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Note

Rapid method for the determination of chlorpheniramine in urine

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Chlorpheniramine maleate is a widely used antihistaminic drug [1—3]. Numerous reports have been published on its disposition, metabolism and pharmacokinetics [4—10].

Various methods have been reported in the literature for the analysis of chlorpheniramine in biological fluids; for example, chloroform extraction followed by spectrophotometric determination [5], gas—liquid chromatographic (GLC) analysis of ethereal extracts [6,7], thin-layer chromatography followed by fluorimetric determination [11] and GLC determination of ketones produced by oxidation of the compound (prior to GLC analysis) [7,12,13]. However, the use of any of these methods has been limited by one or more factors such as lack of sensitivity, non-specificity, non-reproducibility and lack of speed.

Peet et al. [9] described a radioactive tracer procedure for the analysis of chlorpheniramine in urine and blood. Although radioactive tracer techniques are very sensitive, their use in biopharmaceutical studies is also limited.

Single-extraction methods using solvents heavier than water have been reported for various drugs [14—17]. These methods were claimed to be sensitive, specific and fast. We now report a single-extraction method for the analysis of chlorpheniramine in urine.

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EXPERIMENTAL

Compounds and materials

The compounds used were kindly supplied by various firms: chlorpheniramine maleate, mono- and di-desmethylchlorpheniramine by Allen and Hanburys Ltd. (London, Great Britain), and brompheniramine and mono-desmethylbrompheniramine [5] by A.H. Robins Co. (West Sussex, Great Britain). Chlorpheniramine N-oxide was not available as an authentic material.

Gas–liquid chromatography

A Perkin-Elmer F33 gas chromatograph with a flame ionization detector and linked to an Hitachi Perkin-Elmer 1-mV Model 56 recorder was used. Column A (glass column, 2 m × 0.64 cm O.D.) was packed with Chromosorb Q (AW DMCS), 100–120 mesh, and coated with 3% OV-17, and operated under the following conditions: nitrogen, 1.68 kg/cm^2; hydrogen, 0.96 kg/cm^2; air, 2.1 kg/cm^2; oven temperature, 210°C; injection point temperature, 250°C. The column was conditioned at 260°C for 24 h and was silanized with 2 × 5 μl of hexamethyldisilazane (HMDS) before use. The retention times of chlorpheniramine, mono-desmethylchlorpheniramine, di-desmethylchlorpheniramine, brompheniramine and mono-desmethylbrompheniramine were 6.0, 7.5, 8.5, 9.0 and 11.0 min, respectively. Chlorpheniramine N-oxide was analyzed as chlorpheniramine after reduction with titanous trichloride [7].

Determination of chlorpheniramine in urine

To 5 ml of the urine sample in a centrifuge tube were added brompheniramine maleate (10 μg of base per ml, 1 ml) as the internal standard, chloroform (100 μl) and sodium hydroxide (20%, 0.5 ml). The solution was mixed thoroughly on a Whirlimixer (2 min) and centrifuged (5 min). A small globule of clear chloroform solution forms at the bottom of the tube. A 10-μl syringe was inserted through the aqueous phase into the chloroform layer and 5 μl of the solution extracted carefully so that no aqueous solution entered the syringe. The syringe barrel was wiped carefully with a clean tissue and the 5 μl of solution injected on to column A.

The concentration of chlorpheniramine in the urine sample was determined by measuring the ratio of the chlorpheniramine peak (t_R = 6 min) to the brompheniramine peak (t_R = 9 min) and reading the corresponding concentration of chlorpheniramine from a calibration curve. The calibration curve was constructed by repeating the above procedure using solutions of chlorpheniramine of known concentration (5–0.4 μg/ml) in urine and plotting the peak height ratio (PHR) against concentration of chlorpheniramine. The data were subjected to linear regression analysis to give the appropriate calibration factors.

Comparison between chloroform and ether extraction of chlorpheniramine in urine

Twelve samples (5 ml of each sample) of chlorpheniramine maleate solution in urine (4 μg of base per ml) were prepared. Half of these samples were extracted and analysed by the method described above. To each of the other

samples were added brompheniramine maleate (10 μg of base per ml, 1 ml) and sodium hydroxide (20%, 0.5 ml) and the mixture extracted with ether as previously described by Khan [7]. The ethereal extracts were concentrated (42°C, ca. 50 μl) and injected (5 μl) on to the GLC column A. The mean values of the PHR obtained from each extraction method were calculated and the amount (μg) of chlorpheniramine in each sample was determined.

RESULTS AND DISCUSSION

Using the single-extraction method we were able to detect chlorpheniramine in urine (from subjects receiving a 4.0-mg dose of the drug) in amounts of less than 60 ng. Mono-desmethylchlorpheniramine, di-desmethylchlorpheniramine and chlorpheniramine N-oxide could be detected within the limit of 60—70 ng. Sharp and symmetrical peaks were obtained for chlorpheniramine and brompheniramine using the GLC conditions described in the Experimental section (see Fig. 1). The compounds were well separated from each other on the column (see Experimental section). None of the expected metabolic products of chlorpheniramine (mono-, di-desmethylchlorpheniramine and the N-oxide)

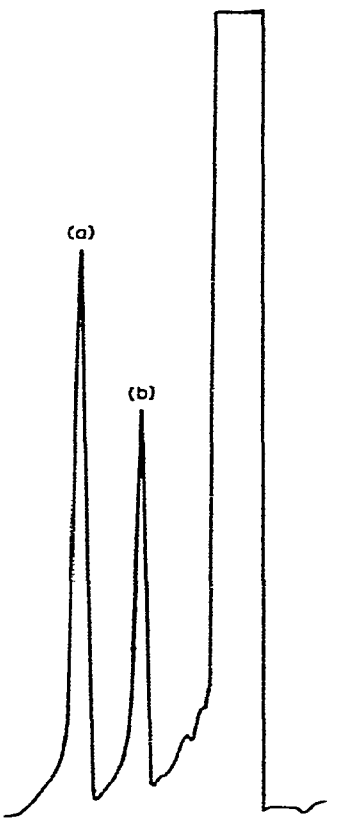

Fig.1. Gas—liquid chromatogram of the chloroform extract of urine obtained from a subject receiving an oral dose of chlorpheniramine maleate (a). Brompheniramine (b) was added to the urine as internal standard.

TABLE I

COMPARISON BETWEEN CHLOROFORM (A) AND ETHER (B) EXTRACTION METHODS FOR CHLORPHENIRAMINE IN URINE

Sample	Amount of chlorpheniramine extracted (μg)	
	Method A	Method B
1	21.1	19.6
2	20.2	20.0
3	20.7	21.6
4	22.1	19.9
5	20.5	20.1
6	21.5	19.8
Mean (± S.E.)	21.0 ± 0.26	20.1 ± 0.27

were detected in the urine of the subjects receiving the drug. Only 20—30% of the dose was excreted unchanged in the urine, in agreement with previous findings [6—9].

The recovery of chlorpheniramine from urine using the ether extraction method (method B, Table I) was more than 90% [7,12]. The amounts of chlorpheniramine extracted using the single-extraction method (method A, Table I) were identical to those obtained using the ether extraction method. This indicates that a recovery of more than 90% can be obtained using the single-extraction method. This extraction method is superior to the ether extraction method because (1) there is no loss of drug due to multiple extractions, evaporation, or adsorption to glass, and (2) it is economical.

The single-extraction method gave reproducible results (Table I). When four calibration curves were constructed (on different occasions) using this method, the corresponding calibration factors were almost identical. The average calibration factor ($\bar{X}$ ± S.E.) was 1.147 ± 0.0133. Linear calibration curves were obtained over the range 0.5—4.8 μg/ml (correlation coefficient = 0.9997).

This method has advantages over the previously described methods for the analysis of chlorpheniramine in urine since it offers the required specificity, sensitivity, reproducibility and speed [5—7,9,11,12]. It is especially useful in routine analysis when a large number of samples are involved and information is required urgently in poisoning cases.

We conclude that the present method is applicable to metabolic, disposition and pharmacokinetic studies of chlorpheniramine, and related compounds (e.g. brompheniramine) and in general to most compounds of high lipid solubility (i.e. with a high chloroform—water partition coefficient).

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