Journal of Chromatography, 525 (1990) 218–224
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5046

Note

High-performance liquid chromatographic procedure for the determination of clozapine, haloperidol, droperidol and several benzodiazepines in plasma

D. WILHELM and A. KEMPER*

Department of Clinical Neurochemistry and Analytic, Central Clinic of Psychiatry and Neurology "Wilhelm Griesinger", Brebacher Weg 15, GDR-Berlin 1141 (G.D R.)

(First received July 26th, 1989; revised manuscript received September 28th, 1989)

Clozapine is an atypical neuroleptic [1,2], which distinguishes it from other neuroleptics (e.g. haloperidol) structurally and clinically. First, clozapine is a dibenzodiazepine derivative whereas haloperidol, for example, is a butyrophenone. However, the main clinical difference is that clozapine has a high antipsychotic activity and may produce a greater improvement in both positive and negative symptoms in schizophrenics but does not produce extrapyramidal side-effects.

Because of reported clozapine-induced hematological complications [3] the drug was withdrawn from the market in all countries. However, the high antipsychotic activity in certain schizophrenic patients who do not respond to other neuroleptics [4–6] has led to renewed interest in clozapine.

It has thus been necessary to develop methods for the determination of plasma levels of clozapine. Some gas chromatographic procedures [7–10] were described ten years ago, but these have the disadvantage of an expensive clean-up procedure to yield the appropriate sample purity and are sensitive only to clozapine.

Recent papers have presented new chromatographic techniques for the determination of clozapine [12–14] and its N-demethylated metabolite [11]. All methods include several liquid–liquid extraction steps for sample cleaning, which are very time-consuming and/or decrease the recovery.

0378-4347/90/$03.50

Attempts have been made to combine clozapine with typical neuroleptics and/or tranquillizers (e.g. diazepam). This paper describes a highly sensitive chromatographic method for the simultaneous determination of plasma levels of clozapine, the typical neuroleptics droperidol and haloperidol and several benzodiazepines after solid phase extractions.

EXPERIMENTAL

Chemicals

Analytical-grade methanol, ethanol and 2-propanol were obtained from VEB Laborchemie Apolda (Apolda, G.D.R.) and distilled before use. HPLC-grade acetonitrile was obtained from VEB PCK (Schwedt, G.D.R.) and used without further purification. Phosphate buffer used for elution was prepared by dissolving 11.41 g of dipotassium hydrogenphosphate trihydrate (Merck, Darmstadt, F.R.G.) in 1 l of tridistilled water and adjusting the pH to 2.9 with phosphoric acid. The 0.5 *M* phosphate buffer for extraction was prepared by dissolving 11.41 g of dipotassium hydrogenphosphate trihydrate in 100 ml of tridistilled water and adjusting the pH to 9 with phosphoric acid.

Apparatus

An HP 1090M high-performance liquid chromatograph from Hewlett-Packard (Vienna, Austria) with a 7010 Rheodyne injection valve (50-μl loop) was used. The separations were performed on a 120 mm$\times$4 mm I.D. column filled with 5-μm Silica 100 RP-18 (Academy of Science of the G.D.R., Berlin) at 45°C, with a 5 mm$\times$4 mm I.D. cartridge dry-packed with the same material. Detection was carried out with a photodiode array spectrophotometric detector built into the chromatograph. For data evaluation by peak areas from 254 and 226 nm, an HP 79994A ChemStation was used, which consisted of an HP 9000 Series 300 computer, a 10-Mbyte Winchester disc drive and a ThinkJet Printer.

Samples were prepared by a solid-phase extraction procedure. The short columns (60 mm$\times$5 mm I.D.) were filled with 100 mg of 100-μm Silica 100 RP-18 material (Academy of Sciences of the G.D.R.).

Chromatographic parameters

The mobile phase flow-rate was 0.80 ml/min. The gradient elution components were eluent I and 2-propanol. Eluent I was 0.05 *M* phosphate buffer (pH 2.9)–ethanol–acetonitrile (70:22:8). The gradient programme is given in Table I.

Standard solutions

All drugs used were donated by VEB GERMED (Dresden, G.D.R.). The stock solutions were prepared by weighing 10 mg of each compound into a 50-ml volumetric flask and dissolving it in methanol. Working solutions contain-

TABLE I

GRADIENT ELUTION PROGRAMME

Step	Eluent I (vol.%)	2-Propanol (vol.%)	Time (min)
1	98.5	1.5	0
2	98.5	1.5	2.5
3	94.0	6.0	10
4	90.0	10.0	10.1
5	90.0	10.0	15
6	98.5	1.5	15.5

ing 10 μg/ml were obtained by diluting the stock solutions with methanol. Clonazepam was used as internal standard (I.S.) and prepared in a similar manner. All standards were stored at 4°C.

Sample preparation

The sorbent was conditioned using 1.5 ml of methanol and 0.5 ml of water. The sorbent was not allowed to dry out at the end of the conditioning step.

Volumes of 500 μl of serum or plasma, 100 μl of a 0.5 *M* dipotassium hydrogenphosphate solution and 10 μl of I.S. solution were vortex-mixed and then passed through the extraction column. The column was then washed with 2.5 ml of water and under vacuum with 30 μl of methanol to remove the rest of the water. The desorption was carried out with 800 μl of methanol followed by evaporation to dryness. The residue was reconstituted with 30 μl of methanol and 40 μl of 0.05 *M* phosphate buffer.

RESULTS AND DISCUSSION

Absolute recoveries were determined to test the efficiency and reproducibility of the extraction procedure. It was performed from serum ($n=10$) and plasma. The concentration added was 50 ng/ml for each compound. No significant difference between the kind of sample (serum or plasma) was observed (Table II).

The precision of the method is presented in Table III. A linear response to the UV detector was obtained for up to 5 μg/ml for each of the selected wavelengths.

The use of reversed-phase packings in liquid chromatography for the separations of benzodiazepines is widely used after extraction from biological liquids [15–17]. In agreement with the reported results it was found that isocratic elution was not suitable for all components. However, to achieve short retention times a gradient programme was selected with the strong eluting component 2-propanol (see Table I).

TABLE II

ABSOLUTE RECOVERIES OF THE DRUGS FROM SERUM

$n = 10$, 50 ng/mg of each drug added.

Compound	Recovery (%)	Coefficient of variation (%)
Droperidol	74.6	8.7
Clozapine	91.1	10.5
Medazepam	67.9	10.7
Haloperidol	101 0	8.9
Carbamazepine	84.3	8.8
Nitrazepam	92.6	6.8
Clonazepam	83.3	8.8
Oxazepam	84.2	7.8
Diazepam	89.6	11.0

TABLE III

PRECISION OF THE METHOD

50 ng/ml of each drug added.

Drug	Within-day ($n=5$)		Day-to-day ($n=10$)	
	Mean concentration (ng/ml)	C.V. (%)	Mean concentration (ng/ml)	C.V. (%)
Droperidol	36.1	8.9	36.5	7.8
Clozapine	45.0	9.5	44.8	10.0
Medazepam	34.5	11.0	33.6	9.3
Haloperidol	51.5	7.8	50.8	8.1
Carbamazepine	42 3	8.9	43.1	9.3
Nitrazepam	45.5	7.0	45.0	6.5
Clonazepam	42.0	9.3	40.9	8.8
Oxazepam	44.5	8.1	42.9	9.0
Diazepam	43.1	10.5	44.0	8.1

Fig. 1 shows typical chromatograms from (A) a blank, (B) a spiked serum extract (100 ng/ml for each drug) and (C) a patient's serum. The peaks are well resolved and, in the case of carbamazepine and haloperidol, identification and quantification are possible from their different UV spectra. Carbamazepine could also be used as an internal standard because it is structurally very similar to clozapine.

The peaks at 4.8 min (X) and 6.5 min (Y) (Fig. 1C) could be metabolites of clozapine because of similarities to clozapine in the UV spectra, whereas the peak at 13.8 min (Z) is probably a metabolite of diazepam. Unfortunately these

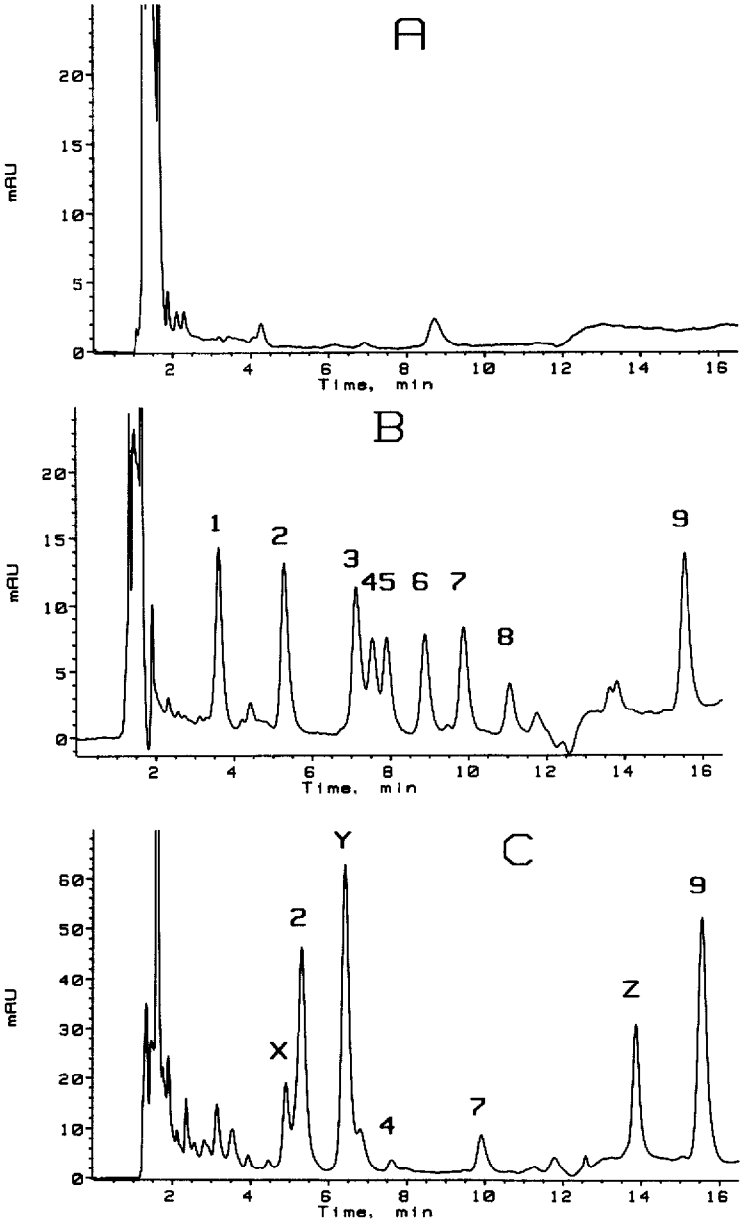

Fig. 1. Chromatogram from (A) a blank, (B) a spiked serum (100 ng/ml for each drug) and (C) a patient's serum (4.22 mg/kg clozapine, 0.38 mg/kg haloperidol, 0.77 mg/kg diazepam). Peaks: 1 = droperidol; 2 = clozapine; 3 = medazepam; 4 = haloperidol; 5 = carbamazepine; 6 = nitrazepam; 7 = clonazepam (I.S.); 8 = oxazepam; 9 = diazepam; X, Y = possible metabolites of clozapine; Z = possible metabolite of diazepam.

metabolites were not available so that identification and co-determination were not possible.

Extraction of clozapine is usually performed by liquid–liquid extraction [7,9,11–14]. Solid-phase extraction is becoming increasingly preferred [15,21,22] because of its easy use, low sample elution volume and good recoveries. Our sample clean-up with solid-phase extraction results in very little interference from endogenous compounds (Fig. 1), and offers good recoveries for all drugs determined. The precision is satisfactory at low concentrations and improves at higher concentrations.

Detection was carried out at two different wavelengths, because many authors present their results for 254 nm and we chose 226 nm as a wavelength where most of the observed drugs have higher absorptions. That allows us to calculate the ratio of the signals for better estimation of the amounts and impurities.

The sensitivity of a method is often expressed as the limit of detection (LOD). The LOD for spectrometric analysis is defined as the S.D. of the baseline noise [18–20]. However, the measurement and determination of the baseline noise are useful only if there is no interference from the matrix, and there are normally peaks present from endogenous compounds during the determination of drugs from biological fluids.

It is thus useful to calculate two parameters, the ILOD and the limit of quantitation (LOQ) [18]. According to the LOD, the ILOD is the amount of a compound giving a peak height three times the S.D. of the baseline noise without any matrix interference, whereas the LOQ is the concentration of an analyte in the matrix that could be determined with a reasonable certainty under the given analytical procedure.

In our case, the ILOD was found to be ca. 250 pg for clozapine and the other reported drugs. The LOQ was ca. 450 pg/ml when volume losses during sample work-up and injection were taken into account. The plasma levels determined in a number of selected patients (with low and high doses) were estimated to be between 50 and 650 ng/ml. These values indicate that this method is sensitive enough for pharmacokinetic studies.

The extraction procedure can be performed in 15 min and chromatography takes ca. 20 min. Under the same conditions monitoring of clozapine plasma levels is possible by isocratic elution only with eluent I (retention time 6.6 min for clozapine). The method is widely used for monitoring clinical cases as well as in fundamental research.

REFERENCES

1 H.Y. Meltzer, J.F. Nash, J.I. Koenig and G.A. Gudelski, J. Clin. Neuropharmacol., 9 (1986) 316–318.
2 J. Lieberman, J. Kane, C. Jones and J. Vital-Herne, J. Clin. Neuropharmacol., 9 (1986) 140–141.
3 J. Indänpään-Heikkilä, E. Alhave and M. Olkinura, Eur. J. Pharmacol., 11 (1977) 193–198.

4 U.J. Povlsen, U. Norling, R. Fog and J. Gerlach, Acta Psychiatr. Scand., 71 (1985) 176-185.
5 S. Kuha and E. Miettinen, Nord J. Psychiatry, 40 (1986) 225-230.
6 L.H. Lindström, Acta Psychiatr. Scand., 77 (1988) 524-529.
7 G.M. Simpson and T.A. Cooper, Am. J. Psychiatry, 135 (1978) 99-100.
8 R. Heipertz, H Pilz and W. Beckers, Arch. Toxicol., 37 (1977) 313-318
9 B. Stock, G. Spiteller and R. Heipertz, Arzneim.-Forsch., 27 (1977) 982-990.
10 M. Ackenheil, H. Bräu, A. Burkhart and A. Franke, Arzneim.-Forsch., 26 (1977) 1156-1158.
11 U. Bondesson and L.H. Lindström, Psychopharmacology, 95 (1988) 472-475.
12 M.G. Choc, R.G. Lehr, F. Hsuan, G. Honigfeld, H.T Smith, R. Borison and J. Volavka, Pharm. Res., 4 (1987) 402-405.
13 C. Haring, C. Humpel, B. Auer, A. Saria, C. Barnas, W. Fleischhacker and H. Hinterhuber, J. Chromatogr., 428 (1988) 160-166.
14 C. Humpel, C. Haring and A. Saria, J. Chromatogr., 491 (1989) 235-239.
15 P. Mura, A. Piriou, P. Fraillon, Y. Papet and D. Reiss, J. Chromatogr., 416 (1987) 303-310.
16 G.L. Lensmayer, C. Rajani and M.A. Evenson, Clin. Chem., 28 (1982) 2274-2278.
17 R. Gill, B. Law and J.P. Gibbs, J. Chromatogr., 356 (1986) 37-46.
18 L.B. Nilsson, J. Chromatogr., 431 (1988) 113-122.
19 J.E. Knoll, J. Chromatogr. Sci., 23 (1985) 422-428.
20 J.P. Foley and J.G. Dorsey, Chromatographia, 18 (1984) 503-511.
21 P.M. Kabra and E.U. Nzekwe, J. Chromatogr., 341 (1985) 383-390.
22 G. Musch and D.L. Massart, J. Chromatogr., 432 (1988) 209-222.