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Determination of imipramine, desipramine and their hydroxy metabolites by reversed-phase chromatography with ultraviolet and coulometric detection

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method is described which analyzes imipramine, desipramine and their corresponding 2-hydroxy metabolites with sequential ultraviolet and coulometric detection from a single common extraction step, so that a wider dynamic range of plasma concentrations can be measured requiring smaller sample volumes. Applicability is broader including single-dose pharmacokinetic studies as well as steady-state concentrations. The extraction procedure gives excellent recoveries for imipramine, desipramine and their metabolites (mean ± S.D.): ultraviolet detection, imipramine 99.5 ± 0.68%, desipramine 100 ± 0.0%, 2-hydroxyimipramine 97.8 ± 3.5% and 2-hydroxydesipramine 93.1 ± 4.22%; coulometric detection, imipramine 97.5 ± 1.9%, desipramine 98.3 ± 1.2%, 2-hydroxyimipramine 90.3 ± 4.0% and 2-hydroxydesipramine 86.6 ± 7.5%.

INTRODUCTION

Various scientific reports have discussed the significance of imipramine and its metabolites in the treatment of depression [1–4]. Previous methods analyzing imipramine were primarily developed for the parent compound and the demethylated metabolite with only a cursory interest in the analyses of the 2-hydroxy metabolites [5–7]. However, the 2-hydroxy metabolites of imipramine have been shown to have neurochemical properties similar to the corresponding parent tricyclics, with other effects being seen both in the hepatic and cardiovascular systems [8–17].

Imipramine has been analyzed by gas chromatography with mass spectrometry (GC–MS) in the selected ion mode for single-dose kinetic studies [18,19]. The GC–MS technique is, however, laborious and time-consuming because many intermediate steps are necessary before the final measurements. The initial high-performance liquid chromatographic (HPLC) methods were also cumbersome and required significant sample volumes to insure analytical sensitivity. However, the newer HPLC procedures have reduced the number of sample-processing steps, but the problems of sample size have remained [20–22]. The limit of quanti-

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tation for the previous HPLC and GC–MS imipramine assays has ranged between 2 and 5 ng/ml with the different techniques reported.

The present method has been designed to be rapid, sensitive and requires much smaller sample volumes. The extraction of imipramine and its corresponding metabolites has excellent recovery so that the method can be used for steady-state or kinetic dose studies. The extraction is also versatile enough so that the protocol samples can be analyzed by two different detection methods, ultraviolet and electrochemical, concurrently.

EXPERIMENTAL

Materials

Ethyl acetate, *n*-heptane and methanol were obtained from Burdick and Jackson (Obetz, OH, USA). Potassium phosphate and concentrated phosphoric acid, HPLC grade, were obtained from Fisher Scientific (Pittsburgh, PA, USA) and tetramethylammonium chloride was purchased from Fluka (Ronkonkoma, NY, USA). The remaining chemicals used were analytical grade. 2-Hydroxydesipramine, 2-hydroxyimipramine and 8-hydroxychloroimipramine were graciously supplied by Drs. A. A. Manian and Stephan Kennedy (NIMH, Rockville, MD, USA). All other drugs were obtained from Sigma (St. Louis, MO, USA). The stripped plasma in this project was obtained from Scantibodies Lab. (Santee, CA, USA).

Apparatus

The analyses were performed on both a Beckman Model 110B pump with a Beckman 163 variable-wavelength ultraviolet detector (Beckman Instruments, Fullerton, CA, USA) at a wavelength of 215 nm and an ESA Coulochem Model 5100A electrochemical detector (ESA, New Milford, MA, USA) with an LKB 2150 HPLC pump (LKB & Pharmacia, Piscataway, NJ, USA). The settings for the electrochemical unit were as follows: detector 1, +0.2 V; detector 2, +0.68 V; guard cell, 0.70 V with a gain of 12 × 10 and response time of 0.4 s. A straight stainless-steel column (120 mm × 4.6 mm I.D.) packed with 5 μm Nucleosil C_{18} was used in both methods (Unimetrics, Shorewood, IL, USA). Both chromatographic systems were also fitted with 7125 and 7010 Rheodyne injectors and 50-μl loops, respectively (Rainin Instruments, Woburn, MA, USA).

Extraction procedure for plasma samples

The analytical method for the HPLC determination of imipramine, desipramine and each of their hydroxy metabolites was as follows: 1 ml of plasma was added to a 10-ml screw-capped polypropylene tube with 10 μl of a 10 mg/ml stock of 8-hydroxychloroimipramine for the ultraviolet method and 10 μl of a 1 mg/ml stock for the electrochemical method added as the internal standard. To each sample, 500 μl of 0.6 *M* carbonate buffer at pH 10.4 and 5 ml of 20% ethyl acetate

in heptane were added. Then this mixture was shaken for 2.5 min and centrifuged at 3000 *g* for 10 min and the organic layer was transferred to another 15-ml polypropylene conical mailing vial (Fred Morrow Scientific, New Milford, NJ, USA) containing 125 μl of 0.025 *M* KH_2PO_4 at pH 2.4. This mixture was again shaken for 2.5 min and centrifuged at 3000 *g* for 10 min. The top organic layer was discarded and the remaining aqueous phosphate layer placed in a 4.5-ml polypropylene conical tube. This tube was then placed in the Savant Concentrating Vac (Savant Instruments, Farmingdale, NY, USA) for 25 min to remove any residue of the organic extraction solvent with 50 μl of this layer being injected into the chromatograph.

Chromatographic conditions

The mobile phase for the separation of imipramine, desipramine, their 2-hydroxy metabolites and 8-hydroxychloroimipramine, the internal standard, was 0.01 *M* KH_2PO_4 (phosphate buffer and 5 m*M* tetramethylammonium chloride adjusted to pH 2.4 with concentrated phosphoric acid, HPLC grade)–acetonitrile (70:30, v/v). The flow-rate was maintained, for both procedures, at 1 ml/min. For separations with both electrochemical and ultraviolet detection, the column was maintained at room temperature.

Human plasma samples

Human plasma samples were obtained from a clinical research protocol approved by the University of Pittsburgh, Institutional Review Board, which involved the random assignment of patients to either a dose of 50–75 mg of imipramine or the same dose of deuterium-labeled imipramine. The study was performed with multiple blood samples being taken during 72 h in both phases to determine the difference of the metabolism between imipramine and its deuterated form for each patient.

Recovery and linearity

Recoveries for the ultraviolet and electrochemical methods were performed with spiked stripped plasma and patient samples at four different concentrations of imipramine and their 2-hydroxymetabolites with 10 μl of 10 mg/ml and 1 μg/ml solutions of 8-hydroxychloroimipramine, respectively. The recovery from the spiked and patient samples would be calculated by standards which were made in 125 μl of 0.1 *M* HCl.

The recovery of 8-hydroxychloroimipramine was also performed at four different concentrations in stripped plasma. The linearity of the ultraviolet assay was determined between 15 to 300 ng/ml, with samples containing 15, 25, 50, 100, 150, 200, 250 and 300 ng/ml imipramine, desipramine and their 2-hydroxy metabolites and 100 ng/ml 8-hydroxychloroimipramine.

The linearity of the electrochemical method was determined between 0.5 to 20 ng/ml, with samples containing 0.5, 1, 2, 4, 8, 10 and 20 ng/ml imipramine,

desipramine and their 2-hydroxy metabolites and 10 ng/ml 8-hydroxychloroimipramine.

RESULTS

Ultraviolet detection at 215 nm of imipramine, desipramine and their 2-hydroxy metabolites was adequately sensitive for therapeutic monitoring of plasma samples. The samples obtained from the maintenance studies had typical steady-state values suitable for measurements by this technique.

The limit of quantitation of imipramine and its metabolites by ultraviolet detection was 15 ng/ml with a signal-to-noise ratio of 4:1 for imipramine and desipramine and of 10:1 for their 2-hydroxy metabolites. The recovery (Table I) at the four different concentrations had an average (±S.D.) of 99.45 ± 0.68% for imipramine, 100 ± 0.0% for desipramine, 93.1 ± 4.22% for 2-hydroxydesipramine, 97.8 ± 3.53% for 2-hydroxyimipramine and 100 ± 0.0% for the internal standard. Recovery studies for both ultraviolet and coulometric detection were also performed to determine the proper solvent system for these compounds and it was found that 20% ethyl acetate in *n*-heptane extracted both the

TABLE I

ASSAY RECOVERY STUDY FOR IMIPRAMINE, DESIPRAMINE AND THEIR HYDROXY METABOLITES FROM SPIKED PLASMA BY ULTRAVIOLET DETECTION (n = 4)

Drug	Concentration (ng/ml)	Mean recovery (%)	C.V. (%)
Imipramine	25	99.2	0.018
	50	98.6	0.032
	100	100	0
	200	100	0
2-Hydroxyimipramine	25	92.6	0.109
	50	98.6	0.032
	100	100	0
	200	100	0
Desipramine	25	100	0
	50	100	0
	100	100	0
	200	100	0
2-Hydroxydesipramine	25	96.4	0.059
	50	87.0	0.137
	100	95.4	0.067
	200	93.4	0.084
8-Hydroxychloroimipramine (internal standard)	25	100	0
	50	100	0
	100	100	0
	200	100	0

standard and patient samples reliably. The linearity of the ultraviolet imipramine assay over a range of 14–300 ng/ml was demonstrated by correlation coefficient of 0.9986 (imipramine, $y = 0.0066x - 0.0001$; desipramine, $y = 0.0099x - 0.0171$; 2-hydroxydesipramine, $y = 0.0154x + 0.0256$; 2-hydroxyimipramine, $y = 0.0199x + 0.0379$). The inter-day reproducibility of spiked plasma samples is shown in Table II. The coefficients of variation (C.V.) ranged between 1.7 and 9.8% for the spiked plasma.

The electrochemical method for imipramine and its metabolites was also sufficiently sensitive for acute kinetic dose studies. The samples obtained from the imipramine deuterated study were also adequately detected by this method. The limit of detection of this assay was 0.5 ng/ml with a signal-to-noise ratio of 4:1 for

TABLE II

INTER-DAY REPRODUCIBILITY OF IMIPRAMINE, DESIPRAMINE AND THEIR HYDROXY METABOLITES FROM SPIKED PLASMA SAMPLES BY ULTRAVIOLET DETECTION ($n = 5$).

Drug	Concentration (ng/ml)	Peak-height ratio	C.V. (%)
Imipramine	15	0.112	9.8
	25	0.174	8.7
	50	0.308	6.3
	100	0.624	4.8
	150	0.946	6.8
	200	1.278	3.0
	300	2.00	4.5
2-Hydroxyimipramine	15	0.282	5.3
	25	0.544	8.8
	50	0.964	7.1
	100	2.09	7.7
	150	3.06	2.6
	200	4.71	2.8
	300	5.94	5.1
Desipramine	15	0.142	5.9
	25	0.244	6.9
	50	0.458	2.9
	100	0.962	1.7
	150	1.41	4.8
	200	1.89	3.5
	300	2.98	5.0
2-Hydroxydesipramine	15	0.228	6.5
	25	0.408	5.9
	50	0.772	3.8
	100	1.87	2.0
	150	2.78	4.4
	200	3.70	3.6
	300	5.05	6.7

TABLE III

ASSAY RECOVERY STUDY FOR IMIPRAMINE, DESIPRAMINE AND THEIR HYDROXY-METABOLITES FROM SPIKED PLASMA BY ELECTROCHEMICAL DETECTION (n = 3)

Drug	Concentration (ng/ml)	Mean recovery (%)	C.V. (%)
Imipramine	0.5	99.7	0.132
	8.0	97.0	0.009
	10.0	96.0	0.078
2-Hydroxyimipramine	0.5	88.0	0.145
	8.0	88.0	0.176
	10.0	95.0	0.034
Desipramine	0.5	99.0	0.025
	8.0	99.0	0.025
	10.0	97.0	0.052
2-Hydroxydesipramine	0.5	87.0	0.098
	8.0	79.0	0.235
	10.0	94.0	0.078
8-Hydroxychloroimipramine (internal standard)	2.0	86.0	0.048
	8.0	85.0	0.123
	10.0	96.0	0.032

imipramine and desipramine and 10:1 for their hydroxy metabolites. The recoveries (Table III) at different concentrations had an average (±S.D.) of 97.5 ± 1.91% for imipramine, 98.3 ± 1.15% for desipramine, 86.6 ± 7.5% for 2-hydroxydesipramine, 90.3 ± 4.04% for 2-hydroxyimipramine and 89 ± 6.08% for the internal standard. The linearity of imipramine by electrochemical detection over the range 0.5–20 ng/ml was also shown with correlation coefficients of 0.997 (imipramine, $y = 0.0869x - 0.0008$; desipramine, $y = 0.1068x - 0.0032$; 2-hydroxyimipramine, $y = 0.180x - 0.081$; 2-hydroxydesipramine, $y = 0.1382x - 0.086$). The detection limit of 0.5 ng/ml was more than adequate to measure the blood levels of the imipramine deuterated study because the plasma levels for some patients were no lower than 1 ng/ml during the required collection time of 72 h. The reproducibility of spiked samples is shown in Table IV.

The inter-day of C.V.s ranges were 0–7.6% for the spiked samples and 0–9.5% for the patient samples. The accuracy of this assay for the ultraviolet and coulometric detection methods was determined with spiked stripped plasma at three different concentrations (Table V). The C.V. range for imipramine, desipramine and their hydroxy metabolites was 0.87–4.65% for the ultraviolet method while the coulometric detection method produced a C.V. range of 1.4–4.8%.

The analytical methods were also tested for potential drug interferences (Tables VI and VII). Figs. 1 and 2 show the chromatograms of a blank and sample to demonstrate the resolution of the compounds of interest for both analytical techniques.

TABLE IV

INTER-DAY REPRODUCIBILITY OF IMIPRAMINE, DESIPRAMINE AND THEIR HYDROXY METABOLITES FROM SPIKED PLASMA SAMPLES BY ELECTROCHEMICAL DETECTION (n = 4)

Drug	Concentration (ng/ml)	Mean peak-height ratio	C.V. (%)
Imipramine	0.5	0.048	4.9
	1.0	0.150	0
	2.0	0.290	3.9
	4.0	0.572	2.2
	8.0	1.05	3.6
	10.0	1.41	3.3
	20.0	2.72	1.4
2-Hydroxyimipramine	0.5	0.040	2.5
	1.0	0.297	4.2
	2.0	0.575	7.1
	4.0	1.01	5.4
	8.0	1.69	7.0
	10.0	2.46	5.1
	20.0	5.36	4.7
Desipramine	0.5	0.075	7.6
	1.0	0.262	4.8
	2.0	0.537	2.3
	4.0	1.02	2.4
	8.0	1.71	3.1
	10.0	2.19	1.6
	20.0	4.60	0.6
2-Hydroxydesipramine	0.5	0.089	2.3
	1.0	0.172	2.9
	2.0	0.385	6.8
	4.0	0.663	5.2
	8.0	1.53	3.5
	10.0	2.03	5.7
	20.0	4.39	7.6

The comparison of the analysis of the samples by both ultraviolet and electrochemical detection methods was also performed and the methods were shown to be interchangeable for the results of the same plasma samples. The blood levels of patient samples determined by both detection methods were analyzed statistically by a Matched-Pair Wilcoxon test and found not to be significant at an α value of less than 0.01. In Fig. 3, graphic representations of these comparisons show linear relationships and no significant differences in the two detection procedures.

TABLE V

ACCURACY FOR IMIPRAMINE, DESIPRAMINE AND THEIR HYDROXY METABOLITES FROM SPIKED PLASMA BY ULTRAVIOLET AND ELECTROCHEMICAL DETECTION (n = 5)

Drug	Concentration spiked (ng/ml)	Concentration determined (ng/ml)	C.V. (%)
Ultraviolet detection			
Imipramine	25	24.4	4.67
	100	99.3	2.85
	200	199.7	2.08
2-Hydroxyimipramine	25	24.7	2.65
	100	100.8	1.74
	200	200.7	0.87
Desipramine	25	24.5	2.26
	100	100.1	3.09
	200	199.55	1.34
2-Hydroxydesipramine	25	24.57	3.45
	100	101.9	1.99
	200	198.9	1.42
Electrochemical detection			
Imipramine	2	2.0	3.8
	4	3.97	1.57
	8	8.04	1.4
2-Hydroxyimipramine	2	1.93	3.0
	4	3.82	4.8
	8	7.93	2.8
Desipramine	2	1.95	4.1
	4	4.02	2.0
	8	7.86	2.2
2-Hydroxydesipramine	2	2.03	2.2
	4	3.74	4.8
	8	7.95	1.4

DISCUSSION

The monitoring of imipramine, desipramine and their hydroxy metabolites in depressed patients is important for interpretation of issues concerning neuropharmacology, cardiovascular pharmacology and compliance. The applicability of the extraction procedure, which can be used on with both an ultraviolet and an electrochemical detection method, is highly advantageous for analyzing samples at different plasma levels. A similar extraction procedure had been previously developed for chloroimipramine by Spreux-Varoquaux in 1987 [23]. The interday reproducibility of these methods is high, so that the pharmacodynamic and pharmacokinetic changes can be assessed for each patient. The plasma volumes

TABLE VI

POTENTIAL DRUGS INTERFERING FOR ULTRAVIOLET DETECTION

Drug	Retention time (min)
Alprazolam	2.49
Amitriptyline	15.02
Chlorodesipramine	21.50
Chloroimipramine	23.18
Desipramine	10.98
Doxepin	6.48
Fluoxetine	16.46
Imipramine	12.44
Mesoridazine	4.33
Nordoxepin	5.90
Norfluoxetine	13.25
Triazolam	2.94
2-Hydroxydesipramine	3.77
2-Hydroxyimipramine	4.09
8-Hydroxychloroimipramine	7.99

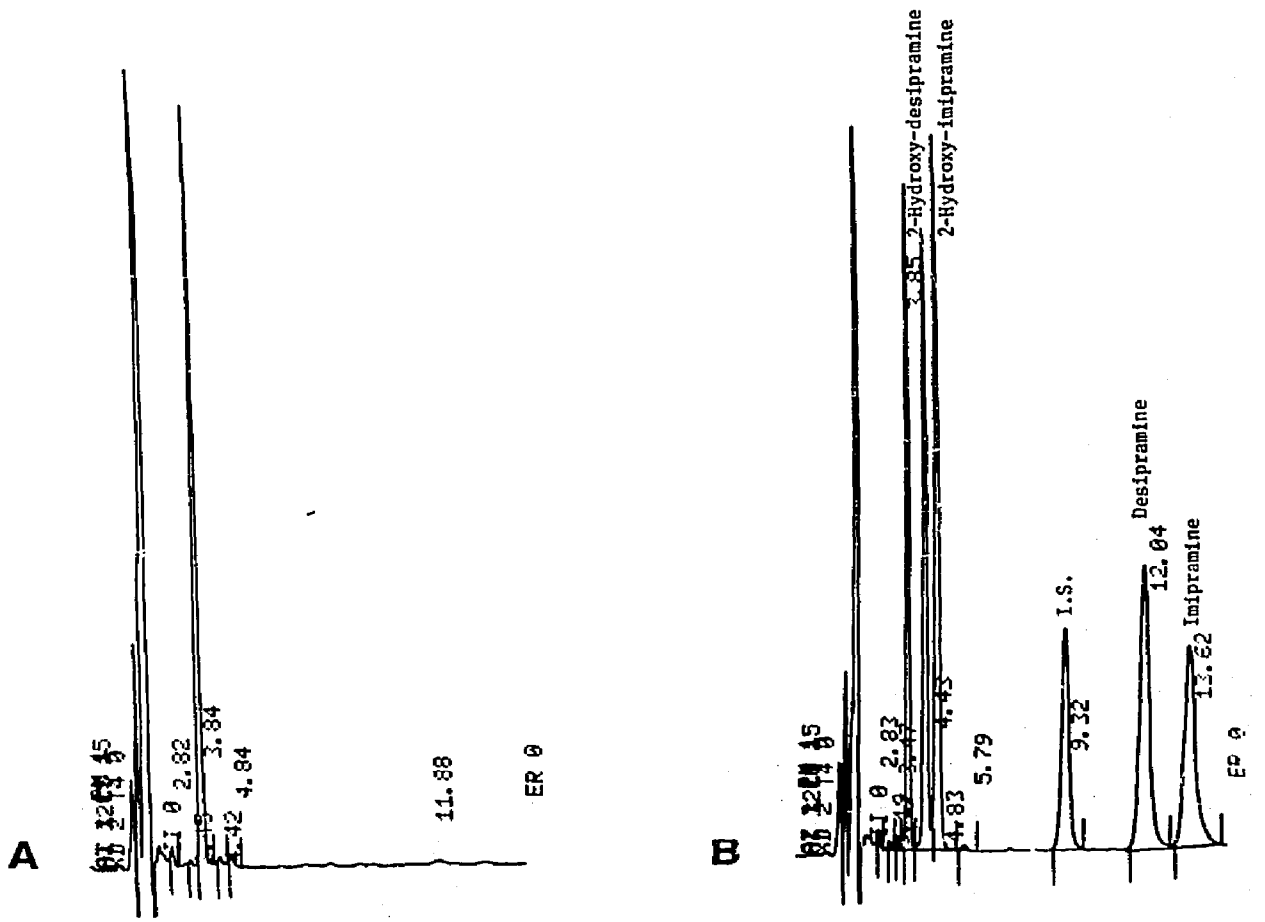

Fig. 1. HPLC–ultraviolet detection profiles of (A) a blank plasma extract and (B) a plasma extract of a 150 ng/ml standard of imipramine, desipramine and their hydroxy metabolites.

TABLE VII

POTENTIAL DRUGS INTERFERING FOR ELECTROCHEMICAL DETECTION

Doxepin, nordoxepin, amitriptyline, fluoxetine, norfluoxetine, triazolam and alprazolam were not detected at the potentials of this assay.

Drug	Retention time (min)
2-Hydroxydesipramine	3.36
2-Hydroxyimipramine	3.62
8-Hydroxychloroimipramine	6.35
Desipramine	8.33
Imipramine	9.39
Chlorodesipramine	15.97
Chloroimipramine	18.21
Mesoridazine	4.50

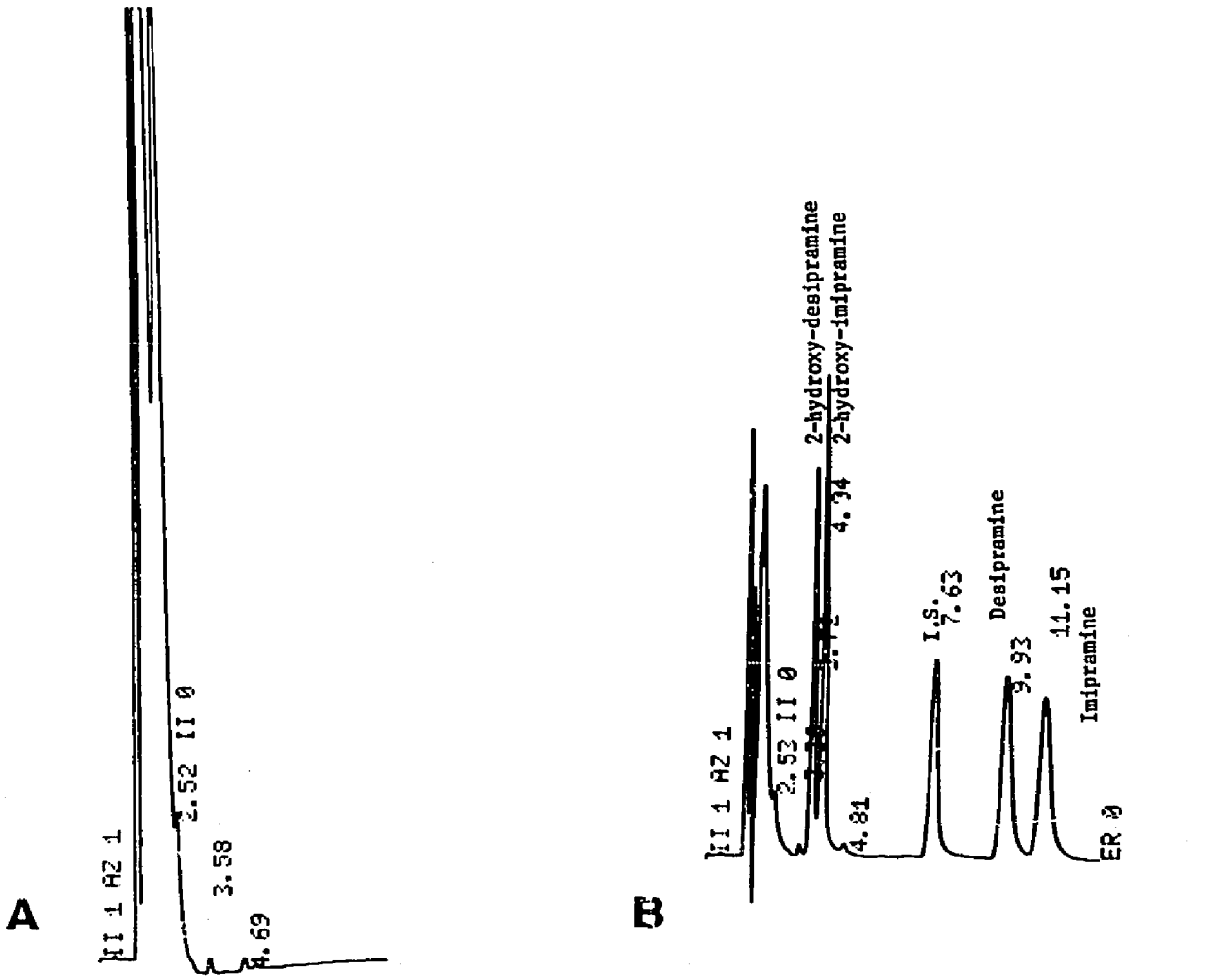

Fig. 2. HPLC–electrochemical detection profiles of (A) a blank plasma extract and (B) a plasma extract of a 10 ng/ml standard of imipramine, desipramine and their hydroxy metabolites.

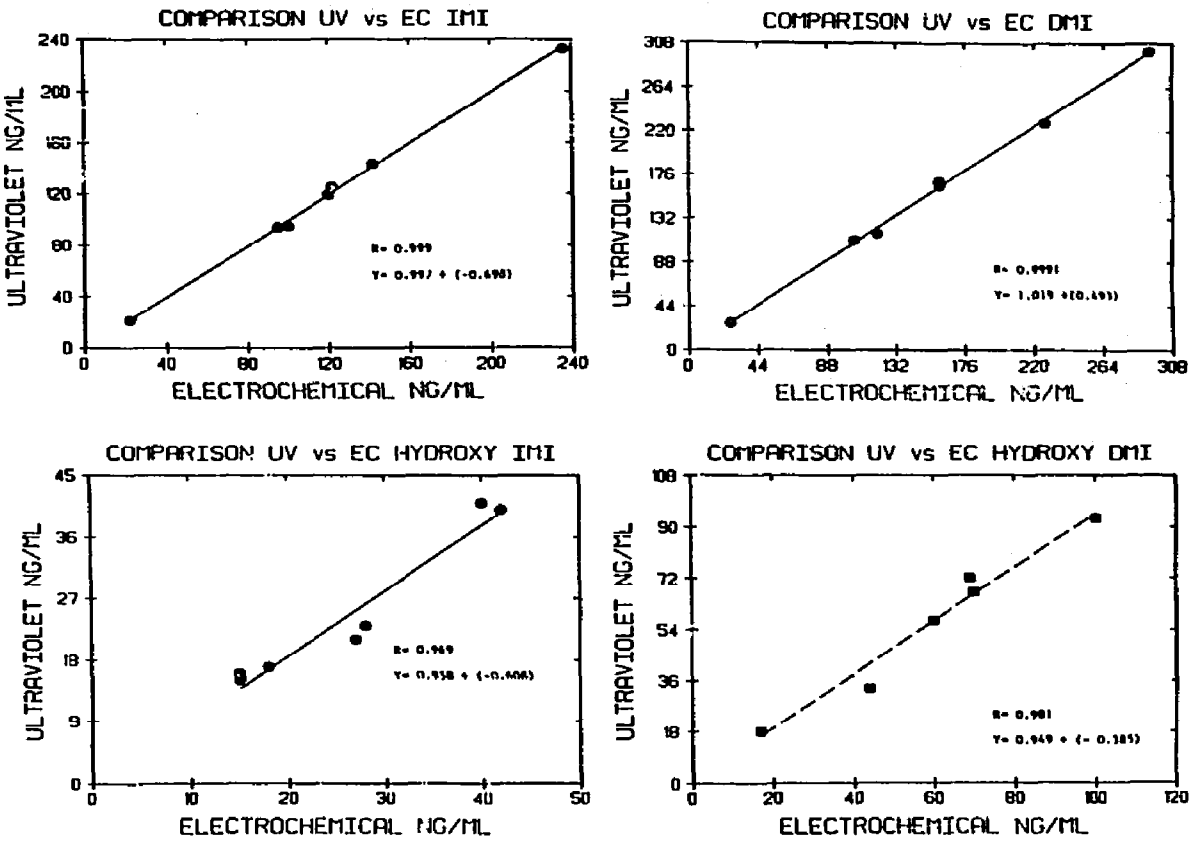

Fig. 3. Graphs of imipramine (IMI), desipramine (DMI) and their 2-hydroxy metabolites showing the linear relationship between the two detection methods.

of this assay may be as low as 50–100 μl for steady-state conditions and 1 ml for kinetic and/or 48-h predictive dosing, which permits more frequent sampling, while the other techniques to date have used 2 or more milliliters of plasma or serum to obtain the same analytical sensitivity. The applicability of these imipramine assays is that concurrent measurements of both steady-state and kinetic plasma levels can be performed within the same protocol.

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