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Quantitative determination of imipramine and desipramine in human blood plasma by direct densitometry of thin-layer chromatograms

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Imipramine and desipramine have been estimated in the plasma of patients receiving therapeutic doses of imipramine. The compounds were extracted as bases into n-heptane, separated by thin-layer chromatography and transferred into coloured spots by an oxidative procedure. The quantification was made with a new densitometric technique of high sensitivity. The coefficient of variation was 11–16% at concentrations of 20 μg litre^{-1} (71·2 nmol and 75·0 nmol litre^{-1} for imipramine and desipramine respectively) and the recovery was 70–81%.

Several analytical methods have been developed to determine the therapeutic concentrations of the tricyclic antidepressive drugs imipramine and desipramine. Hammer & Brodie (1967) devised an *in vitro* isotope derivative technique to assay secondary amines like desipramine by acetylation with [^{3}H]acetic anhydride. A similar method for the estimation of both imipramine and desipramine using [^{14}C]methyl iodide and [^{3}H]acetic anhydride has been described by Harrys, Gaudette & others (1970). Moody, Tait & Todrick (1967) have used a spectrophotofluorometric technique for the determination of imipramine and desipramine. A more selective gas chromatographic method for desipramine has been developed by Ervik, Walle & Ehrsson (1970). There is, however, still a need for quantitative methods to estimate nanogram quantities of desipramine and especially imipramine using a relatively simple technique with a high degree of specificity.

In the present work a new, highly sensitive densitometric technique has been applied for the quantitative determination of both imipramine and desipramine. The compounds were extracted from the plasma of patients treated with therapeutic doses of imipramine, separated by thin-layer chromatography and the chromatogram was then directly evaluated by densitometry.

METHODS AND MATERIALS

Plasma samples

Seven hospitalized patients with various types of mental depression and three healthy volunteers were treated with 150 mg of imipramine (Tofranil) daily, divided into 3 doses. Blood samples were drawn at the earliest one week after the start of the treatment and 7 h after the morning dose. No other drugs were permitted except diazepam and an occasional dose of hypnotic for the night (barbiturates). Blood (10–15 ml) was drawn by venipuncture into heparinized tubes, stored at 4° and centrifuged within 2 h. The separated plasma was stored at — 25° until analysed.

Chemicals

The hydrochlorides of imipramine and desipramine were obtained from Hässle-Ciba-Geigy AB, Mölndal Sweden. The compounds were dissolved in ethanol to give a concentration of 1 g litre^{-1} (3·56 and 3·75 mmol litre^{-1} for imipramine and desipramine respectively) calculated as the free base. These standard solutions were stable for at least one month when stored in the refrigerator and protected from bright light. Working standards were prepared weekly by dilution with n-heptane. Other chemicals and organic solvents were of analytical grade.

Apparatus

A Zeiss Chromatogram Spectralphotometer (Carl Zeiss, Oberkochen, W. Germany) in combination with an integrator (Hewlett Packard, model 3370 A Hewlett Packard, Pennsylvania, USA), and a potentiometric recorder (Compensation Recorder Servogor, model Re 511 Goerz Electro GmbH., Vienna, Austria) was used for the densitometric measurements. A special design of the apparatus reduced baseline fluctuations and increased the sensitivity (Treiber, Nordberg & Lindstedt, 1971).

Extraction of plasma

To plasma (5 ml) in a glass stoppered tube was added n-heptane (5 ml) containing 3% amyl alcohol and 2·5 ml of M $NaHCO_3$. The tube was shaken for 5 min in a mechanical shaker at about 300 strokes min^{-1}, and then centrifuged at 900 *g*. Any emulsion that formed was broken up by placing the tube in a dry ice-acetone mixture followed by thawing and recentrifugation. The organic phase was transferred to another glass stoppered tube and the extraction procedure was repeated twice. The combined organic phases were shaken with 5 ml of 0·1 M NaOH for 5 min and centrifuged. The water phase was then completely removed. The organic phase was evaporated under a stream of nitrogen at 40–50° into a small volume for subsequent application to the t.l.c. plate.

Thin-layer chromatography

The glass plates (20 × 20 cm) were coated with a suspension of silica gel G (E. Merck AG, Darmstadt, W. Germany) (30 g silica gel suspended in 70 ml of water) with a Quick-fit thin layer spreader. The layer thickness was 0·25 mm. After drying the plates were activated at 110° for 45 min and stored over anhydrous calcium chloride. The silica gel layer was divided into lanes of 17 mm by vertical channels of about 1 mm of width. The samples were applied with a capillary pipette as a spot at the origin line, and the solvent was evaporated with a stream of nitrogen. The tubes were rinsed twice with 50 μl of chloroform–methanol (1:1 v/v). The chromatograms were developed at room temperature (20°) in a glass tank by the ascending technique in a solvent system of chloroform–diethyl ether–methanol (85: 15: 20 v/v) (Bonnichsen & Maehly, personal communication) and were removed from the solvent chamber 15 min after the solvent front had reached the upper edge of the plate.

Colour reaction with nitrous gases

The thin layer plates were placed in a chromatography tank together with a beaker containing 0·5–1 g of $NaNO_2$ to which 5 ml of 2 M HCl was added. The lid was put

on and the plate left standing in the nitrous gases for 30–60 min. Imipramine and desipramine gave spots of an intense yellow colour.

Densitometric determination

The densitometric measurement was performed by using transmission and re-reflexion simultaneously as described by Treiber, Nordberg & Lindstedt (1971). The t.l.c. plate was scanned with a speed of 30 mm min^{-1} at the absorption maxima of the compounds (405 nm). Reference substances were run together with the samples on each thin-layer plate in at least 3 different concentrations. In the analysis of samples from patients receiving imipramine, desipramine was also run as a reference. After integrating the peaks a standard curve was constructed.

RESULTS

A standard curve for the quantification of imipramine and desipramine is shown in Fig. 1. Fig. 2 shows a densitometric tracing in the analysis of plasma samples from a subject without treatment, a patient receiving 150 mg of imipramine daily

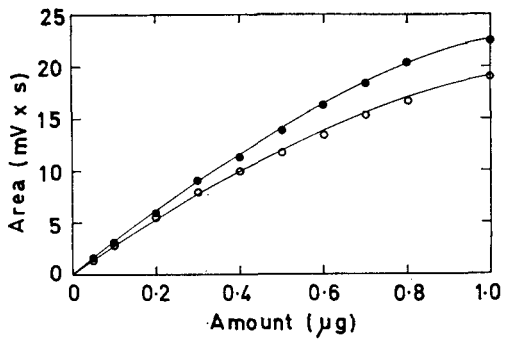

FIG. 1. Standard curve for imipramine (● – ●) and desipramine (○ – ○).

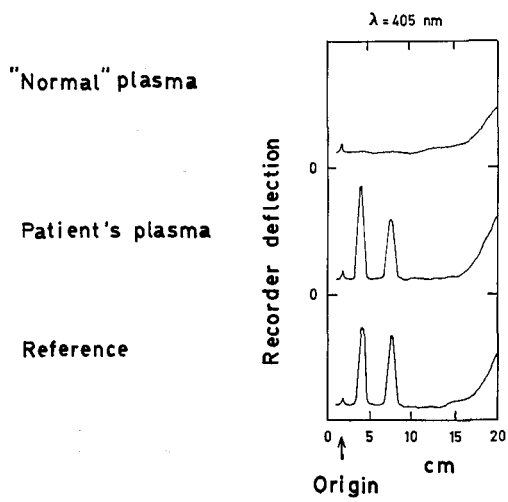

FIG. 2. Densitometry of extracts of normal plasma, patient plasma and 0·2 μg of imipramine (R_F 0·35) and desipramine (R_F 0·12) as references. The patient received 150 mg of imipramine daily and the analysis was made on 5 ml of plasma.

and references. The R_F values of imipramine and desipramine were 0·35 and 0·12 respectively in the solvent system chloroform–diethyl ether–methanol (85:15:20 v/v).

Identification

The method has been devised primarily for the determination of the plasma concentration in patients whose medication is known. Identification was based on the R_F value, characteristic absorption maxima, and the colour reaction with nitrous gases. There were no interfering compounds in the extracts of plasma from subjects not treated with psychotropic drugs (Fig. 2).

Precision of the method and recovery

The precision of the densitometric measurement, expressed as the coefficient of variation of 10 analyses of 0·1 μg of imipramine and desipramine applied as separate spots, was 10·4% for imipramine and 10·2% for desipramine. The precision of the method, evaluated by estimations of 10 plasma samples with a concentration of 20 μg litre^{-1} of both imipramine (71·2 nmol litre^{-1}) and desipramine (75·0 nmol litre^{-1}) (0·1 μg of each compound was added to 5 ml of normal plasma), was 11·4% for imipramine and 15·9% for desipramine. The recovery was 81% for imipramine and 70% for desipramine.

Sensitivity

The lower limit of the method's sensitivity was about 5 μg litre^{-1} (18 nmol litre^{-1}) for both substances, if 5 ml of plasma was used for the extraction. A larger plasma sample gave an increased sensitivity in terms of molarity.

Plasma values on treatment

As blood samples were drawn at the earliest one week after the start of the treatment, it seems evident that the steady state concentration was reached at this time. The values show large variation both in the inter-individual concentrations and in the concentration ratios of imipramine and desipramine indicating, differences in the metabolic rate between the subjects (Table 1).

Table 1. *Plasma levels in 10 patients treated with 150 mg of imipramine daily.* The daily dose was divided into 3 doses and samples were drawn 7 h after the morning administration of the drugs.

Patient	Sex	Age (years)	Weight (kg)	Plasma level Imipramine		Desipramine		Ratio of molarity D/I
				(nmol litre^{-1})	(μg litre^{-1})	(nmol litre^{-1})	(μg litre^{-1})	
I	M	46	73	224	63	277	74	1·2
II	M	53	88	235	66	405	108	1·7
III	M	39	75	256	72	506	135	1·9
IV	M	55	91	28	8	232	62	8·2
V	M	55	72	221	62	60	16	0·3
VI	M	52	77	374	105	176	47	0·5
VII*	F	26	59	160	45	2126	567	13·3
VIII*	M	29	75	164	46	469	125	2·8
IX*	F	26	63	39	11	585	156	15·0
X	M	49	67	167	47	120	32	0·7

* = healthy volunteers

DISCUSSION

The analytical methods now available for the determination of imipramine and its metabolites satisfy the demands for specificity and sensitivity to a varying degree. The isotope derivative techniques developed for desipramine (Hammer & Brodie, 1967) and for imipramine (Harrys & others, 1970) show a satisfactory degree of sensitivity. These techniques are, however, not easy to perform and any primary and secondary amine interferes with the method. Although useful for studies under rigorously controlled conditions they are time-consuming and not well suited for serial determinations in psychiatric praxis. The spectrophotofluorometric method developed by Moody & others (1967) has similar disadvantages. The gas chromatographic method for the estimation of imipramine and its metabolites described by Weder & Bickel (1968) lacks the sensitivity necessary for the measurement of therapeutic plasma levels. Ervik, Walle & Ehrsson (1970) could estimate nanogram quantities of desipramine but not the parent drug imipramine.

With the present procedure both imipramine and desipramine could be estimated with a relatively simple thin-layer chromatographic technique. Nitrous gases were used for the colouring as spraying of the plates with reagents destroyed the silica gel surface making the subsequent densitometric tracing impossible. The new design of the densitometer using reflectance and transmittance simultaneously (Treiber, Nordberg & Lindstedt, 1971) increased the sensitivity 5–10 times compared with earlier models with only reflectance or transmittance. It is possible to detect a spot containing 10–15 ng of imipramine or desipramine and a spot containing about 25 ng of these compounds gives a peak which can be measured with precision.

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