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Note

High-performance liquid chromatographic determination of haloperidol in plasma

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It has been recognized [1—3] that haloperidol is a drug for clarifying the correlation between plasma drug concentrations and clinical effects of psychotropic drugs. Several methods [4—7] for the assay of plasma levels of haloperidol have been described, including gas chromatography [4—6] using an electron-capture detector and radioimmunoassay [7]. Because it has a low sensitivity [4,5] and is time consuming [6], procedures based on gas chromatography are unsuitable for routine monitoring of plasma levels in man. Reagents that are not readily available are required in radioimmunoassay [7].

Bianchetti and Morselli [3] described a method based on gas chromatography using a nitrogen—phosphorus selective detector. We have developed a more rapid and convenient method based on high-performance liquid chromatography (HPLC) using a reversed-phase column and a UV detector.

EXPERIMENTAL

Materials and reagents

Haloperidol, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl)-1-(4-fluorophenyl)-1-butanone, was kindly supplied by Dainippon Pharmaceutical Co., Osaka, Japan. The internal standard, diphenylamine, was obtained from Koso Chemical Co., Tokyo, Japan, and a solution containing 1.0 μg/ml in the mobile

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phase [methanol—water (63:37) containing 0.2 *M* ammonium acetate] was prepared. All other chemicals were of reagent grade and were used without further purification.

Apparatus and chromatographic parameters

A Hitachi 635A liquid chromatograph equipped with a multi-wavelength UV detector (Hitachi 635-0900) and a high-pressure sampling valve (Hitachi 635-5101, 1 μl to about 2 ml) was used. The column was heated at 55° C using a constant-temperature water-bath circulator. Peak heights were calculated using a Takeda digital integrator (Tr 2221 A). Separation was accomplished on a 25 × 0.4 cm microparticulate reversed-phase column (Nucleosil C18, 5 μm: Macherey, Nagel & Co., Düren, G.F.R.).

The mobile phase flow-rate was 0.5 ml/min, and the UV detector was set at 250 nm and was operated at 0.005 a.u.f.s.

Extraction procedure

Aliquots of plasma or serum (1.0 ml) were transferred into 10-ml glass-stoppered centrifuge tubes (1.5 × 10.5 cm) and acidified with 3.5 ml of 0.1 *N* hydrochloric acid. Diethyl ether (4 ml) was added and the mixture was shaken vigorously for 5 min, then centrifuged at 800—1000 *g* for 5 min. The upper, ether layer was aspirated off, then 4 ml of the aqueous layer were transferred into test-tubes containing 1 ml of 1 *N* sodium hydroxide solution and 4.5 ml of chloroform were added. The mixture was shaken gently for 10 min and then centrifuged at 1000 *g* for 5 min.

The aqueous layer was aspirated off then the tubes were shaken by hand or with a Vortex mixer and centrifuged again to obtain the clear chloroform layer. Four millilitres of the chloroform layer were transferred into 10-ml evaporating tubes and dried in vacuo in a water-bath at 30° C. The dry residue was dissolved in 100 μl of elution solvent containing the internal standard, and 30—40 μl of these samples were then chromatographed.

Calibration graph

Standard solutions containing 10, 20, 30, 40, 60 and 80 ng/ml of haloperidol in 0.1 *N* hydrochloric acid were prepared by dilution of a 1.0 μg/ml methanolic stock solution. This stock solution was stable for several weeks if stored at 4° C and protected from light. One millilitre of standard solution and 2.5 ml of 0.1 *N* hydrochloric acid were added to 1.0 ml of drug-free plasma and the samples were processed as described above. The ratios of the peak height of haloperidol to that of diphenylamine were used to construct a calibration graph.

RESULT AND DISCUSSION

HPLC mobile phase

The elution behaviour of haloperidol was examined by using methanol—water containing various amounts of ammonium acetate as the mobile phase. An ammonium acetate-free mobile phase was undesirable as elution of haloperidol did not occur until 35 min, whereas in the presence of ammonium acetate haloperidol eluted rapidly as a sharp, symmetrical peak. The peak height of

haloperidol increased with increasing concentration of ammonium acetate. However, it is well known that a reversed-phase column such as Nucleosil is unstable if the pH of the mobile phase is greater than 7.5; the pH values of methanol—water that contained 0.2 and 0.3 *M* ammonium acetate were 7.3 and 7.5, respectively. In addition, the amount of methanol was carefully controlled, as variations produced changes in the retention time of haloperidol. From the results, methanol—water (63:37) containing 0.2 *M* ammonium acetate was selected as the mobile phase.

Selectivity

Fig. 1 shows the chromatograms obtained for a plasma blank and a plasma sample to which 20 ng/ml of haloperidol had been added. The separation of haloperidol and diphenylamine from endogenous substances was good.

A graph of the ratio of the peak height of haloperidol to that of the internal standard against drug concentration was linear and passed through the origin, with a correlation coefficient of 0.9997. The coefficient of variation of the method at 20 ng/ml was 2.4% (n=8).

The recovery of haloperidol from a plasma sample in comparison with an aqueous sample that contained distilled water instead of plasma was approximately complete. The recoveries of haloperidol from human plasma to which 10 and 20 ng/ml had been added were 102.2 ± 2.9% (n=4) and 100 ± 3.9% (n=4), respectively.

The sensitivity of this method was 5 ng/ml of haloperidol. If a sample of 2 ml or more of plasma was used, it was possible to detect 2—3 ng/ml of haloperidol.

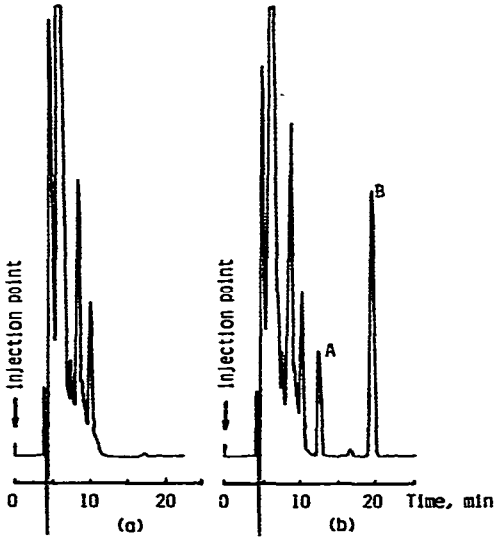

Fig. 1. High-performance liquid chromatograms of (a) human plasma blank and (b) haloperidol in human plasma. Peaks: A = haloperidol; B = internal standard (diphenylamine).

Haloperidol plasma levels in patients

Table I shows the plasma levels in patients undergoing chronic haloperidol treatment. Although only a few results were obtained, it can be seen that the plasma levels increased in proportion to the dose, and these levels of haloperidol were within the range measured by Itoh et al. [8].

TABLE I

PLASMA LEVELS OF HALOPERIDOL IN PATIENTS UNDERGOING CHRONIC TREATMENT

Case	Dose (mg/day)	Plasma level* (ng/ml)
1	6.0	4.6
2	8.0	9.0
3	18.0	22.9

*Plasma levels were determined 3.0 h after oral administration.

Application

In order to examine the application of this method to analogues of haloperidol such as methylperidol and spiroperidol, the retention times and sensitivities for these two drugs were determined by the above method, and the results are given in Table II.

The retention times of these two analogues were similar to that of haloperidol and, as shown in Fig. 1, the peaks of the analogues were not subject to interference from that of drug-free plasma. In addition, there was no marked difference in the peak heights of the three drugs. It therefore seems that the method is applicable to the determination of methylperidol and spiroperidol in plasma samples.

According to our results, the method appears to be suitable for monitoring haloperidol plasma levels during chronic treatment, and also for investigation of the correlation between haloperidol plasma levels and pharmacological effects.

TABLE II

RETENTION TIMES AND RELATIVE PEAK-HEIGHT RATIOS OF HALOPERIDOL, METHYLPERIDOL AND SPIROPERIDOL

Compound	Retention time (min)	Relative peak-height ratio*
Haloperidol	12.5	1.0
Methylperidol	11.6	0.7
Spiroperidol	10.9	0.8

*Ratio of the peak height of the compound to that of haloperidol.

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