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RAPID AND SENSITIVE METHOD FOR DETERMINATION OF HALOPERIDOL IN HUMAN SAMPLES USING NITROGEN–PHOSPHORUS SELECTIVE DETECTION

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SUMMARY

A sensitive gas-chromatographic method for quantitative analysis of haloperidol in human plasma is described. The use of nitrogen–phosphorus selective detection reduces the time required for analysis. Azaperone is used as the internal reference standard. The method is suitable for the determination of haloperidol plasma levels in patients treated with doses ranging from 1.2 to 200 mg/day. No interference from drugs needed in the associated antipsychotic therapy has been found. The simplicity, specificity and sensitivity of the method make it suitable for routine analysis of haloperidol plasma levels in psychotic patients undergoing chronic treatment.

INTRODUCTION

The monitoring of plasma levels of drugs in man during chronic treatment, thus leading to the identification of therapeutic and toxic thresholds in drug plasma concentrations, has permitted a better and a safer therapeutic approach in several areas of medicine.

Haloperidol is currently one of the most widely used psychotropic agents[1,2], but our knowledge of its kinetics in man is very limited[3–6] and practically no data exist on its therapeutic and toxic thresholds. This is due mostly to the lack of suitable analytical methods for easy and rapid monitoring of haloperidol plasma levels in patients undergoing chronic treatment. The available methods lack sensitivity[7,8], are very elaborate and time-consuming[9], or rely on reagents that are not readily available[10].

Our interest in the field of psychotropic drugs[11,12] has prompted us to develop a method suitable for the routine monitoring of haloperidol plasma levels in psychiatric patients. The method described here is based on gas–liquid chromatography (GLC) using nitrogen–phosphorus selective detection (NPD). Because of its simplicity, rapidity and specificity, it offers general advantages over existing methods.

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EXPERIMENTAL

Standard and reagents

Haloperidol [1-{3-(4-fluorobenzoyl)propyl}-4-hydroxy-4-(4-chlorophenyl)piperidine] and azaperone [1-(4-fluorophenyl)-4-{4-(2-pyridinyl)-1-piperadinyl}-butan-1-one], which was chosen as internal reference standard, were kindly supplied by Dr. F. Marcucci (Istituto Mario Negri, Milan, Italy).

The following reagents were used: diethyl ether, acetone and sodium hydroxide (Merck, Darmstadt, G.F.R.).

Stock solutions

Standard solutions in acetone of haloperidol (1 μg/ml) and azaperone (1 μg/ml) were prepared and kept at 4°. Under these conditions the solutions were stable during several weeks.

Calibration curve and quantitation

Haloperidol and azaperone standard curves were established by adding 2.5, 5, 10, 15, 30, 40 and 50 ng of haloperidol and 10 ng of azaperone to 2 ml of drug-free plasma. The samples were processed according to the method described below.

The ratio of the peak areas for haloperidol–azaperone were used to calculate a calibration curve, the slope of which was used in the quantitation of haloperidol in plasma samples.

Extraction procedure

For each analysis 2 ml of plasma were used. 10 ng of a solution of azaperone (1 ng/μl in acetone) were added as internal standard to each sample of plasma in a glass centrifuge tube. 2 ml of distilled water, 200 μl of 2 *N* NaOH and 5 ml of diethyl ether were added, and the tubes were then stoppered and shaken vigorously for 10 sec at room temperature on a Vortex. The tubes were then centrifuged for 2 min at 1000 *g*.

The ether phase was transferred to another series of test tubes, each containing 2.5 ml of 0.2 *N* HCl. The tubes were shaken for 10 sec on a Vortex and centrifuged for 2 min at 1000 *g*. The resulting upper ether phase was discarded. 300 μl of 2 *N* NaOH were added to the aqueous phase, together with 5 ml of diethyl ether, and the tubes were then shaken and centrifuged as before. The ether phase was transferred to a series of evaporating tubes, each with bottom drawn into a capillary pocket of *ca.* 100 μl, and was evaporated under nitrogen in a water bath at 40°. The dry residue was dissolved in 300 μl of acetone, the tubes were shaken on a Vortex for 5 sec and the acetone was evaporated under a nitrogen stream to a volume of 5–10 μl. This concentrated extract was then chromatographed.

The same procedure was employed to determine haloperidol levels in total blood. In this case red cells were haemolysed by diluting 2 ml of blood with 3 ml of distilled water. The blood solution was frozen and thawed to aid lysis.

Apparatus

A Hewlett-Packard gas chromatograph equipped with a nitrogen–phosphorus detector was used with a glass column (2 m × 4 mm I.D.) previously silanized and

subsequently packed with Chromosorb W (80–100 mesh), coated with 3% OV-17 (phenylmethyl silicone) (Applied Sci. Labs., State College, Pa., U.S.A.). The column was initially conditioned by the following temperature programme: from 60° to 300° at 5°/min, with a helium flow-rate of 40 ml/min; then for 4 h at 310° without helium flow; and finally at 285° overnight with a helium flow-rate of 60 ml/min.

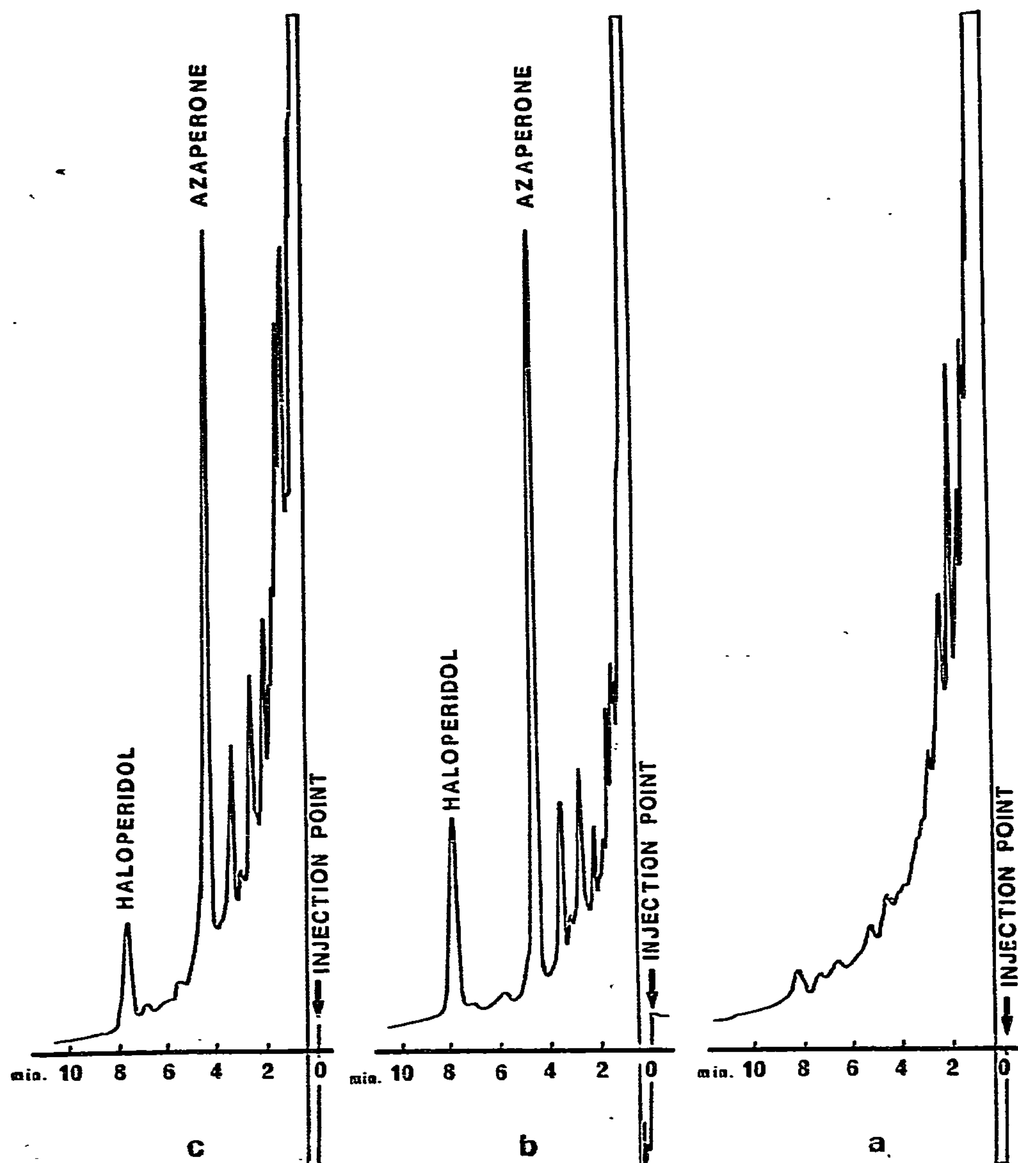

Fig. 1. Chromatography of haloperidol. (a) Extract from 2 ml of human plasma free of drug (blank); (b) extract from 2 ml of human plasma free of drug, to which a known amount of haloperidol has been added; (c) extract from 2 ml of human plasma from a patient receiving haloperidol.

Operating conditions

The column was operated at 285° and the injector port and the detector at 300°. The helium flow-rate was 60 ml/min. The NPD rubidium bead source was heated by a 17 A current at an applied potential of 240 V, and had a hydrogen flow-rate of 3 ml/min and an air flow-rate of 60 ml/min.

RESULTS

The gas chromatogram obtained from a plasma sample to which a known amount of haloperidol was added is shown in Fig. 1, together with those obtained from a plasma blank and from a patient receiving haloperidol therapy plus benzodiazepine treatment.

The peaks of haloperidol and azaperone appear to be resolved, and no interfering peaks from endogenous substances or concomitant therapy are present. The gas-chromatographic characteristics of haloperidol and azaperone, such as retention time, height equivalent to one theoretical plate, resolution and symmetry factor, are reported in Table I. A standard calibration curve obtained after extraction of haloperidol from plasma is shown in Fig. 2, and the coefficient of variation of the method at several concentrations is reported in Table II.

TABLE I

GAS-CHROMATOGRAPHIC CHARACTERISTICS OF HALOPERIDOL AND AZAPERONE

	Azaperone	*Haloperidol*
Retention time (min.)	4.40	7.25
Height equivalent to one theoretical plate (HETP) (mm)	1.40	0.99
Resolution	3.75	3.0
Symmetry factor of peak	0.97	0.96

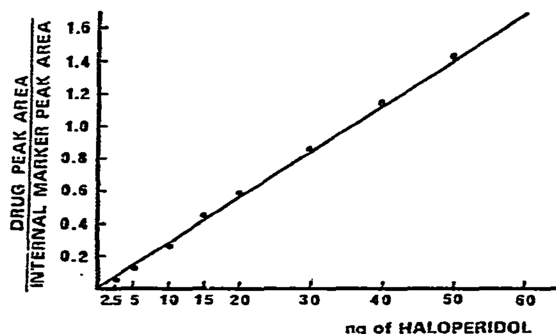

Fig. 2. Standard calibration curve of haloperidol, obtained after extraction from plasma.

The relative recovery of haloperidol (percentage of haloperidol recovered divided by percentage of azaperone recovered) was found to be constant. The absolute recovery of haloperidol, calculated using azaperone as external marker, was found to be 85%. Two consecutive extractions of one sample increased the absolute recovery to 95%.

TABLE II

PRECISION AND ACCURACY OF DETERMINATION OF HALOPERIDOL IN HUMAN PLASMA

The standard deviation is given as a percentage of the mean value.

Amount of haloperidol added to plasma and processed for extraction (ng/ml)	*Mean value of concentration found (ng/ml)*	*S.D. (%)*	*Number of determinations*
2.5	2.37 ± 0.14	6	10
5.0	4.71 ± 0.2	4	10
15	15.5 ± 0.7	4.6	10
30	31.1 ± 1.19	3.9	10
50	51.9 ± 1.67	3.3	10

The absolute sensitivity of the NPD detector was 1 ng for haloperidol and 100 pg for azaperone, which is the response corresponding to 5% full-scale deflection, at an attenuation of ×4. In practical terms it is possible to detect 1 ng/ml using a 2-ml plasma sample.

TABLE III

HALOPERIDOL PLASMA LEVELS IN PATIENTS UNDERGOING CHRONIC TREATMENT

Case	*Dose (mg/kg/day)*	*Plasma level (ng/ml)*
1	0.04	1.4
2	0.05	1.4
3	0.06	2.5
4	0.04	3.5
5	0.25	5.0
6	1.08	22.0
7	2.40	82.0
8	2.50	105.0

TABLE IV

REPEATED MONITORING OF HALOPERIDOL PLASMA LEVELS IN TWO PSYCHOTIC PATIENTS RECEIVING INCREASING AMOUNTS OF THE DRUG

	Dose (mg/day)	*Plasma level (ng/ml)*
Case 6	12	9
	12	7
	12	7
	12	6
	12	7
	24	14
	84	22
	156	100
Case 7	120	82
	180	117
	200	142

Preliminary observations carried out on patients receiving haloperidol at various daily dosages indicate that the method is suitable for monitoring haloperidol plasma levels during chronic administration of the drug. The data are reported in Tables III and IV. It may be observed that a considerable inter-individual variability in drug plasma concentrations may exist, whereas the levels are remarkably constant for one subject receiving a constant daily dose. Drugs given in association, such as tricyclic antidepressants, benzodiazepines and phenothiazines, were found not to interfere.

DISCUSSION

The preliminary assays made on human plasma from patients treated with haloperidol confirm the validity of the method for routine monitoring in psychiatric patients. Our aim was to achieve not only high sensitivity, but also speed and simplicity. The method described by Forsman *et al.*[9] using electron capture detection, is apparently more sensitive than ours, the absolute sensitivity of haloperidol for electron capture being 50 pg or less, but it is more time-consuming. Also, consideration of the absolute sensitivity for the pure product is not necessarily comparable with that for the compound in an extract of plasma. Moreover, by using NPD, we can concentrate the extract to 5–10 μl and inject the total volume (which is practically impossible when using ECD), thereby achieving the same sensitivity, in terms of the concentration per ml of plasma. The very good reproducibility of the method is due to the reliability of the NPD and to the minimal handling of samples. The great improvement attained using NPD is well illustrated by the increasing number of methods based on it, especially for anticonvulsant drugs[13–15]. The high selectivity for nitrogen-containing compounds seems to provide a means for the further simplification of existing methods by reducing the need for purification of plasma extracts prior to chromatography, thus reducing the time required for analysis. To reduce the processing time, we shortened the agitation and centrifugation times without affecting reproducibility and we used diethyl ether, because it is as efficient a solvent as *n*-hexane or *n*-heptane with the advantage of being more volatile. Moreover, diethyl ether does not produce emulsions during agitation if plasma is diluted with an equal volume of water. In this way we reduced the time necessary to prepare 20 samples for GLC injection to less than 1 h. The fact that antipsychotic compounds commonly associated with haloperidol therapy do not interfere with the analysis is another advantage.

Plasma concentrations found in patients treated with haloperidol are in agreement with those available in the literature[3,6,9] at low dosage levels; at high dosages we found concentrations a little less than those measured by Clark *et al.*[10] using radioimmunoassay (RIA). The advantage of RIA with respect to all other methods is the small volume of plasma that is required for analysis. However, the drawbacks of this method are the long time required to obtain all the reagents for antigen–antibody reaction, the time required for the dosage itself, and the fact that unless a kit is made available it cannot be considered of practical use.

Our method requires plasma samples of 2 ml for patients receiving less than 5 mg/day, and only 0.1 ml for patients treated with 200 mg/day. Thus this method, because of its sensitivity, specificity, rapidity and simplicity, appears to offer con-

siderable advantages over the existing methods. It also appears as to be suitable for monitoring haloperidol plasma levels during chronic treatment, thus permitting a more individualized and safer therapy.

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