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DETERMINATION OF BENPERIDOL IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method for the quantitative determination of benperidol in human plasma using haloperidol as internal standard is described. The method involves liquid—liquid extraction, separation of the substances on a reversed-phase column C_{18} followed by ultraviolet detection at 254 nm. The mobile phase consists of 32% acetonitrile in 0.05 *M* potassium dihydrogen phosphate buffer (pH 2.8). The detection limit is 0.5—1.0 ng/ml using 2- or 4-ml plasma samples.

INTRODUCTION

Benperidol (Glianimon®) is an antipsychotic agent of the butyrophenone group. It is one of the most potent neuroleptics, being about eight times more potent than haloperidol, a neuroleptic with related structure (Fig. 1) [1].

No simple and selective method has been described for the determination of benperidol in human plasma, whereas several methods exist for determining haloperidol. The radioimmunoassay developed for haloperidol can be applied to benperidol [2—4]. However, the assay is unspecific, since cross-reaction occurs with other butyrophenones. The radioreceptor assay [5], too, is unspecific, since drugs and metabolites that block dopamine receptors are measured as well.

Gas chromatographic [6—9] and selected-ion monitoring mass spectrometric [10,11] assays have been reported for haloperidol but not for the other butyrophenones. These methods are not suitable for routine monitoring of plasma levels, as they are very time-consuming.

Two methods have been developed for haloperidol using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection [12,13]. Experiments to determine benperidol in human plasma with these methods

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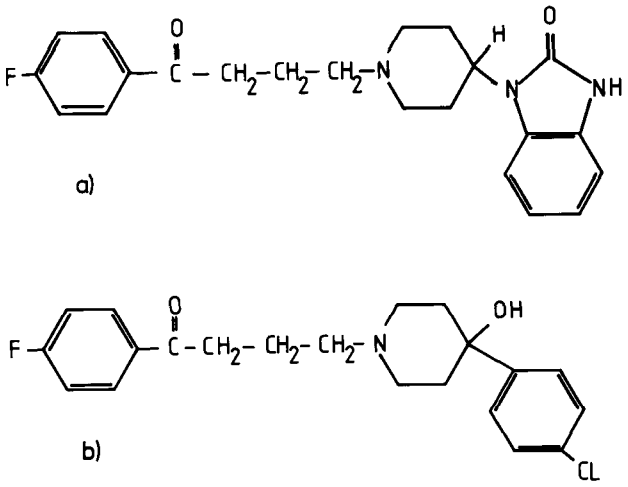

Fig. 1. Chemical structures of (a) benperidol and (b) haloperidol.

have failed in our laboratory. Another HPLC method with electrochemical detection [14] has been reported for haloperidol and reduced haloperidol, not for benperidol.

The method described here, based on HPLC, is selective for benperidol, as well as for haloperidol. It is rapid, and sensitive enough to study the pharmacokinetics of both drugs and to study relationships between plasma concentrations and neuroleptic efficacy.

MATERIALS AND METHODS

Chemicals

Benperidol, 4-[4-(2-oxo-2,3-dihydro-1-benzimidazolyl)-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, was obtained from Tropon (Cologne, F.R.G.) and haloperidol, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone from Janssen-Pharmaceutica (Belgium). *n*-Hexane (Uvasol, Merck), isopropanol (Uvasol, Merck), acetonitrile (LiChrosolv, Merck), sodium bicarbonate (p.a., Merck), phosphoric acid (p.a., Merck) were used without further purification. Aqueous solutions were prepared using deionized water, which had been passed through a Milli-Q water purification system (Millipore).

Standards

Stock solutions were prepared by dissolving 1 mg of benperidol and 1 mg of haloperidol in 1 ml of 0.1 *M* hydrochloric acid. They were stored at 4°C. These solutions are stable for several months. Aliquots of these stock solutions were diluted with 0.05 *M* potassium dihydrogen phosphate buffer (pH 2.8) to a concentration of 1 μg/ml. These solutions served to prepare spiked plasma samples for calibration curves and recovery analyses. Fresh plasma samples were prepared on each day of analysis. Solutions of benperidol in methanol were stable at 4°C for 3—5 weeks only.

Chromatographic system

The chromatographic system consisted of a Waters 6000A solvent delivery

system, a Waters UV detector 440 operating at 254 nm and a Rheodyne 7125 syringe loading sample injector with a 100-μl sample loop. A HP 3390 A integrator was used to determine the concentrations of the neuroleptics by peak area integration. The mobile phase consisted of 32% acetonitrile in 0.05 *M* potassium dihydrogen phosphate buffer (pH 2.8). The buffer solution was passed through a 0.2-μm membrane filter (Schleicher & Schüll) prior to mixing with acetonitrile. The flow-rate was set at 1.0 ml/min. A column (length 250 mm, O.D. 8 mm, I.D. 4 mm) packed with C_{18} Nucleosil (Macherey, Nagel & Co., Düren, F.R.G.; particle size 5 μm) served as stationary phase.

Extraction

The quantity of plasma used for extraction depended on the benperidol concentration. For concentrations >5 ng/ml plasma samples of 1 ml were extracted; for lower concentrations, samples of 2 or 3 ml were used.

Plasma and 50 ng (50 μl) of haloperidol as internal standard were mixed in polypropylene tubes (17.5 ml) with polyethylene stoppers. After adding 1 ml of 2 *M* sodium bicarbonate buffer (pH 10.5) and 5 ml of hexane with 5% or 10% isopropanol (for 1 or 2 ml of plasma, respectively), the samples were shaken for 30 min and centrifuged for 10 min at 1800 *g*. The hexane layer was transferred to another tube containing 100 μl of 0.05 *M* potassium dihydrogen phosphate buffer (pH 2.8). This mixture was shaken again for 30 min and centrifuged for 10 min at 1800 *g*. The hexane layer was aspirated and 90 μl of the aqueous layer were injected into the chromatographic system.

Calibration

Plasma samples of 1 or 2 ml with benperidol concentrations of 1, 3, 5, 10, 20 and 50 ng/ml were prepared. After adding 50 ng/ml haloperidol as internal standard, the samples were treated as described above. Calibration curves were obtained by plotting the peak area ratios of benperidol to haloperidol against the benperidol concentration given.

RESULTS AND DISCUSSION

Extraction

Liquid—liquid extraction of the butyrophenones from plasma in two steps was necessary to separate the drugs from endogenous substances which interfere with the chromatographic analysis. In the first step, the butyrophenones were extracted from alkalized plasma (pH 10.5) with an organic solvent. In the second, they were reextracted with an acidic aqueous solution (pH 2.8). The extractabilities of benperidol and haloperidol were different. The yield of extraction depended on the amount of isopropanol added to hexane (Table I). Plasma samples of 1 and 2 ml, each spiked with 50 ng of benperidol and 50 ng of haloperidol, were extracted with hexane and with hexane + 1%, 5%, 10% or 20% isopropanol.

When the plasma was extracted with pure hexane, a gel was formed in the organic layer; the absolute recoveries were therefore very low and variable. After extraction with hexane + 1% isopropanol the recovery for benperidol was lower than for haloperidol, the ratios being 0.26 for 1 ml of plasma and 0.09

TABLE I

INFLUENCE OF ISOPROPANOL ON THE EXTRACTABILITY OF 50 ng OF BENPERIDOL AND 50 ng OF HALOPERIDOL ADDED TO 1 OR 2 ml OF PLASMA

Values are mean of four extractions.

Plasma volume (ml)	Isopropanol (%)	Extractability (%)		Benperidol/haloperidol
		Benperidol	Haloperidol	
1	0*	8.2 ± 4.5	12.2 ± 5.3	0.67
2	0*	3.2 ± 3.0	26.1 ± 9.3	0.12
1	1	16.5 ± 2.1	63.7 ± 6.1	0.26
2	1	4.8 ± 0.5	56.2 ± 4.6	0.09
1	5	65.3 ± 2.1	75.4 ± 1.6	0.87
2	5	37.4 ± 4.5	73.0 ± 2.8	0.51
1	10	38.8 ± 2.3	29.0 ± 1.9	1.34
2	10	55.2 ± 3.6	68.1 ± 3.2	0.81
1	20**	34.1 ± 3.5	24.0 ± 5.3	1.42
2	20**	19.3 ± 9.8	21.3 ± 9.6	0.91

*Gel formation, extractability very low.
**Peaks very broad and not well separated from other plasma peaks.

for 2 ml of plasma. When 1 ml of plasma was extracted, the best recoveries for both substances were obtained with hexane + 5% isopropanol, with a mean ratio of 0.87. When 2 ml of plasma are extracted, the amount of isopropanol must be increased to 10% in order to obtain comparable recoveries; the ratio was then 0.81. With hexane + 5% isopropanol the recovery for benperidol was too low, the ratio being 0.51.

The addition of isopropanol to hexane is important, especially for the extraction of benperidol; the amount necessary depended on the quantity of plasma used for extraction.

Detection

The absorbances of benperidol and haloperidol at 214 and 254 nm were compared. They were greater at 214 nm than at 254 nm for both drugs.

The ratio of peak area at 214 nm to peak area at 254 nm is 2.0 for benperidol and 1.3 for haloperidol. Nevertheless, the extracted plasma samples were measured at 254 nm, because the signal-to-baseline-noise ratio was better at this wavelength. If there are other drugs in the extracted plasma, the selectivity of analysis can be improved by measuring the absorbances at both wavelengths and comparing the peak area ratios.

Resolution

With the chromatographic conditions described in Materials and methods the retention times are about 6 min for benperidol and about 12 min for haloperidol.

Fig. 2 shows the chromatograms for a blank plasma sample (Fig. 2a), a plasma sample spiked with 5 ng of benperidol and 50 ng of haloperidol (Fig. 2b) and a plasma sample of a patient receiving benperidol 40 mg/day (Fig. 2c). The blank plasma was free of interfering peaks.

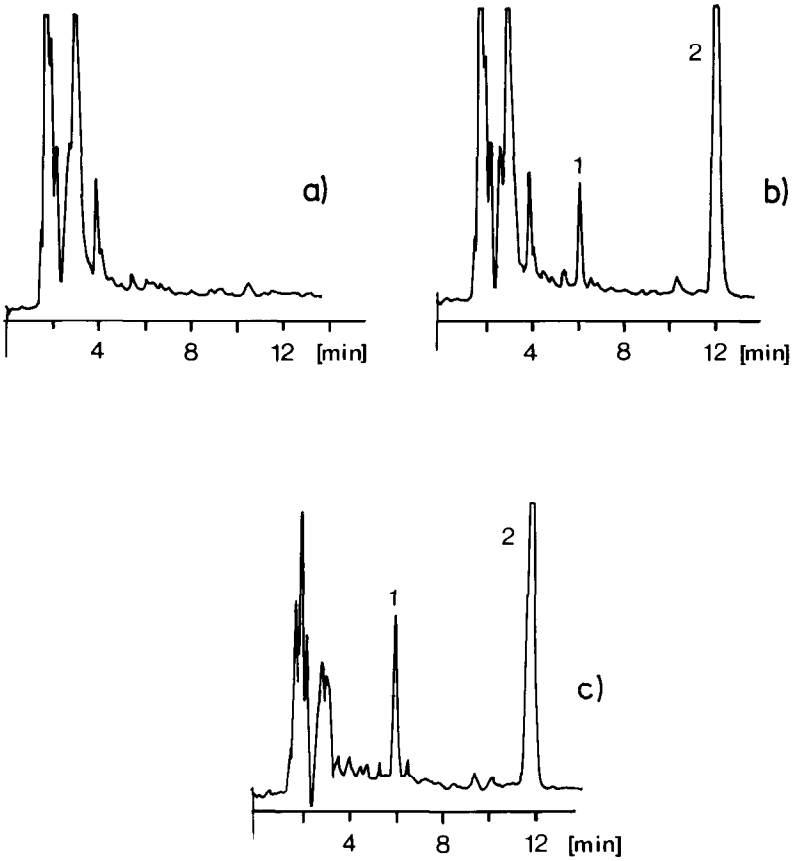

Fig. 2. Chromatograms of blank plasma (a), plasma spiked with 5 ng benperidol + 50 ng haloperidol (b), and plasma taken from a patient treated with 40 mg/day benperidol (c). Peaks: 1 = benperidol; 2 = haloperidol.

The sensitivity of our method was 1 ng/ml if 2 ml of plasma were extracted, and 0.5 ng/ml if 4 ml were extracted.

Calibration and precision

The calibration curves in the range of 1—50 ng/ml benperidol were linear. Mean values of the peak area ratios benperidol to haloperidol with standard deviations and coefficients of variation as well as the regression equation of the line are summarized in Table II. Recovery analyses of spiked plasma samples were done together with each series of patient samples. The results of twelve independent analyses on different days (in the course of two months) are listed in Table III. The coefficients of variation ranged from 3.7% to 6.3%.

Application of the method

Plasma concentrations in patients receiving a high dose of 40 mg of benperidol per day for six or seven days, followed by a low dose of 6 mg/day for fourteen days, were determined. Another group of patients received only a low dose of 6 mg/day for 21 days. The blood samples were collected 14 h

TABLE II

CALIBRATION CURVE DATA

Benperidol concentration (ng/ml)	*n*	Peak area ratio benperidol/haloperidol (mean ± S.D.)	C.V. (%)
1	6	0.029 ± 0.003	10.6
3	6	0.063 ± 0.006	9.1
5	12	0.106 ± 0.005	4.6
10	12	0.204 ± 0.009	4.4
20	12	0.377 ± 0.016	4.3
50	12	0.922 ± 0.036	3.9

Regression equations

$Y_1 = 0.014 + 0.018X$, $r^2 = 0.9998$ (1—50 ng/ml)

$Y_2 = 0.018 + 0.018X$, $r^2 = 0.9999$ (5—50 ng/ml)

TABLE III

RESULTS FOR TWELVE RECOVERY ANALYSES PERFORMED ON DIFFERENT DAYS

Given (ng/ml)	Found (mean ± S.D.) (ng/ml)	C.V. (%)
5	5.14 ± 0.30	5.8
10	9.97 ± 0.63	6.3
20	18.57 ± 0.78	4.2
50	44.97 ± 1.67	3.7

TABLE IV

BENPERIDOL PLASMA CONCENTRATIONS OF PATIENTS RECEIVING 40 mg OR 6 mg PER DAY

No. of patients	Day of blood sampling	Dose (mg/day)	Plasma concentration (ng/ml, mean ± S.D.)	C.V. (%)
10	4	40	15.1 ± 7.3	48.3
10	14	6	4.1 ± 1.4	34.1
7	4	6	3.1 ± 1.2	40.1
7	14	6	4.2 ± 1.2	28.2

after medication on days 2, 4, 7, 14, 21 in Li-heparin tubes. The samples were centrifuged at 1800 *g*; the plasma was then separated and frozen at −40°C.

Table IV shows the mean plasma concentrations on day 4 of ten patients receiving 40 mg/day and plasma concentrations of the same patients on day 14 after reducing the dose on day 6 or 7 to 6 mg/day. The relation between dose and plasma concentration is not linear.

The inter-individual variations of the plasma concentrations were wide. The coefficient of variation for a dose of 40 mg/day on day 4 was 48.3%, the mean plasma concentration being 15.1 ± 7.3 ng/ml. On day 14 the mean plasma concentration was 4.1 ± 1.4 ng/ml with a coefficient of variation of

34.1%. The mean values of seven patients receiving a constant dose of 6 mg for at least 14 days are listed in the same table. Plasma concentrations on day 4 were 3.1 ± 1.2 ng/ml with a coefficient of variation of 40.1%. They were somewhat lower than on day 14 with 4.2 ± 1.2 ng/ml and a coefficient of variation of 28.2%.

The present method developed for the determination of benperidol in human plasma is simple and rapid. With our extraction method and chromatographic conditions other butyrophenones like haloperidol, droperidol or trifluperidol can be detected as well. Their retention times and capacity factors are listed in Table V. The detection limit of benperidol and haloperidol is 1.0 ng/ml using 2 ml of plasma per assay and 0.5 ng/ml using 4 ml. The HPLC method for haloperidol with electrochemical detection [14] is as sensitive as our method. The two HPLC methods with UV detection [12,13] are selective for haloperidol; benperidol could not be determined; the detection limit was 2 ng/ml. Our method is somewhat more sensitive and has the advantage that other butyrophenones can be analysed.

TABLE V

RETENTION TIMES AND CAPACITY FACTORS FOR BENPERIDOL, DROPERIDOL, HALOPERIDOL AND TRIFLUPERIDOL

Compound	t_R (min)	k'
Benperidol	5.9	2.3
Droperidol	5.8	2.2
Haloperidol	11.6	5.4
Trifluperidol	18.3	9.2

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