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REVIEW

ANALYSIS OF TRICYCLIC ANTIDEPRESSANT DRUGS IN PLASMA AND SERUM BY CHROMATOGRAPHIC TECHNIQUES

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CONTENTS

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1. INTRODUCTION

Antidepressant drugs (Fig. 1) represent the first choice in the treatment of moderate to severe depressive illness [1]. Successful therapy with these agents is dependent on many factors and there is no a priori method for the selection of responders and non-responders to treatment. One approach to treatment has been based on the titration of plasma drug concentrations to within narrow therapeutic ranges. This has been, and still remains, a controversial area of clinical practice. Routine monitoring of steady-state concentrations of antidepressants has not been universally adopted [2,3].

A more pragmatic approach has been to suggest that monitoring plasma concentrations may be useful in certain clinical situations e.g. in checking patient compliance, a major reason for therapeutic failure with antidepressants, and these have been discussed elsewhere [4]. A major application of methodology has been in an understanding of the pharmacokinetics and metabolism of these drugs. This use provides a unique challenge to the analyst — to develop a method with adequate sensitivity, usually in the range 1—300 μg/l, and precision for the data generated to be meaningful. A review of chromatographic analytical techniques for tricyclic antidepressants in blood, plasma or serum up to the period May—June 1984 is presented here with the emphasis on application to the clinical situation. Although the topic of the analysis of tricyclic antidepressants has been reviewed before [5—7] recent developments in the application of high-performance liquid chromatographic (HPLC) techniques to the problem merit further discussion.

Abbreviations used in this paper are given in Table 1.

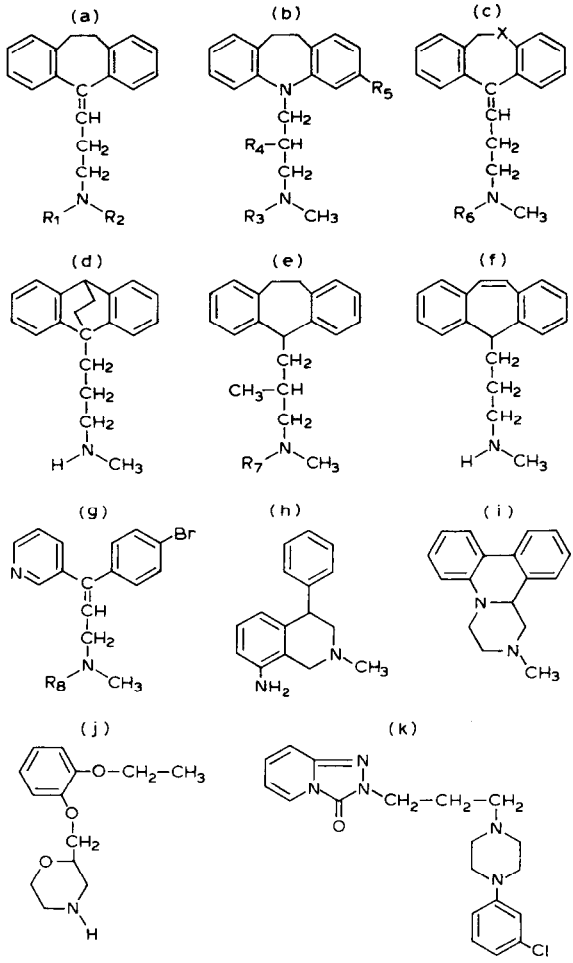
TABLE 1

LIST OF ABBREVIATIONS

AMOX	Amoxapine	LOX	Loxapine
AT	Amitriptyline	MAP	Maprotiline
BUT	Butriptyline	MCLOB	Moclobamide (Ro 11-1163)
CMI	Clomipramine	MIAN	Mianserin
CMN	3-Chloro-4-methoxy nomifensine	β-NA	β-Naphthylamine
DB	2-(Dibenz[*b*,5]azepin-5-yl)-N-methylethylamine	NBUT	Norbutriptyline
		N-DCMI	N-Desmethyl clomipramine
DMI	Desipramine	NOMI	Nomifensine
DCMI	Desmethylclomipramine	NORTH	Northiaden
DEXT	Dextorphan	NT	Nortriptyline
DDOX	Desmethyldoxepin	NZIM	Norzimelidine
DMAP	Desmethylmaprotiline	OXAP	Oxaprotiline
DNT	Desmethylnortriptyline	PT	Protriptyline
DOTH	Dothiepin	TRA-IS	2-[3-(*m*-Chlorophenyl-1-piperazinyl) propyl]-5-methyl-4-phenyl-triazol-3-(2H)-one
DOX	Doxepin		
DSO	Dothiepin sulphoxide		
DTRI	Desmethyltrimipramine	TRAZ	Trazadone
E-NZIM	*E* Isomer of norzimelidine	TRI	Trimipramine
GI	Geometric isomers	VIV	Viloxazine
IMI	Imipramine	ZIM	Zimelidine

1.1. Definition of terms

Any meaningful discussion of analytical techniques is based on a clear understanding of the terminology used. Inevitably the terms accuracy, precision and



(a) Amitriptyline — $R_1 = CH_3$, $R_2 = CH_3$
Nortriptyline — $R_1 = CH_3$, $R_2 = H$
Desmethylnortriptyline — $R_1 = H$, $R_2 = H$
(b) Imipramine — $R_3 = CH_3$, $R_4 = H$, $R_5 = H$
Desipramine — $R_3 = H$, $R_4 = H$, $R_5 = H$
Clomipramine — $R_3 = CH_3$, $R_4 = H$, $R_5 = Cl$
Desmethylclomipramine — $R_3 = H$, $R_4 = H$, $R_5 = Cl$
Trimipramine — $R_3 = CH_3$, $R_4 = CH_3$, $R_5 = H$
(c) Doxepin — $R_6 = CH_3$, $X = O$
Desmethyldoxepin — $R_6 = H$, $X = O$
Dothiepin — $R_6 = CH_3$, $X = S$
Northiaden — $R_6 = H$, $X = S$
(d) Maprotiline
(e) Butriptyline — $R_7 = CH_3$
Norbutriptyline — $R_7 = H$
(f) Protriptyline
(g) Zimelidine — $R_8 = CH_3$
Norzimelidine — $R_8 = H$
(h) Nomifensine
(i) Mianserin
(j) Viloxazine
(k) Trazadone

Fig. 1. Structures of tricyclic antidepressants and related drugs.

sensitivity are invoked to compare or characterise methods. In this review, accuracy is taken to mean the closeness of agreement between the true value and the mean result. Precision is the closeness of agreement between results obtained by applying the analytical procedure several times (this is often expressed as the percentage coefficient of variation, C.V.). Sensitivity is often confused with the detection limit. Sensitivity, as defined by IUPAC, is the change in measured value resulting from a change in concentration of one unit. Detection limit is the smallest amount of substance which can be detected by the analytical procedure.

Selectivity is an important criterion in an evaluation of a drug assay. For the tricyclic antidepressants, which undergo extensive first-pass metabolism leading to a number of metabolites, chromatographic techniques should provide an adequate separation of parent compound and metabolites. In many instances the metabolites are themselves pharmacologically active and should be quantitated by the assay as well.

2. COLLECTION, STORAGE AND STABILITY OF SAMPLES

Most analytical techniques have been applied to the measurement of antidepressants in blood components. Plasma, collected in heparinised glass or polystyrene containers, has been most widely studied, but serum, whole blood or erythrocytes have also been utilised. Once separated by centrifugation, plasma samples are stable at room temperature for several days [8] and when stored at −20°C or below for several months (and probably indefinitely). For example, it has been calculated that the half-life of decomposition of nomifensine plasma concentrations in vitro is $9.4 \cdot 10^4$ days (i.e. 257 years!) at −20°C [9].

The use of Vacutainer tubes has been shown to lower plasma imipramine concentrations compared to those collected in polystyrene or glass tubes [10]. Similarly others have shown, using various analytical techniques, that Vacutainers lower total concentrations of tricyclic antidepressants in plasma [11—13]. This reduction is caused by the inhibition of binding of these compounds to plasma protein, primarily α_1-acid glycoprotein, by tris(2-butoxyethyl) phosphate, a plasticiser contained in the stopper of the Vacutainer tubes [14]. The increased free drug concentration is redistributed between plasma and erythrocytes causing the observed effect.

The practice of using an indwelling needle, flushed with heparin, to collect blood samples (e.g. in pharmacokinetic experiments) has been questioned [7]. While in some studies the free fraction of propranolol [15], diazepam [16,17], chlordiazepoxide [17], oxazepam [17] or salicylates [18] has been shown to increase after heparin injection, others have not observed this effect [19,20]. Because antidepressants are also highly bound to lipoproteins [21,22], heparin-induced increases in free drug concentrations may also occur. If this were the case, low plasma concentrations would be measured since a greater proportion of the drug would be distributed into the erythrocytes. This question does not appear to have been evaluated. Clearly if the effect does occur, then in pharmacokinetic studies the use of whole blood concentrations or a cannula-kept patent with physiological saline would overcome the problem.

Most studies of antidepressant drug concentrations use plasma, but serum

concentrations show a 1:1 correspondence with plasma [23]. On the other hand, erythrocyte concentrations show a variable relationship both for the individual [24] and the drug studied [25]. The choice of biological material should be tempered by the final application. In the following discussion all of the methods discussed have been applied to plasma and/or serum determinations.

2.1. Extraction of plasma samples

It is not possible in the techniques described here to use direct injection of the plasma or serum sample, mainly because this diminishes the lifetime of chromatographic columns and produces a plethora of peaks in the chromatogram, which defy sensible interpretation. Consequently some form of extraction process is necessary. The majority of antidepressants are lipophilic strong bases and accordingly at high pH values the free base can be extracted into an organic solvent from aqueous solutions. High recoveries of radiolabelled antidepressants from plasma at various pH values have been observed (see Fig. 2). At acidic pH the base can be re-extracted into an aqueous phase. This behaviour allows for the separation of antidepressants from plasma constituents and provides a relatively pure sample for chromatographic analysis.

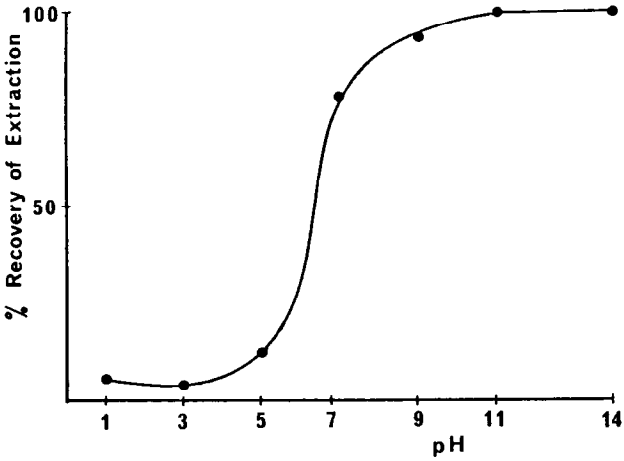


Fig. 2. Recovery of [^{3}H]nomifensine (ca. 900 ng) from 4 ml of plasma as a function of pH. Recovery was complete at a pH of 11 or more. Only a one-step extraction into *n*-hexane was evaluated (unpublished data from authors' laboratory).

Usually a three-step extraction procedure is used, however, as little as one step may be sufficient for some applications. In our laboratories a three-step extraction has been found necessary for sample clean-up. The procedure, outlined in Fig. 3, has been applied to all the major antidepressant drugs. The choice of solvent in the first step is not critical. Most authors select *n*-hexane or *n*-heptane but various fractions of light petroleum, diethyl ether, ethyl acetate or acetonitrile give satisfactory recovery of antidepressants from plasma. A small amount (1—5%) of isoamyl alcohol is sometimes used in the initial extraction solvent to increase the polarity of the medium and to inhibit emulsion formation. The purity of the solvents used is often critical to the results achieved. Distillation of the solvent immediately prior to its use in the extrac-

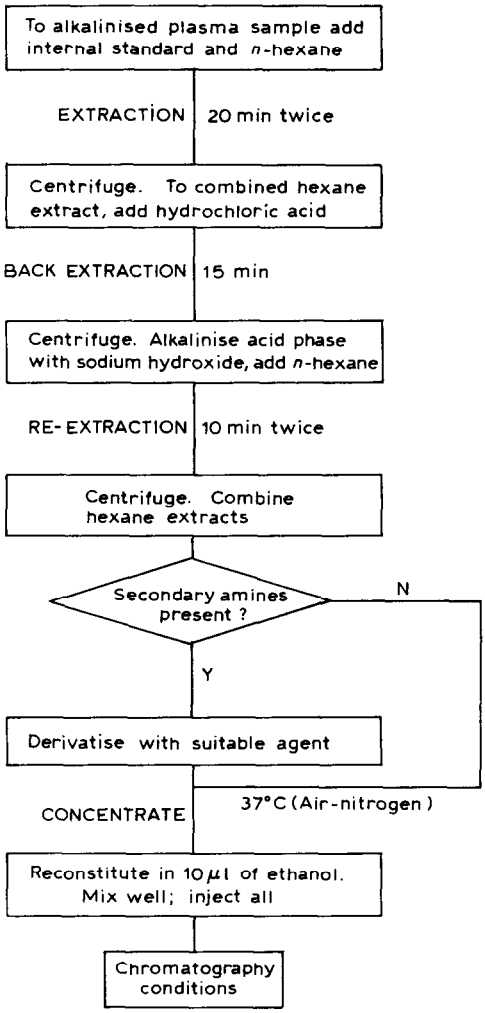


Fig. 3. Scheme for the three-step extraction of antidepressants from plasma. The method is applicable to most antidepressants without modification. Choice of solvent is not critical (see text) but high purity is essential. This general scheme has been applied to sample pretreatment for GC and HPLC in the author's laboratory.

tion is sometimes recommended, but the use of chromatographic grades of solvents (e.g. nanograde or HPLC grade) usually ensures satisfactory chromatograms. For re-extraction into an aqueous phase a mineral acid, usually dilute hydrochloric or sulphuric acid, is appropriate. An organic acid or an appropriate buffer may also be satisfactory, but oxidising acids such as perchloric or nitric acid are probably best avoided. Overall recovery for a three-step extraction is of the order of 60—80% while for the individual steps it is around 90%. Recovery also depends on the biological matrix, so the standard curve should be constructed from samples in a medium appropriate for the applied analysis.

Other extraction techniques have come into vogue for sample clean-up prior to drug analysis and these may have advantages over a three-step procedure, at least in terms of analysis time. The use of C_{18} bonded-phase columns for

sample clean-up has been described [26,27]. Washing with methanol and water activates the columns before the plasma samples are applied; the columns are washed with acetic acid and methanol, then eluted with organic solvent [26] or various ion-pairing mixtures, e.g. ammonium acetate in methanol [27]. Equivalent results for drug concentrations measured by this and the three-step method have been demonstrated [26,27]. An automated separation with a styrene divinylbenzene copolymer in a reversible centrifuge has been used for the extraction of antidepressant drugs [28]. While the effectiveness of this method, its precision and sensitivity were demonstrated in plasma standards it was not applied to clinical samples. The utility of this method awaits further evaluation. A simplified use of CN-bonded silica as an extraction technique has been demonstrated [29]. Amberlite XAD-2 resin has also been used for extraction of a variety of psychotropic drugs, but at the concentrations evaluated it seems that the technique will only be useful for forensic analysis [30]. Differential extraction of amitriptyline and nortriptyline on cellulose has also been demonstrated [31,32]. It seems likely that disposable bonded-phase columns will be increasingly used in routine analysis laboratories for the extraction of antidepressant drugs. As noted, the main advantage is the considerable saving of time. Further evaluations should be carried out, with column extraction techniques applied to the whole range of antidepressant drugs.

2.2. Urine and other biological samples

The analysis of urine, blood, saliva or cerebrospinal fluid samples for tricyclic antidepressants can usually be accomplished using the extraction techniques described for plasma. In routine analysis only blood or saliva will be encountered and occasionally cerebrospinal fluid samples. Urine analysis is most often used for the identification of drug metabolites or drugs used in overdose. In some pharmacokinetic studies 24-h urine samples may be collected in order to quantitate the amount of a dose excreted. In this case, as in overdose, the concentrations of drug are usually high enough (microgram range) as to not present a difficult analytical problem. For drug metabolite studies thin-layer chromatography (TLC) or HPLC is used to separate components and identification can be accomplished by mass spectrometry (MS). A computerised system for identification of tricyclic antidepressants and their metabolites has recently been described [33].

3. ANALYTICAL METHODOLOGIES

Interest in the measurement of antidepressant plasma concentrations grew from the notion that differences in patient responses to psychotropic drugs might be related to concentrations of the drug in various body fluids [34]. This proved to be a formidable task because the analytical techniques of the day had neither adequate sensitivity nor selectivity to accomplish it. The problem of selectivity remains today: because of their extensive metabolism [35] a large number of active metabolites of antidepressants are produced and in assessing relationships to clinical effects these must be quantitated.

Spectrophotometric and fluorimetric techniques lack adequate sensitivity

and/or selectivity for routine application. A number of other methods, not discussed in detail in this review, have demonstrated good precision, sensitivity and selectivity for routine application. Isotope derivative dilution analysis, radioimmunoassay and radioreceptor assay have all been used for analysis of antidepressants [7,36]. Isotope dilution assays are tedious, but no more so than the extractions required for gas chromatography (GC). Radioimmunoassays provide a sensitive and rapid analysis from small plasma volumes. Usually they are direct assays eliminating the need for plasma extraction. They have the disadvantage that they are often not selective, since the antisera raised against a secondary amine will cross-react to varying degrees with tertiary amines [37]. In certain indications or where only a secondary amine antidepressant is prescribed, radioimmunoassay is the method of choice. Radioreceptor assay is another non-selective analysis based on the displacement of a drug from a specific, high-affinity receptor site on (usually) neuronal membranes [38]. The muscarinic—cholinergic receptor has been utilised in an assay for antidepressants [39]. This requires extraction of the plasma samples and separation of secondary and tertiary amines to improve selectivity. Like radioimmunoassay this method would be useful for a rapid check of patient compliance or detection of excessive drug concentrations.

All of the above methods can be applied to analyses in clinically relevant situations. By far the most commonly used methods of antidepressant analysis in plasma are those based on chromatographic techniques. There is a vast literature on gas—liquid chromatography (GLC) and more recently the number of methods based on HPLC have expanded rapidly. It is not the intention of this review to attempt to describe all of the methods in detail but rather to point to some common features. For this reason extensive use is made of tables so that the reader can readily refer to original sources. Papers which are not related to clinical applications are ignored, reflecting a personal bias of the authors. However, methods described here could readily be adapted for other applications, e.g. tablet formulation. The application of plasma drug analysis to clinical psychopharmacology has assumed an important role in therapy.

3.1. Gas—liquid chromatography

GLC is a highly selective technique and based on the type of detector employed, has sufficient sensitivity for use in routine measurement, in pharmacokinetic studies and forensic analysis. An extensive clean-up of plasma samples is necessary and the majority of authors use a three-step procedure, although one step is sometimes employed. A number of GLC methods are summarised in Tables 2 and 3 according to the type of chromatographic detector.

3.1.1. Flame ionisation detector

The flame ionisation detector is the least sensitive of detectors and accordingly relatively large volumes of plasma (3—5 ml) need to be extracted in order to ensure adequate sensitivity and detection limits. The first practical flame ionisation detection (FID) analysis was described for AT and NT [40,41]. In this method, the extracted samples were measured from a standard curve constructed from direct injection of non-extracted solutions into the chromatograph. This

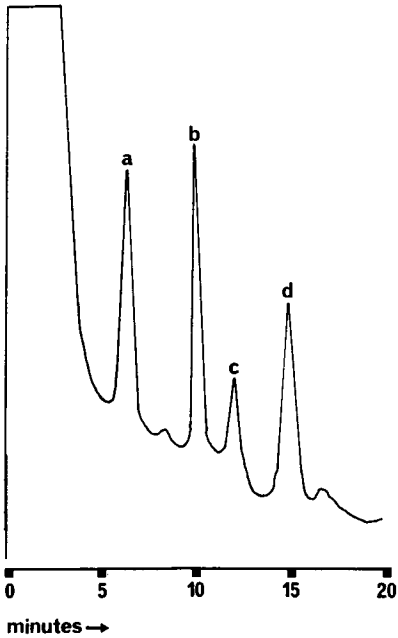


Fig. 4. Separation and flame ionisation detection of doxepin (100 μg/l) and desmethyldoxepin (100 μg/l) from internal standards on a glass column (2 m × 2 mm) packed with 3% OV-17 on Gas Chrom Q 80—100 mesh. Plasma (5 ml) was extracted by a three-step procedure and secondary amines derivatised with trifluoroacetic anhydride (TFA) (J.M.E. Wurm, T.R. Norman, K.P. Maguire, unpublished results). Peaks: a = doxepin; b = clomipramine; c = desmethyldoxepin-TFA; d = maprotiline-TFA.

overestimates plasma concentrations since procedural losses in the assay are not accounted for. The discrepancy is more serious for a three-step than one-step extraction. Further, the internal standard was added at the end of the extraction rather than at the beginning. The internal standard(s) compensates for errors introduced by factors that are otherwise difficult to control, e.g. variation in extraction or derivative formation. Both of these factors are important considerations in setting up new assays and most reported GLC methods take these into account. The choice of column packing is another consideration, a variety being employed. A common choice is 3% OV-17 which provides satisfactory resolution of tertiary and secondary amine drugs in a reasonable run time (see Fig. 4). Derivatisation, e.g. with acetic or trifluoroacetic anhydride, significantly improves the tailing of secondary amine peaks. Table 2 presents a summary of some FID methods for antidepressants. The limit of detection of these methods is around 10—20 μg/l. Selectivity, accuracy, precision and sensitivity of these methods are acceptable for routine monitoring and forensic applications but are not adequate for pharmacokinetic studies.

Thorough cleaning of glassware and high-purity solvents are essential in these methods to obtain good quality chromatograms. Due to the non-selectivity of the detector broad solvent fronts can be expected, which adds to the problem of resolution of peaks.

3.1.2. Electron-capture detector

A lower limit of detection and an increased sensitivity is afforded by the

TABLE 2

SOME GAS CHROMATOGRAPHIC METHODS FOR ANTIDEPRESSANTS BASED ON FLAME IONISATION AND ELECTRON-CAPTURE DETECTION

Drugs*	Reference	Plasma volume (ml)	Extraction steps and solvent	Derivative formed**	Detection limit (μg/l)	Precision (C.V., %)
Flame ionisation detection						
AT, NT	41	5	1; Light petroleum (b.p. 40°C—60°C)	TFA	20	5—7
AT, NT	84	3—5	3; Heptane***—iso-amyl alcohol, diethyl ether	n.d.	20	8—9
DOX, DDOX	85	5	3; Hexane	n.d.	10	11
AT, NT	86	5	3; Heptane	TFA	–	–
AT, NT, IMI, DMI, CMI, DCMI, PT	87	4	3; Hexane—isoamyl alcohol	n.d.	–	7—13
BUT	88	4	3; Hexane	n.d.	10	–
AT, NT	8	1—10	3; Heptane	n.d.	5	3—11
VIV	89	4	3; Hexane	Acetate	200	13
MAP	90	4	3; Hexane—isoamyl alcohol	n.d.	10	4—6
BUT	91	5	3; Heptane—isoamyl alcohol	n.d.	10	–
Electron-capture detection						
DMI	92	4	1; Benzene	TFA	1	3—7
NT and metabolites	93	4	3; Hexane	HFB	10	3—6
MAP	94	1	3; Heptane—isopropanol	HFB	10	3—10
NOMI	95	2	3; Diethyl ether	HFB	1	–
AT, NT	32	0.5	Cellulose column	TEC	1	6—8
ZIM, NZIM	78	2	3; Toluene	n.d.	10	<7
DOX, DDOX	96	1	1; Hexane	TEC	2.5	1—11
AMOX, 8-OH-AMOX, 7-OH-AMOX	97	1—3	3; Ethyl acetate	TFA TMS	1	–
TRI	98	1	3; Heptane	PFBC	< 5.0	7
VIV	99	1	1; Benzene	HFB	20	5

*For abbreviations, see Table 1.

**Abbreviations used are: TFA = trifluoroacetate; HFB = heptafluorobutyrate; TEC = trichloroethylchloroformate; TMS = trimethylsilyl; PFBC = pentafluorobenzyl chloroformate; n.d. = not derivatised.

***Heptane or hexane = *n*-heptane or *n*-hexane unless otherwise specified.

electron-capture detector. A limitation of its use is the need to convert drugs into a species which readily captures electrons, usually a halogenated compound. While this can readily be achieved for secondary amine drugs by conversion to a trifluoroacetyl or heptafluorobutyryl derivative, it represents a more complex analysis procedure for the tertiary amine antidepressants. Methods for DMI, NT, MAP, NOMI and ZIM have been described (see Table 2).

TABLE 3

SOME GAS CHROMATOGRAPHIC METHODS FOR ANTIDEPRESSANT MEASUREMENT BASED ON NITROGEN—PHOSPHORUS DETECTION

Drugs*	Reference	Plasma volume (ml)	Extraction steps and solvent	Derivative formed**	Detection limit (μg/l)	Precision (C.V., %)
IMI, DMI	46	1—3	3; Heptane***—isoamyl alcohol	n.d.	3—5	5—8
AT, NT	100	2	1; Hexane	Acetate	5—15	7
IMI	101	4	1; Heptane	n.d.	1—10	3—10
AT, NT	50	1—3	2; Pentane	Acetate	10—20	11—20
AT, NT	102	1	3; Heptane—isobutanol	n.d.	1	4—13
AT, NT	103	3	3; Hexane—isoamyl alcohol	n.d.	5	5—9
IMI, DMI	104	3	3; Hexane—isoamyl alcohol	TFA	5	5—6
AT, NT	105	2	3; Hexane	n.d.	5	4—19
NOMI	106	2	3; Diethyl ether	HFB	2	5—10
AT, NT	47	1	3; Hexane	n.d.	10	<5
DOX, DDOX *cis/trans* isomers	49	1	1; Hexane	TFA	2	3—8
IMI, DMI	107	2	3; Heptane—isoamyl alcohol	n.d.	1—2	–
DOX	108	2	3; Heptane—isobutanol, diethyl ether	n.d.	3	5—10
IMI, DMI	109	2	2; Cyclohexane	Acetate	5	10
MIAN	110	1	1; Hexane	n.d.	1	7
DMI	111	1	3; Hexane—butanol	n.d.	1	6
AT, NT, IMI, DMI, CMI, DCMI, MAP	51	2	1; Hexane—isoamyl alcohol	HFB	5	3—15
IMI, DMI, AT, NT, DOX, DDOX	112	0.5—4	3; Hexane—isoamyl alcohol, toluene—isoamyl alcohol	n.d.	0.75	3—20
MAP	113	0.5—2	2; Cyclohexane	Acetate	2	–
NOMI	79	1	3; Hexane	n.d.	5	8—20
AT, NT, IMI, DMI, DOX, DDOX, CMI, DCMI, TRI, DTRI	114	2	1; Hexane—isopropanol	n.d.	1	2—8
AT, NT, IMI, DMI	27	0.5	C_{18} Bonded-phase columns	n.d.	1	5—10
MIAN	115	2	3; Hexane—isoamyl alcohol	n.d.	2.5	8
AT, NT, IMI, DMI	116	2	3; Hexane—isopropyl alcohol	n.d.	5	6—14
AT, NT, IMI, DMI, CMI	48	1	3; Heptane—isoamyl alcohol	n.d.	10—20	3—10
MOCLOB	117	0.5—1	3; Ethyl acetate	n.d.	10	6—15

*For abbreviations, see Table 1.

**Abbreviations used are: TFA = trifluoroacetate; HFB = heptafluorobutyrate; n.d. = not derivatised.

***Heptane or hexane = *n*-heptane or *n*-hexane unless otherwise specified.

For the tertiary amines two approaches have been adopted: (1) quantitative oxidation to a species capable of electron capture or (2) derivatisation to form a halogenated compound. Oxidation of AT by ceric sulphate in sulphuric acid and subsequent chromatography has been described [31,42]. A series of other oxidising agents has also been investigated [43] and although the product of the reaction is claimed to be anthraquinone [42] this is disputed [44]. The nature of the product is not important provided quantitative conversion occurs. Conversion of tertiary amines to their carbamates by reaction with various chloroformate derivatives also produces a compound with good electron-capturing characteristics. This technique has been utilised for the determination of AT, IMI, TRI, CMI and DOX (see Table 2). A further method, which is only applicable to the antidepressant lofepramine because of its unusual side-chain, consists of side-chain reduction with sodium tetrahydroborate to the amino alcohol and oxidation to *p*-chlorobenzaldehyde [45]. Procedural losses with this method are probably high. GC with electron-capture detection (ECD) is well suited to all applications of antidepressant measurement, even if it is somewhat more difficult technically.

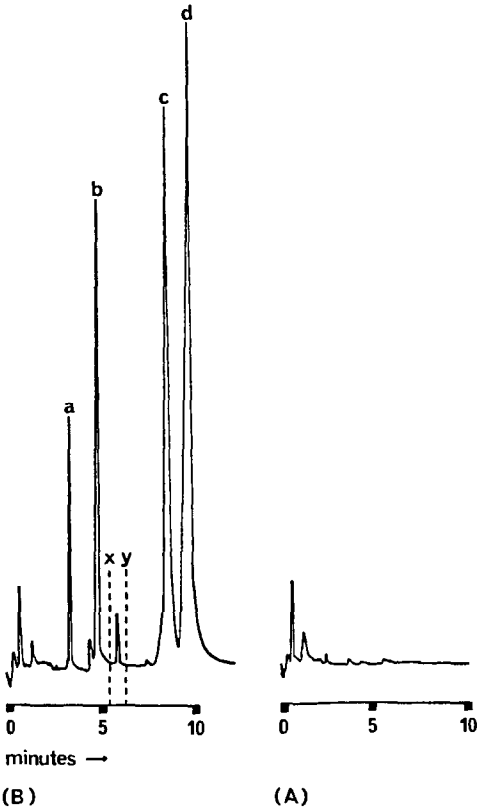


Fig. 5. Gas chromatogram of imipramine and desipramine with a nitrogen—phosphorus detector on 2% OV-101 on Chromosorb W HP (100—120 mesh). A three-step extraction and derivatisation with acetic anhydride was used for 2-ml plasma samples. (A) Blank plasma; (B) sample from a patient receiving 150 mg per day of imipramine (J.M.E. Wurm, T.R. Norman, K.P. Maguire and G.D. Burrows, unpublished results). Peaks: a = IMI; b = dothiepin; c = DMI-acetate; d = northiaden-acetate. y = Retention time of northiaden and x = retention time of DMI.

3.1.3. Nitrogen—phosphorus detector

The nitrogen—phosphorus detector is a modification of a conventional flame ionisation detector with a high selectivity for compounds containing nitrogen or phosphorus and a much lower detection limit than FID. Assay techniques based on FID methods have been published for all the major antidepressants using this detector but requiring smaller volumes of plasma (as little as 1 ml in some methods) (see Table 3). Three-step extraction procedures are usually required and, as before, derivatisation improves the resolution of secondary amine peaks (see Fig. 5).

Some innovations to the extraction process have been introduced when using the nitrogen—phosphorus detector. The final step of the extraction process using a volume of 50—100 μl of organic solvent avoids possible losses during concentration and reconstitution [46,47]. A bubble cap with capillary collector has been described for the recovery of as little as 25 μl of solvent at the final step [48]. The inclusion of two internal standards, one a tertiary and the other a secondary amine, is appropriate for evaluation of losses during derivatisation [49,50].

The majority of recently published methods for antidepressants are based on nitrogen—phosphorus detection (NPD) and this would appear to be the technique of choice for routine monitoring and pharmacokinetic analysis. As with FID a number of stationary phases have been used with 3% OV-17, again popular, although 3% OV-101 and 3% SP-2250 give equivalent results. Capillary columns have not been widely used but in one study [51] a comparison with a conventional-packed column showed higher peak resolution for the capillary column. Both gave reliable results for tricyclic antidepressants and their demethylated metabolites. Some recent reports [52—54] describe methods based on capillary columns for a number of commonly prescribed antidepressants.

3.2. High-performance liquid column chromatography

The principles of HPLC were recognised well before practical applications were made [55]. Separation of antidepressants was achieved by 1975 [56—58] but it was not until 1976 that the practicability of measuring clinical samples by this technique was demonstrated [59]. The technique has been increasingly applied to antidepressant measurement to the extent that it now rivals GLC—NPD as that most commonly used. Table 4 provides a summary of some methods for the common antidepressants and their major metabolites. Various technical aspects of HPLC as applied to therapeutic drug monitoring have recently been reviewed [60,172].

For the antidepressants the majority of methods are very similar, requiring prior extraction of the drug from plasma before application to the column. The type of chromatography performed has varied, but reversed-phase columns appear to be the ones of choice. Separation of drugs, metabolites and internal standards is readily achieved as, for example, in the analysis of imipramine (see Fig. 6). More recently the quantitation of the hydroxylated metabolites of the antidepressants has become important with the demonstration that they are pharmacologically active [61]. Several methods based on HPLC have been described which simultaneously measure parent drug and all active metabolites

TABLE 4

SUMMARY OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS FOR ANTIDEPRESSANT DETERMINATION

Drugs*	Reference	Plasma volume (ml)	Internal standard*	Extraction steps and solvent	Type of HPLC (column)	Detector** wavelength (nm)	Detection limit (μg/l)	Precision (C.V., %)
CMI, DCMI	59	2	TRI	3; Diethyl ether	Ion-pair partition (Diachrom 37—44 μm)	255	?	4.3 (CMI), 6.3 (DCMI)
AT, NT	118	4	AT derivative	3; Diethyl ether	Reversed-phase (Partisil 10 ODS)	254	5	4.7 (AT), 4.5 (NT)
MIAN	119	1	^{2}H	1; *n*-Hexane	Adsorption (μPorasil)	280	–	–
ZIM, NZIM	120	1	GI	1; Diethyl ether	Adsorption (Partisil 5)	258	25	7 (Both)
AT, metabolite	121	1	PT	1; *n*-Hexane	Reversed-phase (RP 8 and Nucleosil 10)	–	0.3	7 (AT)
CMI, DCMI	80	1	TRI	3; Diethyl ether	Ion-pair partition (Partisil 10)	254	–	7.3 (CMI)
NOMI, metabolite	122	2	CMN	1;Diethyl ether	Adsorption (Merckosorb s1100)	242	30	3.5 (NOMI)
AT, NT	123	2	DMI	1; Dichloromethane	Adsorption (Micropak)	240	10	10
CMI, DCMI	124	1—4	IMI	1; *n*-Hexane	Adsorption (LiChrosorb Si 60)	250	2 (CMI), 10 (DCMI)	–
IMI	66	4	^{2}H	1; *n*-Hexane	Adsorption (LiChrosorb Si 60)	250	0.4	8
AT, NT, IMI, DMI	125	2	PT	1; *n*-Hexane—isoamyl alcohol	Adsorption (Silica B/5)	211	10	5—8
AT, NT, DMI, IMI, DOX	62	2	β-NA	2; *n*-Hexane	Ion-pair partition (μBondapak)	254	2	2—7
AT, NT, metabolites	81	1	DB	3; Diethyl ether	Ion-pair partition (Partisil 5)	254	–	10 (AT), 5 (NT)
DOTH, NORTH	126	2	AT	1; Diethyl ether	Reversed-phase (Partisil 10)	231	10	1—11

AT, NT, IMI, DMI, DOX, DDOX	29	1	TRI, PT	Clin-Elut columns	Reversed-phase (various)	214	5	<10
IMI, DMI	127	2	TRI	2; Hexane	Reversed-phase (μBondapak)	252/360 Fluor	1	2—15
ZIM, NZIM	128	1	E-NZIM	2; Diethyl ether	Reversed-phase (Nucleosil C_{18})	254	1	2—9
AT, NT	129	0.5	PT	1; Hexane	Adsorption (Silica B/5)	235	10	5
IMI, DMI		2				270		
IMI, DMI, metabolites	130	1	N-DCMI	1; Hexane—butyl alcohol	Adsorption (Silica B/5)	240 exc. Fluor	1	2—4
AT, metabolites	131	3	DB	2; Hexane	Ion-pair partition (μBondapak C_{18})	254	<5	7
AT, NT, IMI, DMI	132	1	DDOX	3; Hexane—isoamyl alcohol	Reversed-phase (μBondapak C_{18})	210	–	5—7
2-OH-DMI	133	2	2-OH-IMI	2; Methylene chloride	Reversed-phase (Partisil ODS)	254	<10	5
AT, NT, IMI, DMI	134	2	Chloro-AT	2; Ethyl acetate, hexane, methanol	Reversed-phase (MicroPak MCH-10)	210	–	<6
VIV	135	0.5	DEXT	2; Butylchloride—butanol	Reversed-phase (?)	220 exc. Fluor	25	4—9
IMI, metabolites	136	2	None	2; Heptane—isoamyl alcohol	Reversed-phase (Hypersil ODS)	?	1—3	4
CMI, metabolites, IMI, metabolites	137	1	IMI, DMI, CMI, DCMI	2; Heptane—isoamyl alcohol	Reversed-phase (LiChrosorb Si 60)	254	5—10	2—16
IMI, DMI, metabolites	138	1	OH-CMI	3; Diethyl ether	Ion-pair partition (μBondapak C_{18})	ED	5	2—7
AT, NT, IMI, DMI, DOX, DDOX, PT	139	0.5	CMI	2; Isooctane—propanol	Reversed-phase (Bonded silica C_8)	205	6—12	4—17
AT, NT, IMI, DMI, DOX, DDOX	140	2	LOX	2; Butylchloride	Reversed-phase (Ultrasphere ODS)	200	5	4—8
MAP, OXAP	141	1	AT	3; Hexane	Adsorption (Nucleosil C_{18})	214	2	–
IMI, metabolites, AT, metabolites	142	1	NT, IMI	1; Isooctane—methyl-*tert.*-butyl ether	Adsorption (Partisil)	254	25	4
TRI, DOX, AT, IMI, DDOX, NT, DMI	143	1	PT	Automated processor, hexane	Reversed-phase (μBondapak-CN)	254	5	10

(Continued on p. 188)

TABLE 4 (continued)

Drugs*	Reference	Plasma volume (ml)	Internal standard*	Extraction steps and solvent	Type of HPLC (column)	Detector** wavelength (nm)	Detection limit (μg/l)	Precision (C.V., %)
AT, NT, metabolites	144	1	Propranolol	1; Hexane—butanol	Adsorption (Micropak Si 5)	220	2—16	2—10
AT, NT, IMI, DMI, DOX, DDOX, PT	145	1	TRI	1; Hexane—diethylamine	Reversed-phase (Ultrasphere cyano)	245	5	5—10
MIAN, metabolites	146	1	DMI	3; Methyl—*tert.*-butyl ether	Reversed-phase (Partisil 10 ODS-3)	ED	5	5—10
AT, NT, metabolites	147	1	LOX	3; Heptane—isoamyl alcohol	Reversed-phase (LC-1)	254	5	<9
AT, NT, *cis/trans* isomers, 10-OH metabolites	148	2	DMI	2; Hexane—isoamyl alcohol	Reversed-phase (Spherisorb C_6)	205	1	2—8
AT, NT, IMI, DMI, DOX, DDOX	149	2	TRI, PT	1; Hexane—isoamyl alcohol	Ion-pair partition (Silica)	254	≤15	<10
AT, NT, metabolites	150	1	Perazine	1; Hexane—isoamyl alcohol	Adsorption (LiChrosorb)	240	<5	4—10
AMOX, 8-OH-AMOX	151	2	8-MeO-LOX	Clin-Elut columns	Reversed-phase (μBondapak)	254	50	2—11.5
MAP, AMOX, metabolites	152	2	7-OH-LOX	2; Ethyl acetate	Reversed-phase (μBondapak)	214	1—3	5—8
TRAZ, MIAN	153	1	TRA-IS	2; Hexane—isoamyl alcohol	Reversed-phase (μBondapak)	214	5	3—7
AT, NT, IMI, DMI, DOX, CMI, MAP, PT	154	0.5	DCMI, DMI	1; Hexane—isoamyl alcohol	Adsorption (Silica)	214 or 254	≤5	5
AT, NT, IMI, DMI, DOX, DDOX, AMOX, 8-OH-AMOX	155	2	7-OH-LOX	1; Diethyl ether—chloroform	Reversed-phase (Zorbax cyano-propylsilane)	254	5	<4

*For abbreviations, see Table 1. ^{2}H = Deuterium-labeled.

**UV detector unless otherwise stated: detection wavelength given. ED = electrochemical detector; Fluor = fluorescence detector, detection wavelength noted.

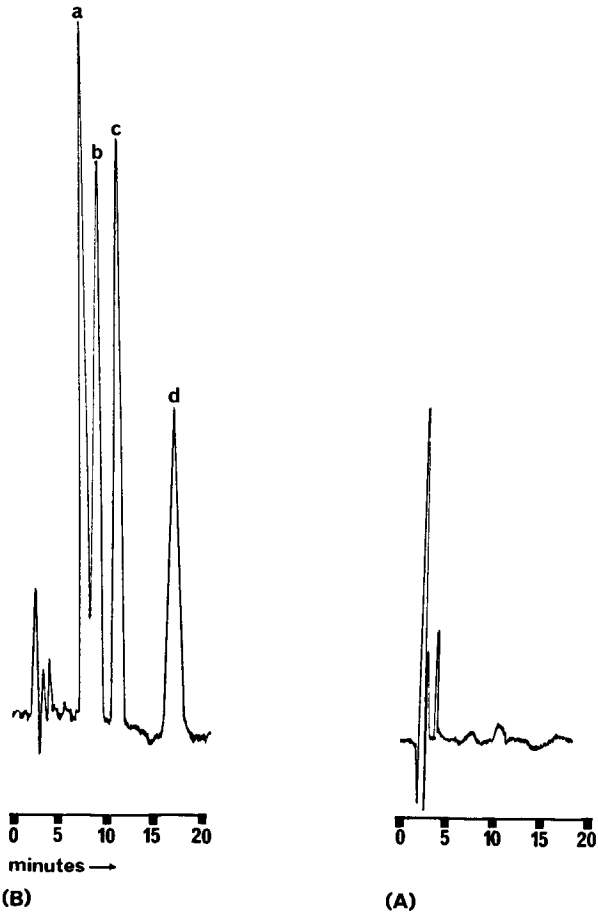


Fig. 6. Separation of imipramine (100 μg/l), desipramine (100 μg/l) from internal standards after a three-step extraction. Chromatography was carried out on a Waters Assoc. HPLC system consisting of a Model 45 solvent delivery system, U6K universal injector and 730 data module. Absorbance of the eluate was measured at 254 nm and a detector sensitivity of 0.005 A f.s. Mobile phase was acetonitrile—1% triethylamine in water (38:62) pH 6.0. A C_{18} Rad-Pak (Waters) column 10 cm × 0.8 cm in a radial compression Z module was used. (A) Plasma blank; (B) plasma standard (T.R. Norman and R.H. James, unpublished results). Peaks: a = desmethylcyanoimipramine; b = desipramine; c = imipramine; d = clomipramine.

(see Table 4). Quantitation using a fixed-wavelength, commonly 254 nm, or variable-wavelength UV detector is most often employed, but examples of fluorimetric or electrochemical detectors are found to be among published methods. Generally lower detection limits can be achieved with the latter detectors, but for routine application and single-dose studies UV detection is quite adequate.

Co-extraction and separation of co-prescribed drugs, i.e. selectivity of HPLC methods, has not always been adequately documented. While it is rare that more than one antidepressant is co-prescribed, benzodiazepines are commonly used for night sedation or anxiety symptoms. In addition non-prescription medications, e.g. codeine, aspirin and cough suppressants, are also used by many patients. The interference of these agents with reported methodologies

TABLE 5

SOME MASS FRAGMENTOGRAPHIC METHODS FOR ANTIDEPRESSANT DETERMINATION

Drugs*	Reference	Plasma volume (ml)	Extraction steps and solvent	Derivative formed**	Type of ionisation***	Detection limit (μg/l)	Precision (C.V., %)
NT, DNT, 10-OH-NT	74	2—3	3; Hexane	HFB	EI	<5	3—6
IMI, DMI	156	1	1; Hexane	Acetate	EI	10	–
CMI, DCMI, IMI	157	1	3; Heptane	PFP	EI	2	4
AT, NT, IMI, DMI, DOX, DDOX, PT	64	4	1; Light petroleum	TFA	EI	10	–
IMI, DMI	158	2	1; Hexane	TFA	CI	–	–
CMI, DCMI	159	2	3; Heptane	PFP	EI	2—5	4—6
MIAN	160	1—2	1; Hexane followed by HPLC	n.d.	EI	1	4 6—7
AT, NT, IMI, DMI, DOX, DDOX, PT	161	2	1; Hexane	TFA	CI	5	4—12
AT, NT	65	1	2; Hexane	TFA	CI	1	–
AT, NT, IMI, DMI, DOX, DDOX	162	2	3; Heptane—isobutanol	HFB	CI	1	5
AT, 10-OH-AT, NT, 10-OH-NT	163	1	3; Heptane—isobutanol	TFA	CI	0.5—1	3—7
CMI	164	0.5	1; Light petroleum followed by gel chromatography	n.d.	EI	–	3—8
CMI, DCMI	165	1	3; Diethyl ether	n.d.	CI	–	5
DOTH	166	1	1; Hexane	n.d.	CI	0.5	–
MAP, DMAP	167	1	3; Benzene	TFA	EI	2	5
AT, NT, IMI, DMI	168	2	1; Toluene—heptane—isoamyl alcohol	n.d.	CI	<10	3—11
DOTH, NORTH, DSO	63	1—2	3; Hexane	TFA	EI	1	4—20
IMI, DMI, 2-OH-IMI, 2-OH-DMI	169	2	1; pH 9, Ethyl acetate, pH 11, hexane—isopropanol	TFA	EI	–	–
MIAN	170	1	3; Hexane	n.d.	EI	<5	3—8
DOX, DDOX	171	1	2; Hexane—isoamyl alcohol	TFA	EI	1	7

*For abbreviations, see Table 1.

**HFB = heptafluorobutyrate; PFP = pentafluoropropionate; TFA = trifluoroacetate; n.d. = not derivatised.

***EI = electron-impact; CI = chemical ionization.

needs to be assessed when setting up HPLC methods (see for example ref. 62).

Further improvements in column and detector technology and wider dissemination of liquid chromatographs should ensure that HPLC remains in the forefront of antidepressant measurement techniques.

3.3. Mass fragmentography

In this technique a mass spectrometer is used as a detector for the effluent from a gas chromatograph. Simultaneous and continuous monitoring of mass spectral ions representing mass fragments of the drugs to be quantitated gives both total-ion (equivalent to a usual GLC trace) and single-ion (characteristic of the drug in the mixture) chromatograms. Most GC—MS combinations can monitor six or more ions simultaneously, this usually being sufficient to quantitate parent drug and metabolites. Mass fragmentography provides a very sensitive technique for drug measurement and has been widely applied to the antidepressants (Table 5). The amount of plasma required is small (usually 1 ml) but a three-step extraction procedure for sample clean-up is necessary. Derivatisation of secondary amines improves peak resolution.

GC—MS allows the use of stable isotope-labeled drugs as internal standard. Deuterium-labeled compounds are most often used although in principle ^{15}N- or ^{18}O-labeled drugs could also be employed. The advantage of deuterium is that these drugs can be readily produced by hydrogen exchange in $^{2}H_2O$ within the facilities available to most laboratories. The use of a deuterium-labeled internal standard ensures extraction, adsorption to glass and chromatographic performance identical to that of the unlabeled substance. In the mass spectrometer the two isotopic substances can be detected separately.

Both electron-impact (EI) and chemical ionisation (CI) procedures have been used to produce mass fragments. At high ionisation energies the tricyclic antidepressants undergo extensive fragmentation and produce a similar mass spectrum with a major peak at *m/e* of 58 due to loss of part of the side-chain [63,64]. For this reason, CI may be the technique of choice since greater structural integrity of the molecule is retained in the spectrum [65]. GC—MS has an obvious application in identification of drugs from overdose cases. Certainly for this use, structural integrity is required, as it is for drug metabolism studies to which the technique is also well suited. The coupling of an HPLC system to a mass spectrometer is a new development [66] but has not yet been extensively applied to drug analysis.

3.4. Thin-layer chromatography

Detection of substances on thin-layer plates can be achieved by an elution process or in situ measurement of compounds on the plates. Only direct evaluation (densitometry) of the plates has sufficient sensitivity, precision and accuracy necessary for quantitative analysis. The proper choice of solvents and plate-coating materials determines the success of separations [67]. Application to antidepressant measurement was first demonstrated for IMI and DMI [68]. After a one-step extraction from 5 ml of plasma, IMI and metabolites were separated on the TLC plate and quantified by densitometry of the intense

yellow spots formed on reaction with nitrous gases. Similar methods for the quantitation of AT and NT [69] and the separation of AT, IMI and their metabolites have been described [70].

So called high-performance thin-layer chromatography (HPTLC) has also been applied to antidepressant determination [71—73]. The plates used in this technique provide a better resolution and sensitivity than conventional TLC plates. As little as 2 μg/l from 1 ml of plasma can be detected. Adequate selectivity, precision and sensitivity has been demonstrated and the methods are widely applied to routine monitoring and pharmacokinetic studies.

4. COMPARISON OF METHODS

Inter- and intra-laboratory comparisons of drug assays are important to establish the validity of an assay. A number of reports have compared results from a series of plasma samples measured by different methods. For NT a comparison between GLC—ECD and mass fragmentography found that the latter was more sensitive [74]. In one study [75] a comparison of GLC and double-isotope derivative dilution assay showed a correspondence for plasma NT concentrations measured by both methods. Similarly radioimmunoassay and GLC show a good correlation for NT [37,76]. For a number of other antidepressants cross-correlations of methods based on widely different physicochemical techniques show good correlations. For IMI [77], ZIM [78], NOMI [79], chlorimipramine [80], AT [81] and MAP (see Fig. 7) comparative studies are available. An external quality control scheme has been attempted but the results were not satisfactory [54]. Further quality control schemes are required in order that reliability of assays can be established across studies.

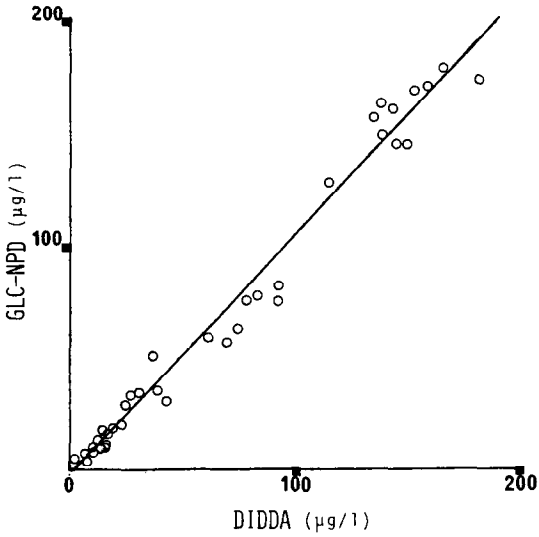


Fig. 7. A comparative study of plasma maprotiline samples dermined by GLC with a nitrogen—phosphorus detector and double-isotope derivative dilution assay (DIDDA). A total of 59 samples were obtained from six patients. A good correlation between the two methods was obtained: $y = 1.07X - 1.95$; $r = 0.99$ (I.M. McIntyre, J.M.E. Wurm, T.R. Norman, K.P. Maguire, B.A. Scoggins and G.D. Burrows, unpublished data).

5. APPLICATION OF METHODS

Some of the applications of the methodologies described here have been mentioned and a more detailed review is to be found elsewhere [82]. The majority of applications have been in two areas: (1) assessing the relationship between plasma concentration at pseudo steady-state and clinical effect and (2) in determining the pharmacokinetic parameters of the drugs. Not all authors agree on the necessity for routine monitoring of antidepressants, since the issue of therapeutic ranges remains controversial [2,4,82,83]. There are some excellent clinical reasons for monitoring of antidepressants. These include: (1) cases of therapeutic failure where non-compliance with drug prescription is suspected; (2) in toxic confusional states where high plasma concentrations are suspected as the cause; (3) in patients with pre-existing cardiovascular disease, where impaired cardiac function may lead to lowered clearance and the possibility of excessive plasma concentrations; (4) assessing the effects of co-prescribed medications on antidepressant levels; (5) in chronic disease states, especially renal and hepatic disease, where moderate drug doses may lead to extraordinary drug concentrations; (6) in the elderly who are more susceptible to the side-effects of drugs at moderate doses; (7) to assess the pharmacokinetic parameters of drugs after single and multiple oral doses.

The indication for measurement will determine which assay technique is chosen. While pharmacokinetic studies will require a method with a low limit of detection, good precision and sensitivity, e.g. GC—MS, most other applications can be readily accommodated with GLC—NPD, HPLC or HPTLC. For overdose studies GLC—FID is more than adequate.

6. CONCLUSIONS

Antidepressant drug monitoring will remain an important part of clinical practice and doubtless as new technologies evolve they will be applied to this purpose. For the present, GLC with NPD or HPLC are the techniques within the reach of most laboratories and, as noted, are well suited to analysis of this important group of drugs. GC—MS is also well suited, but the cost of the apparatus would preclude it from many laboratories. HPTLC may offer advantages in terms of the speed of sample processing, is adequate for routine analysis and the cost is low by comparison to other apparatus. The ultimate decision of which piece of equipment to buy will no doubt be determined by its utility for measuring other drugs and the anticipated demand for antidepressant analysis.

7. SUMMARY

A review of methods for the determination of tricyclic antidepressants in plasma or serum, based on the application of chromatographic techniques, is presented. A general discussion of the techniques in terms of their precision, accuracy, sensitivity and selectivity, with respect to parent drug and metabolites, is used to facilitate a comparison of methods. No one technique can be claimed as the method of choice for these drugs, although gas—liquid chromatography

with nitrogen selective detection has some strong claims, viz. generally good sensitivity and reproducibility of assays and ready availability of equipment in most laboratories. The ultimate choice of a method for determining tricyclics will be determined more by the clinical application (routine monitoring versus pharmacokinetics) than by other factors.

REFERENCES

1 T. Silverstone and P. Turner, Drug Treatment in Psychiatry, Routledge and Kegan Paul, London, 3rd ed., 1982.
2 G.D. Burrows, T.R. Norman, K.P. Maguire, B.A. Scoggins and B.M. Davies, Commun. Psychopharmacol., 2 (1978) 393.
3 L.F. Gram, Clin. Pharmacokinet., 2 (1977) 237.
4 T.R. Norman, K.P. Maguire, B.A. Scoggins and G.D. Burrows, Aust. N.Z. J. Psychiatry, 16 (1982) 73.
5 R. Gupta and G. Molnar, Drug Metab. Rev., 9 (1979) 79.
6 B.A. Scoggins and K.P. Maguire, in G.D. Burrows and T.R. Norman (Editors), Psychotropic Drugs: Plasma Concentration and Clinical Response, Marcel Dekker, New York, 1981, p. 47.
7 B.A. Scoggins, K.P. Maguire, T.R. Norman and G.D. Burrows, Clin. Chem., 26 (1980) 5.
8 J.E. Burch, M.A. Raddats and S.G. Thompson, J. Chromatogr., 162 (1979) 351.
9 I.M. McIntyre, T.R. Norman and G.D. Burrows, Clin. Chem., 27 (1981) 203.
10 D.J. Brunswick and J. Mendels, Commun. Psychopharmacol., 1 (1977) 131.
11 R.C. Veith, V.A. Raisys and C. Perera, Commun. Psychopharmacol., 2 (1978) 491.
12 K.K. Midha, J.C.K. Loo and M.L. Rowe, Res. Commun. Psychol., Psychiatry Behav., 4 (1979) 193.
13 M. Zetin, H.R. Rubin and R. Rydzewski, Amer. J. Psychiatry, 138 (1981) 1247.
14 O. Borga, K.M. Piafsky and O.G. Nilsen, Clin. Pharmacol. Ther., 22 (1977) 539.
15 M. Wood, D.G. Shand and A.J.J. Wood, Clin. Pharmacol. Ther., 25 (1979) 103.
16 P.A. Routledge, B.B. Kitchell, T.D. Bjornsson, T. Skinner, M. Linnoila and D.G. Shand, Clin. Pharmacol. Ther., 27 (1980) 528.
17 P.V. Desmond, R.K. Roberts, A.J. Wood, G.D. Dunn, G.R. Wilkinson and S. Schenker, Brit. J. Clin. Pharmacol., 9 (1980) 171.
18 U.K. Wiegand and G. Levy, J. Pharm. Sci., 68 (1979) 1483.
19 B. Silber, M. Lo and S. Riegelman, Res. Commun. Chem. Pathol. Pharmacol., 27 (1980) 419.
20 C.A. Naranjo, J.G. Abel, E.M. Sellers and H.G. Giles, Brit. J. Clin. Pharmacol., 9 (1980) 103.
21 O. Borga, L. Azarnoff, G.P. Forshell and F. Sjoqvist, Biochem. Pharmacol., 18 (1969) 2135.
22 C.B. Kristensen, Clin. Pharmacol. Ther., 34 (1983) 689.
23 J.J. Saady, V.L. Bloom, N. Narasimhachari, S.C. Goldberg and R.O. Friedel, Psychopharmacology, 75 (1981) 173.
24 K.P. Maguire, G.D. Burrows, T.R. Norman and B.A. Scoggins, Clin. Chem., 26 (1980) 1624.
25 M. Linnoila, F. Dorrity and K. Jobson, Amer. J. Psychiatry, 135 (1978) 557.
26 N. Narasimhachari, J. Chromatogr., 225 (1981) 189.
27 T.C. Kwong, R. Martinez and J.M. Keller, Clin. Chim. Acta, 126 (1982) 203.
28 P. Koteel, R.E. Mullins and R.H. Gadsden, Clin. Chem., 28 (1982) 462.
29 J.J. Thoma, P.B. Bondo and C.M. Kozak, Ther. Drug. Monitor., 1 (1979) 335.
30 H.P. Gelbke, T.H. Grell and G. Schmidt, Arch. Toxicol., 39 (1978) 211.
31 P. Hartvig, S. Strandberg and B. Näslund, J. Chromatogr., 118 (1976) 65.
32 P. Hartvig and B. Näslund, J. Chromatogr., 133 (1977) 367.
33 H. Maurer and K. Pfleger, J. Chromatogr., 305 (1984) 309.
34 B.B. Brodie, J. Amer. Med. Assoc., 202 (1967) 600.

35 L.F. Gram, in G.D. Burrows and T.R. Norman (Editors), Psychotropic Drugs: Plasma Concentration and Clinical Response, Marcel Dekker, New York, 1981, p. 139.
36 W. Reiss, S. Brechbuhler and J.P. Dubois, Prog. Drug. Metab., 3 (1979) 115.
37 K.P. Maguire, G.D. Burrows, T.R. Norman and B.A. Scoggins, Clin. Chem., 24 (1978) 549.
38 I. Creese and S.H. Snyder, Nature, 270 (1977) 182.
39 R.B. Innis, L. Tune, R. Rock, R. Depaulo, D.C. U'Prichard and S.H. Snyder, Eur. J. Pharmacol., 58 (1979) 473.
40 R.A. Braithwaite and J.A. Whatley, J. Chromatogr., 49 (1970) 303.
41 R.A. Braithwaite and B. Widdop, Clin. Chim. Acta, 35 (1971) 461.
42 J.E. Wallace, H.E. Hamilton, L.K. Goggin and K. Blum, Anal. Chem., 47 (1975) 1516.
43 B. Caddy, F. Fish and J. Tranter, Analyst, 101 (1976) 244.
44 A.W. Missen and H.M. Stone, Anal. Chem., 49 (1977) 1864.
45 R. Lundgren, A. Olsson and G.P. Forshell, Acta Pharm. Suec., 14 (1977) 81.
46 T.B. Cooper, D. Allen and G.M. Simpson, Psychopharmacol. Commun., 1 (1975) 445.
47 S. Dawling and R.A. Braithwaite, J. Chromatogr., 146 (1978) 449.
48 G.L. Corona, B. Bonferoni, P. Frattini, M.L. Cucchi and G. Santagastino, J. Chromatogr., 277 (1983) 347.
49 M.T. Rosseel, M.G. Bogaert and M. Claeys, J. Pharm. Sci., 67 (1978) 802.
50 R.N. Gupta, G. Molnar, R.E. Hill and M.L. Gupta, Clin. Biochem., 9 (1976) 247.
51 V. Rovei, M. Sanjuan and P.D. Hrdina, J. Chromatogr., 182 (1980) 349.
52 D.R. Jones, B.J. Lukey and H.E. Hurst, J. Chromatogr., 278 (1983) 291.
53 R.N. Gupta, M. Stefanec and F. Eng, Clin. Biochem., 16 (1983) 94.
54 N. van Brunt, Ther. Drug. Monit., 5 (1983) 11.
55 J.H. Knox, Ann. Rev. Phys. Chem., 24 (1973) 29.
56 J.H. Knox and J. Jurand, J. Chromatogr., 103 (1975) 311.
57 I.D. Watson and M.J. Steward, J. Chromatogr., 110 (1975) 389.
58 M.R. Detaevernier, L . Dryon and D.L. Massart, J. Chromatogr., 128 (1976) 204.
59 B. Mellstrom and S. Eksborg, J. Chromatogr., 116 (1976) 475.
60 R.W. Giese, Clin. Chem., 29 (1983) 1331.
61 W.Z. Potter, H.M. Calil, A.A. Manian, A.P. Zavadil and F.K. Goodwin, Biol. Psychiatry, 14 (1979) 601.
62 H.F. Proelss, H.J. Lohmann and D.G. Miles, Clin. Chem., 24 (1978) 1948.
63 K.P. Maguire, T.R. Norman, G.D. Burrows and B.A. Scoggins, J. Chromatogr., 222 (1981) 399.
64 J.T. Biggs, W.H. Holland, S. Chang, P.P. Hipps and W.R. Sherman, J. Pharm. Sci., 65 (1976) 261.
65 W.A. Garland, J. Pharm. Sci., 66 (1977) 77.
66 H. d'A. Heck, N.W. Flynn, S.E. Buttrill, R.L. Dyer and M. Anbar, Biomed. Mass Spectrom., 5 (1978) 250.
67 A.C. Moffat, The Poisoned Patient: the Role of the Laboratory, Elsevier, Amsterdam, 1974, p. 83.
68 A. Nagy and L. Trieber, J. Pharm. Pharmacol., 25 (1973) 599.
69 D.B. Faber, C. Mulder and W.A. Man in 't Veld, J. Chromatogr., 100 (1974) 55.
70 U. Breyer and K. Villumsen, Eur. J. Clin. Pharmacol., 9 (1976) 457.
71 D.C. Fenimore, C.J. Meyer, C.M. Davis, F. Hsu and A. Zlatkis, J. Chromatogr., 142 (1977) 399.
72 P. Haefelfinger, J. Chromatogr., 145 (1978) 445.
73 N. Sistovaris, E.E. Dagrosa and A. Keller, J. Chromatogr., 277 (1983) 273.
74 D. Borga, L. Palmer, F. Sjoqvist and B. Holmstedt, Proc. Int. Congr. Pharmacol., 3 (1973) 56.
75 G.D. Burrows, B. Davies, K.P. Maguire, T.R. Norman, B.A. Scoggins, R.P. Hullin and J. Burch, Biochem. Med., 20 (1978) 125.
76 J.D. Robinson, R.A. Braithwaite and S. Dawling, Clin. Chem., 24 (1978) 2023.
77 K.K. Midha, J.C.K. Loo, C. Charette, M.L. Rowe, J.W. Hubbart and I.J. McGilveray, J. Anal. Toxicol., 2 (1978) 185.
78 N.E. Larsen and K. Marinelli, J. Chromatogr., 156 (1978) 335.

79 I.M. McIntyre, T.R. Norman, G.D. Burrows and K.P. Maguire, Brit. J. Clin. Pharmacol., 12 (1981) 691.
80 B. Mellstrom and G. Tybring, J. Chromatogr., 143 (1977) 597.
81 B. Mellstrom and R.A. Braithwaite, J. Chromatogr., 157 (1978) 379.
82 B.A. Scoggins, K.P. Maguire, T.R. Norman and G.D. Burrows, Clin. Chem., 26 (1980) 805.
83 S.C. Risch, L.Y. Huey and D.S. Janowsky, J. Clin. Psychiatry, 40 (1979) 4; 40 (1979) 58.
84 H.B. Hucker and S. Stauffer, J. Pharm. Sci., 63 (1974) 296.
85 J.E. O'Brien and O.N. Hinsvark, J. Pharm. Sci., 65 (1976) 1068.
86 G.L. Corona and B. Bonferoni, J. Chromatogr., 124 (1976) 401.
87 G. Nyberg and E. Mårtensson, J. Chromatogr., 143 (1977) 491.
88 T.R. Norman, K.P. Maguire and G.D. Burrows, J. Chromatogr., 134 (1977) 524.
89 T.R. Norman, G.D. Burrows, B.M. Davies and J.M.E. Wurm, Brit. J. Clin. Pharmacol., 8 (1979) 169.
90 S. Karkkainen and E. Seppala, J. Chromatogr., 221 (1980) 319.
91 H. Bruderlein, M. Kraml and D. Dvornik, Clin. Biochem., 10 (1977) 3.
92 M. Ervik, T. Walle and H. Ehrsson, Acta Pharm. Suec., 7 (1970) 625.
93 O. Borga and M. Garle, J. Chromatogr., 68 (1972) 167.
94 U.P. Geiger, T.G. Rajagopalan and W. Riess, J. Chromatogr., 114 (1975) 167.
95 L. Vereczkey, G. Bianchetti, V. Rovei and A. Frigerio, J. Chromatogr., 116 (1976) 451.
96 J.E. Wallace, H.E. Hamilton, R. Olivares and S.C. Harris, J. Anal. Toxicol., 2 (1978) 44.
97 T.B. Cooper and R.G. Kelly, J. Pharm. Sci., 68 (1979) 216.
98 P. Hartvig, W. Handl, J. Vessman and C.M. Svahn, Anal. Chem., 48 (1976) 390.
99 D.E. Case, J. Pharm. Pharmacol., 25 (1973) 800.
100 A. Jorgensen, Acta Pharmacol. Toxicol., 36 (1975) 79.
101 L.A. Gifford, P. Turner and C.M.B. Pare, J. Chromatogr., 105 (1975) 107.
102 J. Vasiliades and K.C. Bush, Anal. Chem., 48 (1976) 1708.
103 D.N. Bailey and P.I. Jatlow, Clin. Chem., 22 (1976) 777.
104 D.N. Bailey and P.I. Jatlow, Clin. Chem., 22 (1976) 1697.
105 H.B. Hucker and S.C. Stauffer, J. Chromatogr., 138 (1977) 437.
106 E. Bailey, M. Fenoughty and L. Richardson, J. Chromatogr., 131 (1977) 347.
107 M. Bertrand, C. Dupuis, M. Gagnon and R. Dugal, Clin. Biochem., 11 (1978) 117.
108 S.E. Reite, Medd. Nor. Farm. Selsk., 37 (1975) 141.
109 K.K. Midha, C. Charette, J.K. Cooper and I.J. McGilveray, J. Anal. Toxicol., 4 (1980) 237.
110 J. Vink and H.J.M. van Hal, J. Chromatogr., 181 (1980) 25.
111 E. Antal, S. Mercik and P.A. Kramer, J. Chromatogr., 183 (1980) 149.
112 D.R. Abernethy, D.J. Greenblatt and R.I. Shader, Pharmacology, 23 (1981) 57.
113 C. Charette, I.J. McGilveray and K.K. Midha, J. Chromatogr., 224 (1981) 128.
114 J.E. Bredesen, O.F. Ellingsen and J. Karlsen, J. Chromatogr., 204 (1981) 361.
115 G.F. Lachatre, G.S. Nicot, L.J. Merle and J.P. Valette, Ther. Drug Monitor., 4 (1982) 359.
116 B. Vinet, Clin. Chem., 29 (1983) 452.
117 K.P. Maguire, T.R. Norman, B.M. Davies and G.D. Burrows, J. Chromatogr., 278 (1983) 429.
118 R.R. Brodie, L.F. Chasseaud and D.R. Hawkins, J. Chromatogr., 143 (1977) 535.
119 J.J. DeRidder, P.C.J.M. Koppens and H.J.M. van Hal, J. Chromatogr., 143 (1977) 281.
120 B. Emanuelsson and R.G. Moore, J. Chromatogr., 146 (1978) 113.
121 J.C. Kraak and P. Bijster, J. Chromatogr., 143 (1977) 499.
122 M. Uihlein and P. Hajdu, Arzneim.-Forsch., 27 (1977) 98.
123 I.D. Watson and M.J. Stewart, J. Chromatogr., 132 (1977) 155.
124 H.G.M. Westenberg, B.F.H. Drenth, R.A. de Zeeuw, H. de Cuyper, H.M. van Praag and J. Korf, J. Chromatogr., 142 (1977) 725.
125 F.L. Vandermark, R.F. Adams and G.J. Schmidt, Clin. Chem., 24 (1978) 87.
126 R.R. Brodie, L.F. Chasseaud, E.L. Crampton, D.R. Hawkins and P.C. Risdall, J. Int. Med. Res., 5 (1977) 387.

127 P.A. Reece, R. Zacest and C.G. Barrow, J. Chromatogr., 163 (1979) 310.
128 D. Westerlund and E. Erixson, J. Chromatogr., 185 (1979) 593.
129 J.T. Streator, L.S. Eichmeier and M.E. Caplis, J. Anal. Toxicol., 4 (1980) 58.
130 T.A. Sutfin and W.J. Jusko, J. Pharm. Sci., 68 (1979) 703.
131 S.H. Preskorn, K. Leonard and C. Hignite, J. Chromatogr., 197 (1980) 246.
132 D.A. Breutzmann and L.D. Bowers, Clin. Chem., 27 (1981) 1907.
133 S.H.Y. Wong, T. McCauley and P.A. Kramer, J. Chromatogr., 226 (1981) 147.
134 J.E. Wallace, E.L. Shimek and S.C. Harris, J. Anal. Toxicol., 5 (1981) 20.
135 R. Gillilan and W.D. Mason, J. Pharm. Sci., 70 (1981) 220.
136 J. Fekete, P. Del Castilho and J.C. Kraak, J. Chromatogr., 204 (1981) 319.
137 J. Godbillon and S. Gauron, J. Chromatogr., 204 (1981) 303.
138 R.F. Suckow and T.B. Cooper, J. Pharm. Sci., 70 (1981) 257.
139 S.J. Bannister, S. van der Wal, J.W. Dolan and L.R. Snyder, Clin. Chem., 27 (1981) 849.
140 P.M. Kabra, N.A. Mar and L.J. Marton, Clin. Chim. Acta, 111 (1981) 123.
141 H.J. Kuss and E. Feistenauer, J. Chromatogr., 204 (1981) 349.
142 R. Dixon and D. Martin, Res. Commun. Chem. Pathol. Psychiatry, 33 (1981) 537.
143 P. Koteel, R.E. Mullins and R.H. Gadsden, Clin. Chem., 28 (1982) 462.
144 G.A. Smith, P. Schulz, K.M. Giacomini and T.F. Blaschke, J. Pharm. Sci., 71 (1982) 581.
145 S.M. Johnson, C. Chan, S. Cheng, J.L. Shimek, G. Nygard and S.K. Whaba Kalil, J. Pharm. Sci., 71 (1982) 1027.
146 R.F. Suckow, T.B. Cooper, F.M. Quitkin and J.W. Stewart, J. Pharm. Sci., 71 (1982) 889.
147 R.F. Suckow and T.B. Cooper, J. Chromatogr., 230 (1982) 391.
148 J.L. Bock, E. Giller, S. Gray and P. Jatlow, Clin. Pharmacol. Ther., 31 (1982) 609.
149 P.K. Sonsella, T.A. Jennison and B.S. Finkle, Clin. Chem., 28 (1982) 457.
150 P.M. Edelbroek, E.J.M. deHaas and F.A. deWolff, Clin. Chem., 28 (1982) 2143.
151 J.J. Tasset and F.M. Hassan, Clin. Chem., 28 (1982) 2154.
152 S.H. Wong and S.W. Waugh, Clin. Chem., 29 (1983) 314.
153 S.H. Wong, S.W. Waugh, M. Draz and N. Jain, Clin. Chem., 30 (1984) 230.
154 T.A. Sutfin, R. D'Ambrosio and W.J. Jusko, Clin. Chem., 30 (1984) 471.
155 S. Yang and M.A. Evenson, Anal. Chem., 55 (1983) 994.
156 G. Belvedere, L. Burti, A. Frigerio and C. Pantarotto, J. Chromatogr., 111 (1975) 313.
157 J.P. Dubois, W. Kung, W. Theobald and B. Wirz, Clin. Chem., 22 (1976) 892.
158 M. Claeys, G. Muscettola and S.P. Markey, Biomed. Mass Spectrom., 3 (1976) 110.
159 G. Alfredsson, F.A. Wiesel, B. Fyro and G. Sedvall, Psychopharmacology, 52 (1977) 25.
160 J.J. DeRidder, P.C.J.M. Koppens and H.J.M. van Hal, J. Chromatogr., 143 (1977) 289.
161 J.M. Wilson, L.J. Williamson and V.A. Raisys, Clin. Chem., 23 (1977) 1012.
162 R.G. Jenkins and R.O. Friedel, J. Pharm. Sci., 67 (1978) 17.
163 W.A. Garland, R.R. Muccino, B.H. Min, J. Cupano and W.E. Fann, Clin. Pharmacol. Ther., 25 (1979) 844.
164 S.J. Gaskell, Postgrad. Med. J., 56 (Suppl. 1) (1980) 90.
165 A. Lapin and M. Karobath, Biomed. Mass Spectrom., 7 (1980) 588.
166 E.L. Crampton, R.C. Glass, B. Marchant and J.A. Rees, J. Chromatogr., 183 (1980) 141.
167 S.P. Jindal, T. Lutz and P. Vestergaard, J. Pharm. Sci., 69 (1980) 684.
168 D.M. Chinn, T.A. Jennison, D.J. Crouch, M.A. Peat and G.W. Thatcher, Clin. Chem., 26 (1980) 1201.
169 N. Narasimhachari, J. Saady and R.O. Friedel, Biol. Psychiatry, 16 (1981) 937.
170 S.P. Jindal, T. Lutz and P. Vestergaard, J. Anal. Toxicol., 6 (1982) 34.
171 T.P. Davis, S.K. Veggeberg, S.R. Hameroff and K.L. Watts, J. Chromatogr., 273 (1983) 436.
172 G.J. Schmidt, in P.M. Kabra and L.J. Marton (Editors), Liquid Chromatography in Clinical Analysis, Humana, Clifton, NJ, 1981, p. 187.