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DETERMINATION OF BROMPERIDOL IN SERUM BY AUTOMATED COLUMN-SWITCHING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A column-switching high-performance liquid chromatographic method with ultraviolet detection is described for the simple and rapid determination of bromperidol, a neuroleptic drug, in dog and human sera The drug in serum and trifluperidol added to the serum as an internal standard were extracted with hexane–chloroform, and the extract was subjected to automated column-switching high-performance liquid chromatography using a hydrophilic metaacrylate polymer column (TSK gel PW precolumn) for sample clean-up and a reversed-phase column (TSK gel ODS-80TM) for separation The detection limit of bromperidol is 0 3 ng/ml serum and the recovery of bromperidol added to serum (2 7–16 0 ng/ml) was satisfactory with a standard deviation of 3% or less

INTRODUCTION

Bromperidol, 4-[4-(*p*-bromophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone (BPD; Fig 1), is a neuroleptic drug [1] that is usually administered as an oral preparation [2] Only a few methods have been reported so far for the determination of the drug in serum or plasma, the metabolism and excretion of BPD in rat, dog and human were investigated using ^{14}C-labelled BPD [3], and its pharmacokinetics in schizophrenic patients were studied by

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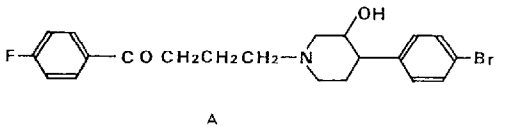

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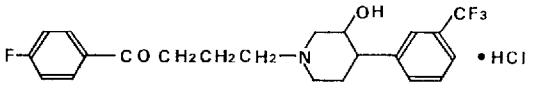

B

Fig 1 Structures of BPD (A) and TFPD HCl (B)

means of gas chromatography–mass spectrometory [2]. A radioimmunoassay method has also been reported [4,5] These methods are sensitive and selective enough to measure BPD in the therapeutic dose range, but they require time-consuming sample clean-up procedures or ^{14}C-labelled BPD

For monitoring the concentration of BPD in serum from patients dosed with BPD and for studying the bioavailability of BPD preparations, a rapid simple, sensitive and selective method for the measurement of BPD in serum is required This paper aims to establish such a method for the determination of BPD in dog and human sera, based on column-switching high-performance liquid chromatography (HPLC) with UV detection Trifluperidol hydrochloride, 4-[4-(*m*-trifluoromethylphenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone hydrochloride (TFPD·HCl, Fig 1) was used as an internal standard.

EXPERIMENTAL

Materials and solutions

BPD and TFPD·HCl were the products of Janssen Pharmaceutica (Beerse, Belgium) Tablets of BPD were prepared in Yoshitomi Pharmaceutical Industries (Osaka, Japan). Deionized water was passed through a Milli-Q system (Millipore, Bedford, MA, U.S A) just before use Other chemicals were of reagent grade. Standard solutions (6–60 ng/ml) were prepared by dissolving BPD in a small amount of acetic acid (final concentration, 4 1–41 μM) and diluting with water. TFPD·HCl solution (45 ng/ml, internal standard solution) was prepared as for BPD standard solutions Serum samples were obtained from dogs (male beagle, 3 years old, 10–11.5 kg weight) and schizophrenic patients (20–21 years old) in the usual manner

Apparatus

The column-switching HPLC system (Fig 2) consisted of a Shimadzu LC-5A high-pressure pump (for eluent A, see below), a Shimadzu LC-3A high-pressure pump (for eluent B, see below), a Tosoh PT-8000 multiple autovalve, an Atto SJ-1700AS autosampler (500-μl injection volume) and a Shimadzu SPD-2A UV monitor fitted with an 8-μl flow cell A TSK gel PW precolumn (a hydrophilic metaacrylate polymer column) for sample clean-up (column I, 35 mm$\times$4 6 mm I.D., particle size 10 μm; Tosoh, Tokyo, Japan) and a TSK gel ODS-80TM column as an analytical column (column II; 150 mm$\times$4 6 mm I.D , particle size 5 μm; Tosoh) were used

Extraction of BPD

To serum (3.0 ml) placed in a test-tube were added 1 0 ml each of the internal standard solution and 2 5 *M* sodium hydroxide, and 15 ml of heptane–chloroform (70 30, v/v). The mixture was shaken vigorously for 15 min and then, centrifuged at 2000 *g* for 10 min A 10-ml portion of the organic layer was evaporated in vacuo at 45 °C to dryness The residue was dissolved in 0 75 ml of eluent A (see below) and used as an extract

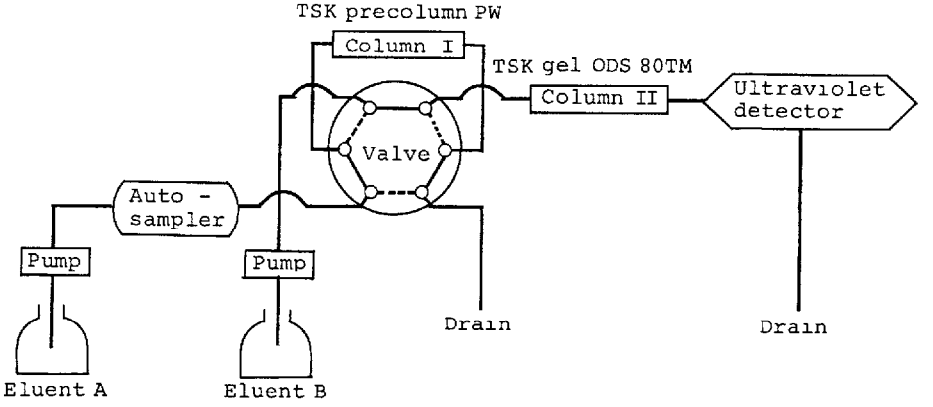

Fig 2 Flow diagram of the column-switching HPLC system

TABLE I

TIME PROGRAMME

Time (min)	Eluent	
	Column I	Column II
0 -10 5	A	B
10 5-12 2	B	B
12 2-32 0	A	B

High-performance liquid chromatography

A 0 5-ml portion of the extract was automatically injected into the chromatograph The column-switching HPLC system was operated according to the time programme depicted in Table I Between 0 and 10 5 min after the sample injection, BPD and TFPD were separated from the interfering substances present in the extract on column I with a mobile phase (eluent A) of acetonitrile and 0 1 *M* potassium dihydrogen-phosphate (pH 2 2, adjusted with 6.0 *M* perchloric acid) (11.5 88.5, v/v) Between 10 5 and 12 2 min after the injection, BPD and TFPD retained on column I were eluted with a mobile phase (eluent B) of acetonitrile and the potassium dihydrogenphosphate solution (47 5 52 5, v/v), and the effluent from column I was switched to column II Then BPD and TFPD were separated on column II by eluting with eluent B (between 12 2 and 32.0 min) The flow-rates of eluents A and B were 1.2 and 0 6 ml/min, respectively The column temperature was 22–26°C The absorbance of the effluent from column II was monitored at 245 nm The peak height was used for the quantification of BPD

RESULTS AND DISCUSSION

Column-switching HPLC

Column I (a hydrophilic metaacrylate polymer column), a TSK SW precolumn (a hydrophilic silica gel column, 35 mm×4 6 mm I D , particle size 15 μm, Tosoh) [6] and a TSK BSA-ODS precolumn (a bovine serum albumin-coated ODS column; 35 mm×4.6 mm I D., particle size 10 μm; Tosoh) [7] were examined as columns for sample clean-up A good separation of BPD and TFPD from the interfering substances that occurred in an extract of serum was achieved using column I When a TSK SW precolumn was used, their separation was unsatisfactory. The drugs trapped on a TSK BSA-ODS precolumn were not completely eluted

Retention of the drugs on column I and their separation from the interfering substances were influenced by the pH of potassium dihydrogenphosphate solution, a component of the mobile phase, and the concentration of acetonitrile in the mobile phase, eluent A (a phosphate solution of pH 2 2 containing 11 5% acetonitrile) gave the best results A much higher concentration of acetonitrile (47 5%) in eluent B was necessary for rapid separation of BPD and TFPD on column II and for sensitive quantification

Other reversed-phase columns [LiChrosorb RP-8 (200 mm×4 0 mm I D , particle size 5 μm, Japan Merck, Tokyo, Japan) and YMC-C8 (150 mm×4 6 mm I D , particle size 5 μm, Yamamura Chemical Labs , Kyoto, Japan)] could be used as analytical columns, but they gave less sensitive detection and poor resolution of peaks

Fig 3A shows typical chromatograms of the extracts of a human serum (a) and a serum spiked with BPD and TFPD·HCl (b) BPD and TFPD were

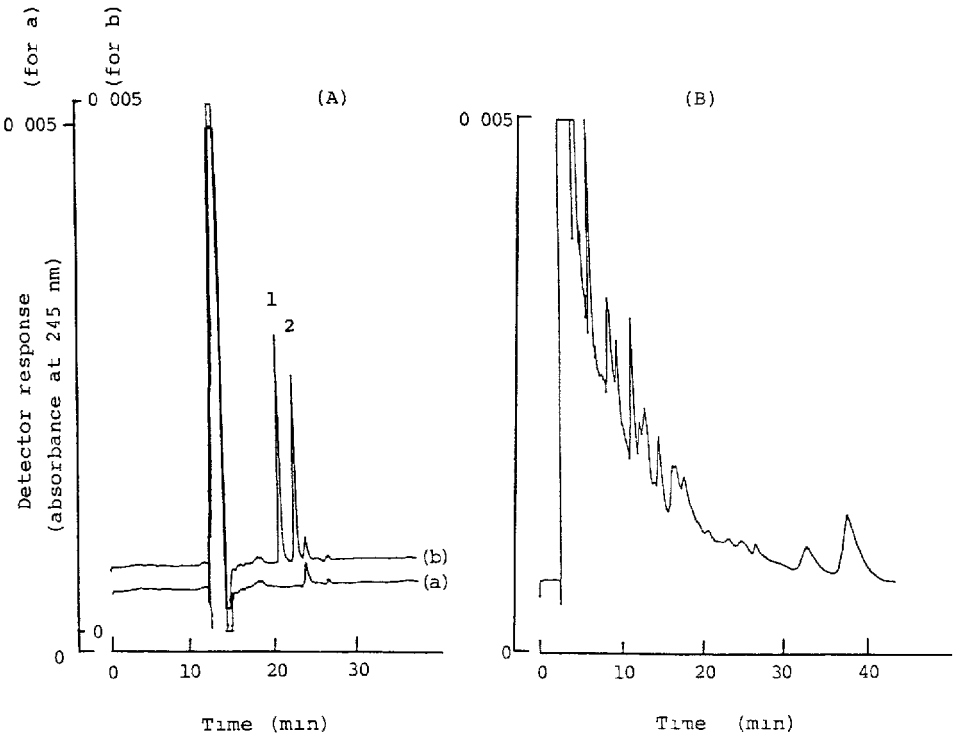

Fig 3 (A) Chromatograms of extracts of a drug-free human serum (a) and serum spiked with BPD (16 ng/ml) and TFPD·HCl (15 ng/ml) (b) (B) Chromatogram of an extract of the drug-free human serum that was injected directly onto column II Peaks 1=BPD, 2=TFPD

separated and detected within 23 min, and no interfering peaks arose around the retention times of BPD and TFPD (21 5 and 23 0 min, respectively) When the same extract as that for Fig 3Aa was directly applied on column II, many interfering peaks were observed (Fig. 3B) This indicates that the use of column I for sample clean-up in the column-switching technique can serve to eliminate the interference from the background components present in the extract. Water for the preparation of all the aqueous solutions should be passed through a Milli-Q system just before use, even though freshly distilled water is employed, otherwise some peaks attributable to UV-absorbing impurities arise around the retention times of BPD and the internal standard and interfere with the high sensitivity detection

Validation of the method

The calibration graph of BPD, constructed by plotting the ratios of the peak height of the spiked BPD to that of the internal standard versus the amount (0–60 ng) of BPD added to serum (3 0 ml), was linear The correlation coefficient (r) of the graph ($n=3$ in each plot) was 0 999 The recoveries of BPD

(2 7, 8 and 16 ng/ml) and TFPD·HCl (15 ng/ml) added to a drug-free human serum were 101 ± (1 3–2 2)% for BPD and 99 3 ± 2 2% for TFPD·HCl (mean ± S D , $n=8$ in each case) The detection limit of BPD in serum was 0 3 ng/ml at a signal-to-noise ratio of 3

The relative standard deviations (R S.D) in within-day precision were 1 7, 2 0 and 1 5% for the mean BPD serum concentrations of 1 1, 7 0 and 10 3 ng/ml, respectively ($n=7$ in each case) The between-day precision was examined for seven days ($n=3$ each day), where the sample serum was kept frozen at −20°C until use The R S D were 4 6, 3.5 and 3 7% for the mean BPD serum concentration of 1 1, 7 0 and 10 1 ng/ml, respectively

BPD concentrations in dog and human sera

Fig. 4 shows a typical time–concentration curve obtained with dog serum after oral administration of a tablet of BPD (6 mg of BPD) The concentration of BPD in serum was at a maximum (8 3 ng/ml) 6 h after administration, and then decreased at a first-order rate The biological half-life was 17 6 h Fig 5

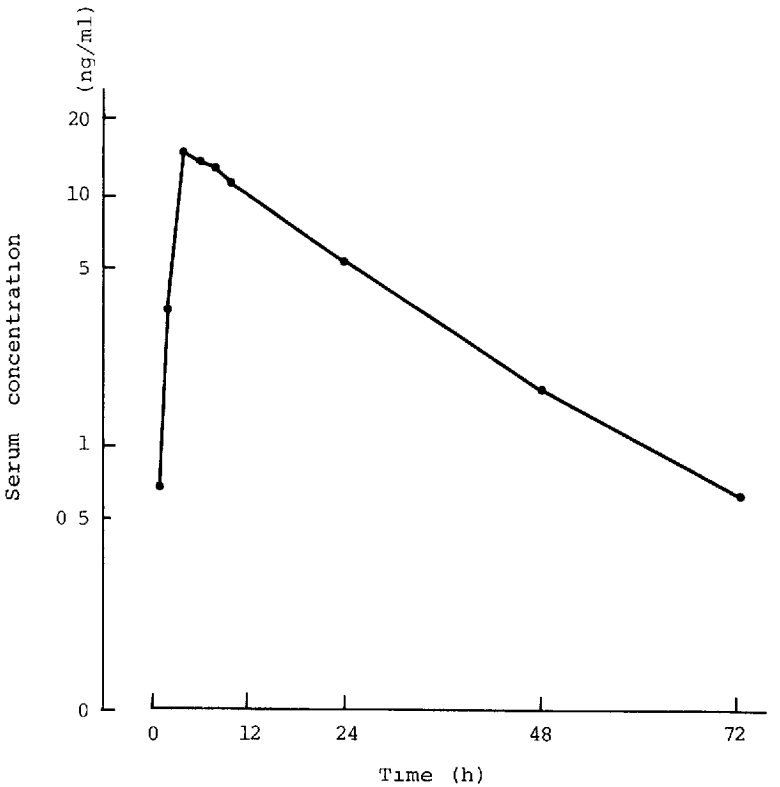

Fig 4 Time–concentration curve of BPD in serum of a dog after oral administration of one tablet of BPD (6 mg)

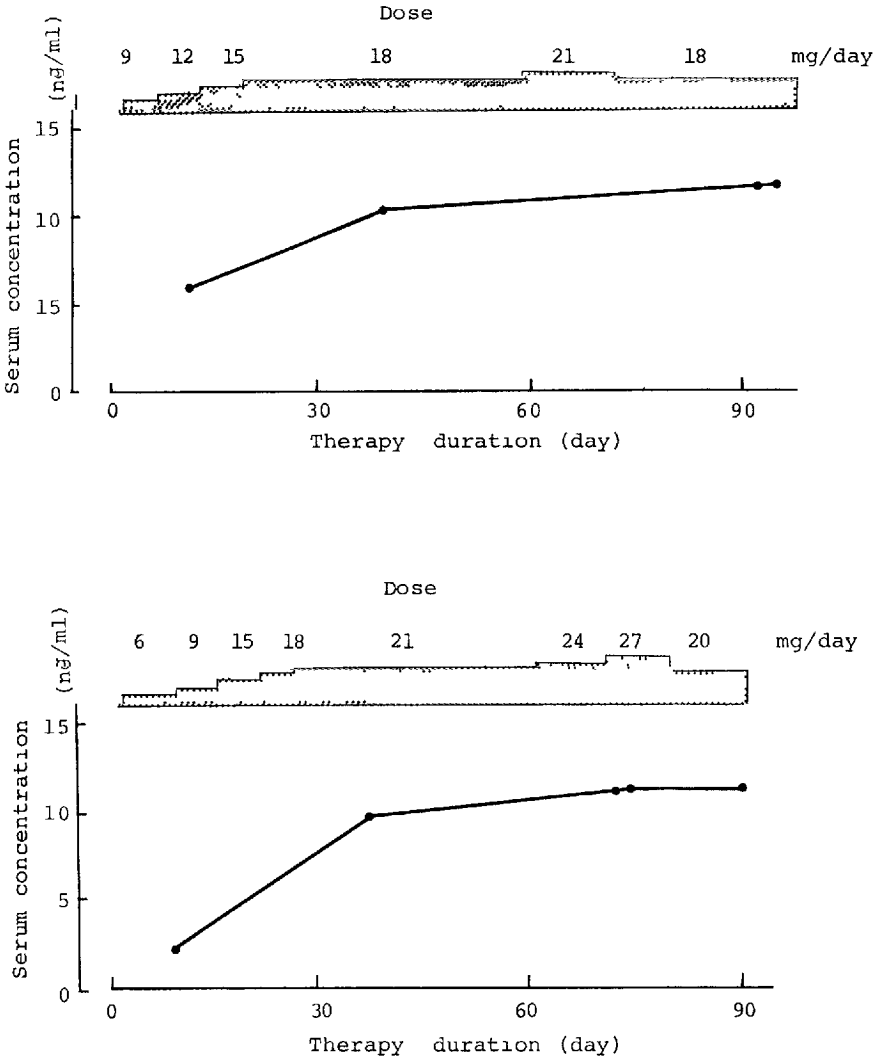

Fig 5 Time-concentration curves of BPD in sera of two patients under long-term therapy Dose regimens are given in the block at the top

shows the time–concentration curves of BPD in sera of two schizophrenic patients under long-term therapy The dose regimen for long-term therapy could be defined by monitoring serum BPD concentrations in the patients by this method

In conclusion, this study has provided the first HPLC method for the determination of BPD in serum The method is simple and rapid and, moreover, offers the necessary sensitivity to permit the monitoring of BPD in serum in

the therapeutic dose range (3–36 mg per day). The method should be useful for biomedical investigations of BPD

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