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Simultaneous determination of imipramine, desipramine and their 2- and 10-hydroxylated metabolites in human plasma and urine by high-performance liquid chromatography

A.G. Chen[a], Y.K. Wing[b], H. Chiu[b], S. Lee[b], C.N. Chen[b], K. Chan[a,*]

[a]*School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK*
[b]*Department of Psychiatry, Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, Hong Kong*

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Abstract

A simultaneous assay for imipramine, desipramine and their 2- and 10-hydroxy-metabolites using high-performance liquid chromatography (HPLC) is described. The drugs and internal standard, pericyazine, were extracted from plasma or urine at pH 9.6 with diethyl ether and back-extracted into 0.1 *M* orthophosphoric acid. The recovery of the compounds ranged from 78.6% for imipramine to 94.3% for 2-hydroxydesipramine. The extracts were analysed by reversed-phase HPLC with electrochemical detection using a mobile phase of 30% acetonitrile in 0.1 *M* K_2HPO_4 at pH 6.0 delivered at 2 ml/min. All compounds were resolved in a run time of 15 min with lower limits of quantification of 1.5 ng/ml for hydroxy-metabolites and 3 ng/ml for imipramine and desipramine. The intra- and inter-day coefficients of variation at 50 ng/ml were 5.2% and 6.8%, respectively ($n=8$).

Keywords: Imipramine; Desipramine; Hydroxyimipramine; Hydroxydesipramine

1. Introduction

Tricyclic antidepressants have been the mainstay of treatment for major depression for over 30 years and imipramine remains one of the most widely used drugs of this class. Various methods for the measurement of imipramine have been described since 1960, usually involving extraction of the drug from biological fluids. The extract or concentrate has been analysed by spectrofluorimetry, isotope-derivative dilution analysis, gas-liquid chromatography (GLC), radioimmunoassay, or high-performance liquid chromatography (HPLC) [1]. In recent years, HPLC has proven to be a useful method for the determination of imipramine in biological fluids.

Until recently, few methods have been reported for the simultaneous determination of imipramine, desipramine and their 2-hydroxy-metabolites, 2-OH-imipramine and 2-OH-desipramine. The methods involved HPLC with either fluorescence [2], amperometric [3], ultraviolet (UV) [4], coulometric [5] or electrochemical detection [6]. None of the methods were sufficiently selective or sensitive to also analyse the 10-hydroxy-metabolites, 10-OH-imipramine and 10-OH-desipramine. We report the simultaneous analysis of these six compounds in plasma and urine samples using reverse phase HPLC with electrochemical detection.

*Corresponding author.

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2. Experimental

2.1. Apparatus

The HPLC system included a Perkin Elmer LC 250 pump and an EDT Chromajet electrochemical detector. The settings for the electrochemical unit were as follows: oxidation cell; +1.00 V; 1 μA sensitivity and 1 s time constant. A PE Nelson 900 series interface linked the detector to a CompuAdd PC 433DLC computer with Turbochrom 3 Quick Tutorial software which was used to control the acquisition, analysis, reporting and plotting of sample data. Analyses were performed on a reversed-phase C_{18} column (Phenomenex Bondclone 10 C_{18}, 300×3.90 mm) linked to a pre-column (RP-18, 10 μm, 40×4.6 mm). Injection was via a Rheodyne 100 μl injector.

Other apparatus included 15 ml capacity centrifuge test tubes with fitting screw caps (Pyrex, Corning, NY, USA).

All glassware was soaked overnight in 3% solution of Extran (E. Merck, Darmstadt, Germany) in water, then rinsed thoroughly with methanol and hot tap water followed by distilled water. The tubes were subsequently silanised by rinsing with a 3% solution of hexamethyldisilazane (HDMS) in distilled chloroform. This treatment of glassware was used to minimise loss of drugs due to adsorption on to the glass walls [7].

2.2. Materials

Imipramine, desipramine, 2-hydroxyimipramine, 2-hydroxydesipramine, 10-hydroxyimipramine, 10-hydroxydesipramine and pericyazine were gifts from Ciba Geigy (Basel, Switzerland). Glass-distilled water was used. Methanol and acetonitrile of HPLC grade and diethyl ether and orthophosphoric acid of Analar grade were from BDH (Poole, UK).

2.3. Standard solutions

Stock solutions (1 mg/ml) of imipramine, desipramine, 2-OH-imipramine, 2-OH-desipramine, 10-OH-imipramine, 10-OH-desipramine and pericyazine (internal standard) were prepared by dissolving accurately weighed quantities of the drugs separately in methanol. The stock solutions remained stable for more than two months when stored at −20°C.

Daily working standards were prepared by dilution of the stock solutions with methanol.

2.4. Chromatographic conditions

The column was operated at ambient temperature (25±1°C) in an air-conditioned room. The mobile phase consisted of 30% acetonitrile in 0.1 *M* K_2HPO_4 buffer at a flow-rate of 2 ml/min. Using concentrated orthophosphoric acid, the pH of the resulting mixture was adjusted to 6.0. The mobile phase was then filtered and degassed before use, using a vacuum filter system equipped with 0.45 μm filter membrane (Phenomenex, Torrance, CA, USA).

The concentrations of imipramine and its five metabolites were calculated using peak height ratios of internal standard and sample peaks.

2.5. Biological samples

With approval from the Medical Ethical Committee of the Prince of Wales Hospital in Hong Kong, five Chinese depressed patients took orally 25 to 200 mg imipramine after overnight fasting. Blood samples were taken 0, 2, 4, 8, 24, 36, 48 and 72 h after administration of the drug. Plasma was separated and stored at −20°C before assay. Bulked urine was collected up to 72 h after ingestion of drug.

2.6. Sample preparation

Aliquots of plasma or urine (1 ml) were pippetted into 15 ml test tubes to which 20 μl of pericyazine in methanol (200 ng) was added as the internal standard. After adding 0.2 ml of 1 *M* sodium carbonate buffer to adjust pH to 9.6, the compounds were extracted by shaking with 10 ml distilled diethyl ether for 10 min using an automatic shaker (Heto, ROTAMIX, RK 20-1) and then centrifuged for 10 min at 1000 *g* using a refrigerated centrifuge (Heraeus instruments, Labufuge 400R). The upper organic layer was qualitatively transferred to another 15 ml test tube by aspiration and 100 μl of 0.1 *M* orthophosphoric acid was added. The solution was shaken for 10 min and centrifuged for 10 min at

1000 *g*. The top layer was discarded and 50 μl of the acid layer was injected on to the HPLC system.

2.7. Quantitation and recovery

Blank plasma or urine were spiked with standard solutions of imipramine, desipramine, 2-OH-imipramine, 2-OH-desipramine, 10-OH-imipramine and 10-OH-desipramine to give concentrations of 15.63, 31.25, 62.5, 125, 250 and 500 ng/ml for imipramine and desipramine, and 7.82, 15.63, 31.25, 62.5, 125 and 250 ng/ml for 2 and 10-hydroxylated metabolites. The samples were then extracted and analysed by HPLC as described in Section 2.4. Calibration curves were constructed by plotting peak height ratios of each drug to that of internal standard against the respective concentrations. Intra- and inter-day variation was assessed at 50 ng/ml. The calibration curve was determined five times during the course of the study.

Absolute recoveries were estimated by comparing the peak heights obtained from spiked samples after extraction with concentrates without the extraction steps of six compounds at the concentration of 125 ng/ml.

3. Results and discussion

3.1. Performance of the HPLC system

The analytical peaks of imipramine, desipramine, 2-OH-imipramine, 2-OH-desipramine, 10-OH-imipramine, 10-OH-desipramine and pericyazine were well resolved with good symmetry (Figs. 1 and 2). The respective retention times were 2.3 min, 2.7 min, 3.2 min, 3.8 min, 6.2 min, 8.7 min and 12.3 min. No endogenous interfering peaks from plasma or urine extracts were observed.

Although acid solution was injected on to the column, the method described in this report has been used for two years in a research setting. Several thousand injections have been made on a single column and it appears no deteriorate. The use of a guard column greatly extends the analytical column life.

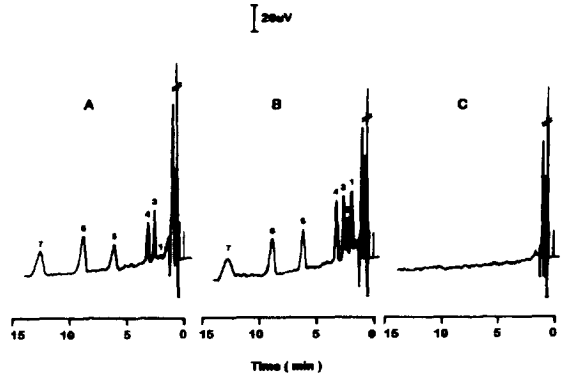

Fig. 1. Chromatograms from an extract of plasma from a depressed patient 2 h after oral dosing of 200 mg imipramine (A); peaks: 1=10-OH-desipramine, 2=10-OH-imipramine, 3=2-OH-desipramine, 4=2-OH-imipramine, 5=pericyazine, 6=desipramine and 7=imipramine; and drug loaded plasma extract after spiking 62.5 ng/ml each of 1, 2, 3 and 4, 200 ng/ml each of 6 and 7 (B); and drug-free plasma extract (C).

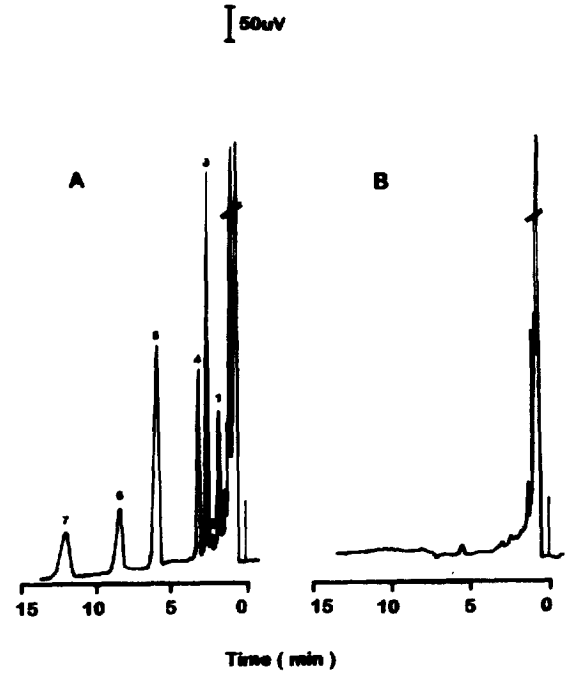

Fig. 2. Chromatograms from an extract of bulked urine from a depressed patient 24 h after oral dosing of 200 mg imipramine (A); peaks: 1=10-OH-desipramine, 2=10-OH-imipramine, 3=2-OH-desipramine, 4=2-OH-imipramine, 5=pericyazine, 6=desipramine and 7=imipramine; and an extract of drug-free urine from the same patient (B).

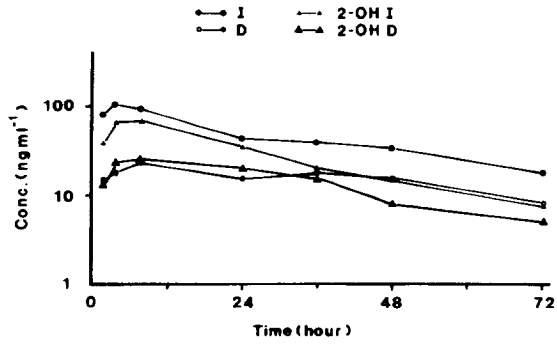

Fig. 3. Plasma concentration–time profiles of imipramine, desipramine and their 2-hydroxylated metabolites obtained from a depressed patient after a single oral dose of imipramine (75 mg).

3.2. Clinical application

Fig. 3 shows plasma concentration–time profiles of imipramine, desipramine, 2-OH-imipramine and 2-OH-desipramine obtained from a depressed patient after an oral dose of 75 mg imipramine. Plasma concentration–time profiles for 10-OH-imipramine and 10-OH-desipramine could not be plotted because the plasma concentrations observed were close to or less than the lower limits of determination of the current method.

3.3. Linearity, sensitivity, precision and recovery

Calibration curves for imipramine and its five metabolites in plasma or urine were linear over the ranges 15.63 to 500 ng/ml for imipramine and desipramine, 7.82 to 250 ng/ml for 2 and 10-hydroxylated metabolites. The linear equations and squared linear regression coefficients for six compounds ($n=5$) were $y=0.00162x+0.00826$ and $r^2=0.997$ for imipramine, $y=0.0022x-0.0068$ and $r^2=0.990$ for desipramine, $y=0.00705x-0.025$ and $r^2=0.999$ for 2-OH-imipramine, $y=0.0102x+0.0601$ and $r^2=0.995$ for 2-OH-desipramine, $y=0.00709x+0.0197$ and $r^2=0.999$ for 10-OH-imipramine and $y=0.00538x+0.0119$ and $r^2=0.997$ for 10-OH-desipramine, respectively. The between-day coefficients of variation for analysis of the six compounds at 50 ng/ml were 6.2% for imipramine, 6.8% for desipramine, 6.4% for 2-OH-imipramine, 5.2% for 2-OH-desipramine, 5.9% for 10-OH-imipramine and 5.7% for 10-OH-desipramine and were considered satisfactory which indicated that the assay was reliable and reproducible.

The limit of detection, based on a signal-to-noise ratio of 5:1, were 3 ng/ml for imipramine and desipramine and 1.5 ng/ml for hydroxy-metabolites.

The recovery of compounds from plasma samples after extraction is high and constant for all compounds (Table 1). No apparent difference was noted in the recovery of drugs from plasma or urine samples. It is interesting to note that since the pK_a of desipramine is 10.2 compared to 9.5 for imipramine, the hydrophillic metabolites appear to have a higher percentage of recovery compared to the parent drug.

3.4. Pharmacokinetics of imipramine in patients

We assume that bioavailability for imipramine is 100% [8]. Plasma concentration data were analysed by a non-compartmental method based on statistical moment theory [9]. The analysis was carried out using a microcomputer program PKCALC which was developed for use in pharmaceutical industry [10].

Table 2 shows pharmacokinetic parameters of imipramine in five depressed patients who took single oral doses of the drug. In this small group of patients, the elimination half-life ($t_{1/2}$) of imipramine ranged from 27.2 to 111.7 h. All of their $t_{1/2}$ are higher than the normal expected range which is 11–25 h. Imipramine was absorbed readily after oral administration and peak plasma concentrations were observed between 4 to 5 h in these patients. The apparent volume of distribution of imipramine ranged from 25.2 to 38 l/kg, and is in agreement

Table 1
Percentage recovery of imipramine and its 2 and 10-hydroxylated metabolites from plasma (125 ng/ml)

Compound	n	Recovery (%)	Standard deviation
Imipramine	3	78.6	4.6
Desipramine	3	86.5	5.5
2-Hydroxyimipramine	3	80.2	2.9
2-Hydroxydesipramine	3	94.3	3.7
10-Hydroxyimipramine	3	88.1	3.8
10-Hydroxydesipramine	3	93.0	5.8

Table 2
Kinetic parameters of imipramine after a single oral dose

Parameter	CKL[a] 20 years, male 72 kg	HPK 33 years, female 61 kg	LWK 49 years, female 41 kg	MFK 56 years, female 76 kg	WK 36 years, female 52 kg
Dose (mg)	200	125	75	75	25
Cl (l/h/kg)	0.83	0.58	0.59	0.54	0.55
V_{ss} (l/kg)	25.2	37.1	28.6	38.0	35.9
Elimination $t_{1/2}$ (h)	27.2	62.3	37.2	111.7	50.6
MRT (h)	30.7	64.2	49.5	159.1	66.1

[a] Age.

with the literature value. The clearance of imipramine, ranging from 0.54 to 0.83 l/h per kg, is lower than the literature value [11,12]. Studies involving a larger number of patients or healthy volunteers are being carried out to substantiate this observation.

3.5. Urinary recovery

Table 3 shows urinary recovery of imipramine and metabolites in five patients over 72 h after different doses of imipramine. Two of them only gave 24 h samples. The amounts of the drugs found in the urine varied among individuals. The renal recovery of 2-OH-desipramine is higher than the parent compound and its other metabolites. Only small amounts of unchanged imipramine and the major metabolite, desipramine, were excreted in the urine. The total dose recovery was well below 100% in the period of collection of urine.

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Table 3
Urinary recovery (% dose) of imipramine and metabolites in five patients over 72 h after a single oral dose

Parameter	CKL[a] 20 years, male	HPK[b] 33 years, female	LWK 49 years, female	MFK[b] 56 years, female	WK 36 years, female
Dose (mg)	200	125	75	75	25
Imipramine	0.85	0.03	0.64	1.90	0.72
Desipramine	5.61	0.39	7.60	3.04	6.44
2-Hydroxyimipramine	0.97	2.14	2.90	0.92	1.24
2 Hydroxydesipramine	18.04	7.26	12.41	6.41	19.38
10-Hydroxyimipramine	0.03	0.03	0.04	0.01	0.16
10-Hydroxydesipramine	0.79	0.12	0.25	0.13	0.86
Total recovery	26.29	10.24	23.84	12.41	28.80

[a] Age.
[b] These patients only gave 24 h samples.

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