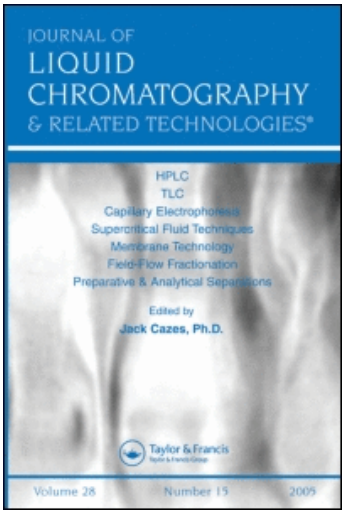

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Simultaneous Determination of Clomipramine and Its Demethylated Metabolite in Plasma and Erythrocytes by High-Performance Liquid Chromatography

P. Marescaux[a]; E. Belan[b]; N. Houdret[a]; M. Goudemand[b]; M. Lhermitte[ac]

[a] Laboratoire de Biochimie Hôpital Calmette, Lille, Cedex, France [b] Service de Psychiatrie Centre Hospitaller Regional de Lille, Lille, Cedex, France [c] Laboratoire de Toxicologie, Faculte de Pharmacie, Lille, Cedex, France

To cite this Article Marescaux, P. , Belan, E. , Houdret, N. , Goudemand, M. and Lhermitte, M.(1994) 'Simultaneous Determination of Clomipramine and Its Demethylated Metabolite in Plasma and Erythrocytes by High-Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 10, 2171 — 2177

To link to this Article: DOI: 10.1080/10826079408013538

URL: http://dx.doi.org/10.1080/10826079408013538

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(10), 2171-2177 (1994)

SIMULTANEOUS DETERMINATION OF CLOMIPRAMINE AND ITS DEMETHYLATED METABOLITE IN PLASMA AND ERYTHROCYTES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

P. MARESCAUX[1], E. BELAN[2], N. HOUDRET[1], M. GOUDEMAND[2], AND M. LHERMITTE[1,3]

[1]*Laboratoire de Biochimie Hôpital Calmette*
rue du Pr. J. Leclercq
59045 Lille Cedex, France
[2]*Service de Psychiatrie Centre Hospitalier Regional de Lille*
avenue de Verdun
59045 Lille Cedex, France
[3]*Laboratoire de Toxicologie*
Faculté de Pharmacie
3 rue du Pr. Laguesse
59045 Lille Cedex, France

ABSTRACT

Clomipramine and its N-demethylated metabolite, N-demethyl-clomipramine were determined simultaneously in plasma or in erythrocytes by a simple hexane-diethyl ether-step extraction procedure followed by reversed-phase liquid chromatography. Baseline separation was achieved by a 5-µm cyanopropylsilane column. The mobile phase consisted of 10mM phosphate buffer-methanol-acetonitrile (25:15:60, v/v/v), at a flow rate of 1.2 ml/min. The eluant was monitored by a UV detector operating at 220 nm. The inter- and intra assay coefficients of variation were within 3.6 and 6.0 % for clomipramine and 4.1 and 6.5 % for N-demethyl clomipramine, respectively. The lowest limit of detection of clomipramine and N-demethylated clomipramine was 5 ng/ml. The method is sensitive, specific and allows for routine analysis in therapeutic controls of patients treated by this antidepressant.

INTRODUCTION

The efficacy of tricyclic antidepressants for treating depression has been substantiated by more than 30 years of experience (1). The correlation between plasma levels of some tricyclic antidepressants and therapeutic effects suggests that measurement of plasma levels may provide valuable information in improving the clinical management of depressed patients (2).

The relationship between the plasma concentration of tricyclic antidepressants and their clinical effect on depressive symptoms is controversial (3). The drugs display marked interindividual differences in the steaty-state concentrations in serum (4), but monitoring therapeutic levels of these drugs is important, since the side effects : anticholinergic effects and cardiac toxicities, are quite common and mainly dose related (5).

Clomipramine is a tricyclic antidepressant medication widely used in western Europe. The drug after absorption, undergoes an important first-pass metabolism to N-demethyl-clomipramine which is pharmacologically active and participates in both therapeutic and unwanted effects (6). After reaching the systemic circulation, clomipramine is further biotransformed into N-demethyl-clomipramine, and both active principles are hydroxylated to metabolites which are further conjugated before being excreted in urine. Hydroxylation of parent drug and metabolite is under polymorphic genetic control by the same cytochrome P450 as debrisoquine and sparteine (6, 7).

Studies concerning blood concentrations of clomipramine and N-demethyl-clomipramine are still conflecting. Clomipramine is an antidepressant with a fairly narrow therapeutic effect. This fact combined, with a high-interindividual variability makes this drug candidate for blood concentration monitoring. Intraindividual variability could be also explained by different distribution in erythrocytes and it will be interesting to know the concentration of the drug in these cells.

A number of analytical methods have been used to determine clomipramine and N-demethyl-clomipramine in biological fluids (2, 8). The most commonly used techniques are based on separations by gas (6, 9-11) or liquid chromatography (5-7, 12-15).

Here we describe an HPLC method which allows the determination of clomipramine and N-demethyl-clomipramine, both in plasma and erythrocytes.

MATERIALS and METHODS

Reagents and chemicals

All chemicals were of analytical grade. Clomipramine, N-demethyl-clomipramine were kindly supplied by Ciba-Geigy laboratory (Rueil-Malmaison, France). Levalorphan, used as internal standard, was a gift from Hoffman-La Roche (Basel, Switzerland). HPLC-grade acetonitrile was obtained from FSA Laboratory, England and HPLC-grade methanol from Scharlau, Spain.

Sample collection and storage.

Blood samples were drawn into red-top vacutainer Tubes (Becton-Dickinson & co., France) and centrifuged within 2h of collection. Plasma and erythrocytes were separated and stored in propylene tubes at -20°C, until assayed

Standard Solutions and Internal Standard

A stock solution containing 2500 ng/ml of clomipramine and N-demethyl-clomipramine was prepared in methanol. The internal standard stock solution of levalorphan (10 µg/ml) was also prepared in methanol. The solutions were stored at -20°C until required. Plasma standard solutions of clomipramine and N-demethyl-clomipramine for the calibration curves were prepared by appropriate dilution of the stock clomipramine and N-demethyl-clomipramine solutions with drug-free plasma so that concentrations of 5, 10, 25, 50, 100, 250 and 500 ng/ml were obtained.

Chromatographic conditions

The HPLC system consisted of a Model 1090, equipped with an automatic injector and a diode array, connected to an HP 85b computer (Hewlett-Packard, Orsay, France), a T5C recorder (Ifelec, Courbevoie, France). Separation was performed on a 5 µm cyanopropyl silane column (4 mm ID x 250 mm) (Société SGE, France) using a mobile phase consisting of 10 mM potassium phosphate buffer (pH 7.0) - methanol - acetonitrile (25: 15: 60, v/v) and a column temperature of 40°C. The flow rate was 1.2 ml/min. The detector was set up at 220 nm.

Sample preparation

- 100 µl of the internal standard (10 µg/ml levalorphan) were introduced in a silanized centrifuge tube and evaporated to dryness under nitrogen at 30°C, 1 ml of plasma standard solutions at different concentrations (5, 10, 25, 50, 100, 250 and 500 ng/ml) or 1 ml of plasma from patients was added. The contents were extracted with 5 ml of a mixture of hexane and diethyl ether (4 : 1, v/v) on a mechanical shaker for 10 minutes and briefly centrifuged (3 000 g, 4°C). The organic layer was tranferred to a clean centrifuge tube and the other phase was again treated by 5 ml of the mixture of hexane and diethyl ether (4 : 1, v/v), after mechanical shaking and centrifugation, the two organic phases were mixed and evaporated to dryness under nitrogen at 30°C. The dried residue was reconstituted with 100 µl of mobile phase and a sample (25 µl) was injected onto the HPLC column.

- For the determination of clomipramine and N-demethyl-clomipramine in erythrocytes, 1ml of erythrocytes was added to a silanized centrifuge tube, containing 1 µg of levalorphan (internal standard), as previously described above. The contents were mixed with hexane-diethyl ether and extracted as previously described for the plasma.

Calibration Curve.

Peak-areas for clomipramine or N-demethyl-clomipramine and the internal standard levalorphan were measured and peak-area ratios (clomipramine or N-demethyl-

Table 1 Drugs tested for possible interference in the HPLC assay of clomipramine and N-demethyl clomipramine

Paracetamol	Oxazepam
Salicylic acid	Prazepam
Carbamazepine	Triazolam
Phenytoin	Amitryptilin
Valproic acid	Imipramine
Digoxin	Fluvoxamine
Theophyllin	Haloperidol
Clonazepam	Thioridazine
Diazepam	Thioproperazine

clomipramine/internal standard) were used for preparation of a calibration curve. The calibration curves were constructed by plotting plasma clomipramine or N-demethyl-clomipramine concentrations (x axis), expressed as ng/ml, versus peak-area ratios (y axis), using linear regression.

This line was then used to calculate the concentration of the drug in the unknown samples (plasma or erythrocytes).

Recovery

Extracts from plasma, prepared as described above, were compared with a direct assay of standards in methanolic solution. These relative recoveries were determined for two different concentrations. The absolute recoveries were also determined for these two different concentrations from extracts of plasma, treated using the procedure described above, except that the internal standard was omitted. All extraction sample residues were reconstituted in 100 µl of the solution of internal standard (1 µg/ml) in mobile phase. In this recovery analysis, levalorphan served as external standard.

Interferences

Interference from endogeneous material and from other drugs was researched. drugs were tested at concentration of 1000ng / ml (Table I).

RESULTS and DISCUSSION

Fig 1 shows the separation and quantitation of clomipramine and N-demethyl-clomipramine in human plasma, using levalorphan as internal standard. In the chromatograms, which were obtained after extraction of 1.0 ml of blank plasma, no additional peaks that could interfere with the determination of the drug, its metabolite and the internal standard are present. Fig 1A represents a chromatogram of blank plasma. Similar result was obtained after extraction of 1 ml of erythrocytes. Blank plasma samples

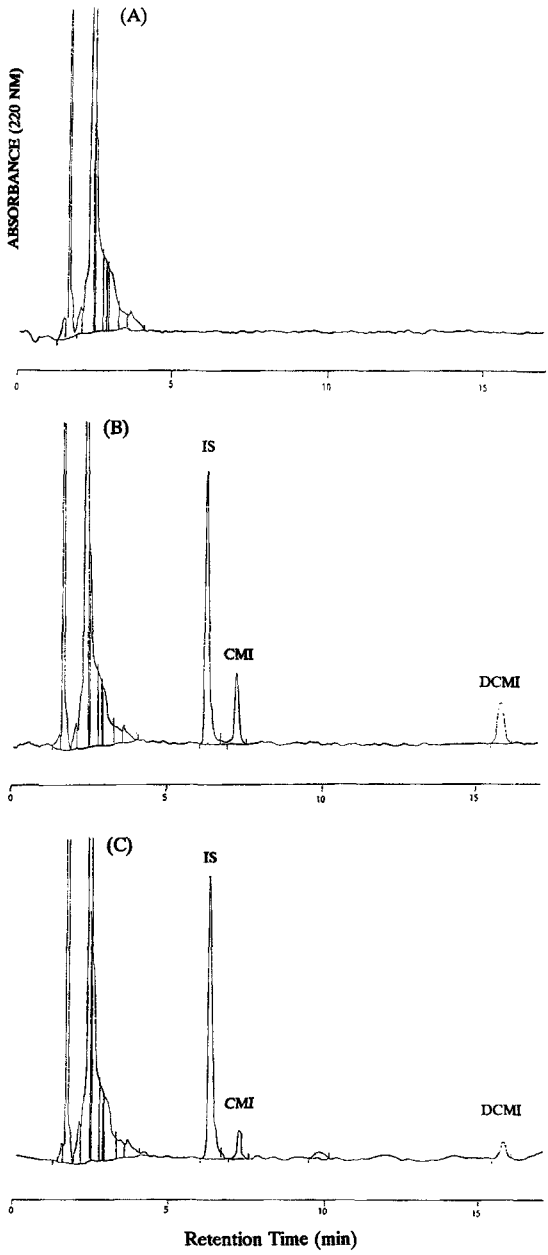

Figure 1 :

HPLC profiles of (A) drug-free human plasma ; (B) spiked drug-free human plasma (1 ml) containing 100 ng clomipramine and N-demethyl-clomipramine and 1000 ng/ml internal standard ; (C) human plasma containing 38 ng/ml clomipramine and 27 ng/ml N-demethyl-clomipramine - Peak CMI = clomipramine ; DCMI = N-demethyl-clomipramine ; IS = internal standard.

and blank erythrocytes sample from 30 subjects were analysed and no plasma or erythrocyte endogeneous peaks, co-eluting with the internal standard, the drug or the metabolite, were detected. Fig 1B is a chromatogram obtained after extraction of 1.0 ml of plasma spiked with 100 ng/ml of clomipramine and N-demethyl-clomipramine. The retention times for the internal standard, clomipramine, and N-demethyl-clomipramine were 6.30, 7.30 and 15.80 min, respectively. Fig 1C shows a chromatogram obtained after extraction of 1.0 ml of plasma from a patient treated by clomipramine.

The calibration curves for clomipramine and N-demethyl-clomipramine were linear over the concentration range of 5 to 500 ng/ml with the square of correlation coefficient (r^2) greater than 0.99. The typical linear relationship for the calibration curve can be expressed by the equations : $y = 0.0033 x - 0.004$, (r^2=0,997), in standard solution and $y = 0.0023 x + 0.015$, (r^2=0,995),in plasma solution for clomipramine and $y = 0.0029 x - 0.016$, (r^2=0,996), in standard solution and $y = 0.0021 x + 0.015$, (r^2=0,996), in plasma solution for demethyl-clomipramine, respectively.

Calibration curves in plasma showed good linearity between peak-area ratios and concentrations from 5 to 500 ng/ml, and the present method is able to detect 5 ng/ml of clomipramine and N-demethyl-clomipramine

Within-run precision (n=5) and day to day reproducibility (n=5), determined by this method were 3.6% and 6.0% for clomipramine and 4.1 and 6.5 % for N-demethyl-clomipramine, respectively, at a concentration of 100 ng/ml.

Recovery of clomipramine and its metabolite was estimated by comparing the peak areas of clomipramine or N-demethyl-clomipramine and the internal standard with those obtained by direct injection of the pure standards of clomipramine or N-demethyl-clomipramine and the internal standard. The mean recovery for clomipramine and for N-demethyl-clomipramine (n = 5) from plasma sample was 85.0 and 90.3 % at 100 ng/ml respectively.

Absolute recovery of the drug and its metabolite was estimated by using levalorphan as external standard. The mean recovery for clomipramine and for N-demethyl-clomipramine (n= 5) from plasma sample was 66.7 and 74.2% at 100 ng/ml, respectively.

Plasma samples or erythrocytes samples stored at -20°C for up to 2 months showed no signs of decomposition and practically the same concentration values were obtained (n = 6). This suggests that clomipramine and its metabolite are stable under these storage conditions, for at least 2 months.

Possible interference by other antidepressants and other drugs at their therapeutic concentrations was evaluated. Commonly used drugs tested (Table I) did not interfered with the assay

The assay is shown to be selective, without interferences from endogeneous materiel and from other drugs commonly used in therapeutic treatment.

The assay is also used for the determination of clomipramine and N-demethyl-clomipramine in erythrocytes.

The method has been applied to many patient samples and is being used routinely in the laboratory for monitoring therapeutic levels. The drug and its metabolite are determined using a simple procedure.

ACKNOWLEDGEMENTS

The authors thank MH Kocmirski for secretarial assistance.

REFERENCES.

1. Goodman W. K. and Charney D. S.
J. Clin. Psychiatry. **40**, 6, 1985.

2. Scoggins B.A., Maguire K.P., Norman T.R. and Burrows G.D.
Clin. Chem. **26**, 805, 1980.

3. Montgomery S.A., McAuley R., Montgomery D.B., Dawling S. and Braithwaite R.A.
Postgraduate Medical Journal, **56 (suppl I)**, 130, 1980.

4. Preskorn S. H., Dorey R. C. and Jerkovich G. S.
Clin. Chem. **34**, 822, 1988.

5. Matsumoto K., Kanba S., Kubo H., Yagi G. and Yuki H.
Clin. Chem. **35**, 453, 1989.

6. Balant-Gorgia A.E., Gex-Fabry M. and Balant L.P.
Clin. Pharmacokinet. **20**, 447, 1991.

7. Balant-Gorgia A.E., Balant L.P., Genet C., Dayer P. and Aeschliman J.M.
J. Clin. Pharmacol. **31**, 449, 1986.

8. Norman T.R. and Maguise K.P.
J. Chromatogr. **340**, 173, 1985.

9. Ninci R. and Sgaragli.
J. Chromatogr. **381**, 315, 1986.

10. Sioufi A., Pommier F. and Dubois J.P.
J. Chromatogr. **428**, 71, 1988.

11. Balant-Gorgia A.E., Balant L.P. and Garrone G.
Ther. Drug Monitoring. **11**, 415, 1989.

12. Sutfin T.A., D'Ambrosio R. and Jusko W.
Clin. Chem. **30**, 471, 1984.

13. Visser T., Oostelbus M.C.J.M. and Toll P.J.M.M.
J. Chromatogr. **309**, 81, 1984.

14. Spreux-Varoquaux O., Morin D., Advenier C. and Pays M.
J. Chromatogr. **416**, 311, 1987.

15 Diquet B., Thomaré P., Becquentin M.and Diviné C.
Biomed. Chromatogr. 7, 59, 1993.

Received: September 8, 1993
Accepted: August 15, 1993