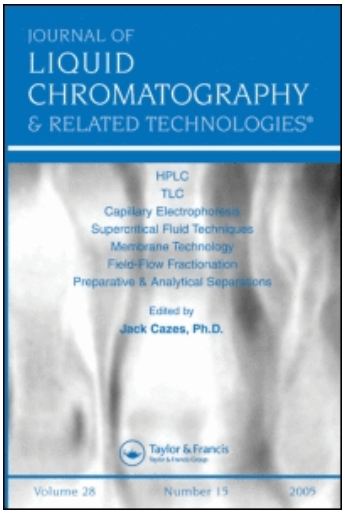

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Tricyclic Antidepressants Analysis by Liquid Chromatography

A. Fazio[a]; E. Spina[a]; F. Pisani[a]
[a] Laboratory of Clinical Neuropharmacology, Neurological Clinic of the University of Messina, Contesse-Messina, Italy

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TRICYCLIC ANTIDEPRESSANTS ANALYSIS BY LIQUID CHROMATOGRAPHY

A. Fazio, E. Spina, and F. Pisani
Laboratory of Clinical Neuropharmacology
Neurological Clinic of the University of Messina
98013 Contesse-Messina, Italy

ABSTRACT

The body of evidence gained up to date is sufficient to indicate that high-pressure liquid chromatography(LC) has versatility and sensitivity in abundance for analyzing tricyclic antidepressants (TCAs), as well as their active metabolites, in body fluids.

The present review deals with an outline of those practical aspects that must be recognized for a correct LC analysis of these compounds in plasma. As examples of application of methodology, selected methods, among those available in the recent literature, are supplied in a summarized form throughout the text.

Extraction of TCAs is carried out by either classical liquid-liquid (involving one,two or three steps) or liquid-solid procedures. Recoveries reported usually range from 60 to 100% of most common TCAs.

Separation modes include either the adsorption or partition chromatography with the possible use of alkylphenyl columns, paired-ion chromatography or CN-bonded columns.

Application of ultraviolet spectrophotometry, usually at a wavelength of around 250 nm, yields a sensitivity of 5-20 ng/ml of most commonly used TCAs, sufficient for both experimental and clinical purposes.

The within-run and day-to-day CVs reported by most methods are below 10 and 15%, respectively.

INTRODUCTION

Tricyclic antidepressants(TCAs) are considered to be the drugs of first choice for the pharmacological treatment of depressive disorders(1,2). Moreover, the recent and growing practice of monitoring drug concentration in blood has opened new prospects for a more rational and effective use of these compounds in the clinical practice. It is now nearly 20 years since it has been demonstrated that the same dose of imipramine or nortriptyline yields markedly different interindividual steady-state plasma drug concentration(3,4). Successively, a large body of studies carried out in different parts of the world has been able to demonstrate a relatively close relationship between blood levels of these drugs and clinical outcome in endogenous depressed patients(5). Data regarding other TCAs, such as amitriptyline, are under continuous accelerating development(5).

In addition,measurement of TCAs in plasma is useful in monitoring patient's compliance, in predicting and preventing side-effects and in documenting clinically important drug interactions.

This area of research has greatly increased the demand of simple and reliable methods for measuring TCAs in body fluids. The techniques used include spectrophotometry, thin-layer chromatography and the more recent gas chromatography, gas chromatography-mass spectrometry, radioimmunoassay and high-pressure liquid chromatography(LC). Due to its versatility and reliability, the latter technique represents the most important new breakthrough in the recent analytical progress.

An excellent review of contemporary methodology, including LC, for the TCAs assay has been published(6) and reports dealing with general aspects of LC and updating its current status are also available in the literature(7,8). The specific purpose of this paper is to give a comprehensive outline of those practical factors

that must be recognized for a correct application of LC to TCAs measurement in plasma. Theoretical aspects have been neglected, while selected,published methods are summarized throughout the text as clear, practical examples of application of methodology.

STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF TCAs

TCAs have a 6-7 ring structure with a two-atom bridge between the two phenyl rings, rendering the molecule to a non-coplanar configu= ration. On the basis of changes in the side chain, TCAs are divided into secondary methylamine(i.e. nortriptyline, desipramine, etc.) and tertiary dimethylamine(i.e. amitriptyline, imipramine, etc.) derivatives(Figure). All these compounds are weak bases(pKa around 10), with high lipid solubility and a molecular weight ranging from 263.5 (nortriptyline and nordoxepine, as free bases) to 315.0 (clomipramine, as free base). All are formulated as hydrochloride salts, while trimipramine is in the form of maleate salt.

TCAs ANALYSIS

Sample Requirement:

TCAs determinations are made with 1 to 2 ml of serum or plasma, both fluids being suitable(9,10,11,12). Serum obtained with the use of gel barrier tubes, however, could yield lower drug values than plasma, probably as a result of an enhanced drug binding to the gelatinous plug used to increase separation of serum(13).

Both glass tubes and evacuated tubes are suitable, but evacuated tubes may be contaminated by the plasticizer(14). In particular, the plasticizer tris(2-butoxyethyl)phosphate displaces basic drugs from their binding sites on α_1-acid glycoprotein with consequent drug distribution into red cells and subsequently lowered plasma concentration(15).

More recently, a similar problem has been documented with the use of indwelling needles, common in multiple blood sampling, flushed with heparinized saline to prevent clotting(16). Although an heparin-induced activation of lipoprotein lipase activity with

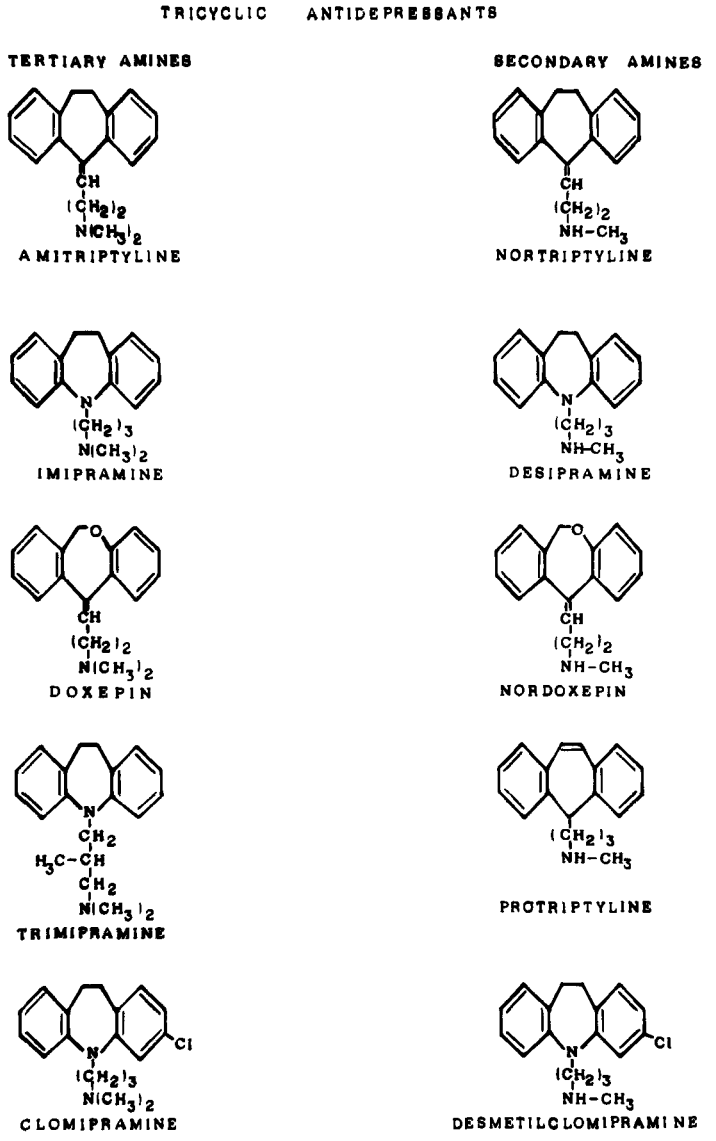

Figure: Molecular structure of common tricyclic antidepressants.

consequent increase in free fatty acids(known displacing agents) has been proposed, the exact mechanism involved is uncertain and still matter of controversies(17).

Samples may be left several days at room temperature(18) and, when frozen at -20°C, for at least six months(19) without changes in TCAs amounts.

Extraction and Recovery:

Classical liquid-liquid extraction procedures are carried out at high alkaline pH with the use of a relatively nonpolar solvent, such as n-hexane, n-heptane, diethyl-ether, etc., and can involve one, two or three steps. Usually, the first extraction step is performed with a suitable solvent followed by re-extraction of TCAs into dilute acid(i.e. ortho-phosphoric , hydrochloric, sulphuric acid, etc.). This removes most acidic and neutral interfering compounds and leads to sufficiently clean extract that requires no further purification. Examples of this type of procedures are summarized in Tables 1-3 (20,21,22).

Extraction recovery is one of the most crucial points in the analysis of TCAs.Due to their physico-chemical properties, in fact,these drugs become easily attached to the glassware and are easily lost during extraction-evaporation procedures. To lessen such problems, all glassware should be carefully silanized or acid-washed and rinsed with a solvent(methanol,hexane or, most commonly, hexane-isoamyl alcohol mixture) before the use. The evaporation temperature should be strictly controlled and tubes promptly removed from the water bath after the solvent had evaporated. To minimize drug losses during extraction, addition of isoamyl alcohol or butanol to the solvent is a commonly made practice. This also helps to prevent emulsion formation. All these precautions have been frequently underlined by different authors(23,24,25,26).As a general rule, since both recovery and reproducibility can vary greatly, any unnecessary manipulation of the samples should be avoided.

TABLE 1

Scheme of a Liquid-Liquid Extraction Procedure Involving One Step and Operative Conditions, according to Sonsalla et al.(20).*

Extraction Procedure	
Plasma or serum	2 ml
Internal standard (3 µg of trimipramine+20 µg of protriptyline/ml of methanol)	100 µl
Borate buffer	1 ml
Hexane/isoamyl alcohol (98/2, v/v)	6 ml
After agitation and centrifugation, transfer solvent and evaporate at 40°C to dryness; reconstitute with 100 µl of mobile phase; inject 25 µl.	

Operative Conditions	
Column	Varian SI-10 (4 x 300 mm)
Mobile Phase	Methanol/NH_4OH/NH_4NO_3 (95/3/2, v/v/v)
Flow rate	1.7 ml/min
Column temperature	25°C
Detector	UV, 254 nm
Sensitivity	0.02 aufs

Recovery: not reported; Precision: within-run CV< 8.8% , day-to-day CV<10.0%

Detection limit: $\leq$ 15 ng/ml^; Linearity: 25 to 1000 ng/ml

* Drugs determined: amitriptyline, nortriptyline, imipramine, desipramine, doxepin, desmethyldoxepin.

^ For all drugs.

Liquid-liquid extraction usually yield recoveries of common TCAs ranging from 60 to 100%(21,22,26,27,28,29).

Liquid-solid extraction procedures have been also described for analyzing TCAs in plasma(30,31,32) and represent an efficient alternative to conventional liquid-liquid extractions. Several steps, e.g. shaking, centrifuging and transfer the extracts, are not required with this type of extractions; therefore, different sources of variability are avoided and the time of analysis is reduced. Columns are filled with either an hydrophilic(e.g. silica) or

TABLE 2

Scheme of a Liquid-Liquid Extraction Procedure Involving Two Steps and Operative Conditions, according to Wong & Mc Cauley(21)*.

Extraction Procedure	
Plasma or serum	2 ml
Internal standard(10 µg of clomipramine/ml of methanol)	160 µl
1N NaOH	2 ml
Hexane/isoamyl alcohol(99/1, v/v)	5 ml

After agitation and centrifugation, transfer the upper, organic phase to a tube containing 200 µl of 0.05% ortho-phosphoric acid; after agitation and centrifugation, transfer the lower phase and inject 50 µl.

Operative Conditions	
Column	uBondapack C-18
Mobile phase	0.05 M KH_2PO_4 at pH 4.7/Acetonitrile(6/4,v/v)
Flow rate	2 ml/min
Column temperature	50°C
Detector	UV, 254 nm
Sensitivity	0.001 aufs

Recovery: 65-75%; Precision: within-run CV < 3.7%; day-to-day CV < 6.7%
Detection limit: ~5 ng/ml^; Linearity: 25 to 800 ng/ml

* Drugs determined: amitriptyline, nortriptyline, imipramine, desipramine.
^ For all drugs.

hydrophobic(e.g. C_{18} bonded phase silica) material. In the former case, all the sample is retained and then the drug is extracted with small volumes of a water-immiscible organic solvent. Recovery values reported with such an extraction range from 75% for desipramine and nortriptyline to 85% for amitriptyline and imipramine and both the within-run and day-to-day CVs are below 10%(30).

Columns packed with an hydrophobic material trap only the drug, which then is eluted with an organic (in this case, water-miscible or water-immiscible) solvent. Recoveries and CVs reported are : 87.1% for desipramine to 96.0% for amitriptyline; within-run CV below 9.8%, day-to-day CV below 11.2%, respectively(31).

TABLE 3

Scheme of a Liquid-Liquid Extraction Procedure Involving Three Steps and Operative Conditions, according to Godbillon & Gauron(22).

Extraction Procedure	
Plasma or blood	1 ml
Internal standard	100 µl
(0.6 µg of clomipramine+1.5 µg of desmethylclomipramine/ or 0.35 µg of imipramine+1 µg of desipramine/ ml of an acidic solution)	
Borate buffer	1 ml
Heptane/isoamyl alcohol (99/1 v,v)	5 ml
After agitation and centrifugation, transfer the organic phase to another tube and add 1 ml 0.1 N H_2SO_4; after agitation and centrifugation, dip it into a mixture of methanol and solid CO_2 and discard the organic phase at room temperature to thaw, add 0.5 ml 1 N NaOH and 5 ml heptane-isoamyl alcohol; transfer the organic phase and evaporate at 60°C to dryness; reconstitute the residue with 300 µl of mobile phase and inject 150 µl.	

Operative Conditions	
Column	5 um LiChrosorb SI 60 (4.6 x 125 mm)
Mobile phase	Ethanol/hexane/dichloromethane/diethylamine ($30/62/8/5.10^{-3}$, v/v/v)
Flow rate	1.5 ml/min
Column temperature	ambient
Detector	UV, 254 nm
Sensitivity	1×10^{-4} AU

Recovery: 95.5-116.4%; Precision: within-run CVs: 2.1 to 16.0%; day-to-day CVs: not reported

Detection limit: 5 ng/ml for clomipramine and imipramine, 10 ng/ml for their mono-demethylated metabolites.

Linearity: 5-50 and 50-400 ng/ml for clomipramine and imipramine; 10-400 ng/ml for the metabolites.

More recently, a method,which circumvent evaporating concentrating step, has been described. This method involves the use of solid-phase cyanopropyl columns and the direct injection of the eluate onto the analytical column(32). The analytical recovery reported is 85 to 100%(mean=94.5%) and the within-run and day-to-day CVs are 0.9-2.2% and 2.1-3.8%,respectively, for all the drugs and metabolites determined(8-hydroxyamoxapine, amoxapine,doxepin,desmethyldoxepin,imipramine,desipramine, amitriptyline and nortriptyline)(32).

Internal Standard:

In most of the published methods, a single internal standard, either a secondary or tertiary amine, is used for the determination of both secondary and tertiary derivatives(26,31). Some authors, however, recommend the use of two internal standards,i.e. a tertiary plus a secondary amine, for a more accurate estimation of the parent drug and its metabolites(20,23,30,32).

LIQUID CHROMATOGRAPHIC SYSTEMS

Separation:

Separations of TCAs are carried out by using either a normal(20, 22,29,31,33,34) or reversed(21,26,27,28,35,36,37) phase column, the latter being probably the most widely used(7,38). As compared to the former,reversed-phase column has greater versatility, resistance to contamination and more rapid equilibration(7,38). In fact,due to the presence of hydrophilic endogenous plasma constituents and hydroxylated metabolites having a high affinity for the polar silica packing material and consequently long retention times, normal phase column necessitates long column-recovery times in between injections(26).Furthermore, some authors have pointed out that silica columns can yield constantly shifting retention times(34,39).

Another advantage,offered by the reversed-phase column over the normal phase column,is that the re-extraction step,necessary

with the latter, can be avoided ,since an aliquot of the aqueous phase can be injected directly into the chromatograph (21,26,27,36).

Problems of ionization can be minimized with the addition of an alkyl amine(i.e. ethyl-, propyl-, nonyl-, butylamine), which competes with the drug for binding to the anion sites on the packing. An improvement of the peak shape is obtained with this practice(40).

The paired-ion technique is another available tool to lessen the problem associated with drug ionization. This technique is currently being performed most successfully on permanently bonded reversed-phase columns and utilizes, as counterions, the sodium salt of alkylsulfonates, with the pH adjusted at low values(i.e. below 5). Drug elution is made at a pH below 7, ensuring a longer column life(26,33,35,41).

In comparison with the more conventional columns, some advantages are reported by using a CN bonded phase column(30,32,42,43). These advantages include shorter column equilibration times, the unnecessity of ion-pairing or ion-suppression with alkyl amines and, consequently, the possibility of using a detection wavelength at 210 nm, at which TCAs exhibit the highest absorption.

Mobile Phase:

For adsorption chromatography, a mixture of solvents, usually made by a nonpolar solvent with a small addition of a highly polar one, is generally required. The choice of these solvents may be very wide; examples of mixtures are those reported in the Tables 1 and 3(see also ref. 20,22,29,31,33,34). On the contrary, in reversed-phase chromatography the selection of a solvent system for drug separation is considerably simplified. In practice,due to its low viscosity allowing a low pumping pressure and giving high column efficiency, acetonitrile mixed with phosphate buffer is the most suitable and commonly used phase(7,38).

TCAs are typical compounds with basic properties and, therefore, an important condition for their optimal separation is the use of a mobile phase at pH $\geq$9. This can be classically used with normal phase columns(20,31), of which silica particles solubility is minimized in absence of water. In reversed-phase columns hydrolysis of the bonded groups occurs at pH $<$2, while at pH $>$7 the silica matrix itself dissolves(7). To extend the column stability at higher pHs, presaturation of the mobile phase with silica before it enters the analytical column has been obtained with the use of a silica-packed precolumn between the pump and the sample valve (32,44,45).

Column Temperature:

Although column may be used at ambient temperature, a temperature control could be preferable. The performance of a number of columns, in fact, is affected by as little as 2-4°C changes at ambient. Moreover, temperatures of 50-70°C can be advantageous, since different physico-chemical factors, such as the mobile phase viscosity and the sample solubility, may be optimized so that the overall system efficiency improves(38).

On the contrary, the stability of the column may be decreased at higher temperatures(7).

Detection:

For routine use, the ultraviolet spectrophotometer detector, at a wavelength of around 250 nm, is currently the most successfull because of reliability and sensitivity. Amounts of 5-20 ng/ml of most common TCAs can be detected with sufficient accuracy. By working at lower wavelength around 200 nm, detection sensitivity for TCAs increases. Under these conditions, however, substances such as ammonia, alkyl amines or alkylsulphonates cannot be added to the mobile phase because of their strong absorption and consequent increase in base-line noise. Methods that avoid these substances and utilize this wavelength are available in literature(27,30).

The use of a fluorimetric or an amperometric detector for the determination of imipramine and its metabolites has been described (35,36). The reported detection limit is around 1 ng/ml.

PRECISION AND ACCURACY

Interferences:

Several basic and neutral compounds might be partially extracted together with TCAs and, therefore, be a potential interference. However, the problem is lessen by different factors, which include very low amounts of the interfering substances in plasma, their poor absorbance at the wavelength used, their rare combination with TCAs in clinical practice. Usually, the authors report in their methods a list of interfering substances.Table 4 shows a number of the most important reported interferences.

TABLE 4

List of the Most Common Interferences with the TCAs Assay Reported in the Literature.

Interfering Drugs	Column used	Reference
Chlorpromazine-Amitriptyline	Varian-SI-10	20
" -Doxepin	"	"
Chlordiazeposside-Doxepin	uBondpack C18	26
Chlorazepate-	"	"
Thioridazine-	"	"
Promazine-Desipramine	"	"
Diethazine-Nortriptyline	"	"
Ethopropazine- "	"	"
Promethazine- "	"	"
Propoxyphene-Amitriptyline	"	21
Chlorpromazine-Clomipramine	"	"
Propoxyphene-Nortriptyline	Ultrasphere-ODS	27
Perphenazine-Amitriptyline	5 um Silica	31
Pyrilamine-Doxepin	"	"
Thioridazine-Imipramine	CN-Bonded	30
Propanolol- "	"	"

It is obvious that, in a laboratory devoted to therapeutic TCAs monitoring, detailed informations on the patient's drug regimen must be available so that potential interferences can be carefully checked.

Reproducibility and Linearity:

The within-run and day-to-day CVs, usually reported also at low drug concentrations, are below 10 and 15%, respectively, that are within the generally accepted limits for drug assays.

Calibration curves for TCAs are linear within a very wide range, from 20 to 2000 ng/ml and more.

CONCLUSIONS

As indicated by a considerable body of evidence, high-pressure liquid chromatography is a reliable and suitable technique for the determination of TCAs in plasma. Among all the LC methods described, no one offers clear advantages as compared to the others; therefore, the choice largely depends on the clinical or experimental situation where the method is being used and on the available equipment in the laboratory. However, if valid TCAs plasma level measurements are to be made, it is much more important, rather than the actual choice of methods, the application of appropriate internal and external quality control procedures(19,46).

As pointed out above, the sources of variability in the analysis of TCAs are numerous and errors can be introduced at several stages, including, for example, inappropriate analytical procedures, a technician's mistake, instrument failure, etc. Interlaboratory variability of TCAs determinations, in fact, still remains high (CV= 29%) (47).

The possibility of fully automated operation of the instrumentation (see the recent "Fast LC" systems,(48) and the availability of columns having a greater efficiency and reducing times of analysis(see,for example, "high speed" columns,(49,50) and microbore columns(51) represent future goals that, once achieved, will open new prospects for a wider and more effective application of LC to TCAs determinations.

REFERENCES

1. Cutler, N.R., Heiser, J.F. The Tricyclic Antidepressants. JAMA, 17, 2264, 1978.

2. Hollister, L.E. Current Antidepressant Drugs: Their Clinical Use. Drugs, 22, 129, 1981.

3. Hammer, W. and Sjöqvist, F. Plasma levels of monomethylated tricyclic antidepressants during treatment with imipramine-like compounds. Life Sci., 6, 1895, 1967.

4. Moody, J.P., Tait, A.C. and Todrick, A. Plasma levels of imipramine and desmethylimipramine during therapy. Br.J.Psychiat., 113, 183, 1967.

5. Task Force on the Use of Laboratory Tests in Psychiatry. Tricyclic Antidepressants-Blood Level Measurements and Clinical Outcome: An APA Task Force Report. Am.J.Psychiat., 142,155,1965.

6. Scoggins, B.A., Maguire, K.P., Norman, T.R., and Burrows, C.D. Measurement of Tricyclic Antidepressants. Part I. A Review of Methodology. Clin.Chem., 26, 5, 1980.

7. Giese, R.W. Technical Considerations in the Use "High-Perfomance" Liquid Chromatography in Therapeutic Drug Monitoring. Clin.Chem., 29, 1331, 1983.

8. Dennis, R. Recent advances in HPLC technology. Pharmacy International., 4, 142, 1983.

9. Nyberg, G., Mortensson, E. Quantitative analysis of tricyclic antidepressants in serum from psychiatric patients. J.Chromatogr., 143, 491, 1977.

10. Kraak, J.C., Bijster, P. Determination of amitriptyline and some of its metabolites in blood by high-pressure liquid chromatography. J.Chromatogr., 143, 499, 1977.

11. Chinn, D.M., Jennison, T.A., Crouch, D.J., Peat, M.A., Thatcher,G.W. Quantitative analysis for tricyclic antidepressant drugs in plasma o serum by gas chromatography-chemical-ionization mass spectrometry. Clin.chem., 26, 1201, 1980.

12. Spina, E., Ericsson, O., and Nordin, C. Analysis of Tricyclic Antidepressants in Serum and Plasma Yields Similar Results. Ther.Drug Monit., 7, 242, 1985.

13. Hullet, F.J., Levy, A.B., and Tachiki, K.H. Serum Imipramine Concentrations Lower Than Plasma Values. J.Clin.Psychiatry., 45,516,1984.

14. Zetin, M., Rubin, H.R., Rydzewski, R. Tricyclic antidepressant sample stability and vacutainer effect. Am.J.Psychiatry., 138, 1247, 1981.

15. Borga, O., Piafsky, K.M., and Nilsen, O.G. Plasma protein binding of basic drugs.Selective displacement from α_1-acid glycoprotein by tris(2-butoxyethyl)phosphate. Clin.Pharmacol.Ther., 22, 539, 1977.

16. Wood, M., Shand, D.G., and Wood, A.J.J. Altered drug binding due to the use of indwelling heparinized cannulas(heparin lock) for sampling. Clin.Pharmacol.Ther., 25, 103, 1979.

17. Naranjo, C.A., Abel, J.G., Sellers, E.M., and Giles, H.G. Unaltered diazepam plasma binding using indwelling heparinized cannulae for sampling. Br.J.Clin.Pharmacol., 9, 103, 1980.

18. Burch, J.E., Raddats, M.A., and Thompson, S.G. Reliable routine method for the determination of plasma amitriptyline and nortriptyline by gas chromatography. J.Chromatogr., 162, 351, 1979.

19. Orsulak, P.G. and Gerson, B. Therapeutic Monitoring of Tricyclic Antidepressants: Quality-Control Considerations. Ther. Drug Monit., 2, 233, 1980.

20. Sonsalla, P.K., Jennison, T.A., and Finkle, B.S. Quantitative Liquid-Chromatographic Technique for the Simultaneous Assay Tricyclic Antidepressant Drugs in Plasma o Serum. Clin. Chem., 28, 457, 1982.

21. Wong, S.H.Y., and McCauley, T. Reversed Phase High-Perfomance Liquid Chromatographic Analysis of Tricyclic Antidepressants in Plasma. J.Liquid Chromatogr., 4, 849, 1981.

22. Godbillon, J., and Gauron, S. Determination of clomipramine or imipramine and their mono-demethylated metabolites in human blood or plasma by high-perfomance liquid chromatography. J.Chromatogr., 204, 303, 1981.

23. Sonsalla, P.K.,Jennison, T.A., Finkle, B.S. Importance of evaporation conditions and two internal standards for quantitation of amitriptyline and nortriptyline. Clin.Chem., 28, 1401, 1982.

24. Gupta, R.N., Molnar, G., Hill, R.E.,Gupta, M.L. Estimation of amitriptyline and its metabolites in serum and urine by G.L.C. using nitrogen-specific detector. Clin.Biochem., 9, 247, 1976.

25. Antal, E., Morcik, S., Kramer, P. Technical considerations in the gas chromatographic analysis of desipramine. J.Chromatogr., 183, 149, 1980.

26. Proelss, H.F., Lohman, H.J., and Miles,D.G. High-Performance Liquid-Chromatographic Simultaneous Determination of Commonly Used Tricyclic Antidepressants. Clin.Chem., 24, 1948, 1978.

27. Kabra, P.M.,Mar, N.A., and Marton, L.J. Simultaneous liquid chromatographic analysis of amitriptyline, nortriptyline, desipramine, doxepin , and nordoxepin. Clin.Chim.Acta, 111, 123, 1981.

28. Brodie, R.R., Chasseaud, L.F., and Hawkins, D.R. Separation and measurement of tricyclic antidepressant drugs in plasma by high-performance liquid chromatography. J.Chromatogr., 143, 535, 1977.

29. Watson, I.D., and Stewart, M.J. Quantitative determination of amitriptyline and nortriptyline in plasma by high-performance liquid chromatography. J.Chromatogr., 132, 155, 1977.

30. Thoma, J.J., Bondo, P.S.,and Korak, C.M. Tricyclic Antidepressants in Serum by a Clin-Elut™ Column Extraction and High Pressure Liquid Chromatographic Analysis. Ther.Drug Monit., 1, 335, 1979.

31. Beierle, F.A. and Hubbard, R.W. Liquid Chromatographic Separation of Antidepressant Drugs:I. Tricyclics. Ther.Drug Monit., 5, 279, 1983.

32. Lensmeyer,G.L. and Evenson,M.A. Stabilized Analysis of Antidepressants Drugs by Solvent-Recycled Liquid Chromatography: Procedure and Proposed Resolution Mechanisms for Chromatography. Clin.Chem., 30, 1774, 1984.

33. Mellström, B. and Braithwaite R. Ion-pair liquid chromatography of amitriptyline and metabolites in plasma. J.Chromatogr., 157, 379, 1978.

34. Vandemark, F.L., Adams, R.F., and Schimdt, G.J. Liquid chromatographic procedure for tricyclic drugs and their metabolites in plasma. Clin.Chem., 24, 87, 1978.

35. Suckow, R.F. and Cooper, T.B. Similtaneous Determination of Imipramine, Desipramine, and their Metabolites in Plasma by Ion-Pair Reversed-Phase High-Performance Liquid Chromatography with Amperometric Detection. J.Pharm.Sci., 70, 257,1981.

36. Reece , P.A., Zacest, R. and Barrow, C.G. Quantification of imipramine and desipramine in plasma by high-performance liquid chromatography and fluorescence detection. J.Chromatogr., 163, 310, 1979.

37. Wallace, J.E.,Shimek, E.L.,Jr., and Harris, S.C. Determination of tricyclic antidepressants by high-performance liquid chromatography. J.Anal.Toxicol., 5, 20-23, 1981.

38. Yost, R.W., Ettre, L.S., Conlon, R.D. Practical Liquid Chromatography. An Introduction. Perkin-Elmer, 1980.

39. Sutheimer, C. Improvements in the liquid chromatographic separation of some tricyclic antidepressants. Chromatogr. Newslett., 7, 38, 1979.

40. Wherli, A., Hildebrand, J.C.,Keller, H.P., Stampfli,R., Frei, R.W. Influence of organic bases on the stability and separation properties of reversed-phase chemically bonded silica gels. J.Chromatogr., 149, 199, 1978.

41. Mellström, B. and Eksborg, S. Determination of chlorimipramine and desmsthylchlorimipramine in human plasma by ion-pair partition chromatography. J. Chromatogr., 116, 475, 1976.

42. Supelco S.A., HPLC Reporter. Supelcosil LC-CN Columns Simplify Analyses for Tricyclic Antidepressants. Vol.III, No.1, 1981.

43. The Supelco Reporter, Monitoring antiarrhythmic drugs and tricyclic antidepressants by HPLC, 1(1), 8, 1982.

44. Atwood, J.G., Schmidt, G.J., Slavin, W. Improvements in liquid chromatography column life and method flexibility by saturating the mobile phase with silica. J. Chromatogr., 171, 109, 1979.

45. Schmidt, G.J., Vandemark, F.L. Liquid chromatographic separation of tricyclic antidepressant drugs using high pH mobile phases. Chromatogr. Newslett., 7, 25, 1979.

46. Jones, S., Turner, P. An external quality control scheme for tricyclic antidepressants. Postgraduate Med.J.Suppl., 56, 94, 1980.

47. Walters, M. Quality control and therapeutic drug monitoring. Pathologist, 37, 247, 1983.

48. Bannister, S.J., van der Wal, S.J., Dolan J.W., Snyder, L.R. Analysis in serum for the commonly used tricyclic antidepressant drugs and their metabolites by high performance liquid chromatography, using the Technicon Fast-LC system. Clin.Chem., 27, 849, 1981.

49. DiCesare, J.L., Doug, M.W., Vandemark, F.L. Instrumentation and columns for very high-speed LC. Am.Lab., 52-64(Aug.), 1981.

50. DiCesare, J.L., Dong, M.W., Ettre, L.S. Very-high-speed liquid column chromatography. The system and selected applications. Chromatographia, 14, 257, 1981.

51. Scott, R.P.W. Microbore columns in liquid chromatography. J. Chromatogr.Sci., 18, 49, 1980.