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THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF IMIPRAMINE AND DESIPRAMINE IN HUMAN PLASMA AND URINE AT SINGLE—DOSE LEVELS

N. SISTOVARIS*, E.E. DAGROSA and A. KELLER

Hoechst AG, Postfach 80 03 20, D-6230 Frankfurt 80 (F.R.G.)

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SUMMARY

Thin-layer chromatographic methods were up-dated for pharmacokinetic studies of imipramine in plasma and urine. The free parent compound and its free desmethyl metabolite desipramine are determined in plasma. Conjugates of both compounds in urine are cleaved on treatment with glucuronidase/arylsulfatase. Following chromatography, intense yellow derivatives are obtained overnight on standing or by exposure to nitrous gases. Detection is performed in the visible range at 405 nm (plasma) or 460 nm (urine).

The methods are selective, accurate and sensitive, with detection limits for plasma of 2 ng/ml imipramine—HCl and 2 ng/ml desipramine—HCl, and 0.06 μg/ml total imipramine—HCl and 0.126 μg/ml total desipramine—HCl for urine. Pharmacokinetic data from plasma and urine results following single oral doses of 50 mg imipramine—HCl to eight volunteers were computed using one-compartment open models.

INTRODUCTION

Imipramine often serves as a standard reference substance in the research and development of new antidepressive drugs. Pharmacokinetic and metabolism data, predominantly in repeated-dose studies, have been reported in the literature [1—5]. By a first-pass effect, the potent desmethyl metabolite desipramine (Fig. 1) is formed to a larger extent following oral administration than after an intravenous dose [5—7]. The increased half-life leads to a higher degree of multiple dose cumulation as compared to imipramine.

In urine, approximately 70% of an oral dose is excreted [1—3], mostly as conjugated 2-hydroxy metabolites. Urinary levels of the free compounds imipramine and desipramine are in the range of the usual detection limits. On the other side, conjugates may be cleaved to allow for determination of total urinary imipramine and desipramine.

Specific single-dose pharmacokinetics could only be determined using gas

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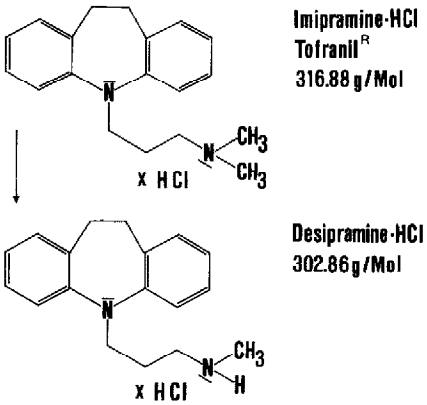

Fig. 1. Structures of imipramine—HCl and desipramine—HCl.

chromatography—mass spectrometry [8]. Using high-performance liquid chromatography (HPLC), Godbillon and Gauron [9] calculated detection limits of 5 ng/ml plasma for imipramine and 10 ng/ml plasma for desipramine, which were not sufficient to calculate single-dose pharmacokinetics. Also using HPLC, Reece et al. [10] reported detection limits of approximately 1 ng/ml for both compounds. Following a single oral dose of 50 mg of drug to one volunteer, plasma imipramine could be measured up to 28 h post administration, whereas only the peak level of 10 ng/ml desipramine (3.5 h post administration) could be quantified precisely (C.V. < 15%).

Using 5-ml plasma aliquots for thin-layer chromatography (TLC) [5], detection limits of 5 ng/ml for both compounds had been achieved. Up-dating the TLC methods improved the detection limits of imipramine and desipramine, allowing the determination of single-dose levels of both compounds in 1-ml samples of plasma and urine.

EXPERIMENTAL

Reagents

The reagents used were carbonate buffer, pH 10.9 (1 mol/l) AR, ethyl acetate AR, chloroform AR, methanol AR, concentrated ammonia solution (25%) AR, β-glucuronidase/arylsulfatase AR (Boehringer Mannheim, No. 15427) and acetate buffer, pH 5.5 (0.2 mol/l) AR. The solvent system was methanol—chloroform—concentrated ammonia (10:1:0.1).

Equipment

A Zeiss KM3 chromatogram spectrophotometer with microoptics and a Servogor® 210 (Metrawatt) recorder were used. Separation was performed on silica gel HPTLC plates without fluorescent indicator (No. 5641, E. Merck, Darmstadt, F.R.G.) in a Camag twin-trough HPTLC chamber 20 cm × 10 cm (No. 25254). For sample clean-up and spotting, a Vortex® mixer, a centrifuge, glass-stoppered tubes (ca. 8 ml), conical glass-stoppered tubes (ca. 8 ml) and a Desaga Autospotter® were used.

Sample preparation

Plasma. In a glass-stoppered tube, 1 ml of plasma was treated with 1 ml of carbonate buffer. The plasma was extracted with 5 ml of ethyl acetate for 30 sec on a Vortex mixer. The phases were separated by centrifugation (5 min), and 4 ml of the organic phase were transferred into a conical tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in 100 μl of chloroform. Using the Desaga Autospotter*, 75 μl were transferred onto the HPTLC plate as a series of consecutive droplets of approximately 100 nl each. Since each of these drops evaporated before the next one was applied, narrow spots were obtained which were suitable for HPTLC.

Urine. In a glass-stoppered tube, 1 ml of urine was treated with 1 ml of acetate bufer and 20 μl of glucuronidase/arylsulfatase for 24 h at 37°C. The subsequent procedure was the same as described for plasma.

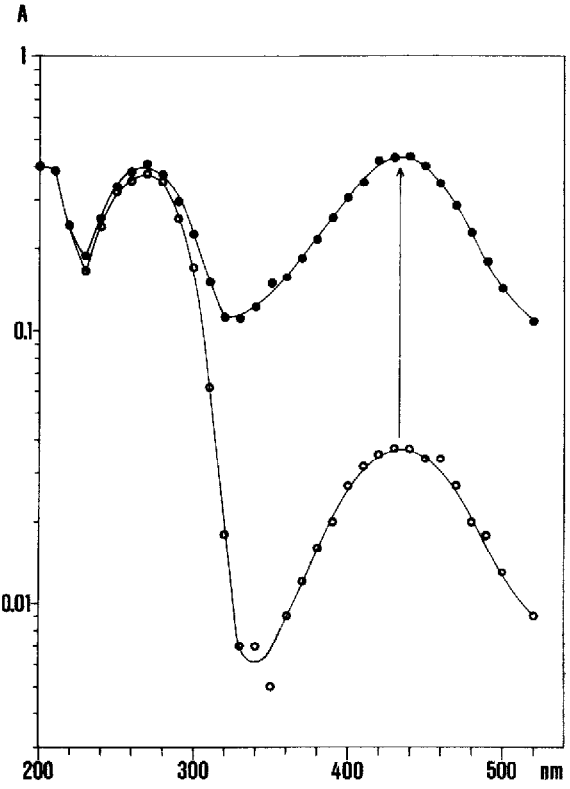

Fig. 2. In situ UV-VIS spectra of imipramine—HCl on a HPTLC plate, 0.7 μg/spot. (○), after chromatography; (●), overnight.

Chromatography

The twin-trough HPTLC developing chamber contained 11 ml of solvent in one compartment. After a 30-min preconditioning period, the plate was developed in the dark over a distance of 5 cm. Intense yellow derivatives (Fig. 2)

*Modified version: Tygon® tubes of larger diameter [Technicon, flow-rated, code 116-0549-09 (white)] and 60 cm long Hostaflon® tubes were used.

are formed on autoxidation overnight or, alternatively, by treatment with nitrous gases [5]. R_F values were imipramine 0.45 and desipramine 0.15.

Measurements were carried out in the direction of the solvent flow with an effective slit (microoptics) of 4.5 mm × 0.15 mm at a wavelength of 405 nm, in the case of plasma, scanning speed 50 mm/min and paper speed 240 mm/min. In the case of urine, measurements were performed at a wavelength of 460 nm in order to improve selectivity*. Peak heights (Figs. 3 and 4) were evaluated and quantified by means of a linear calibration graph based on parallel analysis of standards on the same plate.

RESULTS

Plasma

The compounds were admixed with blank plasma in five concentrations over the range 2—50 ng/ml plasma. Each admixture was split into six portions of 1 ml, so that six equal series were formed. Each series was then analyzed in turn, so that a total of six independent analytical results were available for each concentration.

TABLE I

IMIPRAMINE AND DESIPRAMINE DETERMINATION IN PLASMA BY TLC (RECOVERY, PRECISION AND ACCURACY)

n = 6 determinations, concentrations in ng/ml.

Imipramine—HCl			Desipramine—HCl		
Added	Found (mean ± S.D.)	Accuracy (ng/ml)	Added	Found (mean ± S.D.)	Accuracy (ng/ml)
50.0	50.0 ± 0.0	±0.0	50.0	49.8 ± 0.4	+0.2
20.0	19.7 ± 2.6	+0.3	20.0	20.5 ± 1.4	—0.5
10.0	9.8 ± 1.3	+0.2	10.0	9.8 ± 1.2	+0.2
5.0	5.2 ± 1.0	—0.2	5.0	4.7 ± 0.5	+0.3
2.0	2.0 ± 0.0	±0.0	2.0	1.7 ± 0.5	+0.3
Blank	0		Blank	0	

Quality criteria of an analytical method are selectivity, accuracy, precision and sensitivity [11, 12]. The corresponding parameters were derived from the analytical results given in Table I. As regards selectivity, the assay is free from interference for all substances (Fig. 3). Accuracy was considered to be the deviation (bias) of the mean value of the results from the amount added. For each substance, the average accuracy was approximately 0.1 ng/ml plasma. Regression coefficients were greater than 0.998.

Intra-assay precision was defined in terms of the standard deviation (S.D.), which was constant in the concentration range considered. The detection limit (D.L.) was taken as precision × 2: for imipramine—HCl, precision = 0.8 ±

*Coextracted matter from the enzyme mixture may cause minor interferences at λ = 405 nm, as we experienced.

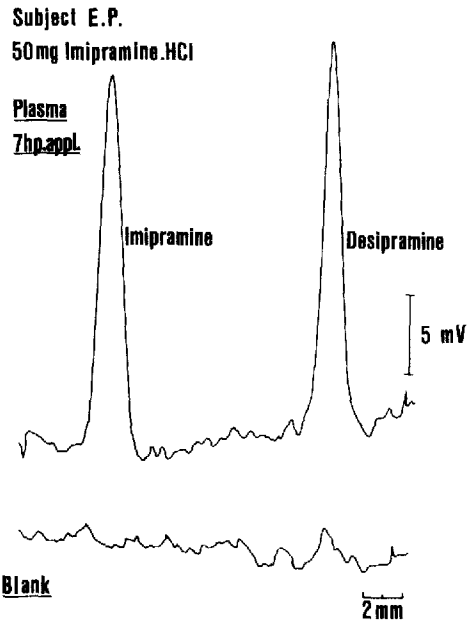

Fig. 3. Determination of imipramine—HCl (22 ng/ml) and desipramine—HCl (13 ng/ml) in plasma, compared to a blank.

1.0 ng/ml, D.L. = 2 ng/ml plasma; for desipramine—HCl, precision = 0.7 ± 0.5 ng/ml, D.L. = 2 ng/ml plasma.

The inter-assay precision was tested in two spiked control plasmas. From values obtained in May 1981, the following degrees of inter-assay precision were determined (ng/ml, mean ± S.D.): for control 1, imipramine—HCl = 21 ± 2, desipramine—HCl = 22 ± 3; for control 2, imipramine—HCl = 5 ± 1, desipramine—HCl = 5 ± 2.

Urine

The compounds were admixed to blank urine in five concentrations over the range 0.2—5 μg/ml urine. Each admixture was split into six portions of 1 ml, so that six equal series were formed. Each series was then analyzed in turn so that a total of six independent analytical results were available for each concentration. Quality criteria of the urine assay were defined by the corresponding parameters abstracted from the analytical results given in Table II. As regards selectivity, the assay is free from interference for both substances (Fig. 4).

For both imipramine and desipramine, the average accuracy was <0.03 μg/ml urine. Regression coefficients were greater than 0.998. The means of concentrations measured were linearly correlated to the S.D. values and in this way the intra-assay precision was defined.

The detection limit (D.L.) was taken as the intercept × 2: for imipramine—HCL, precision = 1.7% of the result + 0.03 μg/ml, D.L. = 0.06 μg/ml urine; for desipramine—HCl, precision = 1.2% of the result + 0.08 μg/ml, D.L. = 0.16 μg/ml urine.

The inter-assay precision was tested in two spiked control urines. From

TABLE II

IMIPRAMINE AND DESIPRAMINE DETERMINATION IN URINE BY TLC (RECOVERY, PRECISION AND ACCURACY)

n = 6 determinations, concentrations in μg/ml.

Imipramine—HCl			Desipramine—HCl		
Added	Found (mean ± S.D.)	Accuracy (μg/ml)	Added	Found (mean ± S.D.)	Accuracy (μg/ml)
5.00	5.00 ± 0.22	±0.00	5.00	5.10 ± 0.13	—0.10
2.00	2.00 ± 0.06	±0.00	2.00	2.00 ± 0.10	±0.00
1.00	0.98 ± 0.04	+0.02	1.00	1.03 ± 0.14	—0.03
0.50	0.53 ± 0.05	—0.03	0.50	0.50 ± 0.18	±0.00
0.20	0.20 ± 0.02	±0.00	0.20	0.22 ± 0.04	—0.02
Blank	0		Blank	0	

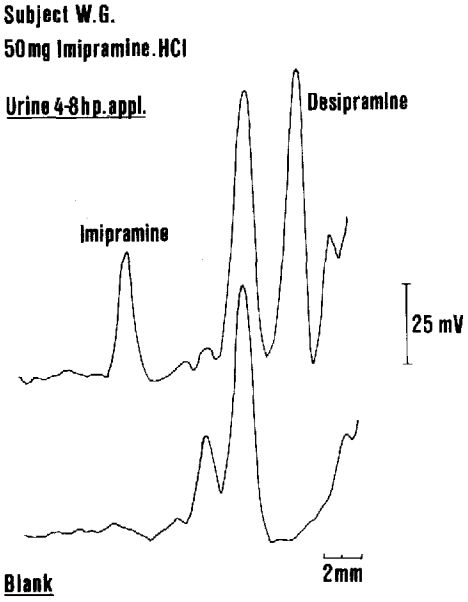

Fig. 4. Determination of total imipramine—HCl (0.7 μg/ml) and total desipramine—HCl (1.8 μg/ml) in urine, compared to a blank.

values obtained in May 1981, the following degrees of inter-assay precision were determined (μg/ml, mean ± S.D.): for control 1, imipramine—HCl = 2.0 ± 0.08, desipramine—HCl = 2.0 ± 0.12; for control 2, imipramine—HCl = 0.49 ± 0.08, desipramine—HCl = 0.51 ± 0.25.

Pharmacokinetics

Imipramine—HCl was administered in a single oral dose of 50 mg of Tofranil® to eight healthy male volunteers (age 21—42 years, height 172—190 cm, weight 63—83 kg). Blood was sampled up to 24 h and urine collected up to 48 h post administraton.

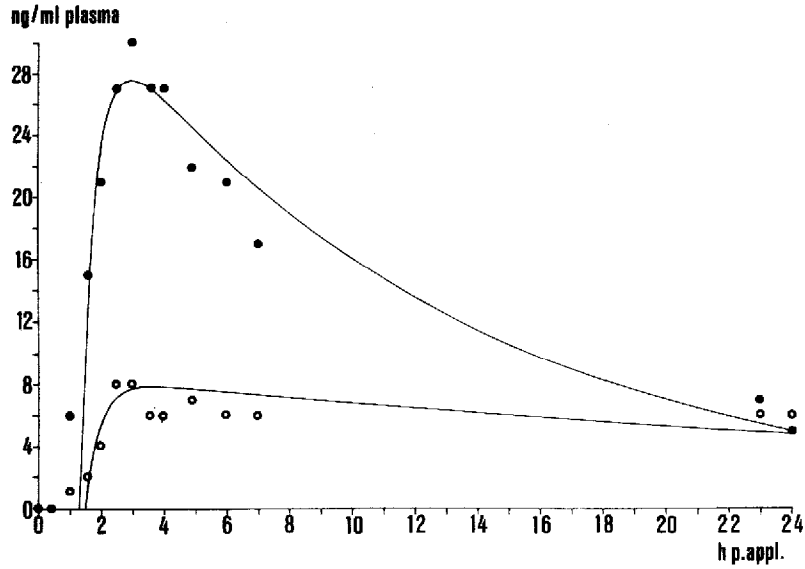

Fig. 5. Plasma pharmacokinetics from mean plasma levels of imipramine—HCl and desipramine—HCl after a single oral dose of 50 mg of imipramine—HCl to eight volunteers. (•), imipramine—HCl; (○), desipramine—HCl.

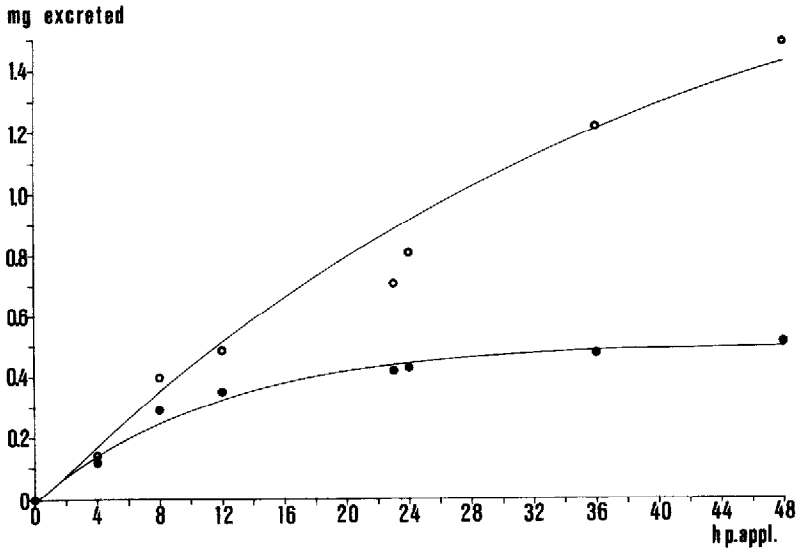

Fig. 6. Mean cumulative renal excretions of total imipramine—HCl and total desipramine—HCl after a single oral dose of 50 mg of imipramine—HCl to eight volunteers. (•) total imipramine—HCl; (○), total desipramine—HCl.

Using TLC, plasma levels were found in the ranges 0—80 ng/ml imipramine—HCl and 0—15 ng/ml desipramine—HCl, and urine levels were found in the ranges 0—1.0 μg/ml total imipramine—HCl and 0—3.0 μg/ml total desipramine—HCl.

Pharmacokinetic profiles were calculated based on one-compartment open

models. This is demonstrated by the plasma kinetics from the mean plasma levels in Fig. 5 and by the mean cumulative excretions shown in Fig. 6.

After a lag time of 1.3 ± 0.5 h, imipramine was absorbed with a half-life of 0.5 ± 0.3 h. Maximum plasma levels of 28 ± 17 ng/ml imipramine—HCl (range 10–83 ng/ml) were reached 3.4 ± 0.1 h post administration. The drug was eliminated with a half-life of 8.4 ± 1.1 h.

Desipramine plasma levels rose after a lag time of 1.5 ± 0.5 h with a half-life of 0.6 ± 0.3 h. Maximum levels of 8 ± 3 ng/ml desipramine—HCl (range 4—14 ng/ml) were reached 4.8 ± 1.5 h post administration. The metabolite was eliminated with a half-life of approximately 40 h.

Areas under the curves were: for imipramine—HCl 330 ± 184 ng h/ml (range 146—744 ng h/ml), for desipramine—HCl 123 ± 57 ng h/ml (range 58—208 ng h/ml). From these data, a steady-state level of approximately 60 ng/ml of both compounds may be expected for the oral doses of 3 × 50 mg imipramine—HCl per day. Renal clearance of both substances was calculated from data up to 24 h post administration as 24 ± 14 ml/min in the case of imipramine and 131 ± 63 ml/min in the case of desipramine.

From the mean cumulative excretions (Fig. 6), urinary half-lives were determined to be 8 h in the case of imipramine and 28 h in the case of desipramine. In Table III, the means of cumulative renal excretion 24 and 48 h post administration and the extrapolated values are given.

TABLE III

CUMULATIVE RENAL EXCRETION OF TOTAL IMIPRAMINE AND TOTAL DESIPRAMINE FOLLOWING A SINGLE ORAL DOSE OF 50 mg OF TOFRANIL® TO EIGHT VOLUNTEERS

	Total imipramine—HCl		Total desipramine—HCl		Sum of percentages of dose (mean ± S.D.)
	Amount excreted (mg, mean ± S.D.)	Percentage of dose (mean ± S.D.)	Amount excreted (mg, mean ± S.D.)	Percentage of dose (mean ± S.D.)	
24 h	0.4 ± 0.2	0.9 ± 0.4	0.8 ± 0.2	1.8 ± 0.4	2.6 ± 0.7
(range)	(0.2—0.9)		(0.6—1.2)		
48 h	0.5 ± 0.4	1.0 ± 0.8	1.5 ± 0.6	3.4 ± 1.4	4 ± 2
(range)	(0.2—1.4)		(0.8—2.7)		
Extrapolated values	0.5 ± 0.4	1.1 ± 0.8	2.0 ± 1.2	4 ± 3	5 ± 3
(range)	(0.2—1.5)		(0.8—4.2)		

DISCUSSION

The results were in accordance with values published previously. Following a single oral dose of 50 mg of imipramine—HCl to eight volunteers, peak levels of 28 ± 17 ng/ml plasma agreed with the results of Godbillon and Gauron [9] (18 ± 6 ng/ml). The drug plasma half-life of 8 h was in the range given by the same authors (approx. 6 h).

From single-dose data, the estimated steady-state plasma levels of approximately 60 ng/ml and approximately 60 ng/ml for drug and metabolite,

respectively, were in the ranges observed by Nagy and Treiber [5] (53 ± 30 ng/ml imipramine—HCl and 84 ± 49 ng/ml desipramine—HCl). Within 24 h post application, the cumulative renal excretion of 0.2—0.9 mg total imipramine—HCl and 0.6—1.2 mg total desipramine—HCl were in the ranges expected when considering those established by Crammer et al. [3] (0.6—0.7 mg total imipramine—HCl and 0.6—1.4 mg total desipramine—HCl).

Imipramine assay in plasma and urine was carried out to demonstrate quantitative TLC as a practicable, selective and sensitive analytical tool in drug/metabolite research. This technique may be used in most cases where low drug levels have to be determined. Since automatization of TLC [13] has become possible, even large series of samples can be processed with reasonable work and cost factors.

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