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A method for the gas chromatographic determination of butyrophenones

Haloperidol and trifluperidol are potent and specific neuroleptics belonging to the butyrophenone series whose chemical structures are illustrated in Fig. 1.

The pharmacology of the butyrophenones has been extensively reviewed[1-4] and the clinical use of these compounds is continuously increasing. However, there

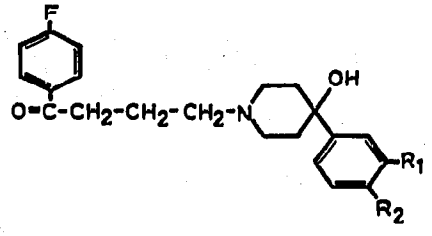

Fig. 1. Chemical structures of the butyrophenones: haloperidol: R_1 = H; R_2 = Cl; trifluperidol: R_1 = CF_3; R_2 = H.

are relatively few publications concerning the distribution and excretion of these drugs and their metabolites[5-9]. Tissue distribution of butyrophenones in animals has usually been studied by radioisotope techniques[10-12].

Although these techniques are very sensitive they cannot always answer the question whether the radioactivity found is wholly due to the administered labelled drug or to its metabolites.

In order to overcome the limitation of the above mentioned methods a simpler and more rapid procedure for the analysis of plasma and tissue levels of butyrophenones has been developed. The gas chromatographic (GC) method described here takes advantage of the presence of halogens in the chemical structure of butyrophenones which permits the use of an electron capture detector. The method described gives an excellent resolution and allows a quantitative estimation of haloperidol and trifluperidol intact.

Experimental

Reagents. All reagents must be of reagent grade purity. All inorganic reagents were made up in triply distilled water. 1 *M* KH_2PO_4 buffer, pH 7.2; diethyl ether: analytical reagent grade ether, containing not more than 0.00005% peroxides, was used. The bottle must be opened on the day of the extraction.

Extraction from blood. 2 ml of whole blood, 2 ml of buffer, 4 ml of H_2O and 10 ml of diethyl ether are placed in a glass-stoppered centrifuge tube. The tubes are shaken on a reciprocating shaker for 10 min and then centrifuged at 0° for 5 min. The ether phase is transferred to another glass-stoppered centrifuge tube. The water phase is re-extracted with 10 ml of ether and the ether extracts are combined.

5 ml of 6 *N* HCl are added to the ether extracts and the tubes are shaken for 10 min and then centrifuged at 0° for 5 min. The ether phase is discarded. The acidic phase is adjusted to a basic pH (10) and then is re-extracted twice with 10 ml of diethyl ether. The combined ether extracts are evaporated to dryness at 45° in a water bath.

The residue is dissolved in a suitable volume of a solution of internal standard in acetone. A suitable aliquot, from 1 to 3 μl, is injected in the GC column.

Extraction from tissue. Tissues are homogenized in cold absolute ethanol (1:10, w/v). The homogenate is then centrifuged at 9,000 $\times$ *g* for 20 min. The supernatant is evaporated to dryness and the residue is dissolved in 5 ml of 6 *N* HCl and extracted five times with 10 ml of diethyl ether. The ether phases are discarded. The acidic phase is adjusted to a basic pH (10) and then the procedure is identical to that described for blood extraction.

Gas chromatographic conditions. The gas chromatograph used was Model G I (Carlo Erba, Milan) equipped with a Ni-63 electron capture detector (voltage 50 V). The stationary phase was 3% OV-17 on Gas-Chrom Q (100–120 mesh) packed into a 2 m glass column (I.D. 2 mm, O.D. 4 mm). The flow rate of the carrier gas (nitrogen) was 40 ml/min or 60 ml/min and the column temperature was 270 or 280°, respectively, for the analysis of trifluperidol or haloperidol.

TABLE I

RELATION BETWEEN PEAK AREA AND AMOUNT OF BUTYROPHENONES

Butyrophenone	*Amount (ng)*	*R*[a]
Haloperidol	2	1.352
	5	2.544
	7	3.333
	10	4.391
Trifluperidol	1	0.246
	2	0.410
	3	0.556
	5	0.895

[a] R = [butyrophenone area (cm^2)]/[0.2 ng internal standard area (cm^2)].

Quantitative analysis. For identification and calculations the internal standard technique was used. 2-N-Benzylamino-5-chlorobenzophenone was chosen as an internal standard because of its suitable retention time. The area of the peaks was calculated by measuring, in convenient units, the height and width of the peak at half height. Butyrophenones can be quantitated by GC when the relative peak area is used as an index of concentration, since a linear relationship exists between relative peak area and haloperidol or trifluperidol concentration in the range of 2 to 10 ng and 1 to 5 ng, respectively, as reported in Table I.

Typical gas chromatograms showing the separation of butyrophenones analyzed and internal standard are illustrated in Fig. 2. Preliminary studies of the partition coefficient of butyrophenones between water and diethyl ether have shown that these drugs are substantially quantitatively extracted from the aqueous media with this organic solvent. The recovery from water and from rat blood, brain and adipose tissue is reported in Table II. Blanks from extracts of rat blood or tissue do not show peaks that interfere with a biological application of the GC procedure.

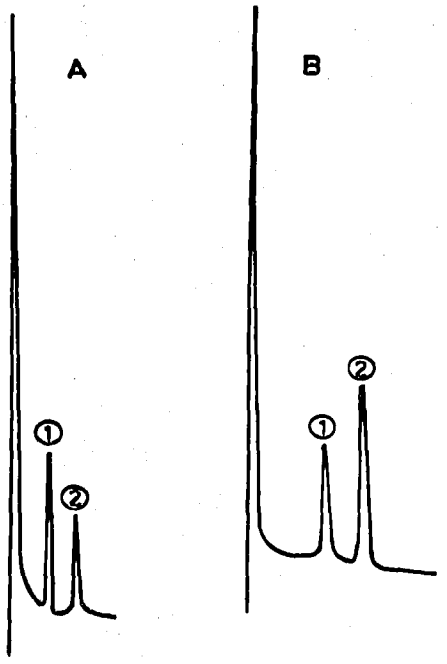

Fig. 2. Gas chromatographic separation of a mixture of: (A) internal standard (1) and haloperidol (2); (B) trifluperidol (1) and internal standard (2).

TABLE II

RECOVERY STUDIES

Compound	*% Recovery ± S.E. from*			
	Water	*Rat blood*	*Rat brain*	*Rat adipose tissue*
Haloperidol	90 ± 2	72 ± 2	73 ± 3	70 ± 1
Trifluperidol	92 ± 3	82 ± 2	83 ± 1	90 ± 1

The method described may be extended to the analysis of other members of the butyrophenone chemical series. The high sensitivity of the method suggests its application to the determination of the level at butyrophenones in the blood of animals and humans.

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