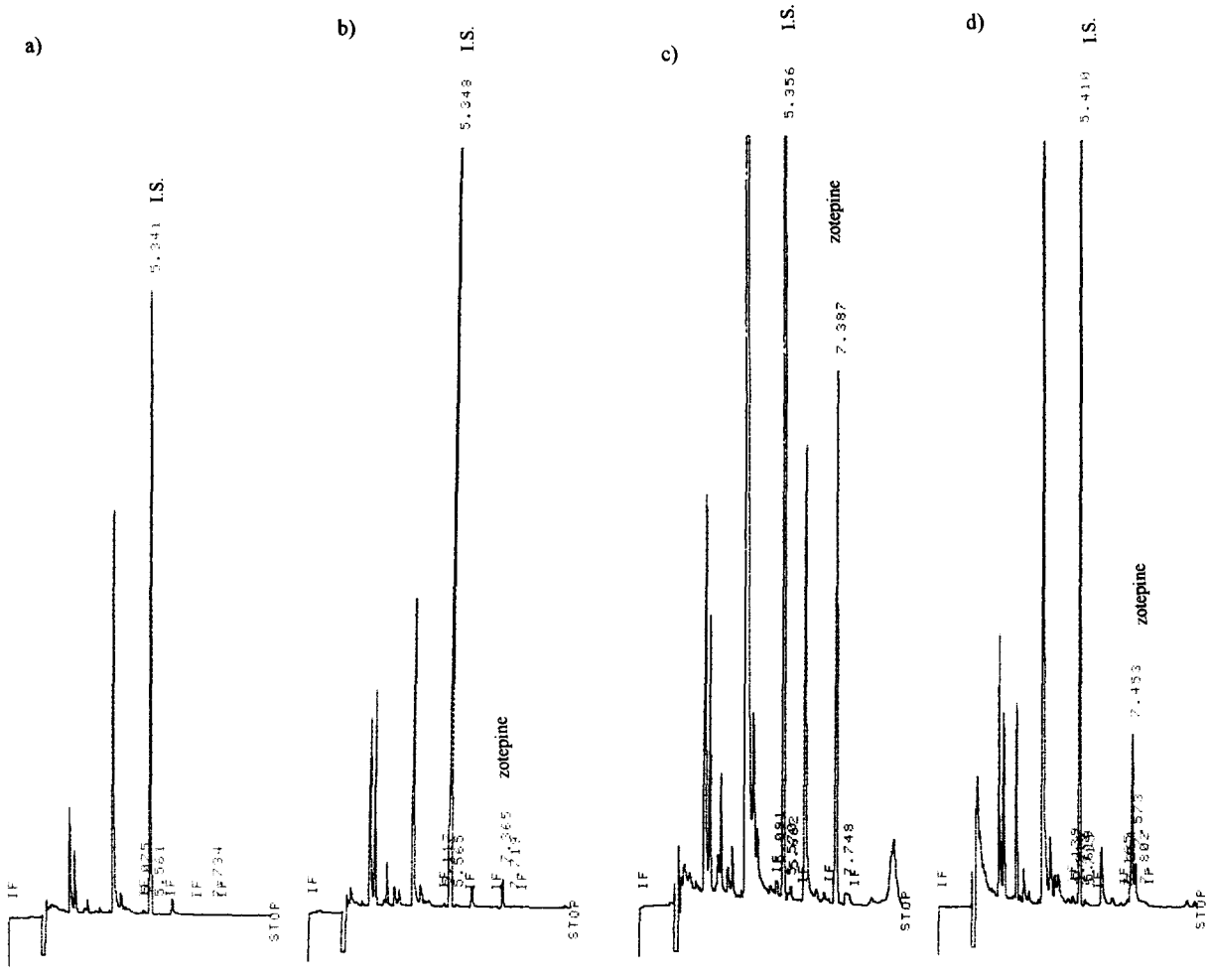

Fig. 2. Typical chromatograms obtained from (a) blank serum, (b) serum spiked with 5 ng ml^{-1} zotepine, (c) serum spiked with 40 ng ml^{-1} zotepine and (d) serum from a patient with 25 ng ml^{-1} zotepine, all with internal standard: t_r (I.S.) = 5.34–5.41 min, t_r (zotepine) = 7.36–7.45 min. Attenuation of the integrator: $at = 4$ in (a) and (b) and $at = 3$ in (c) and (d); a decrease of one unit in at doubles the plotted peak heights; chart speed = 0.5 cm min^{-1}.

3. Results and discussion

Fig. 2 shows typical chromatograms obtained from extracts of blank serum, serum spiked with 5 ng ml^{-1} zotepine, serum spiked with 40 ng ml^{-1} zotepine (all with internal standard, I.S.) and a

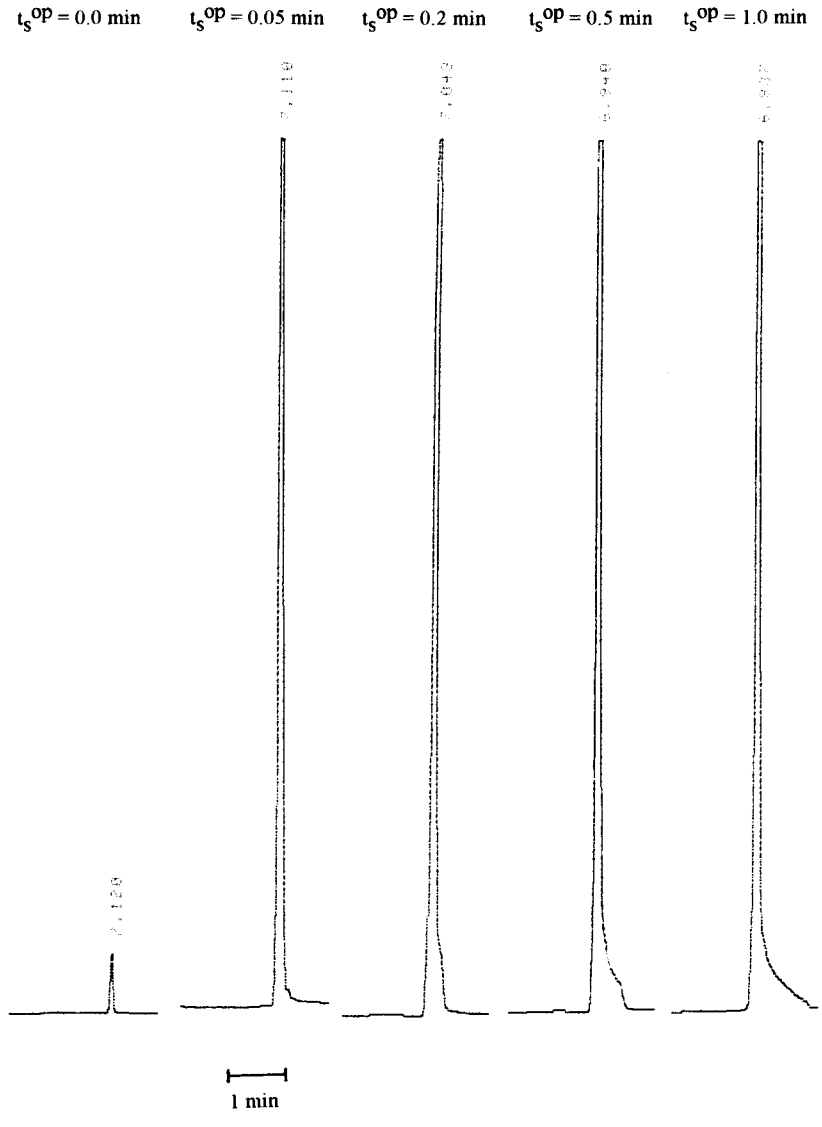

Fig. 3. Chromatograms of 20 ng of zotepine in 2 μl of n-heptane isopropyl alcohol (9: 1, v/v) at different times of opening the split, $t_s^{op} = 0$, 0.05, 0.20, 0.50 and 1.00 min. Attenuation = 2; chart speed = 1 cm min^{-1}.

patient's serum calculated to contain 25 ng ml^{-1} zotepine. Zotepine (retention time $t_r = 7.36$ min) and the I.S. ($t_r = 5.35$ min) exhibited well separated, narrow and symmetrical peaks under the chromatographic conditions described. The time of opening the split, $t_s^{op} = 0.05$ min, was tested to be the optimum (Fig. 3). In the split mode ($t_s^{op} = 0$), the peak area was considerably decreased. If t_s^{op} was increased above 0.1 min in the split–splitless mode, an increased tailing of the peak appeared.

The recoveries of zotepine and the I.S. were determined by comparison with direct injection of the compounds into the chromatograph. The recovery of the examined extraction procedure was 42% for zotepine at 100 ng ml^{-1}. For comparison, for the assay of psychotropic drugs extraction recoveries of up to 90% have been reported in

Table 1
Accuracy and precision of the assay

Concentration added (ng/ml)	Measured concentration (mean ± SD) (ng ml^{-1})		Accuracy (%)		Precision (RSD (%))	
	Within-day	Between-day	Within-day	Between-day	Within-day	Between-day
5	4.56 ± 0.24		91		5.3	
10	8.96 ± 0.32	9.42 ± 0.87	90	94	3.6	9.3
25 [a]	23.82 ± 1.24		95		5.2	
100	102.4 ± 6.2	102.0 ± 5.2	102	102	6.1	5.1

[a] Data for light-sensitivity test ($n = 5$).

literature. In the present method, about 25% (1 ml of 4 ml) was normally already lost in the first extraction step. Separation of more than 75% of the organic phase from the aqueous phase is difficult because of the relatively high polarity of the organic solvent. However, it was found for concentrations below 10 ng ml^{-1} that, possibly because of the suppression of adsorption, the cited solvent mixture was advantageous compared with less polar solvent mixtures, e.g. *n*-hexane–isoamyl alcohol (98.5:1.5, v/v). Although the extracts obtained when testing *n*-hexane isoamyl alcohol (98.5:1.5, v/v) were considerable purer, owing to the good separation of the 0.2 mm i.d. capillary no interfering peaks eluted in the chromatograms of multiple blank serum samples with *n*-heptane–isopropyl alcohol (9:1, v/v) as the extraction solvent. Therefore, the examined extraction procedure was regarded as a good compromise when it was used for sample preparation in the described chromatographic method.

Calibration curves were typically described by the equation $y = 0.0049x + 0.0011$, where y is the peak-area ratio $A_Z/A_{I.S.}$ and x is the concentration of zotepine (ng ml^{-1}). The calculated peak-area ratios and the added concentrations displayed a good linear relationship between 0 and 100 ng ml^{-1} with a correlation coefficient $r = 0.9993$. The assay of zotepine was accurate and precise, as summarized in Table 1. The within-day and between-day RSDs for samples of 5, 10 and 100 ng ml^{-1} were consistently less than 7–10%. The concentrations of drug measured in samples of 5, 10 and 100 ng ml^{-1} relative to the known concentrations, were in the range 90–102%.

The capability of the method for the assay of zotepine at very low concentrations is illustrated in Fig. 4. Extracts from serum samples containing 5.0 ng ml^{-1} exhibit peaks well differentiated from the baseline noise. The limit of detection was estimated to be 1.0 ng ml^{-1} under the described conditions at a signal-to-noise ratio of 2. Owing to the accuracy and precision found at 5.0 ng ml^{-1} and the limit of detection of 1 ng ml^{-1}, 3.5 ng ml^{-1} can be regarded as the minimum quantifiable concentration. Therefore, the method is capable of measuring serum levels of zotepine in single-dose kinetics after a 25–50 mg dose.

In extracts of serum samples from patients treated with zotepine, a peak very close to the zotepine peak was always found (Fig. 2d). Because this peak was never found in blank serum samples or in spiked serum and also as co-medication can be excluded as a source of this peak, it is concluded that this peak represents a metabolite of zotepine. Metabolization pathways involve *N*-demethylation, *S*-oxidation, *N*-oxidation and 7,8-hydroxylation [2]. However, the identification of which metabolite corresponds to this peak must be confirmed by the preparation and analysis of suitable reference substances.

In the clinical practice, multiple concomitant medication is usual when neuroleptics such as zotepine are administered. This includes benzodiazepines (e.g. diazepam, lorazepam, nitrazepam, chlordiazepoxide), high-potency neuroleptics (e.g. haloperidol, flupenthixol, clopenthixol, fluphenazine) and antidepressants (e.g. amitriptyline, trimipramine, clomipramine, trazodone, dibenzepine). Also metabolites of these drugs, which

can occur in concentrations comparable to that of the drug, have to be taken in consideration (e.g. desmethyl metabolites of antidepressants, 10-hydroxyamitriptyline and -nortripytline, reduced haloperidol). Other low-potency neuroleptics (e.g. levomepromazine, chlorprothixene, perazine, promethazine) can occasionally also be co-administered to zotepine. Furthermore, interferences are possible on changing the medication. Therefore, it has to be ascertained that these

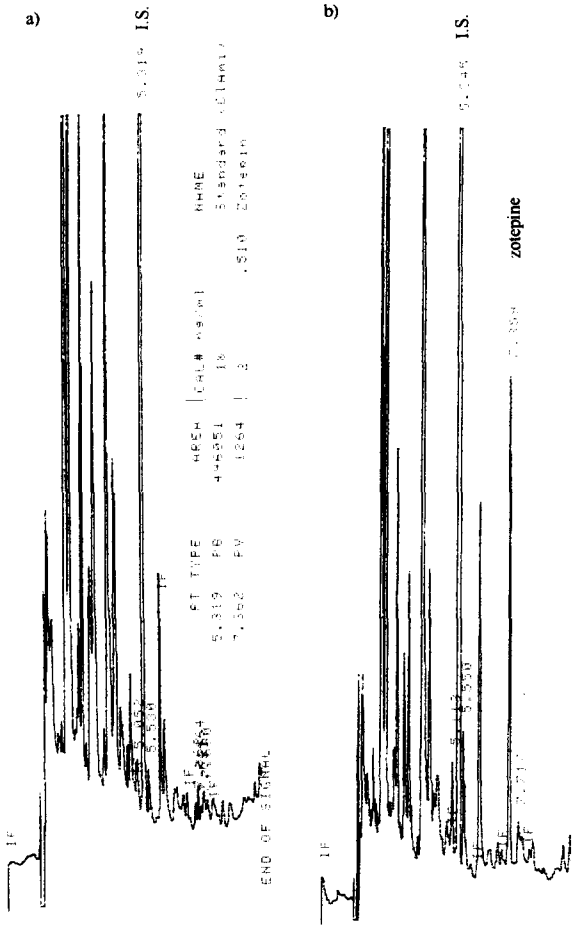

Fig. 4. Typical chromatograms obtained from (a) blank serum and (b) serum spiked with 5.0 ng ml^{-1} zotepine, both with internal standard: t_r (I.S.) = 5.31–5.35 min; t_r (zotepine) = 7.359 min. Attenuation = 0, therefore peak hights were plotted 16 times higher than in the chromatograms in Fig. 2a and b; chart speed = 0.5 cm min^{-1}.

compounds give no interferences with zotepine or the I.S. in the chromatograms. The drugs and metabolites tested are shown in Table 2. From the relative retention times, it is obvious that no drug or metabolite interferes with zotepine or the I.S.

There have been numerous reports of light sensitivity of drugs, which can interfere considerably with quantitative assays in serum or plasma [11]. Therefore, serum samples containing 25 ng ml^{-1} zotepine were tested in a simple experiment by allowing them to stand for 0, 1, 2, 3 and 4 h under daylight conditions (on a window ledge). No decrease in the measured concentration with increased exposure time and also no additional peak of a photoproduct of zotepine in the chromatograms were found. When a solution of zotepine in *n*-heptane iso-propyl alcohol (9:1, v/v) was irradiated with a UV lamp (365 nm) and analysed chromatographically, also no decrease in peak areas or additional peaks were detected. Thus, with respect to light sensitivity zotepine can be regarded as a stable analyte.

Fig. 5 shows the weekly serum levels of four patients as a function of the daily dose. A linear relationship resulted with intercept = 0.48, slope = 0.21 and $r = 0.829$. the slope of 0.21 (in ng ml^{-1} mg^{-1}) represents the mean concentration–dose ratio corresponding to reports of 0.24 [6], 0.28 [7] and 0.21 ng ml^{-1} mg^{-1}, [11]. Therefore, in the steady state no difference in the mean concentration–dose ratio in comparison with investigations on Asian patients [6,7,11] was found, as for the c_{max} after a single dose to Asian patients [2] and healthy Caucasian volunteers [10]. However, because two variables differed in Ref. [10] compared with Ref. [2], it is not possible to decide whether the ethnicity or the psychopathology was responsible for the considerable differences in c_{max}; e.g. for haloperidol, increased serum levels have been found in Asians compared with a Caucasian population [12,13]. The different sensitivities of various ethnological groups to effects of psychotropic durgs are well known [14]. Pharmacokinetic reasons should be at least in part responsible for the important implication.

Table 2
Lack of interferences with other psychotherapeutics and metabolites

Drug or metabolite	Retention time (min)	Relative retention time
Zotepine	7.40	1.00
Chloramitriptyline (I.S.)	5.36	0.72
Clomipramine	5.51	0.74
Desmethylclomipramine	5.79	0.78
Amitriptyline	3.92	0.53
Nortriptyline	4.07	0.55
(*E*)-10-Hydroxyamitriptyline	5.00	0.68
(*E*)-10-Hydroxynortriptyline	5.22 ($R_s = 0.91$ from I.S.) [a]	0.70
Mianserine	4.06	0.55
Doxepine	3.92 + 4.05	0.53 + 0.55
Dibenzepine	6.05	0.82
Trazodone	15.20	2.05
Haloperidol	13.75	1.86
Fluphenazine	> 10	> 1.35
cis(*Z*)-Clopentixol	> 10	> 1.35
cis(*Z*)-Flupentixol	15.79	2.13
Chlorprothixene	6.41 + 6.70	0.78 + 0.90
Perphenazine	28.00	3.78
Perazine	10.60	1.43
Alprazolam	13.75	1.86
Diazepam	5.90	0.80
Chlordiazepoxide	6.80 + 11.55	0.92 + 1.56
Lorazepam	5.63	0.76
Nitrazepam	10.20	1.38
Midazolam	7.62 ($R_s > 1$ from zotepine) [a]	1.03
Oxazepam	5.00	0.68
Carbamazepine	4.99	0.68
Zolpidem	10.76	1.45

[a] R_s = peak separation factor

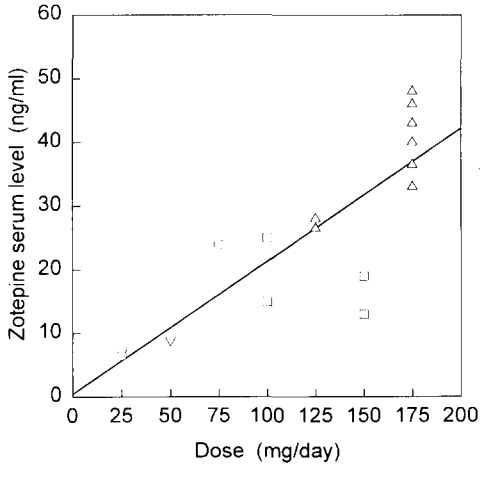

Fig. 5. Weekly steady-state serum levels of zotepine as a function of the daily doses for four schizophrenic patients. Each type of symbol represents the data for one individual. Linear regression: intercept = 0.48, slope = 0.21, $r = 0.829$.

4. Conclusion

Compared with other neuroleptics, the pharmacokinetics of zotepine in a Caucasian population have rarely been investigated. The method presented here can be a means to fill this gap.

Acknowledgements

This work was supported by the Bundesministerium für Forschung und Technologie of the FRG under the code number 07NBL04. The authors thank H. Lundbeck A/S (Copenhagen, Denmark) for providing reference substances of neuroleptic drugs and metabolites and Mrs. Helma Kühne for technical support.

References

[1] S. Uchida, F. Honda, M. Otsuka, Y. Sato, J. Mori, T. Ono and M. Hitomi, Arzneim.-Forsch., 29 (1979) 1588–1594.

[2] K. Noda, A. Suzuki, M. Okui, H. Noguchi, M. Nishiura and N. Nishiura, Arzneim.-Forsch., 29 (1979) 1595–1600.

[3] K. Fukuhura, Y. Emi, T. Furukawa, T. Fujii, K. Iwanami, N. Watanabe and Y. Tsubura, Arzneim.-Forsch., 29 (1979) 1600–1606.

[4] W.W. Fleischhacker, C. Barnas, C.H. Stuppack, B. Unterweger, C. Miller and H. Hinterhuber, Psychopharmacol. Bull., 25 (1989) 97–100.

[5] C. Barnas, C.H. Stuppack, C. Miller, C. Haring, B. Sperner-Unterweger and W.W. Fleischhacker, Int. Clin. Psychopharmacol., 7 (1992) 23–27.

[6] K. Otani, T. Hirano, T. Kondo, S. Kaneko, Y. Fukushima, K. Noda and Y. Tashiro, Br. J. Psychiatry, 157 (1990) 128–130.

[7] K. Otani, T. Kondo, S. Kaneko, T. Hirano, K. Mihara and Y. Fukushima, Hum. Psychopharmacol., 7 (1992) 331–336.

[8] T. Kondo, K. Otani, M. Ishida, K. Mihara, O. Tanaka, S. Kaneko and Y. Fukushima, Hum. Psychopharmacol., 8 (1993) 133–139.

[9] T. Kondo, K. Otani, M. Ishida, O. Tanaka, S. Kaneko and Y. Fukushima, Ther. Drug Monti., 16 (1994) 120–124.

[10] B. Saletu, J. Grünberger, P. Anderer and K. Chwatal, Fortschr. Neurol. Psychiatr., 59 (1991) 45–55.

[11] Y. Higashi, Y. Momotani, E. Suzuki and T. Kaku, Pharmakopsychiatrie, 20 (1987) 8–11.

[12] S.G. Potkin, Y. Shen, H. Pardes, B. Phelbs and D. Zhou, Psychiatry Res., 12 (1984) 167–172.

[13] K.M. Lin, R.E. Poland, J.K. Lau and R.T. Rubin, J. Clin. Psychopharmacol., 8 (1988) 195–201.

[14] T. Okuma, Folia Psychiatr. Neuron. Jpn., 35 (1981) 79–81.