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Note

Determination of zimelidine and its demethylated metabolite in human plasma by gas chromatography

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Medical treatment of endogeneous depression has been improved during the past ten years using monitoring of the plasma concentration of the active drug[1–3].

Many potent anti-depressants are currently available. Unfortunately, they all give more or less severe side-effects, of which the most frequently seen is a substantial reduction in salivation[4]. Electrocardiographic disorders have been reported during treatment with these drugs[5].

A new anti-depressant, zimelidine (ZMD), has been introduced as an effective drug without side-effects. Therefore, therapeutic control of plasma concentrations of the parent compound (ZMD) and of the main metabolite, norzimelidine (NZMD), might be important from a clinical point of view if a correlation between these concentrations and the effectiveness of the drug could be established.

This paper describes a gas chromatographic method for the determination of ZMD and NZMD with a sensitivity sufficient for the concentrations that are present after conventional therapeutic doses.

EXPERIMENTAL

Reagents and glassware

Toluene of analytical grade from E. Merck (Darmstadt, G.F.R.) was distilled once before use. Undiluted borate buffer (Titrisol buffer) of pH 10 (E. Merck) was used to buffer the solutions. To prevent adsorption to glassware, N,O-bis(trimethylsilyl)acetamide (BTSA) of specially purified grade from Pierce (Rockford, Ill., U.S.A.) was added to the ethanolic stock solutions. Glassware was cleaned with detergent in an ultrasonic bath and rinsed twice with redistilled water and once with methanol.

Reference substances

Structural formulae are shown in Fig. 1. Stock solutions (1 g/l) in ethanol were prepared of ZMD, NZMD, and of the internal standard, N-methyl-3-(4-bromophenyl)-3-phenylallylamine (CPK 191). To all stock solutions 100 μl BTSA were added per 10 ml solution. If the solutions are kept in a refrigerator, they are stable for 1 year.

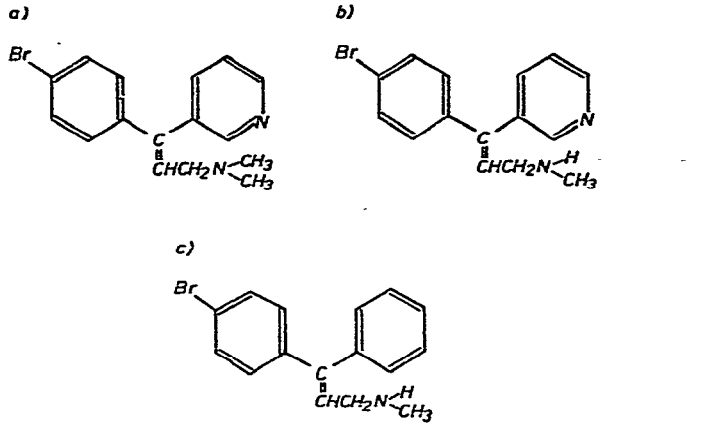

Fig. 1. Structural formulae of (a) zimelidine, (b) norzimelidine and (c) the internal standard (CPK 191).

Extraction procedure

To a centrifuge tube containing 2.0 ml of plasma, 500 μl of borate buffer (pH 10) were added. The internal standard (300 ng) was added and the compounds were extracted with 6 ml of toluene by mixing for 5 min in a rotary mixer (20 rpm). After centrifugation for 5 min, the organic phase was transferred to a 10-ml glass-stoppered tube containing 1 ml of 0.1 *N* sulphuric acid. The compounds were extracted into the aqueous phase by mixing for 5 min. After centrifugation, the organic phase was discarded. The aqueous phase was made alkaline by adding 100 μl of 6.6 *N* sodium hydroxide solution. The compounds were extracted into 3 ml of toluene by mixing for 5 min in a rotary mixer. After centrifugation, the organic phase was transferred to a tapered tube moistened with ethanol. From this solution 2 μl were injected into the gas chromatograph.

Gas chromatography

A Pye series 104 Model 74 gas chromatograph equipped with an electron-capture detector was used. The pre-heater temperature was 250°, the column temperature 240°, and the detector temperature 350°. A glass column, 0.9 m × 4 mm I.D., packed with 1% (w/w) OV-17 on Chromosorb 75 (100–120 mesh) was used. The amount of column material was 5 g. The column was conditioned at 350° for 14 h. The carrier gas (argon–methane, 90:10) flow-rate was 50 ml/min. The pulse interval was 150 μsec and the attenuation 10^3.

Calculations

The plasma concentrations were read from standard curves constructed from chromatograms of plasma samples containing varying but known amounts of ZMD and of NZMD (Fig. 2). The compounds were added to plasma, giving concentrations up to 100 and 300 μg/l for ZMD and NZMD, respectively. The peak height ratios (*R*) of ZMD to CPK 191, and of NZMD to CPK 191, were plotted against the concentrations (Fig. 3).

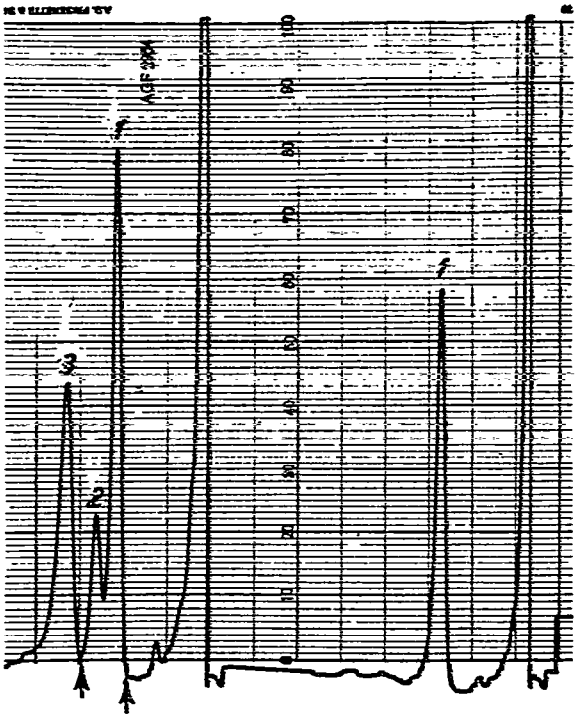

Fig. 2. Chromatograms of two plasma samples. The right-hand chromatogram illustrates a blank plasma with added CPK 191 (1). The sample in the left-hand chromatogram contained ZMD (2) and NZMD (3) in concentrations of 15 and 50 μg/l, respectively. The retention times for (1), (2) and (3) are 2.0 min, 2.6 min and 3.3 min, respectively.

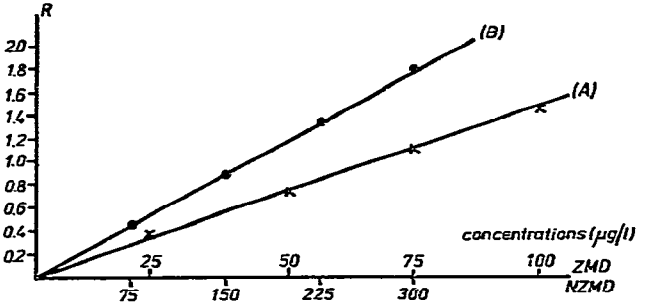

Fig. 3. Calibration graph constructed on the basis of chromatograms from plasma samples containing varying but known amounts of ZMD (A) and of NZMD (B).

RESULTS AND DISCUSSION

Quantitative determination of ZMD and NZMD is complicated by the strong adsorption of the two compounds on glass surfaces. All initial attempts to construct a plasma standard curve by adding the substances from ethanolic solutions at concentrations of 1 and 3 mg/l for ZMD and NZMD, respectively, failed to give a linear plot. To prevent adsorption of ZMD and of NZMD on the glass surfaces in the

TABLE I

ACCURACY TEST FOR ZIMELIDINE AND NORZIMELIDINE FROM PLASMA

Concentration added (μg/l)		*No. of samples*	*Calculated concentrations (μg/l)*	
ZMD	*NZMD*		*ZMD*	*NZMD*
25	75	10	24.7 ± 1.8	72.2 ± 4.8
50	150	10	49.3 ± 2.7	144.6 ± 7.4
75	225	10	74.6 ± 4.0	225.4 ± 5.3
100	300	10	101.8 ± 2.5	306.0 ± 8.2

micropipettes BTSA (1%, w/w) was added to the solutions. Mass fragmentographic analysis has confirmed that no reaction occurs between BTSA and ZMD or NZMD. Later steps in the extraction procedure include transference of organic phases with pasteur pipettes from one tube to another. Considerable loss of ZMD and NZMD occurs in this step due to adsorption if the inner surface of the pipette is not moistened with ethanol. When these precautions are taken, a linear calibration curve can be obtained (Fig. 3).

The chromatograms in Fig. 2 illustrate that the internal standard (1) and ZMD (2) are incompletely separated. However, the peak height ratio between (2) and (1) increases with increasing concentration when using the same baseline for the two peaks, drawn between the two arrows.

Accuracy tests were performed on 40 plasma samples containing ZMD and NZMD in varying concentrations within the therapeutic concentration range, and errors were no greater than 7% (Table I). The lower limit for quantitation (sensitivity) was found to be below 10 μg/l when a plasma volume of 2.0 ml was used. At the research laboratory of the Astra Pharmaceuticals AB, a high-performance liquid chromatographic (HPLC) method has been developed [6]. To compare the two methods, 10 plasma samples from patients undergoing therapeutic treatment with ZMD were analysed by both methods. The results (Fig. 4) indicate that the two methods give mostly identical values for NZMD, whereas the ZMD values deviate a little more (18.5%). However, both methods seem to be adequate for the daily clinical monitoring of plasma concentrations.

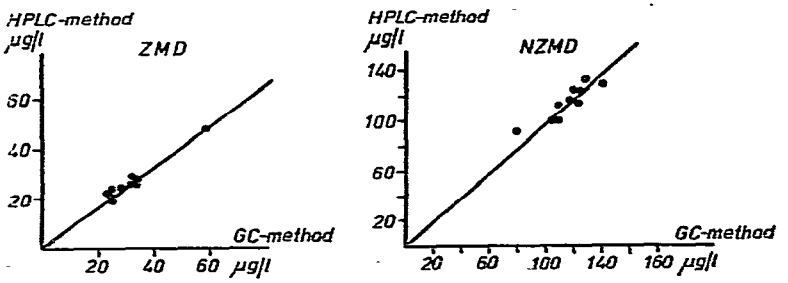

Fig. 4. The correlation between the GC and HPLC methods. The left-hand graphs gives correlating ZMD values ($r = 0.98$). The right-hand graph gives correlating NZMD values ($r = 0.90$).

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