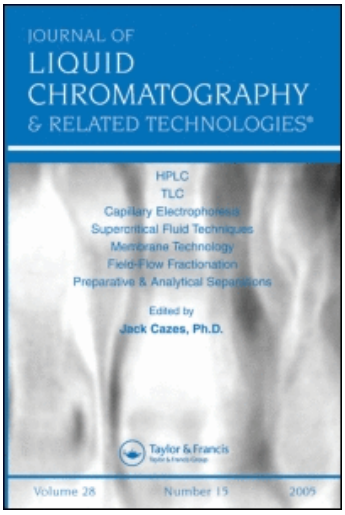

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Automated Determination of Trimipramine and N-Desmethyl-Trimipramine in Human Plasma or Serum by HPLC With On-Line Solid Phase Extraction

Sebastian Härtter[a]; Birgit Hermes[a]; Christoph Hiemke[a]
[a] Department of Psychiatry, University of Mainz Untere Zahlbacher Strasse, Mainz, Germany

To cite this Article Härtter, Sebastian , Hermes, Birgit and Hiemke, Christoph(1995) 'Automated Determination of Trimipramine and N-Desmethyl-Trimipramine in Human Plasma or Serum by HPLC With On-Line Solid Phase Extraction', Journal of Liquid Chromatography & Related Technologies, 18: 17, 3495 — 3505
To link to this Article: DOI: 10.1080/10826079508010465
URL: http://dx.doi.org/10.1080/10826079508010465

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(17), 3495-3505 (1995)

AUTOMATED DETERMINATION OF TRIMIPRAMINE AND N-DESMETHYL-TRIMIPRAMINE IN HUMAN PLASMA OR SERUM BY HPLC WITH ON-LINE SOLID PHASE EXTRACTION

SEBASTIAN HÄRTTER, BIRGIT HERMES, AND CHRISTOPH HIEMKE*
Department of Psychiatry
University of Mainz
Untere Zahlbacher Strasse 8
D-55131 Mainz, Germany

ABSTRACT

A fully automated method including column-switching and isocratic high performance liquid chromatography (HPLC) was developed for determination of the tricyclic antidepressant trimipramine (T) and its N-demethylated metabolite N-desmethyltrimipramine (DT).
The limit of quantification was below 10 ng/ml for T and DT. The assay revealed linearity between detector response and drug concentration in a therapeutically relevant range of 10 to 500 ng/ml. The mean intra- and interassay variabilities were 6.2 and 12.3 % , respectively, for T and 4.7 and 8.7 % respectively, for DT. The method can be applied to therapeutic drug monitoring of patients under T therapy and may be useful for pharmacokinetic studies.

INTRODUCTION

Trimipramine (T) is a tricyclic antidepressant (TCA) which has been applied to the therapy of depression for over 20 years. Although, the chemical structure of T is closely related to other TCAs, like imipramine, it differs from the other TCAs in some pharmacological aspects [1]. The most striking difference is the induction of supersensitivity of the noradrenergic system after long term treatment with T [2]. This casts doubts on the

concept that receptor down-regulation by TCAs is relevant for the antidepressant effect [3, 4]. Moreover, preliminary clinical studies suggest that high doses of T have antipsychotic efficacy without inducing extrapyramidal side effects [5].

Supervision of T treatment by therapeutic drug monitoring (TDM) of blood concentrations is rare. Only few methods are so far available for the determination of T in body fluids. Methods reported in the literature use gas chromatography [6, 7] or high performance liquid chromatography (HPLC) [8-11]. They require laborious and time consuming sample pretreatment by either solvent-solvent extraction [6-10] or off-line solid phase extraction [11]. The assay described here enables simple and rapid quantification of T and DT by direct injection of plasma or serum samples on a column-switching system coupled to isocratic HPLC.

METHODS AND MATERIALS

Chemicals

Trimipramine methansulfonate and N-desmethyltrimipramine (free base) were kindly donated by Rhône-Poulenc (Vitry sur Seine, France). Clomipramine and its main demethylated and hydroxylated metabolites, maprotiline, and carbamazepine were gifts from Ciba Geigy AG (Basel, Switzerland). Fluvoxamine maleate was donated by Duphar (Weesp, Netherlands), paroxetine by SmithKline Beecham (Worthing, UK), haloperidol and risperidone by Janssen (Beerse, Belgium), chlorprothixene by Tropon (Köln, Germany), moclobemide and diazepam by Hoffmann -La Roche AG (Basel, Switzerland), lorazepam by Wyeth (Münster, Germany), and biperidene by Nordmark (Uetersen, Germany).
Acetonitrile and methanol were of LiChrosolve® quality and di-potassium hydrogenphosphate and orthophosphoric acid of analytical grade (Merck, Darmstadt, Germany). Water was deionized and filtered by a Milli-Q water processing system (Millipore, Eschborn, Germany).

Standards

Stock solutions were prepared by dissolving 10 mg T and 10.3 mg DT in 10 ml methanol, each. They were diluted with deionized water and mixed with drug free plasma or

serum to obtain calibration standards with concentrations of free bases ranging between 10 and 500 ng/ml (10, 50, 100, 300, and 500 ng/ml).

Quality control samples (QCs) were prepared by mixing the 1 mg/ml methanolic stock solutions with plasma or serum to acchieve concentrations of 20, 100, and 250 ng/ml T and DT, respectively.

All standards were stored at -20°C until use.

Plasma or serum samples

Plasma or serum samples were obtained from either healthy non-medicated volunteers to obtain drug-free plasma or from patients treated with T for at least 7 days. Patients' blood for the preparation of plasma or serum was collected in the morning immediately before the first daily dose.

Instrumentation

The HPLC system consisted of a CMA/200 autosampler (CMA/Microdialysis AB, Stockholm, Sweden), a six-port switching valve (Besta, Wilhelmsfeld, Germany) coupled to the autosampler and two HPLC pumps A and B, both from Bischoff, type 2200 (Leonberg, Germany). Pump A eluted the analytical mobile phase, pump B was used for loading plasma or serum sample onto the clean-up column and subsequent washing. Moreover, a variable wavelenght ultraviolet (UV) detector, type SPD-10A (Shimadzu, Kyoto, Japan) was included. Chromatograms were recorded and integrated by a Kontron chromatogram integration software PCIP (Kontron, Milan, Italy).

Analytical columns (250 x 4.6 mm) and pre-columns (20 x 4.6 mm I.D.) were packed with Spherisorb CN (5 µm particle size) by MZ-Analysentechnik (Mainz, Germany). Clean-up columns (10 x 4.0 mm I.D.), packed with Hypersil CPS (10 µm particle size), were also supplied by MZ-Analysentechnik.

Chromatographic procedure

0-5 min: After centrifugation (3000 g for 5 min), 100 µl plasma or serum was injected automatically onto the clean-up column. Proteins and other interfering compounds were

washed to waste by deionized water containing 5 % (V/V) acetonitrile at a flow rate of 1.5 ml/min by pump B.

5-8 min: After the six port valve had been switched at 5 min, the analytes to be determined were eluted onto the analytical column (foreward flush) and separated by a mobile phase (pump A) consisting of 23 % (v/v) 8 mM phosphate buffer (adjusted to pH 6.2 with orthophosphoric acid) / 58 % acetonitrile / 19 % methanol (V/V) at a flow rate of 1.5 ml/min. The compounds were detected by UV-absorption at 214 nm.

8-16 min: Eight min after start of the analytical run the switching valve was reset, 16 min later, the next sample was processed.

The clean-up column was replaced after having processed about 80 plasma or serum samples.

Interferences

Drugs that are frequently used in combination with T were checked for interferences. The following drugs were included: tricyclic antidepressants and metabolites, maprotiline, paroxetine, fluvoxamine, and moclobemide. The neuroleptics haloperidol, perazine, chlorprothixene, and risperidone were included. Moreover, the tranquilizers diazepam, flunitrazepam, and lorazepam, and the anti-parkinsonian drug biperidene were tested.

Calculations

Peak heights versus nominal concentrations of the calibration samples were submitted to weighted ($1/y^2$) linear regression to calculate correlation coefficients, slopes and intercepts. Concentrations of drugs in patients' samples or QCs were calculated from the regression lines.

Precision and accuracy were determined from 5 replicate runs of QCs for assessment of intraassay variability and by analyses of QCs on different days (at least 10 for each concentration) for assessment of interassay variability.

Recovery was determined by comparing the peak heights of standard samples containing three different concentrations (20, 100, and 250 ng/ml; N=5 for each

concentration) by processing the samples without and with inclusion of column-switching and a clean-up precolumn.

RESULTS

Chromatographic separation and inferences

The chromatographic conditions described here provided baseline separation of the analytes within less than 20 min including sample purification on the clean-up column. No interferences were detectable either with plasma or serum constituents or with suggested trimipramine metabolites as shown in Fig.1. Both, T and DT were also well separated from most drugs that were tested for interference. The only interference observed was 8-hydroxy-N-desmethylclomipramine which was not separated completely from DT.

Recovery

The recovery rates of T were 79 ± 5, 78 ± 6, and 85 ± 9% for 20, 100, and 250 ng/ml, respectively (N = 5). The recovery rates for DT were 100 ± 6, 89 ± 7, and 99 ± 12& for 20, 100, and 250 ng/ml, respectively (N = 5).

Linearity and Limit of Quantification Limit

Linear regression analyses of calibration curves of serum specimen supplemented with T and DT and measured on 6 independent days revealed linearity between peak heights and concentrations with correlation coefficients always greater than 0.99 for both, T and DT. Concentrations as low as 10 ng/ml could be quantified with a mean CV< 20% (N = 6) for both analytes.

Precision and accuracy

Table 1 shows precision and accuracy data according to the determination of within-run variabilities and between-run variabilities which ranged from 3 to 15% and from 0.4 to 6.8%, respectively.

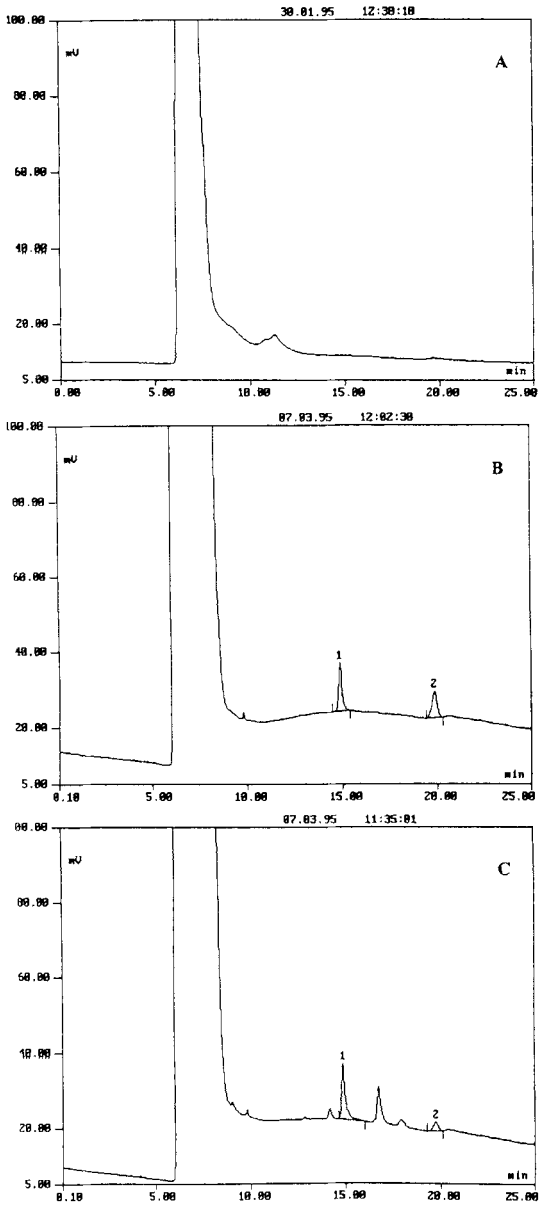

Figure 1. Representative chromatograms of serum samples analysed by the described column-switching HPLC system. (A) Blank serum obtained from a nonmedicated healthy volunteer; (B) blank serum supplemented with 100 ng/ml of each, trimipramine (1) and desmethyltrimipramin (2); (C) serum obtained from a patient treated for two weeks with daily oral doses of 100 mg trimiprmine. The calculated concentrations in sample C were 113 ng/ml for trimipramine and 37 ng/ml for desmethytrimipramine.

TABLE 1

Assay reproducibility and accuracy according to the analysis of quality control samples.

Intraassay precision and accuracy

Analyte	Nominal Conc. (ng/ml)	Precision (%)	Inaccuracy (%)	N
Trimipramine				
	20.00	10.8	-6.8	5
	100.0	6.1	+2.4	5
	250.0	4.9	-5.4	5
N-Desmethyltrimipramine				
	20.00	7.1	+3.6	5
	100.0	3.9	+4.1	5
	250.0	3.1	+2.7	5

Interassay precision and accuracy

Analyte	Nominal Conc. (ng/ml)	Precision (%)	Inaccuracy (%)	N
Trimipramine				
	20.00	15.1	+2.5	10
	100.0	11.2	+1.2	11
	250.0	10.6	+0.4	10
N-Desmethyltrimipramine				
	20.00	11.5	+2.3	11
	100.0	6.1	+0.7	10
	250.0	8.4	+1.5	10

TABLE 2

Resulting T and DT plasma or serum levels of patients treated with different doses of T.

Pat. No.	T-dose (mg/day)	Co-medication	T conc. (ng/ml)	DT conc. (ng/ml)
1	175	no/	87	<10
2	150	haloperidol,lorazepam	219	27
3	100	paroxetine, fluvoxamine, lorazepam	297	51
4	200	risperidone	90	7
5	100	paroxetine	38	14
6	100	no	113	37
7	150	perazine	293	319
8	100	fluvoxamine,clomipramine,carbamazepine	274	<10
9	150	no	343	517
10	200	moclobemide	128	38
11	25	clomipramine, haloperidol, lorazepam	42	<10
12	100	no	20	<10
13	150	no	63	27
14	200	risperidone, biperidene	82	12
15	100	no	123	347
16	50	moclobemide	148	46
17	125	diazepam, chlorprothixene	102	29
18	150	perazine, diazepam	250	195
19	50	moclobemide, maprotiline	265	76
20	100	no	153	289
21	75	paroxetine	80	265

Analysis of patients' plasma or serum

The analyses of plasma or serum samples from 21 patients who were treated for at least 7 days with different oral doses of T revealed highly variable blood levels without obvious correlations between blood level and the given dose (table 2). Seven of these patients received T monotherapy while all other patients had concomitant medications.

DISCUSSION

The method described were seemed advantageous over methods described by others [6-11]. The column-switching technique allowed fully automated processing of plasma or serum samples. Determinations could be accomplished within 20 min. The assay yielded sufficient precision, accuracy and sensitivity. It can therefore be applied to pharmacokinetic studies and also to therapeutic monitoring of patients under T therapy.

Analysis of patient samples indicated poor correlation between the given dose and the resulting blood concentrations. This was in agreement with findings reported for other tricyclic antidepressants [12, 13]. However, the poor correlation might also have been due to the co-medication with other drugs. Some comedications are suggested to interact with the metabolism of T [14]. Nevertheless, variabilities in the metabolic capacities for degradation of T must also be considered [15].

In conclusion, the described HPLC method established for the determination of T and its N-demethylated metabolite in plasma or serum is to our knowledge the first fully automated method that enables a reliable and sensitive determination of these two compounds without time-consuming sample pre-purification. The method enabled measurements of therapeutically relevant T and DT blood concentrations. It is suitable for pharmacokinetic studies and may also be helpful to investigate suggested drug interactions between T and other psychotropic drugs [14].

ACKNOWLEDGEMENTS

This research project was supported by the Stiftung Rheinland-Pfalz fuer Innovation (grant 836-386261/51). We thank Dr. W. Fischer, Rhone-Poulenc (Köln, Germany) and the pharmaceutical companies for supplying us with pure drug substances.

REFERENCES

1. Gross, X. Xin, and M. Gastpar, Neuropharmacol. 30: 1159-1166 (1991)

2. Hauser, H.-R. Olpe and R.S.G. Jones, Eur.J.Pharmacol. 111: 23-30 (1985)

3. Sulser, In Usdin et al. (Eds.) Frontiers in biochemical and Pharmacological Research in Depression Raven Press, New York pp 249-261 (1984)

4. Duncan, I.A. Paul, K.R. Powell, J.B. Fassberg, W.E. Stumpf and G.R. Breese, J. Pharmacol. Exp. Ther. 248: 470-77 (1989)

5. Eikmeier, K. Muszynski, M. Berger and M. Gastpar, Pharmacopsychiatry 23: 212-214 (1990)

6. Abernethy, D.J. Greenblatt and R.I. Shader, Clin. Pharmacol. Ther. 35: 348-352 (1984)

7. Hatori, E. Takashima, T. Yamada and O. Suzuki, J. Chromatogr. 529: 189-193 (1990)

8. Visser, M. Oosterblos and P. Toll, J. Chromatr. 309: 81-93 (1984)

9. Eap, L. Koeb, E. Holsboer-Trachsler and P. Baumann, Ther. Drug Monit. 14: 380-385 (1992)

10. Gulaid, G.A. Jahn, C. Maslen and J. Dennis J. Chromatogr. 556: 228-233 (1991)

11. Mazhar and S.R. Binder, J Chromatogr. 497: 201-212 (1989)

12. Preskorn, S.H., J. Clin. Psychiatry 54 (Suppl.): 14-34 (1993).

13. Richelson, E., May Clin. Proc. 69: 1069-1081 (1994).

14. Seifritz, Holsboer-Trachsler, E., Hemmeter, U., Eap, C.P. and Baumann, P. Europ. Neuropsychopharmacol. 4: 15-20 (1994)

15. Bolaji, O.O., Coutts, R.T. and Baker, G.B., Res. Comm. Chem. Pathol. & Pharmacol. 82: 111-120 (1993).

Received: April 19, 1995
Accepted: May 9, 1995