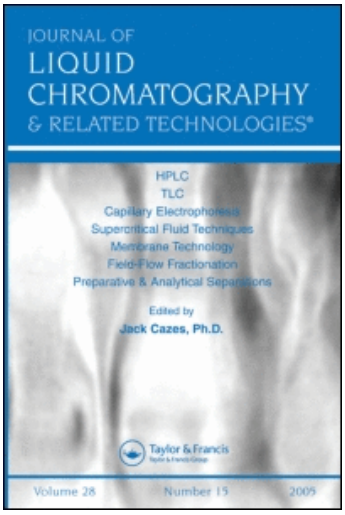

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SENSITIVE DETERMINATION IN PLASMA OF IMIPRAMINE AND DESIPRAMINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING ELECTROCHEMICAL DETECTION

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ABSTRACT

A sensitive high-performance liquid chromatographic method, using electrochemical detection, has been developed for the analysis of both imipramine and desipramine in plasma.

This involved many n-hexane-isoamyl alcohol extractions, in the presence of an internal standard (trimipramine). Analysis was performed by isocratic elution with 27 % 0.01M potassium dihydrogen phosphate, 40 % acetonitrile, 33 % methanol, with the pH adjusted to 9, on a Nucleosil 5 μm, CN column, with electrochemical detection at + 0.9 Volt.

The limit of sensitivity of the assay was 1.0 ng/ml. The method was applied to a single dose (75 mg) pharmacokinetic study of imipramine, in healthy volunteers, with a good accuracy (95 - 105 %) and precision (less than 11.5 %).

INTRODUCTION

Imipramine was largely used in the treatment of depression. This drug is extensively demethylated by first-pass metabolism

in the liver, to its primary active metabolite, desipramine (1).

At steady-state, it is well-known that plasma levels of the hydroxy-metabolites of imipramine may be in relation with strong cardiovascular toxicity (2). They are present in central nervous system in acute overdose cases (3), but their levels are not detectable after a single oral dose administration of the parent drug.

A large number of H.P.L.C. methods have been developed, mostly for therapeutic drug monitoring purposes (4, 5, 6, 7, 8, 9, 10). They have been characterized by rather poor sensitivity on both imipramine and desipramine, achieving detection limits of only 10 to 50 ng/ml. Only one H.P.L.C. method employing an electrochemical (E.C.) detector has been published (11), with a limit of quantification at 5 ng/ml.

The procedure described here provides an E.C./H.P.L.C. assay. The sensitivity of which (1.0 ng of imipramine and desipramine per milliter of plasma), enables plasma concentrations to be monitored over 48 h , after administration of a single imipramine oral dose (75 mg) in a pharmacokinetic study.

MATERIALS AND METHOD

Chemicals

Imipramine and desipramine chlorhydrate were supplied by Ciba-Geigy laboratories (Rueil-Malmaison, France), and trimipramine maleate by Rhône-Poulenc-Rorer (Paris, France).

H.P.L.C. grade acetonitrile, methanol and n-hexane, were obtained from Carlo-Erba (Milan, Italy).

R.P.E. grade isoamyl alcohol and potassium dihydrogen phosphate were obtained also from Carlo-Erba (Milan, Italy).

Analytical grade boric acid (99.8%), potassium chloride (99.5%), and suprapur grade sulfuric acid (96%), sodium hydroxide (27%) were obtained from Merck (Darmstadt, Germany).

Bi-distilled water was obtained before use, through a water purification system (Milli-Q, Millipore Corp., U.S.A.).

Chromatography

A Waters system was used. It consisted of a model 510 pump, a WISP 710 B automatic sample injector, a 460 electrochemical detector and a 746 integrator-recorder.

The column (300 x 4.6 mm i.d.) was packed with Nucleosil CN, 5µm particle size (Touzart & Matignon, France).

The mobile phase consisted in a mixture of aqueous potassium dihydrogen phosphate solution (0.01M), acetonitrile and methanol in the ratio 135 / 200 / 165 (V/V/V), which was adjusted at pH 9 with 2N NaOH. Prior to use, the mobile phase was filtered through an HVLP 04700 Durapore membrane (Millipore Corp., U.S.A.) and helium-degassed. This was carried through the column at 1.0 ml/min.

All separations were carried out at room temperature. Once set up, the system was run continuously to ensure stability and sensitivity.

The glassy-carbon working electrode was set at + 0.9 Volt potential, versus a KCl reference electrode.

Standard solutions

Stock solutions (100 µg/ml) of imipramine (IMI.), desipramine (DMI.), and trimipramine (I.S.) were weekly prepared in 0.01N HCl solution, and stored at + 4°C.

The working solutions were prepared by dissolving stock solutions in 0.01N HCl, at final concentrations of 1000 - 500 - 100 - 25 ng/ml for both IMI., DMI. and 250 ng/ml for I.S..

Calibration standards (0 - 1 - 2 - 4 - 8 - 10 - 20 - 40 - 60 - 80 ng/ml) were daily made in pooled human plasma. These were prepared according to the sample preparation.

Sample preparation

In a 10 ml screw-capped tube, 1 ml of plasma was supplemented with 1 ml of 0.032M borate buffer pH 10.5, 50 µl of I.S. solution (0.25 µg/ml) and 6 ml of n-hexane-isoamyl alcohol (97:3, V/V). The tube was shaken mechanically for 10 min. and centrifuged for 10 min. at 3000 r.p.m.. In a 10 ml screw-capped tube, 5.0 ml of organic phase were added to 1 ml of 0.1N sulfuric acid. After shaking for 10 min. and centrifuging for 5 min. at 3000 r.p.m., the organic phase was carefully discarded using a Pasteur pipette. The aqueous phase was made alcaline with 500 µl of 1N NaOH and extracted with 6 ml of n-hexane-isoamyl alcohol (99:1, V/V). The mixture was shaken and centrifuged. The upper organic layer, transferred into another 10 ml screw-capped tube, was evaporated to dryness under a gentle stream of nitrogen at 30°C.

The residue was dissolved in 200 µl of the mixture acetonitrile-water (40:60, V/V). An aliquot of 70 µl was injected into the chromatograph.

RESULTS AND DISCUSSION

Analytical conditions

For iminobibenzyl compounds, correlation was found between the electrochemical response and the functional tertiary amine group (12). Tricyclic antidepressant agents can be oxidized at potential of + 1 Volt and consequently detected electro - chemically. However, others compounds present in the extract will be oxidized as well and may give rise of interference. Therefore, we selected a + 0.9 Volt working potential because the signal-to-noise ratio was the best. The sensitivity of the working electrode gradually declined, and we cleaned the electrode every day. Also it is essential to stabilize the electrode until the response is constant.

Apparently each manipulation of the extraction procedure increased the chances of variable losses due to glassware adsorption. The use of I.S. and reference compounds in acidic solutions, associated with the addition of isoamyl alcohol to n-hexane, improved day-to-day precision sufficiently (4,7). In our extraction procedure, the recoveries of IMI. and DMI. were included in 68 - 72 % range.

Chromatograms

Figure 1 shows chromatograms obtained from extracts of plasma spiked with 10 ng/ml IMI. and DMI. (figure 1a), blank plasma (figure 1b), and plasma sample obtained after the administration of an imipramine single oral dose (75mg), in a healthy volunteer (figure 1c).

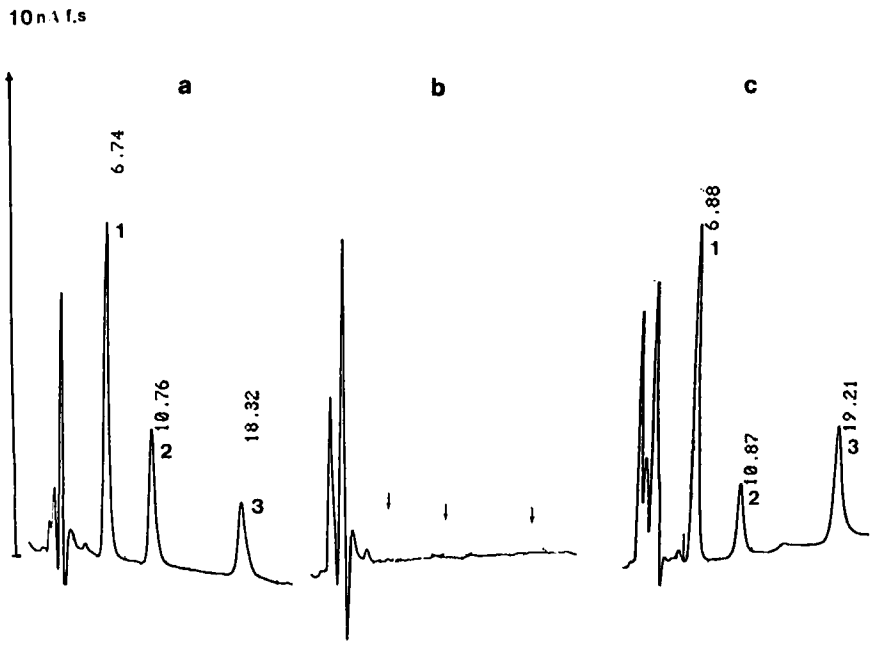

FIGURE 1 : Chromatograms obtained from extract of :

(a) plasma spiked with imipramine (IMI.), desipramine (DMI.)(10 ng/ml both) and internal standard (I.S.).

(b) human blank plasma.

(c) plasma sample from a healthy volunteer included in a pharmacokinetic study : IMI. : 15.3 ng/ml, DMI. : 14.2 ng/ml.

peaks : 1 = I.S. ; 2 = IMI. ; 3 = DMI.

Concentrations were calculated comparing the ratio of peak areas of samples (major peak / I.S.), with calibration curve. Trimipramine (I.S.) was eluted in 6.7 min., then imipramine and desipramine in 10.8 min. and 18.3 min., respectively.

The limit of detection for both IMI. and DMI. was 1.0 ng/ml, allowing a signal-noise ratio of 4, when 1.0 ml of

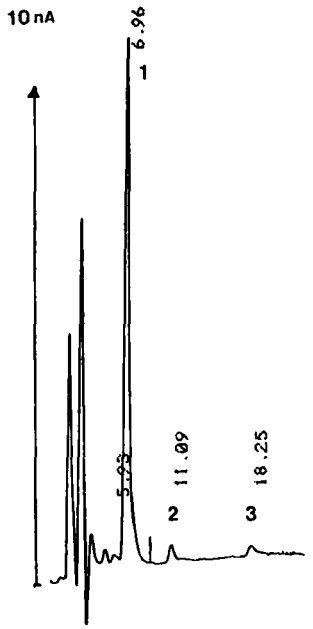

FIGURE 2 : Chromatogram obtained from extract of a blank plasma sample spiked at the limit of detection (1.0 ng/ml of IMI. and DMI.).

peaks : 1 = I.S. ; 2 = IMI. ; 3 = DMI.

plasma was used (figure 2). These values were sufficient for pharmacokinetic studies.

Linearity

The calibration curves which give the ratio of areas major peak / I.S., as a function of concentration were linear over the range 0 - 80 ng/ml and can be expressed by the equations :

TABLE 1

Within-day Variability and Accuracy in Measured Imipramine and Desipramine Concentrations in Plasma.

concentration spiked (ng/ml)	concentration found mean ± SD (ng/ml)	accuracy (%)	coefficient of variation (%)
for imipramine			
10 (n = 10)	10.06 ± 0.27	100.6	2.7
20 (n = 8)	20.08 ± 0.68	100.4	3.4
40 (n = 8)	40.00 ± 0.95	100.0	2.4
for desipramine			
10 (n = 10)	9.92 ± 0.73	99.2	7.3
20 (n = 10)	20.18 ± 1.31	100.9	6.4

TABLE 2

Between-day Variability and Accuracy in Measured Imipramine and Desipramine Concentrations in Plasma.

concentration spiked (ng/ml)	concentration found mean ± SD (ng/ml)	accuracy (%)	coefficient of variation (%)
for imipramine			
0 (n = 3)	N.D.	-	-
4 (n = 4)	3.86 ± 0.30	96.5	7.9
10 (n = 8)	9.51 ± 0.84	95.1	8.8
20 (n = 5)	20.90 ± 1.03	104.5	4.9
for desipramine			
0 (n = 3)	N.D.	-	-
4 (n = 4)	3.73 ± 0.43	93.3	11.5
10 (n = 7)	9.69 ± 0.41	96.9	4.2
20 (n = 5)	20.68 ± 1.40	103.4	6.9

N.D. = Not Detected

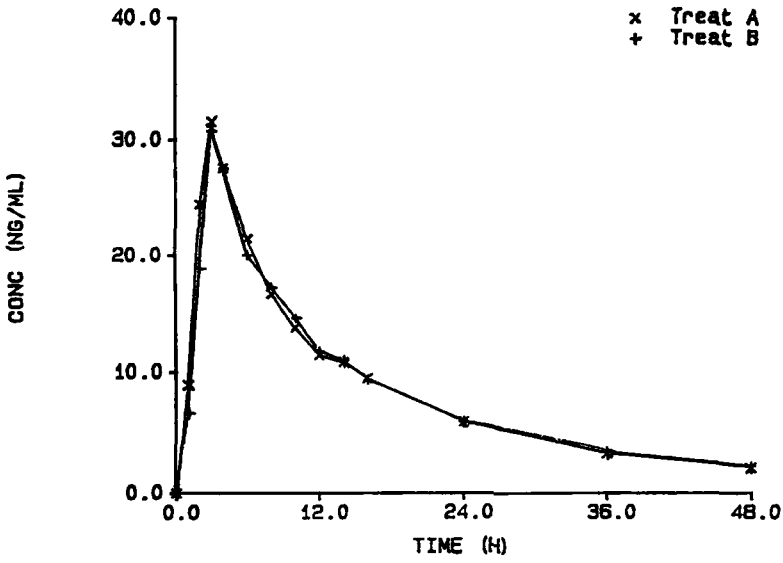

FIGURE 3 : Mean plasma levels of imipramine after oral administration of 75 mg of IMI. to twelve volunteers.
treat A (x) = IMI. + suriclone
treat B (+) = IMI. + placebo

for imipramine :

$y = 0.047 x - 0.025$ $r = 0.9988$ (8 df) ($p < 0.001$)

for desipramine :

$y = 0.0308 x - 0.038$ $r = 0.9987$ (8 df) ($p < 0.001$)

Accuracy

As shown in the table 1, the within-day variability of the method was illustrated by coefficients of variation less than 3.4 % and 7.3 % for IMI. and DMI., respectively.

TABLE 3

Retention Time for Tricyclic Drugs and Other Psychiatric Drugs and Metabolites.

Drug	Retention time min.
buspirone	N.D.
carbamazepine	N.D.
clobazam	N.D.
diazepam	N.D.
droperidol	N.D.
suriclone	N.D.
triazolam	N.D.
tropatepine	N.D.
zolpidem	N.D.
haloperidol	4.9
pipothiazine	6.3
trimipramine (I.S.)	**7.8**
trihexyphenidyle	9.8
chlorpromazine	10.5
amitryptiline	10.7
clomipramine	11.0
imipramine	**12.5**
desipramine	**19.4**

N.D. = Not Detected

Reproductibility assays were performed on 3 series of human plasma samples containing 4.0, 10.0 and 20.0 ng/ml of both IMI. and DMI. (table 2). The coefficients of variation were inferior to 11.5 %, and the accuracy inside 95 - 105 % range.

Specificity

This method has been only used in the study of pharmacokinetic interaction between suriclone and imipramine in man (13), (figure 3).

However, it may be used for the therapeutic drug monitoring in depressed patients. So, the table 3 lists the retention time of psychotropic drugs, which are often concurrently administered to patients receiving imipramine treatment. The most were not detected, and no interferences appeared if peaks occurred on chromatograms.

CONCLUSION

The present H.P.L.C./ E.C. procedure for rapid determination of both imipramine and desipramine, is sufficiently sensitive, specific and suitable to be applied in pharmacokinetic studies, and the therapeutic drug monitoring.

In our study, the automation permitted the analysis of about 55 samples a day.

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