Journal of Chromatography, 565 (1991) 363–373
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5733

Determination of benperidol and its reduced metabolite in human plasma by high-performance liquid chromatography and electrochemical detection

STEPHAN SÜSS, WALTHER SEILER and CHRISTOPH HIEMKE*

Department of Psychiatry, University of Mainz, Untere Zahlbacher Strasse 8, 6500 Mainz (Germany)

GÜNTER SCHÖLLNHAMMER

Troponwerke, Biochemische Entwicklung, Berliner Strasse 156, 5000 Köln 180 (Germany)

and

HERMANN WETZEL and ANDREAS HILLERT

Department of Psychiatry, University of Mainz, Untere Zahlbacher Strasse 8, 6500 Mainz (Germany)

(First received July 10th, 1990; revised manuscript received October 31st, 1990)

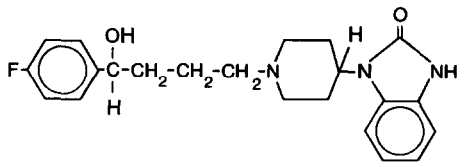
ABSTRACT

An isocratic high-performance liquid chromatographic method with electrochemical detection for the quantification of benperidol and its suggested reduced metabolite TVX Q 5402 in human plasma is described. The method included a two-step solid-phase extraction on reversed-phase and cation-exchange material, followed by separation on a cyanopropyl silica gel column (5 μm; 250 mm × 4.6 mm I.D.). The eluent was 0.15 *M* acetate buffer (pH 4.7) containing 25% acetonitrile (w/w). Spiperone served as internal standard. The inclusion of the cation-exchange step provided sample purity higher than those achieved with other methods. After extraction of 1 ml of plasma, concentrations as low as 0.5 ng/ml were detectable for both benperidol and the metabolite. In plasma samples collected from a schizophrenic patient treated with a single oral dose of 6 mg of benperidol, plasma levels of benperidol and of the metabolite could be measured from 20 min to at least 12 h after administration.

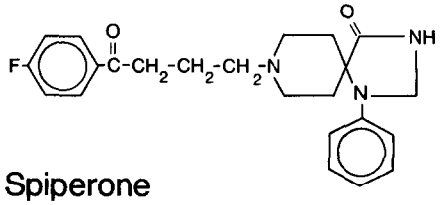
INTRODUCTION

Benperidol (Glianimon™, Fig. 1) is a highly potent neuroleptic drug of the butyrophenone group. Despite its widespread use, conclusive information on both its metabolism and its pharmacokinetics is scarce owing to technical difficulties in accurately determining low concentrations of the drug and its metabolites in human plasma. It has been suggested that, similar to the metabolism of the closely related haloperidol [1], a major metabolite of benperidol is formed by reduction of the 4-oxybutyl group to form TVX Q 5402 (I) (Fig. 1), whose neuroleptic efficacy remains largely unstudied. This indicates the need for an analytical method for the quantification of both of these compounds, which combines high sensitivity and selectivity as well as simplicity for routine use.

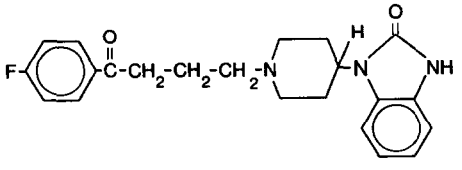
0378-4347/91/$03.50



TVX Q 5402



Spiperone



Benperidol

Fig. 1. Structures of benperidol, its reduced derivative TVX Q 5402 (I) and spiperone.

None of the methods previously described for the quantification of benperidol includes measurement of its reduced derivative I, and indeed they all have major disadvantages that prevent their extension to compound I. Radioimmunoassays described for the determination of haloperidol [2] and modified for the determination of benperidol [3] are sensitive but lack sufficient selectivity. Likewise, sensitive radioreceptor assays do not provide satisfactory selectivity [4]. Gas chromatographic (GC) [5] or GC–mass spectrometric methods [6,7] as reported for haloperidol might be adaptable for measuring benperidol. However, as well as being costly, these methods are very laborious and unsuitable for routine laboratory use. A high-performance liquid chromatographic (HPLC) method for determining benperidol using liquid–liquid extraction and UV detection requires large sample volumes to achieve sensitivities sufficient for pharmacokinetic studies [8]. Instead, as recently reported for haloperidol [9], the combination of HPLC with solid-phase extraction and electrochemical detection (ED) appears to be more suitable to achieve the sensitivities required. Furlanut *et al.* [10] described a sensitive HPLC method for quantifying benperidol in human plasma using one-step solid-phase extraction and ED. However, this assay does not include determination of I. In addition, for separation it uses expensive microbore material not readily available in many laboratories. Moreover, repetition of the extraction procedure described by Furlanut *et al.* [10] failed to provide sample purification sufficient for the maintenance of ED sensitivity.

This paper describes the establishment of an improved sensitive and selective assay for the quantification of benperidol in human plasma using HPLC and ED. In addition, this assay can also determine the suggested reduced metabolite I with similar levels of sensitivity and selectivity. Thus, for the first time, a single method can be used for investigations on both the pharmacokinetics and the metabolism of benperidol. The method included a two-step extraction of small sample volumes with reversed-phase and cation-exchange material, yielding highly purified analytes for separation and detection.

EXPERIMENTAL

Chemicals

Benperidol, 1-[1[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one, and its reduced derivative I, 1-[1-[4-(4-fluorophenyl)-4-hydroxybutyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one, were obtained from Tropon (Cologne, Germany). Spiperone, 8-[4-(4-fluorophenyl)-4-oxobutyl]-1-phenyl]-1,3,8-triazaspiro[4,5]decan-4-one, was kindly donated by Janssen Pharmaceutica (Beerse, Belgium) (Fig. 1). Methanol (LiChrosolv, Merck, Darmstadt, Germany) and acetonitrile (Lichrosolv; Merck) were used without further purification. The water used in this study was purified and deionized by a Milli-Q water-processing system (Millipore, Eschborn, Germany). All other chemicals were of analytical grade purchased from commercial sources.

Standards

Stock solutions of benperidol, I, spiperone (internal standard), and all other drugs tested were prepared by dissolving 10 mg in 10 ml of a mixture of 10% 1 *M* HCl and 90% methanol (v/v). These solutions could be stored for several months in the dark at −20°C without measurable decomposition, which is in agreement with other reports [8]. Similar stabilities were observed for appropriately spiked plasma samples stored at −20°C.

For spiperone, the internal standard solutions to be added to the plasma samples were prepared by diluting the stock solution with methanol to a final concentration of 1 μg/ml. For benperidol and I, the standard concentrations for the preparation of calibration curves were obtained by adding 0.15 *M* acetate buffer (pH 4.7) to stock solutions for final concentrations of 10, 50, 70, 100 and 1000 ng/ml.

Plasma used for calibration curves was spiked by adding 100 μl each of the internal standard solution and the appropriate drug standard solutions to 1 ml of blank plasma, to give final concentrations of 100 ng of spiperone and 1, 5, 7, 10 and 100 ng of benperidol and I per millilitre of plasma.

In order to investigate possible interference from coadministered drugs with the analysis of the neuroleptics of interest, a series of chromatographic runs was performed with 25 commonly used neuroleptics, tranquillizers, and antidepres-

sants. Stock solutions of these drugs (Table I) were diluted with 0.15 *M* acetate buffer (pH 4.7) to a final concentration of 1 μg/ml for direct injection.

Plasma samples

Blood samples were withdrawn from the antecubital vein of healthy volunteers

TABLE I

RETENTION TIMES AND PEAK AREAS OF SELECTED PSYCHOPHARMACOLOGICAL AGENTS

Drug (1 μg/ml)	Retention time (min)	Peak area for 1-ng values divided by 1000 (integrator units)
Neuroleptics		
TVX Q 5402 (I)	9.60	290
Benperidol	13.17	360
Spiperone	17.96	100
Haloperidol	22.69	74
Bromperidol	25.22	67
Triflurperidol	31.13	35
Pimozide	70.93	167
Fluspirilen	N.D.[a]	–
Antidepressants		
Desmethyldoxepin	15.09	9.5
Doxepin	18.08	28
Desmethylimipramine	19.28	35
Protriptyline	20.04	2.7
Nortriptyline	20.42	2
Desmethyltrimipramine	22.48	27
Imipramine	23.12	59
Desmethylperazinedimaleate	23.17	52
Amitriptyline	24.76	25
Trimipramine	26.00	42
Desmethylclomipramine	29.40	18.5
Perazinedimaleate	29.74	67
Clomipramine	35.32	210
Desmethylmaprotiline	44.36	2
Maprotiline	54.49	1.2
Tranquillizers		
Chlordiazepoxid	10.7	0.75
Lorazepam	N.D.	–
Diazepam	N.D.	–
Desmethyldiazepam	N.D.	–
Bromazepam	N.D.	–

[a] N.D. = not detected.

or schizophrenic patients. Plasma was obtained by centrifugation of heparinized blood samples at 3000 *g* for 10 min, and was stored at −20°C until assayed.

Extraction

For extraction of the neuroleptics, 1 ml of centrifuged plasma (3000 *g* for 30 min) was transferred into 10-ml glass tubes and mixed with 100 µl of spiperone internal standard solution (leading to a final concentration of 100 ng of spiperone per millilitre of plasma).

The solid-phase extraction procedure involved sequential subjection of plasma samples to 3-ml (300 mg) C_{18} reversed-phase and 1-ml (100 mg) benzenesulphonic acid cation-exchange (SCX) columns (both Bond Elut™ cartridges; ICT, Frankfurt, Germany) mounted on a vacuum-driven Vac-Elut™ column holder, (Analytichem, Harbor City, CA, U.S.A.). Prior to use, the reversed-phase columns were conditioned by sequentially drawing three column volumes of methanol, two column volumes of 1 *M* acetic acid in methanol and one column volume of water through the columns. The cation-exchange columns were conditioned with two column volumes of methanol, three column volumes of 2.5 *M* NH_3 in methanol, two column volumes of methanol, two column volumes of 5 *M* HCl in methanol and one column volume of water.

For reversed-phase extraction the samples were mixed with 7 ml of 0.1 *M* NaOH before transfer to the C_{18} columns. After sample loading without vacuum, the upper parts of the cartridges were rinsed five times with water. Removal of polar residues was performed by drawing two column volumes of water and one column volume of 40% methanol in water (w/w) through the column. Elution of the analytes with 1 ml of 1 *M* acetic acid in methanol was done by centrifugation of the cartridges (0 to 3000 *g* within 10 min). The eluates were collected in 10-ml glass tubes.

For cation-exchange extraction the samples eluted from the C_{18} columns were mixed with 7 ml of acetic acid (10%). Sample loading and rinsing of the cartridges were performed as outlined above. Remaining contaminants were removed by drawing three column volumes of water and three column volumes of methanol through the columns. The analytes were eluted with 1 ml of 2.5 *M* NH_3 in methanol by centrifugation as described above. Subsequent evaporation of the solvent was performed at 40°C using a vortex evaporator (Haake Buchler, Saddle Brook, NJ, U.S.A.). For injection into the chromatographic system the dried analytes were redissolved in 750 µl of 0.15 *M* acetate buffer (pH 4.7) using a vortex mixer (30 s).

Chromatography

The chromatographic system comprised a Constametric III pump (LDC, Milton Roy, Hasselroth, Germany), a pulse damper (Bischoff, Leonberg, Germany) and an automatic injector, Model Promis (Spark Holland, Emmen, The Netherlands) with a Rheodyne 7010 injection valve and a 100-µl sample loop. A 5020

ESA guard cell (Bischoff) was installed ahead of the autosampler in order to oxidize chemical impurities in the mobile phase. One 0.2-μm filter ESA element (Bischoff) was placed between the pulse damper and the guard cell, and another between the analytical column and the dual coulometric–amperometric detector cell, Model ESA 5011 (Bischoff). Separation was performed on an analytical column (250 mm × 4.6 mm I.D.) with an integrated precolumn (20 mm × 4.6 mm I.D.), both containing 5-μm Hypersil-CPS (CTI, Idstein, Germany). An ESA Coulochem Model 5100 A controller (Bischoff) was used to set the potentials of the electrodes: the guard cell at +0.8 V and the analytical cell at +0.55 V (detector 1) and at +0.7 V (detector 2), respectively. The signals from detector 2 were registered and integrated by a D-2000 chromato-integrator (Merck-Hitachi, Darmstadt, Germany). Isocratic elution was performed at a flow-rate of 1.5 ml/min using a mobile phase of 0.15 *M* acetate buffer (pH 4.7) containing 25% acetonitrile (w/w). Prior to use, the eluent was filtered through a 0.2-μm membrane filter (Millipore). The entire system was operated at room temperature.

RESULTS

Chromatography

After extraction of aqueous standard solution or spiked blank plasma the chromatographic conditions described above provided a baseline separation of spiperone as internal standard and both analytes within less than 20 min (Fig. 2A–C). Chromatograms of extracted unspiked blank plasma did not reveal any interfering peaks (Fig. 2D). Of the 25 drugs tested for interference, only doxepin showed a retention time similar to that of a compound of interest. The latter was spiperone, which was used as internal standard (Table I).

An electrode potential of +0.7 V for detection provided the best sensitivity while maintaining a low baseline noise. Voltammograms for benperidol, I and spiperone are presented in Fig. 3.

The assay linearity was checked by linear regression analyses of seven independent calibration curves derived from 1-ml blank plasma samples spiked with 1–100 ng/ml for both analytes. Using relative peak areas, correlation coefficients were computed as ranging between 0.986 and 0.999 for benperidol and between 0.964 and 0.999 for I. The curve intercepts were calculated as −0.022 to 0.01 for benperidol and −0.007 to 0.011 for I.

The detection limits for both benperidol and for I were found to be below 0.5 ng/ml of plasma. Lower levels could be detected (Fig. 4), although the ability to quantify them accurately has not been evaluated. Using the control plasma samples comprising the calibration curves that were run with each series of patient samples, the reliability of the assay based on independent analyses from seven different days was obtained (Table II).

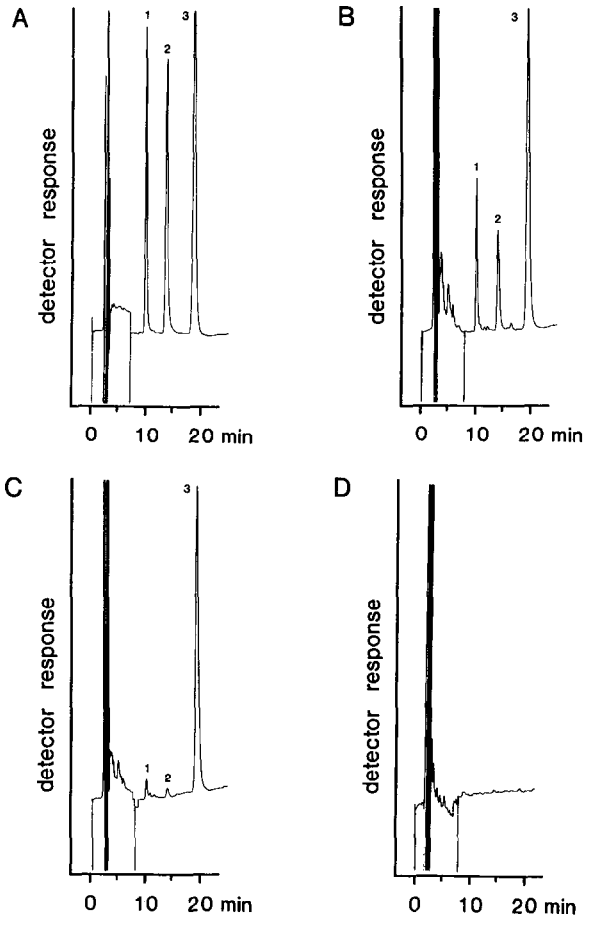


Fig. 2. Representative chromatograms of samples including spiperone (100 ng/ml) as internal standard (I.S.). (A) A standard solution containing 20 ng/ml each of benperidol and I; (B)–(D) extracted blank plasma samples spiked with 10 (B), 1 (C), and 0 (D, no I.S. added) ng benperidol and I per ml of plasma. Peaks: 1 = I; 2 = benperidol; 3 = spiperone.

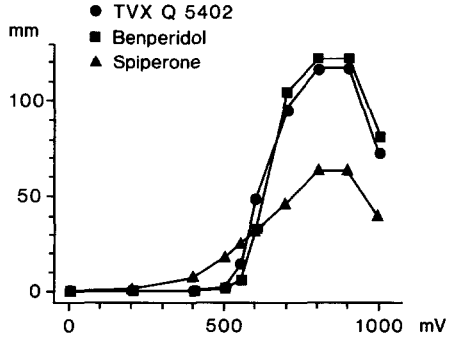


Fig. 3. Voltammograms of benperidol, I, and spiperone. The detector response is given as peak height (mm).

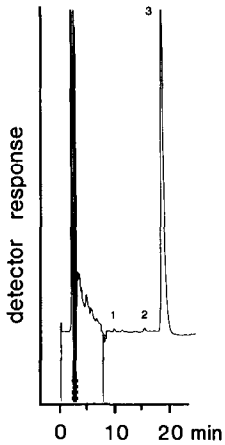


Fig. 4. Chromatogram of a patient's extracted plasma sample collected 48 h after oral administration of 6 mg of benperidol. Peak labelling as in Fig. 2. Plasma concentrations for benperidol and I were determined as 0.3 and 0.2 ng/ml, respectively.

Extraction

Extracts of blank plasma after only a single extraction step on the C_{18} cartridge contained substantial amounts of impurities (Fig. 5), which were effectively removed by subsequent cation-exchange extraction (Fig. 2D). Extractability values for the entire procedure were 96.0 ± 2.1% for I, 73.6 ± 2.5% for benperidol and 57.8 ± 1.9% for spiperone (all $n = 5$).

Application

The applicability of the method for investigation into the pharmacokinetics and metabolism of benperidol can be judged from preliminary results of a more

TABLE II

ASSAY RELIABILITY AS FOUND FOR INDEPENDENT ANALYSES ON SEVEN DIFFERENT DAYS

Data are presented as means.

Given (ng/ml)	TVX Q 5402			Benperidol		
	Found (ng/ml)	C.V. (%)	*n*	Found (ng/ml)	C.V. (%)	*n*
1	1.1	19.0	12	1.0	14.2	12
5	4.9	6.2	7	4.8	4.0	7
7	6.3	6.5	7	6.7	4.3	7
10	9.6	8.4	9	9.9	6.8	9
100	104.9	–	2	103.2	–	3

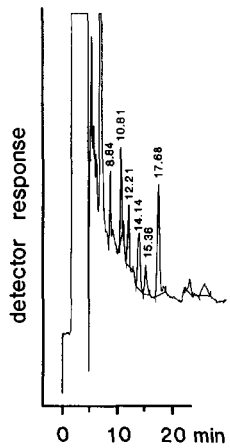


Fig. 5. Chromatogram of a blank plasma sample after single-step solid-phase extraction on a C_{18} reversed-phase column. Note the various interfering peaks from unknown contaminants.

detailed study currently in progress. Analyses of plasma samples obtained from a schizophrenic patient treated with a single oral dose of 6 mg of benperidol are presented in Fig. 6. Benperidol was detectable in the plasma within 20 min after

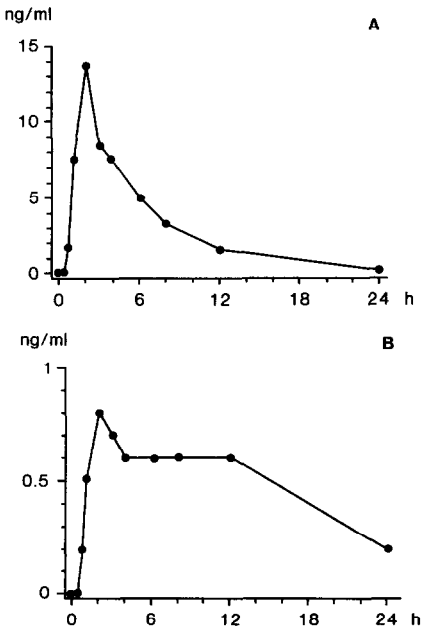


Fig. 6. Concentration profiles of (A) benperidol and (B) its reduced metabolite I measured in plasma samples obtained from a schizophrenic patient treated with a single oral dose of 6 mg of benperidol. Note the different ordinate scales.

drug administration for at least 12 h. Also clearly visible was the parallel appearance of I.

DISCUSSION

This study had two major aims: first, the detection of benperidol with a sensitivity sufficient for pharmacokinetic investigations by means of a reliable and fast analytical method, second, the simultaneous quantification of the suggested reduced metabolite of benperidol, I.

The use of an ED system as described in this paper allowed reproducible and reliable quantification of benperidol at concentrations below 1 ng/ml, which is satisfactory for monitoring plasma levels of benperidol even below therapeutically active concentrations. This detection limit was achieved without increasing the sample volume, which would have been necessary if less sensitive methods such as UV detection had been used [8]. The restriction to sample volumes not exceeding 1 ml of plasma is necessary for clinical applications if study designs relying on more extensive blood sampling schedules are needed.

An extraction procedure with high recovery was a prerequisite to achieve the sensitivity required. The two-step solid-phase extraction described here gives higher recovery rates than the liquid–liquid extraction methods previously reported [8]. A further advantage of the improved extraction procedure is its superior purification capability, which leads to a high degree of selectivity. Moreover, highly purified analytes are required because of the potential vulnerability of the ED system to impurities. The electrodes of electrochemical detectors lose sensitivity after continued exposure to complex samples, such as biological fluids, without sufficient prior purification. The two-step solid-phase extraction procedure described here was found to be advantageous compared with a single-step extraction on C_8 as used in a recently published benperidol assay [10]. Nonetheless, in order to maintain the sensitivity of the ED system described here the eluent must be changed regularly, and the entire device, must be frequently rinsed, even if exposed only to highly purified samples.

The inclusion of the reduced metabolite of benperidol, I, in the assay was an important expansion compared with all previously published methods for the determination of benperidol. Using this assay the occurrence of I in the blood plasma of patients treated with benperidol was clearly visible, thus indicating that benperidol is metabolized *in vivo* by reduction of the 4-oxobutyl group. In the absence of any published information on that metabolic pathway this is the first report on the formation of I in humans. Therefore, with regard to the unknown neuroleptic efficacy of this reduced derivative the analytical procedure described here provides a useful tool for further studies on the bioavailability of benperidol and the *in vivo* formation of its metabolite.

ACKNOWLEDGEMENTS

The authors thank the nursing staff of the Psychiatrische Klinik (Mainz, Germany) for helping with the blood sampling. Gabriele Stroba is gratefully acknowledged for her skillfull technical assistance.

REFERENCES

1 A. Forsman and M. Larsson, *Curr. Ther. Res.*, 24 (1978) 567.
2 H. Itho, Y. Fujii and K. Ichikawa, *Adv. Hum. Psychopharmacol.*, 3 (1984) 29.
3 G. Schöllnhammer, H. Spechtmeyer, C. Hesse, J. Husser, H. Ebeling and H. M. Parish, Pharmacopsychiatry, 18 (1985) 54.
4 I. Creese and S. H. Snyder, *Nature*, 270 (1977) 180.
5 D. R. Abernethy, D. J. Greenblatt, H. R. Ochs, C. R. Willis, D. D. Miller and R. I. Shader, *J. Chromatogr.*, 307 (1984) 194.
6 H. Maurer and K. Pfleger, *J. Chromatogr.*, 272 (1983) 75.
7 M. A. Moulin, R. Camsonne, J. P. Davy, E. Poilpre, P. Morel, D. Debruyne and M. C. Bigot, *J. Chromatogr.*, 178 (1979) 324.
8 R. Krüger, I. Mengel and H. J. Kuss, *J. Chromatogr.*, 311 (1984) 109.
9 N. D. Eddington and D. Young, *J. Pharm. Sci.*, 77 (1988) 541.
10 M. Furlanut, A. Perosa, P. Benetello and G. Colombo, *Ther. Drug Monit.*, 9 (1987) 343.