GLC Determination of Doxepin Plasma Levels

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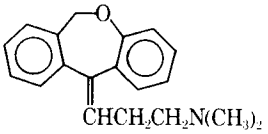
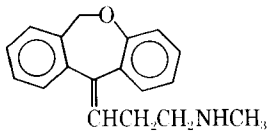
Abstract □ A procedure is described for extracting doxepin, a tricyclic antidepressant, from plasma and subsequently measuring its concentration by GLC. The developed technique permits the resolution and quantitative determination of the *cis*- and *trans*-isomers of doxepin as well as its desmethyl metabolite. The method allows precise, reliable measurement of the drug and one of its metabolites in concentrations as low as 10 ng/ml of plasma.

Keyphrases □ Doxepin—GLC analysis of *cis*- and *trans*-isomers and desmethyl metabolite, plasma □ GLC—analysis, *cis*- and *trans*-isomers and desmethyl metabolite of doxepin, plasma □ Antidepressants—doxepin, GLC analysis, plasma

Doxepin, *N,N*-dimethyl-3-dibenz[*b,e*]oxepin-11(6*H*)ylidene-1-propanamine (I) hydrochloride, is a therapeutic agent used in the treatment of psychoneurotic anxiety and depression. It is a tricyclic dibenzoxepine derivative used as an isomeric mixture with a distribution of approximately 15% *cis* and 85% *trans*. Oral administration of an efficacious drug may result in a therapeutic failure unless it is sufficiently bioavailable. To compare the absorption of doxepin formulated in different dosage forms, a method was developed to measure plasma levels of doxepin (I) and its desmethyl metabolite (II) in humans who had received oral, therapeutic doses.



I



II

Previous efforts to produce a bioassay of doxepin by GC (1, 2) did not provide adequate sensitivity for the measurement of normal plasma drug levels. More recently, however, a technique for tricyclic antidepressants (3) demonstrated suitable sensitivity. The procedure described here provides not only sensitivity but also simultaneous resolution and measurement of the *cis*- and *trans*-isomers of doxepin as well as its desmethyl metabolite.

EXPERIMENTAL

Reagents—The following were used: 20% NaOH, *n*-hexane[1], 1 *N* HCl, and 0.02% triethanolamine in chloroform.

Instrumentation—A biomedical gas chromatograph[2] equipped with a flame-ionization detector was used. The glass column was 1.83 m × 2 mm i.d., packed with 3% OV-225 on 100–120-mesh Gas Chrom Q[3].

The column was operated isothermally at 215°; the detector temperature was 280°, and the injection port temperature was 200°. The helium flow rate was 40 ml/min with an inlet pressure of 40 psig, the hydrogen flow was 35 ml/min, and the air flow was 300 ml/min. The electrometer range was 1 with an attenuation of 16–64.

[1] Mallinckrodt Nanograde.
[2] Hewlett-Packard 7610A.
[3] Applied Science Laboratories.

Table I—Recovery of Doxepin from Human Plasma

cis-Isomer			*trans*-Isomer		
ng Spike/ml Plasma	Recovery, ng	%	ng Spike/ml Plasma	Recovery, ng	%
8	4.8	60	45	31	69
8	6.8	85	45	32	71
8	5.0	62	45	32	71
8	5.4	68	45	32	71
8	6.8	85	45	32	71
16	11	69	90	57	63
16	11	69	90	61	68
16	12	75	90	57	63
16	11	69	90	63	70
32	25	78	180	134	74
32	24	75	180	133	74
32	22	69	180	126	70
32	26	81	180	133	74
		$\bar{x}$ = 73			$\bar{x}$ = 70
		SD = 8			*SD* = 4

Extraction of Doxepin from Plasma—A 5-ml sample of plasma is pipetted into a 50-ml round-bottom test tube fitted with a plastic stopper. The plasma is made basic with 0.5 ml of 20% NaOH and then extracted twice with 5 ml of *n*-hexane by shaking the tube, in a horizontal position, on a wrist-action shaker for 20 min each time.

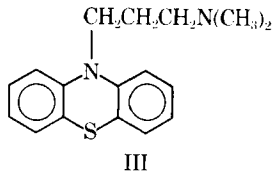
The sample is centrifuged after each extraction, and the organic layers are combined in a 15-ml centrifuge tube. Then 1.0 ml of 1 *N* HCl is added to the combined hexane fractions and mixed on a wrist-action shaker for 15 min to extract the drug into the acid aqueous phase.

The hexane containing lipid-soluble material is discarded. Then 0.3 ml of 20% NaOH is added to the 1-ml aqueous acid extract, and the sample is reextracted twice with 1-ml portions of *n*-hexane on a wrist-action shaker for 5 min each. The extracts in which the doxepin has been distributed are combined in a 13-ml centrifuge tube.

The overall purpose of this three-step extraction procedure is to provide a "chromatographically clean" sample with minimum background contamination.

Preparation of Sample for Chromatography—One milliliter of 0.02% (v/v) triethanolamine in chloroform is added to the sample extract. It acts as a "scavenger" and minimizes the adsorption of the doxepin on the glass surfaces during the concentration step. The extract is then evaporated to dryness at 50° in a heating block[4] under a gentle stream of dry nitrogen.

The residue is reconstituted with 100 μl of *n*-hexane containing 1 mg % promazine (III). (Promazine was chosen as an internal standard because of its structural similarity to doxepin and its chromatographic characteristics.) The tube and contents are mixed on a vortex mixer for approximately 20 sec, and then 5 μl is withdrawn and injected into the chromatograph.



III

The feasibility and efficiency of the extraction and measurement were tested by adding known quantities of doxepin[5] to plasma and following the described procedure.

[4] Temp-Blok module heater, Lab Line Instruments.
[5] Adapin, Pennwalt Corp., Rochester, N.Y.

Table II—Doxepin Levels in Chronically Treated Subjects

Subject	Age, years	Sex	Doxepin or Placebo Regimen[a]	Patient Response	Plasma Level, ng/ml		
					cis	*trans*	Desmethyl
1	68	Female	25 mg tid–16 weeks	Moderately good	11	42	87
2	66	Female	25 mg qid–6 weeks	Good	9	43	Nil
3	68	Female	100 mg bid–37 weeks	Very good	10	141	100
4	31	Female	25 mg qd–5 weeks	No change	Nil	Nil	Nil
5	60	Female	50 mg qid–3 weeks	Moderately good	9	63	Nil
6	33	Female	50 mg tid–3 weeks 25 mg tid–4 days	No change	Nil	Nil	Nil
7	35	Male	50 mg tid–5 weeks	No change	11	46	Nil

[a] Double-blind study; not complete.

RESULTS AND DISCUSSION

Typical chromatograms of human plasma extracts are shown in Fig. 1. The blank plasma extract is from a subject receiving a placebo, while the patient plasma is from a chronically treated subject approximately 3 hr after a 25-mg dose (given three times daily). The chromatographic technique developed permits the resolution and quantitative determination of the *cis*- and *trans*-isomers of doxepin and its desmethyl metabolite.

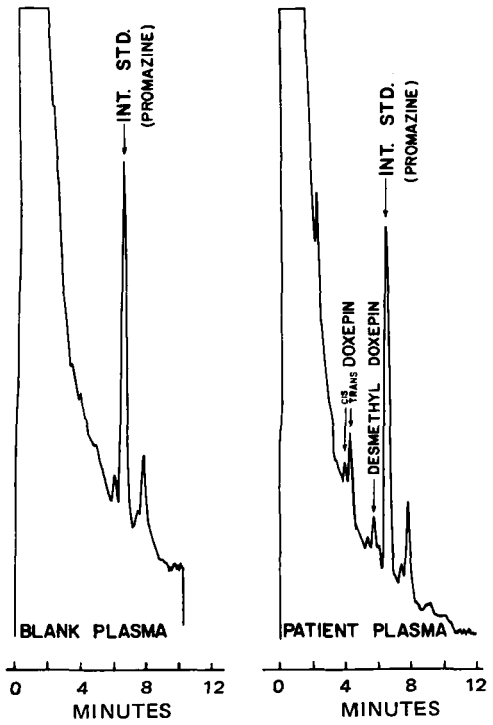


Figure 1—*Typical chromatograms of extracts of human plasma.*

To determine that the methodology provided a reliable measure of plasma drug levels, human plasma was spiked with varying concentrations of doxepin hydrochloride and assayed according to the procedure (Table I). As can be seen from the data, the technique allows precise and reliable measurement of the drug in concentrations as low as 10 ng/ml of plasma.

Table II shows the doxepin and metabolite levels in the plasma of chronically treated subjects undergoing therapy according to the indicated regimen. The values show that the method has adequate sensitivity for measuring drug levels in the blood of patients receiving therapeutic doses.

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