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Note

High-performance liquid chromatographic determination of mepixanthone in serum

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Mepixanthone (3-methoxy-4-piperidinomethylxanthone, Fig. 1) is an analeptic drug used in respiratory and cardiorespiratory insufficiency [1-4]. An antidoping screening procedure for mepixanthone is reported in the literature [5], but quantitative methods for the determination of this drug are not available. In this paper a simple and sensitive high-performance liquid chromatographic (HPLC) method is described which has also been used for the determination of mepixanthone after intravenous administration.

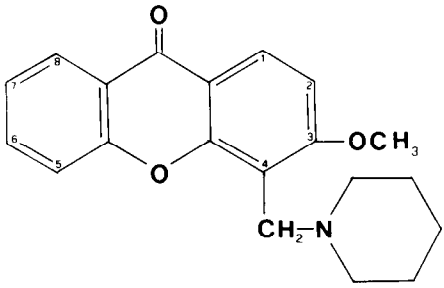

Fig. 1. Structure of mepixanthone.

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EXPERIMENTAL

Chemicals and reagents

Mepixanthone was supplied by Dott. Formenti, Milan, Italy; chlorpromazine [2-chloro-10-(3-dimethylaminopropyl)phenothiazine] (internal standard) was from Farmitalia Carlo Erba, Milan, Italy. Acetonitrile and isopropyl alcohol were HPLC grade (E. Merck, Darmstadt, F.R.G.). Diethylamine was analytical grade (Merck). The extraction tubes Toxi Tube A, active ingredients of which are sodium carbonate (3.5%), sodium bicarbonate (3.5%), dichloromethane (18%) and dichloroethane (17%), buffered at pH 9.0, were supplied by Analytical Systems (Laguna Hills, CA, U.S.A.).

Instrumentation

A Perkin-Elmer Series 3B liquid chromatograph equipped with a 5-μm silica Si 60 column 250 × 4 mm (Merck) was used. The mobile phase was acetonitrile–isopropyl alcohol–diethylamine (50:50:0.004) at a flow-rate of 1 ml/min. The column effluent was monitored with a Perkin-Elmer LC-75 variable-wavelength detector set at 237 nm.

The samples were injected using an automatic sampler (Perkin-Elmer Model LC-420) fitted with a 50-μl sample loop. The 2-ml vials for automatic sampling (Supelco, Bellefonte, PA, U.S.A.) were adapted by inserting 250-μl microtest tubes (LP Italiana, Milan, Italy) to allow the automatic injection from samples having a total volume of only 200 μl [6].

All the chromatographic data were processed with a Perkin-Elmer Model Sigma 10 data system.

Preparation of standards

Standard calibration solutions, containing 0, 5, 20, 50, 200, 400, 800, 2000 μg/l mepixanthone, were prepared in human serum by dilution of an aqueous solution of mepixanthone.

Chlorpromazine was used as a 0.9 mg/l solution in bidistilled water.

Extraction procedure

Serum or plasma samples (1 ml) and chlorpromazine solution (4 ml) were added to a previously mixed Toxi Tube A extraction tube and mixed for 5 min on a rotary mixer. After centrifugation (3000 ***g*** for 5 min) the organic layers (upper phase) were transferred to glass tubes and evaporated at room temperature under a stream of dry nitrogen. The residues were reconstituted in 200 μl of acetonitrile–diethylamine mixture (500:0.02) and transferred into the automatic sampler vials.

The sample concentrations were calculated by comparing the sample's peak area ratio of mepixanthone to chlorpromazine with those of calibration standards.

The Toxi Tube A extraction tubes are reproducible and time-saving. Care should be taken to continue the full extraction operation until the final rotary mixing, completing one sample extraction before the next one, thus avoiding the deposition of salts.

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms of serum extracts from a patient receiving mepixanthone intravenously. For the system used, the number of theoretical plates was 7160 and the capacity ratio of mepixanthone was 2.66. The limit of sensitivity for mepixanthone is 1 μg/l.

The within-assay variation was measured by replicate analyses (n = 20) of mepixanthone standard solutions in human serum, containing 5, 50 and 400 μg/l. The coefficients of variation (C.V.) were 5.4%, 3.4% and 3.0%, respectively. Between-assay C.V. values were determined by comparing the results obtained on different days (n = 10) over a period of three months, for three control sera containing 50, 400 and 800 μg/l mepixanthone. The C.V. values were 6.3%, 5.9% and 5.8%, respectively.

The extraction recovery of mepixanthone from human serum (Table I) was

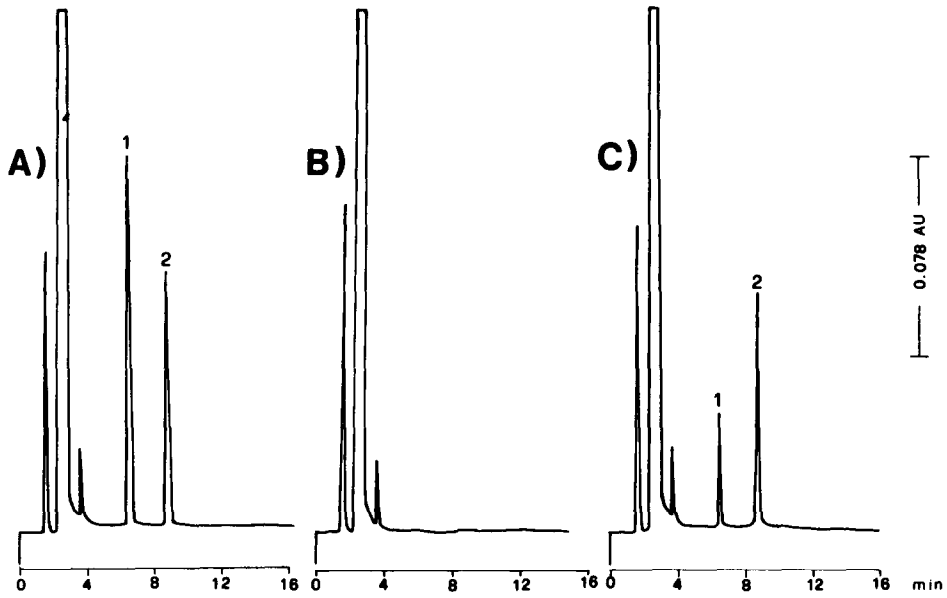

Fig. 2. Chromatograms of (A) an extract from a plasma sample spiked with 800 μg/l mepixanthone and 3.6 mg/l chlorpromazine, (B) plasma blank, and (C) plasma after the administration of mepixanthone to the patient. Peaks: 1 = mepixanthone, 2 = chlorpromazine. AU = absorbance unit.

TABLE I

EXTRACTION RECOVERY

Mepixanthone concentration (μg/l)	Mean recovery (n = 20)	
	%	S.D.
5	71.2	6.2
50	81.7	3.6
400	88.3	3.1

calculated at three concentrations by comparing the sample's peak area of mepixanthone with that of an unextracted equivalent concentration.

The correlation coefficient between the found (Y) and theoretical (X) concentration for serum spiked with 50, 200, 400, 800 and 2000 μg/l mepixanthone was $r = 0.99991$, and the correlation curve was described by the equation $Y = 1.069X - 16.18$.

The reliability of the proposed method was tested in a patient by determination of plasma mepixanthone following intravenous administration of 50 mg. Blood samples were taken at 0, 15 and 30 min, and 1, 3, 6, 12 and 24 h after administration of the drug. The pharmacokinetic behaviour with related parameters is shown in Fig. 3.

The method described has the advantages of a small sample requirement, sensitivity, selectivity and speed. No interferences from endogenous serum constituents were seen and it was therefore possible to operate at a wavelength of 237 nm, at which mepixanthone absorbs more than at the other analytical wavelength of 302 nm (absorbance ratio, 237/308 nm = 3.24).

Retention times of other drugs that were extracted under the assay conditions described and studied as potential sources of interference are shown in Table II. The only drug that has a retention time close to that of mepixanthone is dimefline [5], but the two peaks are separated with a resolution of 1.257 and a column selectivity of 1.087. Moreover, a patient who is treated with mepixanthone is not expected to be treated also with dimefline, since the two drugs belong to the same pharmacological class and are alternative.

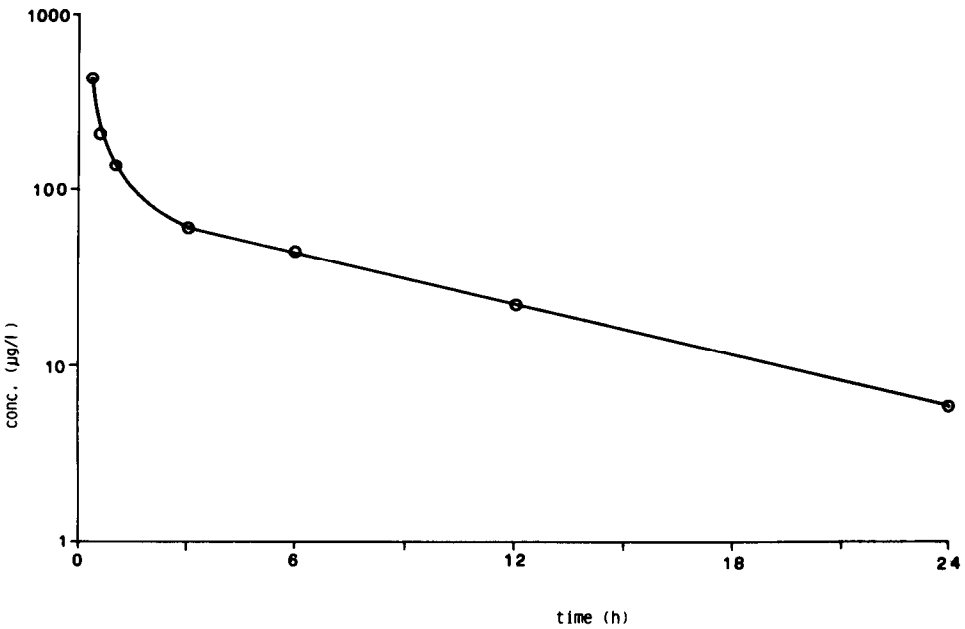

Fig. 3. Serum concentration curve following intravenous administration of mepixanthone (50 mg). Kinetic parameters: K_e = 2.01 h^{-1}, K_{21} = 0.81 h^{-1}, K_{12} = 4.48 h^{-1}, V_d = 234.71 l, V_1 = 26.99 l, $V_{d\beta}$ = 317.22 l, $t_{1/2}$ = 3.00 h.

TABLE II

RETENTION TIMES OF MEPIXANTHONE AND OTHER POTENTIAL INTERFERING COMPOUNDS

The conditions are as described in the text. The resolution between mepixanthone and chlordiazepoxide, nikethamide and dimefline is 2.341, 2.020 and 1.257, respectively.

Compound	Retention time (min)	Relative retention time*
Fluphenazine	2.74	0.32
Levomepromazine	4.02	0.47
Doxapram	4.02	0.47
Chlordiazepoxide	5.39	0.63
Nikethamide	5.48	0.64
Mepixanthone	6.06	0.71
Dimefline	6.44	0.75
Promethazine	8.05	0.94
Chlorpromazine	8.56	1.00
Dipyrone	9.12	1.06
Thioridazine	11.98	1.40
Amitriptyline	12.24	1.43
Promazine	12.68	1.48
Imipramine	15.41	1.80

*Chlorpromazine = 1.00.

In agreement with other authors, the analytical column shows a long useful lifetime [7, 8] due to the predominancy of organic solvent in the mobile phase.

The method seems to be suitable for kinetic studies as it can detect very low concentrations found 24 h after administration.

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