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QUANTITATION OF ZIMELIDINE AND NORZIMELIDINE IN PLASMA USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A method is described for the quantitation of a new non-tricyclic antidepressant, zimelidine, and its pharmacologically active, N-demethylated metabolite, norzimelidine, in plasma. The method involves a single extraction of basified plasma with diethyl ether, concentration of the ethereal extract, chromatography on a high-performance liquid chromatograph and quantitation using a variable-wavelength UV detector.

The respective geometric isomers of zimelidine and norzimelidine are used as internal standards for quantitation. Resolution is effected using a 5-μm silica gel column with an aqueous methanolic solution of ammonium nitrate as the mobile phase. The minimum quantitated amount was 25 ng and the coefficient of variation for the method did not exceed 7% in the range 25 to 1000 ng/ml for both compounds. The method has been applied in monitoring the plasma concentration of zimelidine and norzimelidine in plasma from depressed patients and an example of this application is presented.

INTRODUCTION

Zimelidine (I) is a new anti-depressant drug which is presently at an early stage of clinical trial in several countries. Animal studies have revealed that its N-demethylated metabolite, norzimelidine (II), has similar pharmacological properties and potency to the parent drug (I). Consequently, in

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studies of the efficacy of this new drug in humans, it was necessary to monitor the plasma concentration of both zimelidine and norzimelidine.

A method for quantitating zimelidine and norzimelidine in plasma using high-performance liquid chromatography (HPLC) based on the method of Schill et al. [2] has been applied clinically [3]. This method effected resolution using ion-pair partition chromatography and employed a mobile phase which consisted of an organic solvent (methylene chloride—butanol) saturated with an aqueous solution of perchlorate salts. The whole system required precise thermostating to prevent variable retention times and emulsion formation which resulted in intolerable detector noise. This requirement could not be achieved reliably with the instrumentation available to us. The method reported here describes an alternative HPLC system which simultaneously resolves the geometric isomers of both zimelidine and norzimelidine and which is also compatible with a simple procedure for their extraction from plasma.

EXPERIMENTAL

Reagents and materials

The molecular structures of the quantitated compounds are presented in Fig. 1. The dihydrochloride monohydrates of I, II and III, and the oxalate of IV were gifts from Astra Chemicals (Sydney, Australia). The ammonium nitrate, sodium hydroxide and methanol were analytical grade substances (Ajax Chemicals, Sydney, Australia) and were used without further purification. The diethyl ether was B.P. anaesthetic grade and freshly distilled before use.

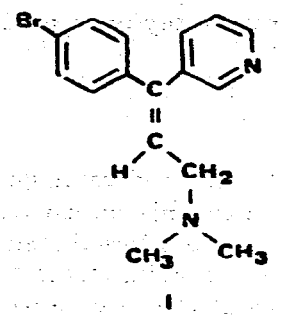
All glassware was cleaned with a chromic acid mixture and washed with distilled water. The glass evaporation tubes were silylated with Siliclad (Clay Adams, Parsippany, N.J., U.S.A.) washed with distilled water and dried.

Extraction from plasma

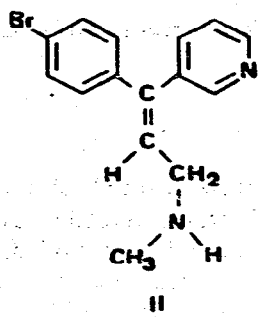
Plasma (1 ml) and an aqueous solution of internal standards (500 ng of III · 2HCl · H_2O and 500 ng of IV · oxalate per 100 μl) was made alkaline (0.5 ml, 5 *M* NaOH) and was shaken with diethyl ether (10 ml) on a vortex mixer for 2 min. After centrifugation at 1500 *g* for 2 min the diethyl ether phase was transferred to an evaporation tube — a 15-ml glass tube with a 100-μl capillary at the base. The extract was concentrated on a water-bath at 40° using an anti-bumping granule (BDH, Melbourne, Australia). When no diethyl ether remained the stoppered tube was immersed in an ice—water bath. This allowed the diethyl ether to condense and wash down the internal walls of the evaporation tube. The remaining diethyl ether was evaporated under a gentle stream of nitrogen and the residue redissolved in 60 μl of the mobile phase by shaking on a vortex mixer for 15 sec. All of this extract was injected onto the column using a loop-injection valve.

Chromatography

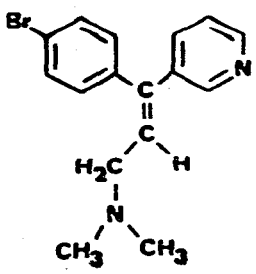
A Varian Aerograph Model 8500 high-performance liquid chromatograph equipped with a Spectra-Physics Model 770 variable-wavelength UV detector



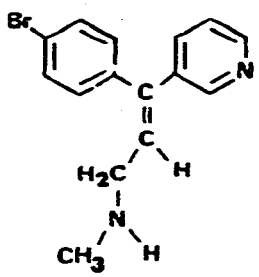
ZIMELIDINE



NORZIMELIDINE



GEOMETRIC ISOMER OF ZIMELIDINE



GEOMETRIC ISOMER OF NORZIMELIDINE

Fig. 1. Molecular structures of zimelidine, norzimelidine and their respective geometric isomers.

operated at 258 nm was used. Samples were injected using a Valco 7000-p.s.i. injection valve fitted with a 60-μl loop. The column was 120 × 4.5 mm I.D. stainless steel tubing packed with silica gel having an average particle diameter of 5 μm (Partisil 5). The column temperature was maintained at 30° with a thermostated water jacket. The mobile phase consisted of methanol and an aqueous solution of ammonium nitrate (0.1 *M*) in the ratio 100 : 5 and its flow-rate was 50 ml/h. Minor decreases in the ratio of aqueous ammonium nitrate to methanol were required occasionally to optimize the resolution and at these times another calibration curve was prepared.

Calibration and reproducibility

Known quantities of zimelidine and norzimelidine (25—1000 ng of each as the dihydrochloride monohydrate) were added to blank plasma which were then analysed. Calibration curves were constructed by plotting peak height ratios between zimelidine (I) and its geometric isomer (III), and norzimelidine (II) and its geometric isomer (IV) versus the respective amounts of added zimelidine and norzimelidine. The reproducibility of the assay was determined for both zimelidine and norzimelidine by adding a known amount of

each compound to a bulk plasma sample and then carrying out replicate analyses on this sample.

RESULTS AND DISCUSSION

Initial effort was directed toward the development of a method using gas chromatography with electron capture detection. Although this approach was satisfactory for the quantitation of zimelidine, no suitable internal standard could be found for the quantitation of norzimelidine. Irregular losses of norzimelidine occurred when ethereal extracts were concentrated by gentle evaporation. The molar response on electron capture of norzimelidine com-

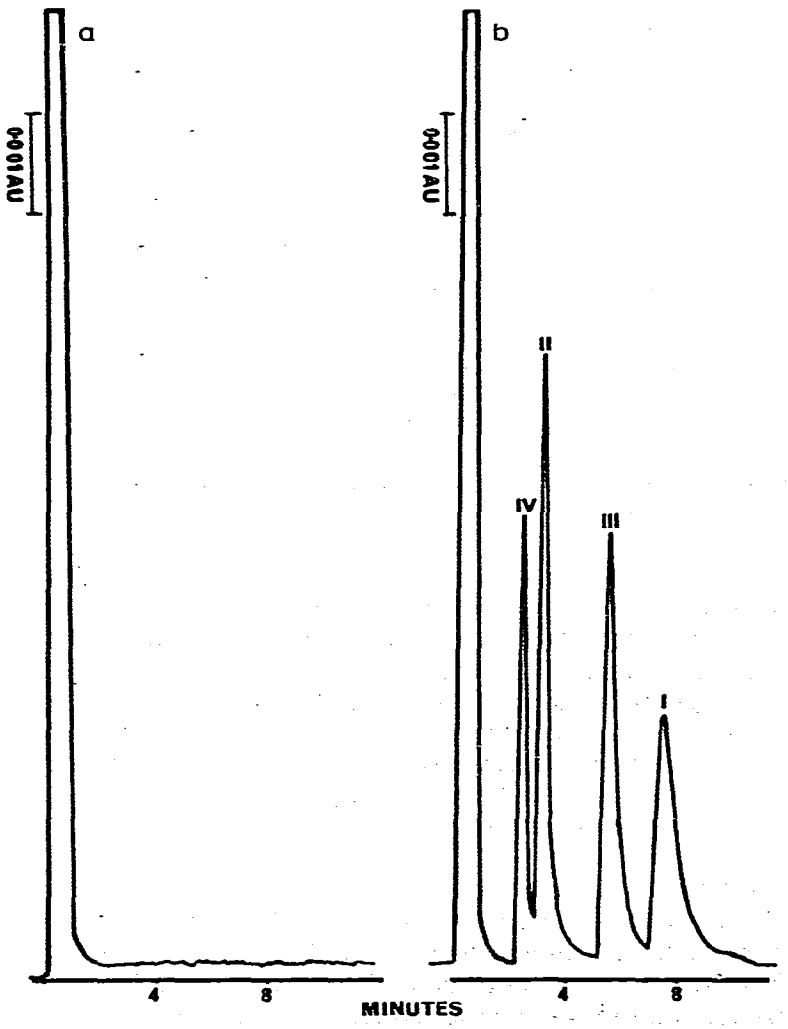


Fig. 2. Chromatograms of blank human plasma and human plasma containing 200 ng of zimelidine and 300 ng of norzimelidine. Chromatographic conditions are given in the text and compound identification in Fig. 1.

pared to zimelidine, chromatographed on a glass column packed with 3% OV-1 or 3% OV-17 on Gas-Chrom Q, was both erratic and low (< 10%). The geometric isomers were unsuitable as internal standards on these systems because neither compounds I and III, nor II and IV could be resolved. The possibility of resolving these geometric isomers on HPLC stimulated our interest in this approach.

Preliminary work using tritiated material [4] had shown that diethyl ether could extract 100% of zimelidine and norzimelidine from basified plasma. This factor, combined with the ease of concentration of diethyl ether, made it a most suitable solvent for extraction. Compounds II and IV were also completely extracted into diethyl ether from alkaline plasma. When diethyl ether extracts of blank plasma were chromatographed, there were no interfering endogenous compounds with the same retention time as compounds I to IV (Fig. 2a). In fact, all the detected endogenous compounds eluted before them. No other drugs which co-chromatograph with compounds I to IV have been observed in extracts from plasma of patients (Table I).

The chromatography system that has been developed is based on that reported by Jane [5]. Applications of the original systems to forensic problems have been reviewed more recently [6]. The mechanism that determines the separation with the use of a polar mobile phase and microparticulate silica-packing is not understood. Wheals [6] has suggested that processes such as ion exchange and hydrogen bonding may be contributing. The ability of this system to resolve the geometric isomers is illustrated in the chromatogram in Fig. 2b.

When the optimum composition for the mobile phase was being sought, it became evident that a balance had to be achieved between adequate resolution of norzimelidine and its geometric isomer, and the peak shape of zimelidine and its geometric isomer. This balance was influenced by the strength of the aqueous ammonium nitrate solution and the ratio of methanol to water in the mobile phase. The retention times of all four components were decreased by increasing either the ammonium nitrate concentration or the water-to-methanol ratio. The resolution of the four components illustrated in Fig. 2b was found to be adequate for accurate and reproducible quantitation.

The sensitivity of this method was adequate for monitoring clinically relevant concentrations of zimelidine and norzimelidine. The sensitivity could be

TABLE I

DRUGS WHICH DID NOT INTERFERE IN THE ANALYSIS OF ZIMELIDINE AND NORZIMELIDINE IN PLASMA

Chlorpromazine	Paracetamol
Danthron	Penicillin
Diazepam	Prednisone
Dioctyl sodium succinate	Sodium salicylate
Indomethacin	Theophylline
Meclozine	Thioridazine
Nitrazepam	

TABLE II

REPRODUCIBILITY DATA FOR THE QUANTITATION OF ZIMELIDINE AND NORZIMELIDINE

Compound	Plasma concentration (ng/ml)	No. of assays	Peak height ratio (mean ± S.D.)	Coefficient of variation (%)
I	1000	6	2.488 ± 0.059	2.4
II	1000	6	3.217 ± 0.052	1.6
I	500	6	1.147 ± 0.040	3.5
II	500	6	1.503 ± 0.077	5.1
I	300	6	0.728 ± 0.044	6.0
II	300	6	1.011 ± 0.067	6.6
I	100	6	0.232 ± 0.008	3.2
II	100	6	0.359 ± 0.011	3.0
I	25	6	0.075 ± 0.005	6.4
II	25	6	0.150 ± 0.007	4.3

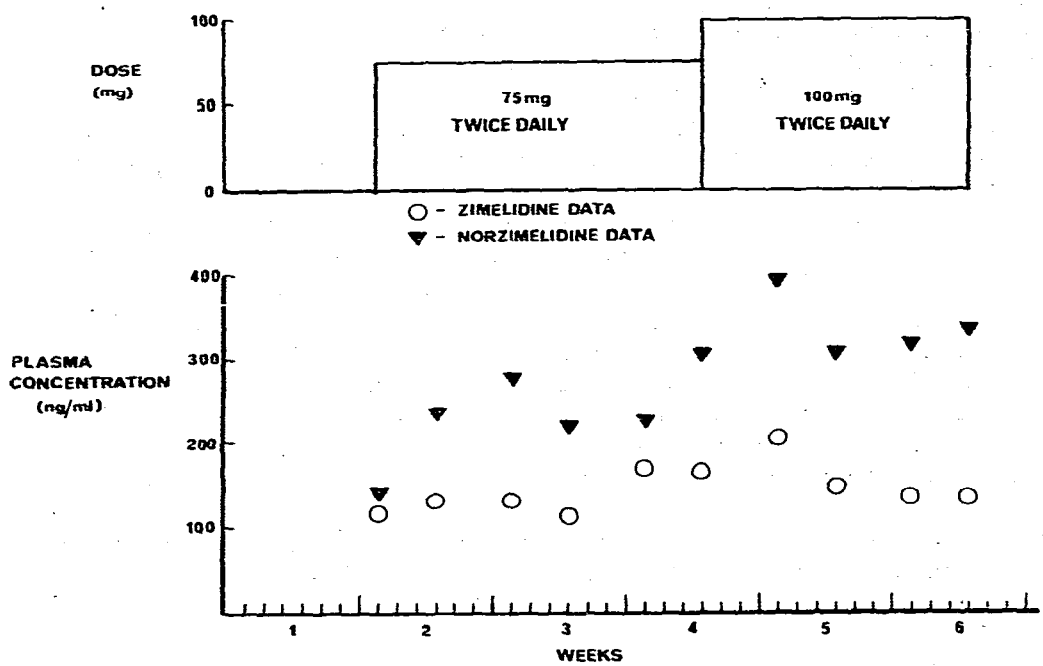


Fig. 3. Some typical plasma concentration—time data for a patient on oral zimelidine. Blood samples were collected twice weekly, 6 h after the 8 a.m. dose. The patient took placebo tablets during the first week.

extended easily for more sophisticated pharmacokinetic studies by increasing the volume of biological fluid assayed and by using a more sensitive, fixed-wavelength UV detector.

The reproducibility of the technique is demonstrated by the linearity of the calibration plots (see also the data in Table II). The slight positive devia-

tion of the norzimelidine calibration curve from the origin was caused by the presence of a trace amount of norzimelidine as an impurity in its internal standard (IV). The presence of this impurity has been observed previously [4]. The reproducibility data in Table II show that the coefficient of variation for quantitation of both components in plasma is less than 7% in the range 25 to 1000 ng/ml.

This method has been applied to analysis of over 300 plasma samples from patients. Typical plasma concentration—time profiles for zimelidine and norzimelidine for a patient on different dosages of zimelidine are illustrated in Fig. 3.

The high efficiency of microparticulate silica columns operated with polar mobile phases has permitted the resolution of geometric isomers so that they can be quantitated reproducibly. The similarity in physico-chemical properties between geometric isomers may make them an ideal internal standard for quantitation now that they can be rapidly resolved by HPLC.

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