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Note

Quantitative determination of doxepin and desmethyldoxepin in rat plasma by means of gas–liquid chromatography–mass fragmentography

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Doxepin [11-(3-dimethylaminopropylidine)-6*H*-dibenzo(*b*,*e*)oxepin hydrochloride] is a drug clinically useful in the treatment of anxiety and depression[1]. It is generally assumed that the therapeutic action of tricyclic antidepressants is mediated by the desmethylated metabolites[2–8] and it is therefore important to have a method for the simultaneous measurement of doxepin and desmethyldoxepin. Gas chromatographic and radioisotopic methods have been described[9], but they suffer from a lack of sensitivity and specificity, particularly when applied to biological specimens.

The method described here does not have the above disadvantages because it combines the high resolving power of the gas chromatograph with the high sensitivity and specificity of the identification provided by the mass spectrometer[10–12]. The procedure involves separation by gas–liquid chromatography and detection by mass fragmentography.

MATERIALS AND METHODS

Standards and reagents

Doxepin and desmethyldoxepin were supplied by Penwalt Corp. (Rochester, N.Y., U.S.A.) and promazine (used as an internal standard) by Pierrel (Milan, Italy).

The following reagents were used: acetic anhydride and sodium hydroxide (Merck, Darmstadt, G.F.R.), pyridine, dichlorodimethylsilane, *n*-hexane, toluene, absolute ethanol and isoamyl alcohol (Carlo Erba, Milan, Italy).

Glassware

All glassware was silanized by soaking for 6 h in a 5% solution of dichlorodimethylsilane in toluene and dried in an oven at 80°, then rinsed with ethanol prior to use.

Gas chromatography–mass fragmentography

A Finnigan Model 3100 quadrupole mass spectrometer equipped with a gas chromatograph and a Model 6000 computer system was used.

The chromatographic conditions were as follows: column, glass tube, 1 m × 2 mm I.D., packed with 3% OV-1 on Gas-Chrom Q, 100–120 mesh (Applied Science

Labs., State College, Pa., U.S.A.); oven temperature, 240°; injection port temperature, 260°; carrier gas (helium) flow-rate, 20 ml/min.

The mass spectrometer was operated under the following conditions: separator temperature, 250°; ion source temperature, 100°; energy of ionization beam, 70 eV; ionization current, 200 μA.

Measurements were performed by multiple ion detection, focusing the mass spectrometer on the ions at *m/e* 220 and 277 for doxepin, *m/e* 234 for desmethyldoxepin N-acetyl derivative and *m/e* 238 for promazine, which was used as an internal standard for quantitation.

Extraction procedure and derivative formation

A 1-ml volume of heparin-treated plasma, previously acidified with 0.5 ml of 0.1 *N* hydrochloric acid and stored at −20°, was made alkaline by adding 0.5 ml of 1 *N* sodium hydroxide solution and extracted with 3 ml of *n*-hexane by shaking for 30 min on an automatic shaker.

After centrifuging at 2000 *g* for 10 min, 2.5 ml of the organic layer were carefully transferred into conical glass-stoppered tubes with pipettes that had been rinsed with isoamyl alcohol just before use. Then 50 and 100 μl of acetic anhydride reagent were added and the stoppered tubes allowed to stand for 30 min in a sand-bath at 70°. The reaction proceeded with complete derivatization and the derivative was found to be very stable in slightly acidic or alkaline aqueous solutions. The stopper was then removed and the reaction mixture evaporated to dryness under a gentle stream of nitrogen at 70°. After cooling to room temperature, the dry residue was redissolved in 100 μl of absolute ethanol containing promazine (1 μg/ml) and 1, 2 or 3 μl of this solution were injected on to the gas chromatographic column.

Measurement of doxepin

A range of standard solutions, each containing 100 ng of promazine and 10–500 ng of doxepin (free base), were prepared in 100 μl of absolute ethanol. The standard solutions were found to be stable over a period of several months when stored

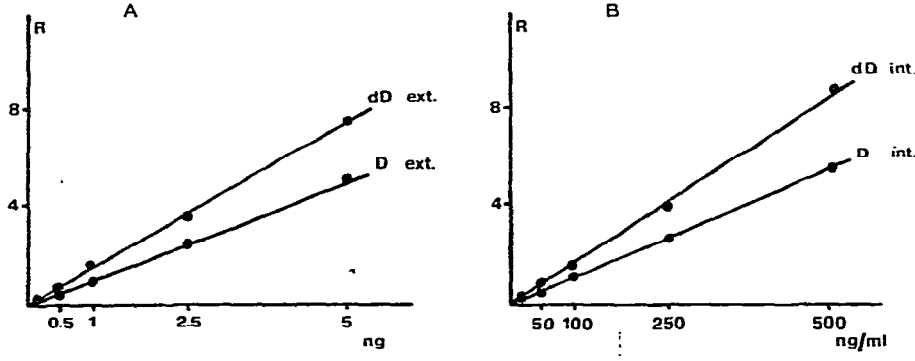

Fig. 1. (A) External calibration graphs for doxepin (D) and desmethyldoxepin (N-acetyl derivative) (dD). (B) Calibration graphs obtained by adding known amounts of doxepin and desmethyldoxepin to 1 ml of rat plasma and processing them as described under *Extraction procedure and derivative formation*. R = ratio of the mass fragmentographic peak areas of doxepin and desmethyldoxepin (N-acetyl derivative) to that of the internal standard promazine.

at −4° in a refrigerator. A calibration graph was prepared by injecting 1-μl aliquots of these solutions prior to determining doxepin in the test samples (Fig. 1A).

The ratio of the peak area of doxepin to that of promazine was linear over the range 100 pg to 5 ng of doxepin on injection.

Measurement of desmethyldoxepin

As this drug is determined as its N-acetyl derivative, standard solutions were prepared as follows. To a range of samples, each containing 10–500 ng of desmethyldoxepin, 50 μl of pyridine and 100 μl of acetic anhydride were added. After reaction, as previously described, 100 μl of a 1 μg/ml solution of promazine in ethanol were added to the dry residue. The standard solutions were found to be stable for several days when stored in a refrigerator at −4°. A calibration graph (yield of reaction 100%) was prepared by injecting 1- or 2-μl aliquots of these solutions (Fig. 1A).

The ratio of the peak area of desmethyldoxepin to that of promazine was linear over the range 100 pg to 5 ng of desmethyldoxepin on injection.

Recovery studies

The separate and combined addition of doxepin and desmethyldoxepin hydrochloride to drug-free plasma at concentrations ranging from 10 to 500 ng/ml for each drug resulted in overall recoveries of 83 ± 5% for doxepin and 74 ± 4% for desmethyldoxepin. A linear response was found over the range 10–500 ng/ml in plasma (Fig. 1B).

Kinetic parameters

These parameters were obtained by means of the BMD-X-85 program[13] on a PDP 11/45 digital computer.

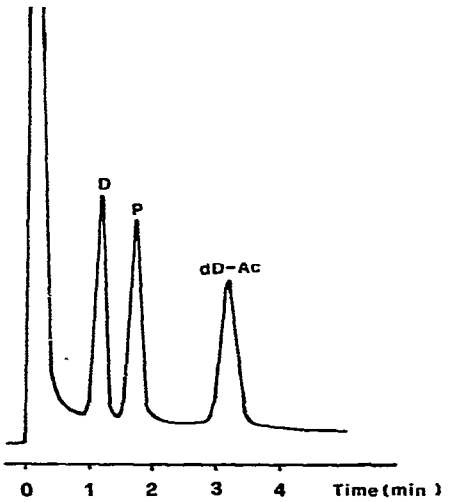

Fig. 2. Gas chromatographic separation of doxepin (D), desmethyldoxepin N-acetyl derivative (dD-Ac) and promazine (P), used as an internal standard.

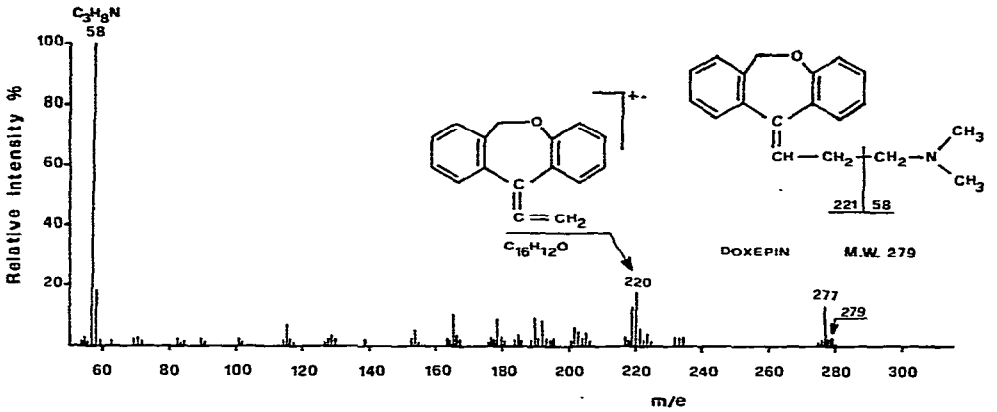

Fig. 3. Mass spectrum of doxepin.

RESULTS AND DISCUSSION

The gas chromatogram of doxepin, desmethyldoxepin N-acetyl derivative and their internal standard promazine is shown in Fig. 2. The acetylation reaction was chosen because of the suitable retention time of the derivative formed, and in order to avoid absorption of desmethyldoxepin on the stationary phase of the gas chromatographic column. The identity of the gas chromatographic peaks was checked by means of mass spectrometry.

The mass spectrum of doxepin (Fig. 3) shows a molecular ion at *m/e* 279, the base peak at *m/e* 58, corresponding to a β-bond fission with respect to the nitrogen atom of the side-chain, giving rise to N,N-dimethylformimine with retention of the positive charge. The ion at *m/e* 220 arises as shown in Fig. 3.

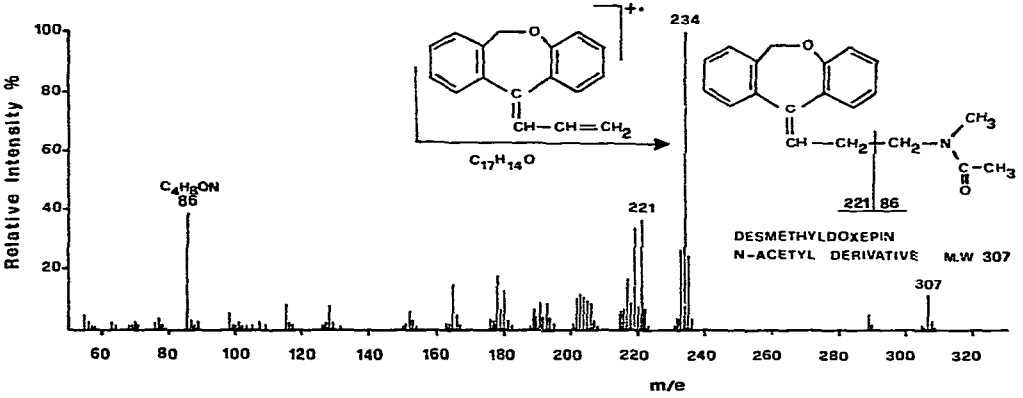

Fig. 4. Mass spectrum of desmethyldoxepin N-acetyl derivative.

The mass spectrum of desmethyldoxepin N-acetyl derivative (Fig. 4) shows a molecular ion at *m/e* 307. Other intense peaks are present at *m/e* 234, 221 and 86.

The mass spectrum of promazine shows a molecular ion at *m/e* 284, and other peaks at *m/e* 238, 85 and 58 (base peak), as shown in Fig. 5.

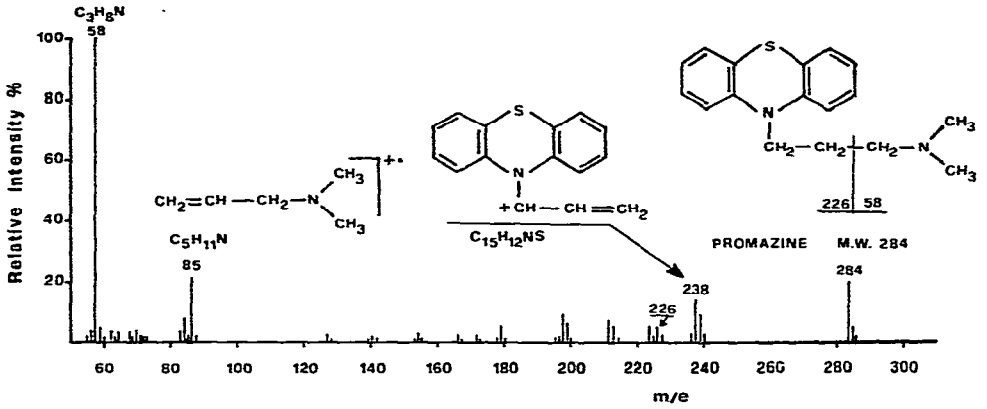

Fig. 5. Mass spectrum of promazine.

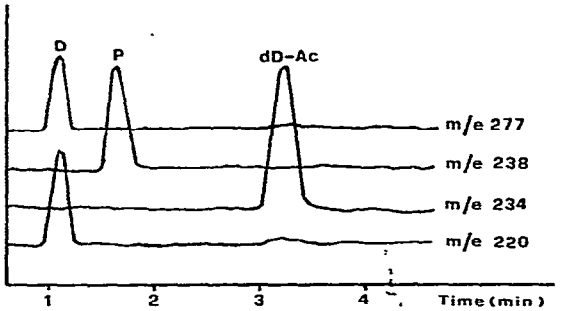

Fig. 6. Mass fragmentogram of doxepin (D), desmethyldoxepin N-acetyl derivative (dD-Ac) and promazine (P), used as an internal standard, obtained from a rat plasma extract.

In Fig. 6 is reported a typical mass fragmentogram of a rat plasma extract. No interferences from endogenous substances were found. Quantitation was performed by focusing the instrument (at 70 eV) upon the ions at *m/e* 277 and 220 for doxepin, 234 for desmethyldoxepin N-acetyl derivative and 238 for promazine (internal standard).

The validity of this analytical procedure for *in vivo* determinations of doxepin has been demonstrated by studying the disappearance curve of the drug in rat plasma. Male Sprague Dawley rats (200–220 g body weight) were injected intravenously with a single dose of 5 mg/kg of doxepin. Groups of three animals were sacrificed and plasma samples collected at 5, 10, 20, 30, 60, 120 and 180 min after doxepin injection. The results are shown in Fig. 7; each point is the average of three determinations. It appears that the elimination of doxepin can be described by means of a two-compartment open model, after intravenous injection. No detectable amounts of desmethyldoxepin were found in rat plasma.

The pharmacokinetic parameters of doxepin are reported in Table I.

In conclusion, the specificity and sensitivity of the gas chromatographic–mass fragmentographic method described appear to be satisfactory for pharmacokinetic

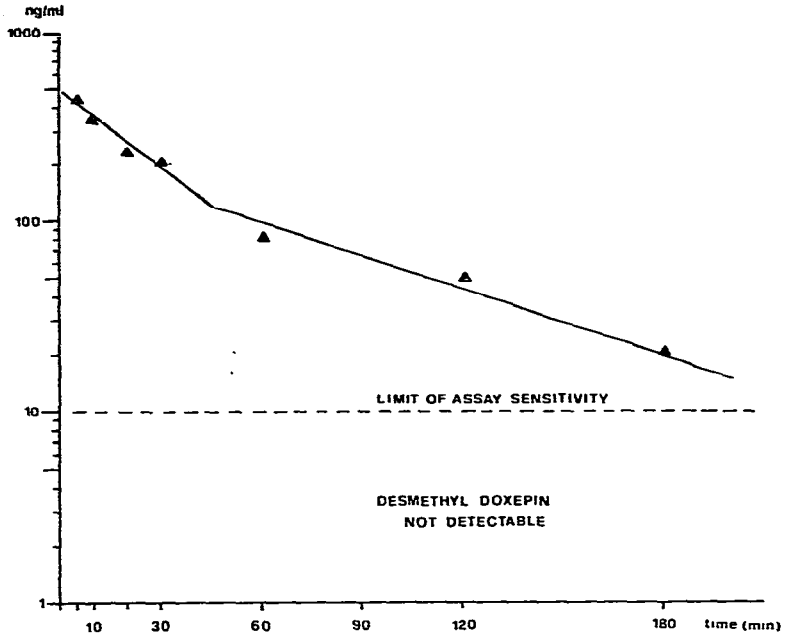

Fig. 7. Doxepin levels in rat plasma at different times after intravenous injection (5 mg/kg). Each point is the average of three determinations.

TABLE I

PHARMACOKINETIC PARAMETERS[13] OF DOXEPIN

Kinetic parameter	*Value*	*Kinetic parameter*	*Value*
A (ng/ml)	371	K_{el} (min^{-1})	0.0622
α (min^{-1})	0.064	K_{12} (min^{-1})	0.0196
$(t_{\frac{1}{2}})\alpha$ (min)	10.82	V_1 (l/kg)	8.695
B (ng/ml)	204	V_2 (l/kg)	5.533
β (min^{-1})	0.0126	$(V_d)\beta$ (l/kg)	18.048
$(t_{\frac{1}{2}})\beta$ (min)	55	CL (l/min·kg)	0.227
K_{21} (min^{-1})	0.0303	AUC (ng/ml·min)	21980

studies with doxepin. This method is specific, as endogenous substrates were found not to interfere, and it is sensitive, covering a range of drug plasma concentrations usually present during single or repeated administration of doxepin.

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