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# Review

# Drug level monitoring: antidepressants

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ABSTRACT

The determination of antidepressant drugs which act by blocking neuronal uptake of biogenic amines, because of their widespread use and high toxicity, remains one of the most commonly requested drug assays in clinical laboratories. Easy to use immunoassay reagents for the estimation of these drugs are commercially available. However, immunoassays have not been universally accepted because of high probability of these reagents producing false negative and false positive results. At present, column liquid chromatography with absorbance detection and coupled with solid-phase extraction is the most viable technique for a general procedure for the identification and determination of these drugs. The technique of liquid chromatography is economical, environmental friendly since water-miscible and biodegradable solvents can be used for extraction of drugs and their chromatographic separation, and amenable to full automation. New techniques of separation, such as supercritical fluid chromatography and capillary zone electrophoresis, have not yet been applied for the determination of therapeutic concentrations of antidepressants.

CONTENTS



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LIST OF ABBREVIATIONS

AMOX Amoxapine
AT Amitriptyline
CI Chemical ionization
CMI Clomipramine
DCMI Desmethylclomipramine
DDOX Desmethyldoxepin
DMAP Desmethylmaprotiline
DMI Desipramine
DOX Doxepin
DTRI Desmethyltrimipramine
ECD Electron-capture detector
EI Electron impact
EMIT Enzyme-multiplied immunoassay technique
FID Flame ionization detector
FLU Fluoxetine
FLUV Fluvoxamine
GC Gas chromatography
IMI Imipramine
I.S. Internal standard
LC Column liquid chromatography, high-performance liquid chromatography
MAP Maprotiline
MS Mass spectrometer
N.A. Not available
NFLU Norfluoxetine
NPD Nitrogen–phosphorus detector, nitrogen-sensitive detector
NT Nortriptyline
PT Protriptyline
RIA Radioimmunoassay
RP Reversed phase
SPE Solid-phase extraction
TLC Planar liquid chromatography, thin-layer chromatography
TRAZ Trazodone
TRI Trimipramine

## 1. INTRODUCTION

Drug therapy is the first choice for the treatment of depression, one of the most frequent conditions presenting in psychiatric and medical practices. Antidepressant drugs are of two types and are based upon the hypothesis that some types of depression result from impaired function of central noradrenergic, dopaminergic and serotonergic pathways.

One type of antidepressant drugs is commonly called "tricyclic antidepressants" because the

most commonly prescribed drugs of this class, *i.e.* amitriptyline and imipramine, have fused tricyclic structures. These drugs and their active metabolites are believed to act by blocking the uptake of neuronal norepinephrine and serotonin in varying degrees. For simplicity, drugs like maprotiline and fluoxetine, which do not have a typical tricyclic structure, but which are believed to be amine uptake inhibitors, are still commonly referred to as tricyclic antidepressants.

The other less commonly used class of drugs to treat depression is monoamine oxidase inhibitors. These drugs act by blocking the enzyme monoamine oxidase, which by oxidation of the amino group deactivates the biogenic amines (norepinephrine and serotonin) to inactive metabolites. The use of these effective and useful drugs was limited because of their potential to cause hypertensive crises after ingestion of some types of food containing tyramine. In the last few years, there has been a resurgence in the use of this class of drugs for the treatment of atypical depression and in patients with cardiac disease who may not tolerate tricyclic antidepressants.

There is an increasing trend to use laboratory procedures to improve diagnosis and treatment of psychiatric disorders. Monitoring of therapeutic drug concentrations of tricyclic antidepressants continues to increase despite the fact that there is not a well established relationship between drug concentration and therapeutic response. The subject of therapeutic drug monitoring in the management of depression was discussed in detail in a conference held in Dallas, TX, USA on October 30–31, 1987. The proceedings of this conference have been published [1]. Tricyclic antidepressant drug assays are among the frequently requested emergency drug assays in suspected cases of overdoses despite the fact that there is no antidote for these drugs and their removal by dialysis or hemoperfusion is inefficient.

Therapy with monoamine oxidase inhibitors is generally monitored by the determination of inhibition of the enzymatic activity of monoamine oxidase rather than by the determination of plasma drug concentrations [2].

In this paper I have critically reviewed the current literature (from 1980 to June 1991) in the light of our own experience, the analytical methods currently used for the determination of tricyclic and related antidepressants in biological fluids. The structures of drugs covered in this review are shown in Fig. 1. The subject of analytical methodology for the determination of tricyclic antidepressants has been previously reviewed [3–7].

## 2. SAMPLE COLLECTION

Plasma (or serum) is the most widely used specimen for monitoring therapeutic drug concentrations. Whole blood or hemolyzed specimens are avoided as the concentration of drug in red blood cells in most cases is different from its concentration in plasma. Monitoring drug concentration in washed red blood cells, in an at-

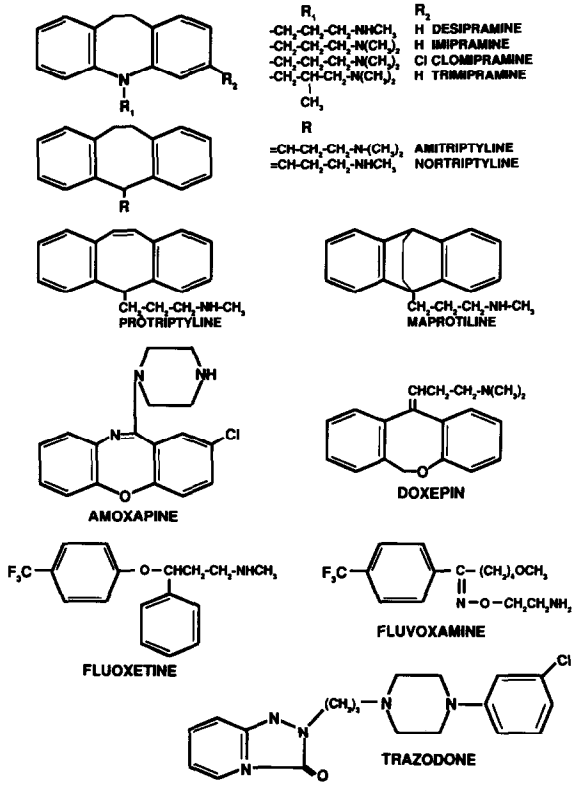


Fig. 1. Chemical structures of antidepressants discussed in this review.

tempt to find a better correlation between drug concentration and clinical response, has now been abandoned.

Pre-analytical considerations of sample collection and storage have been recently reviewed by Orsulak [6]. It appears that the debate during the 1970s about the loss of antidepressant drugs, due to additives in the blood collecting tubes, has now been settled due to changes in the formulation of evacuated blood collecting tubes. There was no significant difference in imipramine (IMI) and desipramine (DMI) concentrations when blood was collected in Vacutainer or Venoject brands of red-stoppered (no anticoagulant) or green-stoppered (heparin as anticoagulant) evacuated tubes [8]. Initially, blood collection in heparinized tubes was preferred because more plasma could be obtained than the amount of serum from the same volume of blood collected in a tube without any anticoagulant. However, now serum is the preferred specimen so that fibrin strands in plasma may not clog pipette tips of analyzers used for immunoassays or cartridges used for solid-phase extraction (SPE). Use of serum separators or use of blood-collecting tubes with gels is not recommended as it may lead to the loss of drug.

Serum should be separated from the clot within a few hours of sample collection. Serum can be stored at room temperature for two to three days and at 4°C for up to a week as antidepressant drugs are quite stable. For long-term storage, freezing of serum at −20°C is adequate. Bupropion is an exception as it exhibits both a temperature- and pH-dependent degradation in plasma [9]. Urine is still analyzed in some laboratories for the identification of antidepressant drugs and their metabolites in suspected cases of overdose. However, urine analysis may not always be helpful to decide if the drug has been ingested in therapeutic or toxic doses. Urine is generally extracted without prior hydrolysis of conjugates as unconjugated drugs and metabolites are excreted in urine in adequate amounts. Urine is stored at 4°C without any preservative.

## 3. DETERMINATION OF METABOLITES

Most of the antidepressant drugs undergo rapid and extensive metabolism in man and other animals. The main routes of metabolism are N-demethylation and ring hydroxylation. The popular drugs amitriptyline (AT) and IMI are tertiary amines which are N-demethylated to corresponding secondary amines nortriptyline (NT) and DMI, respectively, which are pharmacologically active and are themselves commonly prescribed antidepressants. By inference, secondary amine metabolites of other tertiary amine antidepressants, *e.g.* doxepin (DOX), clomipramine (CMI) and trimipramine (TRI) are also considered pharmacologically active and measured together with the parent tertiary amines. Secondary amines are further N-demethylated to produce primary amines. However, at present, primary amine metabolites are not measured mainly because their pharmacological effect is not clearly established. Fluoxetine, a secondary amine drug, is an exception. Norfluoxetine, a primary amine metabolite of fluoxetine, is routinely measured.

The relationship between the serum concentration of hydroxy metabolites of antidepressants and clinical response does not appear to have been clearly established. In any case, hydroxy metabolites are not routinely monitored in clinical laboratories because of the analytical problems for the simultaneous determination of hydroxy metabolites along with the parent drug and secondary amine metabolites. Amoxapine is an exception. 8-Hydroxyamoxapine is determined along with the parent drug.

Antidepressant drugs are highly protein-bound and the therapeutic concentrations of the "free" fraction of these drugs are quite low [10,11]. Routine determination of the free fraction of antidepressant drugs is an analytical challenge. As a result no serious effort appears to have been made to relate clinical response with the concentration of free drugs.

## 4. SAMPLE PREPARATION

It has not been possible to analyze biological samples directly by any chromatographic technique without any prior clean-up or concentration. The chromatographic system is operated at a sensitivity where direct injection of the biological samples produces a plethora of peaks in the chromatogram. The approach of simple precipitation of proteins with organic solvents, commonly used for the determination of antiepileptic drugs, is also not used for the determination of antidepressants. However, some of the immunoassays for the determination of antidepressants is carried out without prior extraction or concentration.

### *4.1. Liquid–liquid extraction*

Until recently, extraction of antidepressants from biological samples with water-immiscible organic solvents has been the most popular technique. A number of different approaches have been used. In isolation, some of the published suggestions appear contradictory. Some of the parameters for liquid–liquid extraction are compared below.

#### *4.1.1. Equipment*

Glass culture tubes with PTFE-lined screw caps have been invariably used to extract biological samples. In some publications, the size of the tubes is not described while in others, only the capacity rather than the dimensions of the tubes are given. A narrow tube allows the collection of a larger fraction of the organic phase than allowed by the use of a wide tube. There is a tendency for the adsorption of these drugs on to the glass surface after extraction from alkalinized biological samples [12]. Primary amine metabolites are particularly prone to adsorption. This adsorption of drugs leads to artifactually low results and non-linear curves. The percentage loss of the drug due to adsorption increases as the drug concentration in the sample decreases.

A number of procedures have been suggested to minimize adsorption of drugs. One of the commonly used methods is to silanize the glassware with dichloromethylsilane. In some cases the glassware is soaked overnight in a dilute solution of dichloromethylsilane in toluene [13], while in others, contact of glass surface with the silanizing solution for as little as 5 min is suggested by Gupta *et al.* [14]. Silanized glassware can be used with alcohols and hydrocarbons. However, solvents like ethyl acetate and halogenated hydrocarbons may dissolve the silyl coating. The silanized glassware was reused by Kristinsson [13] for up to three to four weeks without re-silanization. For this purpose, glassware was washed with methanolic hydrochloric acid. Washing the glassware with alkaline detergents removes the silyl film. In an alternative approach for minimizing the adsorption of drugs on glass surfaces, the glassware was rinsed by Bredesen *et al.* [15] with methanolic solution of volatile amines, *e.g.* triethylamine. The volatile amine is removed during drying of the tubes and it is believed that active sites on the glass surface remain deactivated during the extraction process. In some procedures, trimethylamine [15] or triethylamine [16] has been added to the sample itself, and adequate amounts of added amine are carried through the extraction process.

Some investigators have found silanization and use of volatile amines inadequate to completely eliminate the problem of drug adsorption [12] and recommend the addition of an excess of an analogue of the drug to be measured to the sample. However, such an approach can cause additional strain on the chromatographic separation or prolong the analysis time.

In another simple approach to avoid adsorption of drugs, extraction of biological samples has been carried out in plastic, generally polypropylene tubes. However, there is always a risk of introduction of contaminants due to slow dissolution of plastic by solvents and other chemicals used for extraction. In any case it is good practice to check different brands and even different batches of the same brand of plastic tubes for their resistance to different chemicals prior to use.

Mixing of the aqueous sample and the organic

solvent has been carried out by vortex-mixing or by mixing with different kinds of mechanical shakers. It is not clear from different publications if vortex-mixing is carried out with a single-tube mixer or a multi-tube mixer. Mixing a large number of tubes manually may be tiring and non-reproducible. There is a wide range of time suggested for vortex-mixing the sample with the organic solvent ranging from 30 s by Kiel *et al.* [16] to 15 min by Abernethy *et al.* [17]. Mixing, particularly high-speed vortex-mixing of plasma with organic solvents, leads to the formation of emulsions. Use of rotary mixers at slow speed allows simultaneous mixing of a large number of samples uniformly with reduced emulsion formation. To avoid mixing and emulsion formation, the sample was adsorbed on Extrelut and the drugs were recovered with ethyl acetate by Sasaki and Baba [18]. This approach has not been commonly used for the extraction of antidepressants. It appears that these drugs are firmly bound to Extrelut, and highly polar solvents such as ethyl acetate are required for their elution. The resulting extract is not as clean as that obtained by the use of non-polar solvents.

*4.1.2. Choice of solvent*

*n*-Hexane remains the most commonly used solvent for the extraction of antidepressant drugs. A review of twenty publications showed that thirteen procedures used *n*-hexane, two used *n*-heptane, two used diethyl ether, one used chlorobutane, one used methylene chloride, one used ethyl acetate and one used pentane. In some multi-step procedures, different solvents were used for different extractionsteps. Use of diethyl ether as a common laboratory solvent is being discouraged because of safety considerations. Diethyl ether is quite polar and can extract many polar compounds; the extract has to be dried over anhydrous sodium sulfate to remove appreciable amounts of the aqueous phase dissolved in diethyl ether. Hexane is free from these problems. The problem with hexane, however, is that is has a relatively high boiling point. Pentane is quite similar to hexane in all its desirable properties and also has a relatively low boiling point. However, pentane has failed to gain any popularity.

In a majority of the procedures, hexane has been mixed with low-molecular-mass alcohols in concentration ranging from 0.1 to 5%. The primary purpose of the addition of an alcohol to *n*-hexane is to adjust the polarity of hexane so as to make the partition of antidepressant drugs from the aqueous phase to the organic phase more favourable. However, the possibility of co-extraction of unwanted compounds also increases. Other claimed advantages of alcohols in hexane include prevention of emulsion formation and adsorption of drugs. Dixon and Martin [19] used a mixture of isooctane–methyl *tert.*-butyl ether (9:1) for the extraction of antidepressants.

Many procedures use a three-step protocol for the extraction of antidepressant drugs from biological samples. The sample is made alkaline (pH $>$ 10) with the use of a variety of reagents, *e.g.* sodium hydroxide solution, solid sodium carbonate and carbonate or borate buffer etc., and then extracted with the organic solvent. The organic phase is collected avoiding all traces of the aqueous phase and back-extracted into an aqueous acid phase. The organic phase is discarded. The aqueous phase is made alkaline and extracted into a new aliquot of organic phase which is collected and evaporated. In recent years, the third step of extraction, *i.e.* the extraction of the acidic aqueous phase with organic solvents, has been avoided in a number of cases. Aliquots of the aqueous phase are directly injected into the liquid chromatographic (LC) apparatus. Van Brunt [7] evaporated the acidic phase itself. For ease of evaporation, the organic phase is back-extracted into methanolic hydrochloric acid rather than by aqueous acid.

An alternative two-step extraction scheme has been suggested by Gupta *et al.* [14] to reduce the time required for extraction and to minimize the use of glassware. In this approach, the sample is made acidic (pH $<$ 2) and extracted with an organic solvent which is discarded. The sample is then made alkaline and re-extracted with a new aliquot of the organic solvent.

In rare cases, only one-step extraction has also been used. Chinn *et al.* [20] extracted the sample

at alkaline pH with the organic solvent, an aliquot of which is directly injected into the gas chromatography. Dixon and Martin [19] evaporated the extract obtained by one-step extraction. The specificity is provided by the detector.

*4.1.3. Evaporation*

For gas chromatographic (GC) analysis, the final extract has to be highly concentrated and free from moisture. In most of the procedures, 5–10 ml of the second hexane extract is evaporated at 45–50°C in a stream of nitrogen. Sonsalla *et al.* [21] used a stream of air to aid the evaporation of hexane. In another procedure, Lapin [22] evaporated diethyl ether *in vacuo*. Evaporation of organic solvents is a slow and tedious process. Efficient fume hoods are required to avoid contamination of the laboratory atmosphere with the vapours of organic solvents. A supply of extra pure nitrogen is required to minimize the contamination of the drug extract. A number of approaches have been used to eliminate evaporation or to simplify the process of evaporation. It has already been mentioned that for LC aliquots of the aqueous acid phase are directly injected. For GC, the second extraction is made with a very small volume of the organic solvent, *i.e.* 25 μl [23] or 50 μl [17], aliquots of which are directly injected into the GC system. Pentane can be evaporated at a relatively low temperature of 45°C without the aid of nitrogen or reduced pressure.

*4.1.4. Recovery*

For adequate sensitivity of detection and analytical reproducibility, it is essential that extraction recovery of the analyte is at least 50%. Therefore, most of the publications do report the important parameter of extraction recovery. However, it is not clear how the extraction recovery was calculated. In liquid–liquid extraction, it is not practical or desirable to collect 100% of the layers. A significant fraction of the desired layer is lost during the separation of the layers at each step of extraction. It appears that the reported extraction recovery of 95–100% of antidepressant drugs by a three-step extraction procedure has been corrected for the loss of layers. Most of the tertiary and secondary amine antidepressant drugs are quite lipophilic and can be efficiently extracted into non-polar solvents. When proper precautions to avoid adsorption of drugs are taken, theoretical extraction recovery should be quantitative. However, practical extraction recovery may range from 60 to 75% due to loss of layers during their manipulations.

*4.1.5. Hydroxy metabolites*

In a majority of publications, determination of hydroxy metabolites of antidepressant drugs has not been investigated. These metabolites have not been identified in the chromatograms of extracts of serum obtained from patients receiving antidepressant therapy because of lack of authentic standards. However, there are a few publications which report the optimized determination of hydroxy metabolites. The same general procedure of liquid–liquid extraction used for AT or NT has been applied for the extraction of hydroxy metabolites of these drugs. In a majority of cases, the extraction recovery of AT, NT and 10-OH-AT is similar in the range 85–100% but that of 10-OH-NT is significantly low in the range 50–65% [24–26]. However, in another publication [27], the extraction recovery of 10-OH-NT (~90%) is actually higher than that of AT (73%). There is some difference in the recovery of *cis*- and *trans*-isomers of 10-OH-NT [27]. Hydroxy metabolites of AT and NT are easily dehydrated by treatment with acid to produce corresponding 9,10-dehydro-AT and 9,10-dehydro-NT. These dehydro compounds have extraction properties similar to those of AT and NT.

Hydroxy metabolites of IMI and DMI are more polar than the corresponding parent drugs. Therefore solvents of relatively high polarity such as diethyl ether [28], methylene chloride [29] or a mixture of ethyl acetate–hexane–isoamyl alcohol (50:49:1) [30] have been used, whereas the parent drugs have been efficiently extracted with non-polar solvents. The recovery of OH-DMI (60%) is still lower than that of IMI (90%) [28].

*4.1.6. Amitriptyline-N-oxide*

Amitriptyline-N-oxide is a significant metabo-

lite in rat and mouse microsomes and is present in dog urine but has not been detected in human liver microsomes or human plasma. Amitriptyline-N-oxide is also a pro-drug of AT and is used therapeutically in Europe. Amitriptyline-N-oxide is believed to act by its conversion into AT. It produces adequate concentrations of AT in the brain at low plasma concentration. Therefore, this pro-drug shows a reduced incidence of adverse side-effects as compared to AT itself. It has been claimed that amitriptyline-N-oxide cannot be extracted into hexane or diethyl ether from alkalinized plasma [31], but can be recovered in good yield by two [31] or three [32] extractions with methylene chloride. However, Kiel *et al.* [16] report that amitriptyline-N-oxide can be extracted in a yield similar to that of NT by a single extraction with hexane–1-butanol (9:1).

*4.1.7. Automation*

Liquid–liquid extraction is slow and tedious, particularly when a large number of samples have to be processed. A fully automated liquid–liquid extraction system coupled with an LC system was developed by Bannister *et al.* at the Technicon Instrument Corporation [33]. The system was based on Technicon autoanalyzer principle using air bubbles to separate samples. The sample was extracted with isooctane–1-propanol (77.5:22.5), the organic phase back extracted into 50 m*M* sulfuric acid and the aqueous phase then directly injected into the LC system. The adsorption of drugs onto the extraction device was minimized by the use of special brand "Acidflex" pump tubing. It seems, however, that this instrument failed to gain any popularity. There is no published report by any independent laboratory describing the use of this instrument for the extraction of antidepressants.

*4.2. Off-line solid-phase extraction*

In the last few years a number of SPE procedures have been described for the determination of antidepressants. SPE is based on the principle of the adsorption of the analytes on about 50–300 mg of a suitable sorbent packed in disposable plastic cartridges or columns. The sorbent bed is conditioned (activated) with suitable washes to remove contaminants from the sorbent bed and to improve the retention of the analyte. After the passage of the sample, the sorbent is selectively washed to remove undesirable impurities without the loss of desired compounds. Finally, the desired compound is eluted in a minimum volume of a solvent which could be either injected directly or evaporated rapidly and easily.

Bonded-phase silica, *e.g.* octadecyl, octyl, phenyl and cyanopropyl, have been the commonly used supports for the extraction of drugs. Cation-exchange and mixed-phase bonded silica are also being used for the extraction of basic drugs including antidepressants.

In some procedures, cartridges packed with diatomaceous earth, *e.g.* ClinElut (Varian, Harbor City, CA, USA) or Extrelut (EM Science, Gibbstown, NJ, USA) have been used for the extraction of antidepressants [18]. Use of this type of solid material is considered as liquid–liquid extraction rather than solid-phase extraction. These cartridges are not activated, can accept a fixed volume of sample only according to its capacity, retain the entire sample, are not washed to remove impurities, are eluted by water-immiscible solvents by gravity only and cannot be reused. Different parameters for the SPE of antidepressants are examined briefly in the following sections.

*4.2.1. Extraction cartridge*

Until a few years ago the choice of the sorbent and the size of the extraction cartridge was limited. In some of the early papers, details about the size of the cartridge or the amount of the sorbent in the extraction cartridge are not fully described. Now a wide selection of cartridge sizes containing different amounts of sorbents are available. Therefore, a complete description of the extraction cartridge is desirable. Use of syringes to apply suction or positive pressure are slow and rarely used. Now vacuum devices to apply suction for the simultaneous processing of ten or more cartridges are commonly used. In the papers published since 1980 describing SPE of antidepressants, three procedures use Sep-Pak $C_{18}$ (Waters,

Milford, MA, USA) [34–36], three BondElut $C_{18}$ (Varian) [37–39], one Bio-Rad $C_{18}$ (Hercules, CA, USA) [40], one BondElut CN [41], one BakerBond CN [42] and one BondElut $C_2$ silica extraction cartridge [43]. All of these SPE procedures claim to give clean extracts with excellent recoveries of the desired drugs. However, a described procedure optimized for a particular brand of extraction column may not be applicable to another brand of the extraction column even when packed with the same type of material, *e.g.* $C_{18}$ silica [44,45]. The binding of basic drugs to the sorbent depends upon the undeclared characteristics of residual silanols and percentage of carbon loading of bonded silica. In another approach, Carfagnini *et al.* [46] used a combination of two extraction cartridges to get clean extracts. The sample was first passed through a Carbopack B cartridge, the drugs were eluted with methylene chloride–methanol (6:4) and the eluate was passed through a silica-based sulfonic acid-type cation-exchange cartridge (Supelco, Bellefonte, PA, USA). However, the benefits of increased cost of using a two-cartridge system over the procedures using only a single cartridge have not been clearly presented.

*4.2.2. Conditioning of extraction cartridge*

Different published procedures describe different protocols to prewash the extraction cartridge prior to the application of the sample. However, it is not clear if the nature of the solvent used for the final wash has any effect on the extent of the adsorption of antidepressant drugs. Thus Kobayashi *et al.* [36] applied the serum sample to a Sep-Pak $C_{18}$ cartridge without any prewashing of the cartridge. In a number of cases, the extraction cartridge has been washed with different volumes of methanol and water. In addition, Bidlingmeyer *et al.* [35] washed the Sep-Pak $C_{18}$ cartridge with 5 ml of 0.1 *M* ammonium hydroxide prior to the application of the sample. Gupta and Steiner [39] washed the BondElut $C_{18}$ column with 1 ml of 1 *M* hydrochloric acid and Mazhar and Binder [40] washed the Bio-Rad $C_{18}$ column with 3 ml of elution reagent (methanol–diethylamine, 100:0.6) followed by 3 ml of 1% potassium bicarbonate in 10% acetonitrile. It is preferable to wash the extraction cartridge at least once with the elution reagent or a solvent of similar pH to that of the elution reagent at some stage prior to the application of the sample. This wash step minimizes extraneous peaks introduced by the extraction cartridge. Roberts and Hann [43] treated the BondElut $C_2$ cartridge with 0.25 ml of a 2 m*M* aqueous solution of carbamazepine followed by two washes of 2 ml methanol and two of 2 ml water. The authors claim that the pretreatment of the extraction column with carbamazepine dramatically increased the recovery of antidepressants from serum. The mechanism of this beneficial effect of carbamazepine pretreatment on the recovery of antidepressants is not clear. Washing with two portions of 2 ml methanol is expected to remove the bulk of carbamazepine from the extraction column.

The manufacturers of the extraction cartridges recommend that the cartridges be used only once. Some of the compounds present in biological samples may be adsorbed permanently by the sorbent and affect the adsorption of analytes in subsequent samples. Extraneous compounds or the analyte itself from the previous sample may be introduced into the subsequent extract. However, Kwong *et al.* [37] have reported that the extraction cartridges could be used at least three times without any significant effect on the recovery of antidepressants or on the cleanliness of the extract.

*4.2.3. Sample application*

In a majority of procedures for SPE of antidepressants, cartridges of 1 ml capacity packed with 100 mg of sorbent are used. In some cases [37–39], the total volume of the sample, buffer and internal standard is about 1 ml, and therefore can be loaded directly onto the extraction cartridge. When the total sample volume is greater than the capacity of the column, a large-volume reservoir is coupled to the extraction cartridge with an adaptor [40].

For efficient adsorption of drugs on non-polar adsorbents, the drug should be in the non-ionized form. In some cases, 0.1 *M* ammonium hydrox-

ide [35], 0.1 *M* sodium carbonate [39], carbonate buffer, pH 9.8 [34] or phosphate buffer, pH 7 [41] have been used to make these drugs non-ionized. In other cases, the serum sample is considered adequately, buffered and no additional buffer is used [37,38,40].

Antidepressant drugs are highly protein-bound. Recovery of these drugs by SPE can be variable and low due to incomplete liberation of drugs bound with proteins. The simplest approach to minimize the effect of protein binding is to pass the sample through the sorbent at a low rate [38]. Mazhar and Binder [40] diluted the sample with an equal volume of water, and Bidlingmeyer *et al.* [35] diluted the sample with one volume of 0.1 *M* ammonium hydroxide. Dilution of serum weakens the protein binding of drugs and increases the time of contact of the drug with the adsorbent. Kobayashi *et al.* [36] used four volumes of methanol to precipitate proteins and the resultant supernatant was applied to the extraction cartridge. Musch and Massart [42] treated the sample with two volumes of acetonitrile to precipitate the proteins and after processing the supernatant by SPE recovered imipramine in a 97% yield. However, it appears that methanol may not be a suitable solvent to precipitate proteins as it is an efficient solvent to elute basic drugs adsorbed on $C_{18}$ silica sorbents. In any case, protein precipitation is not necessary to recover antidepressant drugs in excellent yield by SPE.

*4.2.4. Elution*

The choice of the elution reagent to recover antidepressant drugs adsorbed on extraction cartridges depends partly on the mode of subsequent chromatography. For use with GC, the extract has to be evaporated. Therefore, a small volume of solvent with a relatively low boiling point containing volatile salts, *e.g.* ammonium acetate, is desirable [37]. However, for use with LC, evaporation of the extract should be and can be avoided [39,40]. To increase the sensitivity of detection, the elution volume should be kept to a minimum [39,40]. Mazhar and Binder [40] have diluted two parts of eluate with one part of water to inject 50 $\mu$l of the final solution. With proper choice of chromatographic conditions, dilution of the eluate can be avoided resulting in improved sensitivity of detection.

Antidepressant drugs are quite hydrophobic and are strong bases. They are bound to $C_{18}$- or CN-silica by adsorptive property of bonded silica for hydrophobic molecules and by ionic forces of residual silanols for the amino groups. In general, organic solvents alone are inefficient in eluting the adsorbed drugs from the extraction cartridges [47]. Narasimhachari [34] used a relatively large volume (10 ml) of a mixture of hexane–isopropyl alcohol (9:1) to elute antidepressants from a Sep-Pak $C_{18}$ cartridge. Trazodone is an exception. It has been eluted efficiently with 0.5 ml of methanol from a 1-ml BondElut $C_{18}$ column [48]. The efficiency of elution can be increased by adding compounds like triethylamine to methanol to neutralize silanol groups of the extraction cartridge [40] or by making the organic solvent acidic [39,42]. At acidic pH, basic drugs become ionic and have reduced affinity for bonded silica. However, antidepressant drugs cannot be eluted with aqueous acid alone. Musch and Massart [42] used 50% methanol and Gupta and Steiner [39] used 66% acetonitrile at acidic pH to elute antidepressant drugs from the extraction cartridges.

In most cases, the final eluate is collected by placing collection tubes in a rack under the outlets of the vacuum manifold. These outlets are made of steel which can be attacked by acidic reagents. To avoid contamination, the eluate can be collected easily and efficiently by centrifugation [39]. Disposable plastic adapters are now available commercially (Worldwide Monitoring, Horsham, PA, USA) which can be fitted on the extraction cartridges just prior to the elution step to avoid the contact of the eluate with metallic tubes.

*4.2.5. Selectivity of solid-phase extraction*

Strong affinity of antidepressants for bonded silica has allowed to obtain very clean extracts. Adsorption of acidic drugs has been minimized by selecting a high-pH matrix. Acidic, neutral and weakly basic drugs are adsorbed along with

antidepressants. However, these drugs have been eliminated by washes with organic solvents which do not elute antidepressants [39,40]. It is particularly useful to remove benzodiazepines which are commonly prescribed as sedatives along with antidepressants. However there are many other basic drugs, *e.g.* antiarrhythmic drugs and phenothiazines, which are strong bases and are coextracted with antidepressants.

Hydroxy metabolites are also coextracted with the parent antidepressant drugs. Introduction of a hydroxyl group into the molecule of AT or IMI does not change the basic nature of these drugs or significantly affect their hydrophobicity as shown by their extraction into solvents like hexane–butanol [27]. However, it should be possible to elute them selectively from the extraction column. Pankey *et al.* [49] reported that hydroxy metabolites were removed from their parent drugs (AT, NT, IMI, DMI) by their proprietary SPE procedure developed to improve the specificity of their commercial enzyme-multiplied immunoassay technique (EMIT) assay for these major antidepressant drugs. Mazhar and Binder [40] get the hydroxy metabolites in their final eluate. The hydroxy metabolites elute early during chromatographic separation and thus do not interfere with the assay of antidepressant drugs.

*4.2.6. Automation for solid-phase extraction*

For the extraction of a large number of samples, manual SPE is also tedious and somewhat slow. A number of manufacturers have marketed instruments which allow automatic performance of some or all of the steps for a SPE procedure. Koteel *et al.* [50] described the use of Dupont's PREP I automated sample processor and disposable cartridges packed with Type W styrene–divinyl copolymer for the extraction of antidepressants. However, this instrument has been withdrawn from the market and is not discussed further. Another semi-automated system for SPE called "AASP" was marketed by Varian (Walnut Creek, CA, USA). This instrument requires special cassettes of extraction cartridges to fit into a modified-vacuum manifold. Each cassette has ten cartridges containing 50 mg of sorbent as compared to 100 mg of sorbent in each cartridge used for manual SPE. Washing of cartridges, loading of samples and washing of impurities are carried out manually under a positive pressure of nitrogen. The cassette is then placed in the AASP system where each cartridge is sequentially eluted and the eluate injected automatically onto the analytical column. The manufacturer has published an application note for the extraction of antidepressants. Ni *et al.* [51] have described the determination of CMI and IMI using AASP. The production of this instrument has also been suspended recently. Gilson Medical Electronics (Middleton, WI, USA) has introduced a system called ASPEC which allows fully automated extraction of drugs from biological matrices. The system also acts as an autoinjector. Regular SPE cartridges marketed by a number of companies can be used with this system. The samples are processed sequentially. In some procedures, the extraction time for a given procedure may be quite long depending upon the number of conditioning and washing steps. The manufacturer of this device has published two procedures using BakerBond 100-mg CN cartridges for the extraction of antidepressants. The two procedures differ only in the elution of drugs.

It appears that automated SPE devices have yet to gain any popularity. A comparison of benefits resulting from the increased cost of an automated device for the determination of antidepressants has not yet been published.

*4.3. On-line solid-phase extraction*

In this technique, analytes are concentrated on a pre-column which is an integral part of the liquid chromatograph. After washing, the precolumn is automatically back-flushed with the mobile phase to transfer the analytes on to the analytical column. The same pre-column can be used repeatedly [52]. However, the precolumn deteriorates after the injection of only a few samples due to the precipitation of proteins. Protein precipitation with acidic or alkaline reagents prior to the injection of the sample on the pre-column was attempted by Dadgar and Power [53] to prolong

the life of the pre-column. However, this approach gave only poor recovery of AT. To prolong the life of the precolumn, Matsumoto *et al.* [54] have packed their pre-column with a metacryl polymer gel for size exclusion (exclusion size = 5000) for the concentration of antidepressants. After the passage of the sample, the precolumn is washed with water for 10 min and the analytes are then introduced onto the analytical column by back-flushing the pre-column. In this procedure, the recovery of antidepressants is quantitative and the entire analytical procedure is highly reproducible. Similarly, Supelco has published an application note using a Supelcosil Hisep pre-column coupled with a Hisep analytical column for direct injections of serum samples for the determination of antidepressants. A fully automated system "REMEDi" incorporating on-line SPE for the screening of antidepressants and other basic drugs in urine or serum is being marketed by Bio-Rad Labs. (Hercules, CA, USA).

The system for on-line SPE is generally fully automated where all the steps are computer-controlled and the sample is injected with an autosampler. At least two pumps are used to deliver different solvents [53]. However, Svensson *et al.* [10] used a manual system by simply replacing the loop of the valve injector with a pre-column. Sample injections and washings of the pre-column are made manually with a syringe.

It is evident from the paucity of published procedures that on-line sample preparation is not yet a popular technique for the determination of antidepressants. McDowall *et al.* [55] have summarized the problems of this column-switching technique. One of the major disadvantages of on-line pre-concentration is that some material may become irreversibly bound to the packing material of the pre-column, thus decreasing its capactiy to retain analytes from subsequent samples. There is a limited flexibility to wash the precolumn with different solvents for the regeneration of the pre-column. Above all, the approach of automated on-line sample preparation requires additional costly equipment. Manual on-line SPE systems are slow and tedious.

## 5. GAS CHROMATOGRAPHY

It appears the GC, which was the only practical technique for therapeutic monitoring of antidepressants, has lost its popularity since the mid-1980s. Now only a few laboratories engaged in routine monitoring of therapeutic concentrations of antidepressants use GC. However, GC remains a highly sensitive and specific technique. For some applications such as determination of the "free" concentration of antidepressants, GC may be the only feasible technique. Some of the variables for GC methods are discussed briefly.

### *5.1. Choice of internal standard*

For chromatographic analysis, particularly for GC procedures, it is common practice to add one or more compounds as internal standard(s) (I.S.) to the sample to avoid precise measurement of volumes during extraction and analysis. There are exceptions, however. Hsu [56] has determined AT and NT in rat brain by GC without the addition of any I.S. The choice of a proper I.S. has been difficult as suitable compounds to be used as I.S. are often unavailable. In some procedures, one commonly prescribed drug, *e.g.* AT, has been used as an I.S. for the assay of another popular drug, *i.e.* IMI [7]. Such an approach may lead to complications. It has been observed that quite often the order for an assay is for a different drug than which is being actually prescribed to the patient. Further, in many overdose cases, the identity of the drug is not precisely known.

In some procedures, promazine, which has some structural similarities to tricyclic antidepressants and which is rarely prescribed as a drug, has been used as an I.S. for the determination of antidepressants [15,44]. However, promazine, like other phenothiazines is photosensitive and therefore may not be a suitable I.S. for the assay of antidepressants which are photostable.

In a majority of GC procedures a single I.S., either a tertiary amine or a secondary amine, has been used for the estimation of both tertiary and secondary bases. This is so because it is difficult to obtain appropriate compounds to be used as

multiple I.S., and there is a problem of separation of multiple compounds from one another, from analytes and from other potential interferences. However, in our experience the use of two I.S., a tertiary amine and a secondary amine, provides improved precision for the simultaneous estimation of tertiary and secondary bases. Secondary amine drugs behave differently from tertiary amine drugs in their extraction efficiency and their losses due to adsorption. The use of two I.S. assumes additional importance if the GC procedure requires derivatization of secondary amines. A secondary amine I.S. monitors the extent of derivatization. On the other hand use of multiple I.S. for one type of drug as suggested by Burch *et al.* [57] appears to be unnecessary and may lead to confusion in the calculation and interpretation of results.

### *5.2. Derivatization*

In general, there is a tendency for adsorption of primary and secondary amines on packed columns and to a lesser extent on capillary columns. This adsorption can be minimized by preparing suitable derivatives. However, there is a trend to avoid the preparation of derivatives even if it merely involves the addition of a few microliters of a volatile reagent to the final extract prior to its evaporation. Peak tailing and adsorption of underivatized secondary amines have been minimized by the deactivation of active sites of the GC injector and column [7,17]. In some cases derivatives are prepared for use with an electron-capture detector (ECD). Thus trifluoroacetyl, pentafluoropropionyl [58] and heptafluorobutyryl [59] derivatives of primary and secondary amines have been prepared when an ECD is used. With the use of other detectors, acetyl and trifluoroacetyl derivatives are commonly prepared. Trifluoroacetyl derivatives have higher volatility than the corresponding acetyl derivatives and produce sharp peaks. Trifluoroacetyl derivatives are generally prepared with trifluoroacetic anhydride [25]. However, trifluoroacetic anhydride is very reactive and in some cases may produce multiple products. Sasaki and Baba [18] have used N-trifluoroacetylimidazole to prepare trifluoroacetyl derivatives of DMI.

### *5.3. Choice of column*

A majority of GC procedures for the determination of antidepressants published in the 1980s have used 1–2-m-long packed glass columns. The liquid phase most commonly used for the GC separation of antidepressants remains OV-17 or an equivalent packing, *e.g.* SP-2250 [17,43]. It is a fairly selective liquid phase and most of the commonly prescribed antidepressants are separated. However, primary amine metabolites of these drugs are not well separated from their secondary amine precursors with or without derivatization on these columns [13,57]. To separate primary amine metabolites, Kristinsson [13] used OV-225 as the liquid phase. Burch *et al.* [57] removed primary amines by reaction of the extract with salicylaldehyde and used an OV-17 column to separate secondary amines from their tertiary amine precursors.

There is an increasing trend to use quartz capillary columns with bonded liquid phases for the GC determination of drugs [7,14,18,25,27,58]. With proper choice of parameters, Jones *et al.* [27] achieved adequate separation using a 15 m × 0.32 mm-I.D. DB-5 column. Non-polar stationary phases such as SE-30 or equivalent phases like DB-1 or DB-5 are commonly used. These columns allow the separation of primary amines from their secondary amine precursors with or without derivatization. However, a commonly prescribed benzodiazepine (diazepam) may elute close to CMI on such a column. In such a case a capillary column bonded with a polar liquid phase, *e.g.* OV-17, may be required to achieve the desired separation.

Helium is the recommended carrier gas used to exploit the high efficiency of the capillary columns. Use of hydrogen, which can provide even higher efficiency than helium, cannot be used with the commonly used detectors, nitrogen-phosphorus detector (NPD) and mass spectrometer for the determination of antidepressants.

In our experience, splitless injection provides

high sensitivity and good reproducibility for the determination of antidepressants. Use of the split injection mode suggested by Van Brunt [7] to reduce the solvent peak appears to be unnecessary with the use of NPD. With proper choice of flow-rates of detector gases and make-up gas, the solvent peak can be kept to a minimum in the splitless mode. In fact, even septum purge is not required with a splitless injector and a NPD, if the injection volume of the extract is kept to about 1 μl.

### 5.4. Detectors

Use of a flame ionization detector (FID) for the determination of antidepressants is of historical importance only. Many extraneous peaks are produced with the use of a FID when serum extracts are analyzed at the instrument sensitivity required for the detection of antidepressant drugs. However, Burch *et al.* [57] have described a clean chromatogram for the determination of considerably low concentrations of AT and its metabolites with the use of FID.

It appears that presently ECD is also rarely used. Antidepressant drugs do not respond to ECD and have to be derivatized. Derivatization of a tertiary amine is somewhat involved and produces the same product as the corresponding secondary amine. Thus two analyses are required to determine the concentrations of a tertiary amine and its corresponding secondary amine metabolite. Karlsson [60] has described a modified procedure for the derivatization of AT for its detection by an ECD. In recent years, however, determination of fluoxetine (FLU) and its corresponding primary amine metabolite norfluoxetine (NFLU) has been carried out with the use of an ECD after the preparation of pentafluoropropionyl or heptafluorobutyryl derivatives [58,59]. Theoretically, therapeutic concentrations of FLU and NFLU can be determined with the use of a NPD, with or without derivatization.

It is now certain that NPD is the most suitable detector for GC detection of psychoactive drugs which are present in plasma in low concentrations. Currently available NPDs are easy to use and quite robust. In many cases, the optimal flow-rates for the detector gases are factory-set. The quality of alkali beads has been improved and now have a long life. The problem of extraneous peaks due to phosphorus-containing contaminants in blood-collecting tubes has also been solved by changing the formulation of these tubes. However, the NPD also has some limitations. Alkali beads have a limited lifespan and it is costly to replace them periodically. Some of the liquid phases which provide excellent separation, such as OV-225, cannot be used with this detector. The baseline is not stable due to the bleeding of the nitrogen-containing liquid phase. Even capillary columns with bonded liquid phases containing nitrogen are not compatible with the use of a NPD for high-sensitivity analysis.

The mass spectrometer is the most sensitive and specific detector for GC. Until recently, a mass spectrometer was available in only a few laboratories because of high initial and operating costs. Now a number of companies are marketing simplified benchtop mass spectrometers which are adequate for drug analysis.

### 5.5. GC data

Determination of some of the commonly prescribed antidepressants is summarized in Table 1.

## 6. COLUMN LIQUID CHROMATOGRAPHY

The phenomenal growth in the application of LC techniques for the determination of antidepressants in biological fluids has continued since the early 1980s. There have been a number of improvements in the LC instrumentation and column technology. Some of the variables for LC methods for the determination of antidepressants are discussed below.

### 6.1. Choice of internal standard

In LC, a relatively large volume (10–50 μl) of the final extract is injected. Such volumes can be injected accurately and with good precision with autosamplers or with manual syringes. Therefore

TABLE 1

GAS CHROMATOGRAPHIC DETERMINATION OF ANTIDEPRESSANTS

| Drug[a] | Sample volume[b] (ml) | Extraction | Column[c] dimensions and packing | Oven temperature (°C) | Detector | Derivatization | Detection limit (μg/l) | Ref. |
|---|---|---|---|---|---|---|---|---|
| AT<br>NT<br>N-7084★<br>MAP★ | 1 (dialysate) | Toluene–isoamyl alcohol (85:15) | 25 m × 0.3 mm, OV-101 | 160°, 30°/min → 270° | EI-MS | Trifluoroacetyl | 2 | 11 |
| AT<br>10-OH-AT<br>N-7084★<br>NT[d] | 1 | *n*-Hexane | 1 m × 2 mm, 2% OV-17 Chromosorb W, 80–100 mesh | 235° | EI-MS | Trifluoroacetyl | 2 | 25 |
| AT<br>NT<br>PT★<br>10-OH-E-AT<br>10-OH-E-NT<br>10-OH-Z-NT<br>Chlorprothixene★ | 1 | Hexane–2-butanol (98.2) → 0.001 *M* HCl → pH 14 → 100 μl of *n*-butyl acetate | 15 m × 0.3 mm, DB-5 | 120°, 32°/min → 250° | NPD | – | 0.5 | 27 |
| AT<br>TRI<br>IMI<br>NT<br>DTRI<br>DMI<br>PT<br>DDOX★<br>MAP<br>CMI<br>DCMI | 1 | Pentane wash at acidic pH → alkaline pH, extract twice with hexane → 1% methanolic formic acid, evaporate | 1 m × 2 mm, 3% OV-225 Supelcoport, 100–120 mesh | 215° | NPD | – | 10–20 | 13 |
| AT<br>IMI<br>DOX<br>CMI<br>N-7084★<br>Benzoctamine★ | 1 | Pentane wash at acidic pH → alkaline pH, extract with pentane → evaporate with acetic anhydride | 15 m × 0.3 mm, DB-1 | 200°, 5°/min → 255° | NPD | Acetyl | 10 | 14[e] |

*(Continued on p. 198)*

TABLE 1 *(continued)*

| Drug[a] | Sample volume[b] (ml) | Extraction | Column[c] dimensions and packing | Oven temperature (°C) | Detector | Derivatization | Detection limit (μg/l) | Ref. |
|---|---|---|---|---|---|---|---|---|
| NT<br>DDOX<br>DMI<br>PT<br>MAP | | | | | | | | |
| AT<br>TRIM<br>IMI<br>DOX<br>NT<br>DTRI<br>DMI<br>DDOX<br>Promazine★<br>CMI<br>DCMI | 2 | Hexane–isoamyl alcohol (98.3:1.7) → evaporate | 1.8 m × 2 mm, 3% OV-25 Gas-Chrom Q, 100–120 mesh | 245° | NPD | – | 10 | 15 |
| AT<br>IMI<br>NT<br>DOX<br>DMI<br>DDOX<br>CMI★ | 0.5–4.0 | Hexane–isoamyl alcohol (98:2) → 0.1 *M* HCl → alkaline pH → 50 μl of toluene–isoamyl alcohol (85:15) → organic layer directly injected | 1.8 m × 2 mm, 3% SP-2250 Supelcoport, 80–100 mesh | 230° | NPD | – | 1 | 17 |
| AT<br>IMI<br>NT<br>DMI | 2 | Extraction with 200 μl of toluene–heptane–isoamyl alcohol (70:20:10) → organic layer directly injected | 0.6 m × 4 mm, 2.5% Carbowax–0.5% KOH Gas-Chrom Q, 80–100 mesh | 225° | CI-MS (methane) | – | 10 | 20 |
| AT<br>IMI[f]<br>NT<br>DMI<br>MAP<br>DCMI<br>CMI | 1 | Diethyl ether → 0.1 *M* sulfuric acid → alkaline pH → diethyl ether → evaporate | 0.4 m × 2 mm, 3% OV-17 Chromosorb HP, 80–100 mesh | 240° | CI-MS (methanol) | – | <1 | 22 |
| AT<br>IMI<br>NT<br>DMI | 1 | *n*-Heptane–isoamyl alcohol (60:3) → 0.2 *M* HCl → alkaline pH → 25 μl of heptane → organic layer directly injected | 1.2 m × 3 mm, 10% OV-17 Gas Chrom Q, 100–120 mesh | 235° | NPD | – | 10–20 | 23 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MAP★<br>CMI | | | | | | | | |
| AT<br>IMI<br>NT<br>DMI<br>CMI★ | 0.5 | BondElut $C_{18}$ (1 ml) column; wash with 4 × 1 ml of 10 m*M* acetic acid, 1 × 1 ml of 90% methanol, elute with 2 × 200 μl of 5 m*M* dicthylamine in methanol, evaporate elutate | 1.8 m × 2 mm, 3% SP-2250 Supelcoport, 80–100 mesh | NA[g] | NPD | – | 20 | 37 |
| AT<br>TRIM<br>IMI<br>NT<br>DOX<br>DMI<br>DDOX<br>Promazine★ | 1 | BondElut $C_2$ (3 ml) column, wash with 2 × 2 ml of 0.2 *M* sodium carbonate, 2 × 2 ml of water; elute with 1 × 2.5 ml of methanol–chloroform (1:9); evaporate eluate | 1.2 m × 2 mm, 3% SP-2250 Supelcoport, 80–100 mesh | 235° | NPD | – | 30 | 43 |
| CM<br>PT★<br>DCMI | 2 | Sep-Pak $C_{18}$ cartridge wash with 1 × 0.5 ml of water; elute with 1 × 10 ml of acetone-*n*-hexane (3:7), evaporate eluate | 2 m × 2 mm, 3% SP-2250 Supelcoport, 80–100 mesh | 265° | NPD | Trifluoroacetyl | 2 | 87 |
| CMI[f]<br>DCMI | 1<br>(blood) | *n*-Heptane–isoamyl alcohol (99:1) → 0.5 ml of 0.05 *M* sulfuric acid → pH 14 → *n*-heptane → derivatization → evaporate | 12.5 m × 0.2 mm, Hewlett-Packard methyl silicone | 200°, 0.5′, 50°/min → 280° | EI-MS | Pentafluoropropionyl | 1 | 88 |
| AT★<br>DOX<br>NT★<br>*cis*-DDOX<br>*trans*-DDOX | 1 | Hexane–isoamyl alcohol (9.5) → 0.5 ml of 0.1 *M* HCl → evaporation | 1.1 m × 2 mm, 3% OV-17 Gas-Chrom Q, 100–120 mesh | 220° | EI-MS | Trifluoroacetyl | 1 | 89 |
| NFLU<br>FLU<br>Tomoxetine★ | 1 | Butyl chloride – 5 ml of 0.2 *M* HCl → pH 9.9 → butyl chloride → evaporation | 1.2 m × 3 mm, 3% SP-2100 Supelcoport, 80–100 mesh | 190° | ECD | Pentafluoropropionyl | 20 | 90 |
| FLU<br>NFU<br>8-Methoxyloxapine★ | 1 | Petroleum ether (3.2 ml) + dichloromethane (0.5 ml) + 60% aqueous isopropanol (2 ml) → 1 ml of 0.05 *M* HCl → pH 14 → 0.2 ml of dichloromethane → derivatization → evaporation | 15 m × 0.32 mm, DB-17 | 130°,1′, 15°/min → 300° | NPD | Butyryl | N.A.[h] | 91 |

*(Continued on p. 200)*

TABLE 1 *(continued)*

| Drug[a] | Sample volume[b] (ml) | Extraction | Column[c] dimensions and packing | Oven temperature (°C) | Detector | Derivatization | Detection limit (μg/l) | Ref. |
|---|---|---|---|---|---|---|---|---|
| FLU<br>NFLU<br>Tomoxetine★ | 1 | BondElut Certify (1 ml) column at pH 6.0, wash with methanol (2 ml), acetonitrile (2 ml); hexane–ethyl acetate (1:1, 2 ml); elute with dichloromethane–isopropanol ammonia (80:20, 2 ml) → evaporation | 30 m × 0.25 mm, DB-1 | 110°, 10°/min → 200° | ECD | Pentafluoropropionyl | 20 | 58 |
| IMI[f]<br>DMI | 2 | Extrelut (2.4 g) column → elute with 20 ml of ethyl acetate → 3 × 2 ml of 0.01 *M* HCl → pH 10 → 2 × 2 ml of *n*-hexane → evaporation | 25 m × 0.33 mm, CBP1 (Shimadzu) | 80°, 3°/min → 240° | CI-MS (isobutane) | Trifluoroacetyl | 0.1 | 18 |
| TRAZ[i]<br>Benperidol★ | 0.5 | Benzene (10 ml) → evaporation, addition of I.S. | 1 m × 3 mm, 3% OV-1 Gas-Chrom Q, 80–100 mesh | 270° | NPD | – | 50 | 92 |
| Chlorohaloperidol★<br>TRAZ | 0.1–1 | Hexane–isoamyl alcohol (98:2) → evaporation | 1.2 m × 2 mm, 3% OV-101 Chromosorb W, 80–100 mesh | 260° | NPD | – | 5 | 93 |
| Trimethobenzamide★<br>TRAZ | 5 (blood) | Butyl chloride–isopropanol (99:1) → 2.5 ml of 0.05 *M* sulfuric acid → pH 10 → 50 μl of chloroform → extract directly injected | 4 m × 0.25 mm, DB-1 | 265° | FID | – | N.A. | 94 |

[a] Drugs, metabolites and internal standards (indicated by an asterisk) are listed according to their elution order.
[b] Plasma or serum unless indicated otherwise.
[c] Columns are made of glass or quartz unless stated otherwise.
[d] NT and 10-OH-NT were determined separately.
[e] Modified procedure.
[f] Deuterated analogues were used as internal standards.
[g] Refer to Supelco Technical Bulletin No. 782.
[h] The procedure was applied for the analysis of autopsy samples in a fatal overdose case of fluoxetine.
[i] The metabolite, 1-*m*-chlorophenylpiperazine, was determined separately after the preparation of heptafluorobutyryl derivative.

addition of an I.S. just prior to injection [46] appears to be unnecessary with the use of absorbance detectors. The use of I.S. is quite helpful to monitor various extraction steps. However, I.S. may not be required if the extraction procedure is simple. Thus, Matsumoto *et al.* [54] have obtained good precision without the use of any I.S. In their procedure, serum samples were injected directly on a pre-column for purification. It is advisable to use an I.S. in multi-step liquid–liquid and SPE procedures. A proper I.S. helps to correct the variability in the packings of SPE cartridges. A single I.S. is considered adequate for the determination of both tertiary and secondary amines because there is no derivatization step in LC procedures. However, there is an exception. Sonsalla *et al.* [21] have used a tertiary amine (TRI) and a secondary amine protriptyline (PT) as I.S. to monitor the losses of drugs during extraction. The use of two internal standards appears to be unnecessary now, as in a number of cases, the evaporation of extract is not required for LC analysis.

### *6.2. Choice of column*

The choice of the column for LC analysis is more or less empirical. In earlier papers, 25–30-cm-long columns packed with 10 μm silica particles were commonly used. In some publications 25-cm-long columns packed with 5-μm particles have also been used [16,24]. In most of the applications the I.D. of the column is 3.9–4.6 mm. In one publication [40] a 10 cm × 2.1 mm I.D. column packed with 3 μm particles has been used. Use of such columns requires special precautions regarding plumbing and sample injection. However, there is a saving of the mobile phase with the use of short and narrow-bore columns.

In about half of the LC procedures published in the 1980s, columns packed with a polar stationary phase such as unbonded silica or silica bonded with cyanopropyl groups (CN) have been used in the reversed-phase mode. The mobile phase in these procedures is polar and based upon water-miscible solvents. The rational of the popularity of CN-silica columns for the determination of antidepressants is not clear. Columns packed with a non-polar stationary phase, *i.e.* $C_8$ or $C_{18}$ silica, are preferred because of their long life unless a better separation can be achieved by an alternative stationary phase. Columns packed with silica bonded with small carbon chains, *e.g.* $C_3$ [24], may have a shorter life than a $C_8$ or $C_{18}$ column because the underlying silica matrix is more exposed to attack by mobile phase chemical additives and their susceptibility to loss of bonded phase. The choice between a $C_{18}$ and a $C_8$ column is difficult. With a given mobile phase, retention of antidepressants on a $C_{18}$ column is more than on a $C_8$ column of the same brand and similar dimensions without necessarily improving the separation. Therefore, a $C_8$ column may be better than a $C_{18}$ column for faster analysis. Wong *et al.* [61] have described the use of phenyl columns for improved separation of some drugs. However, complete separation from potentially interfering co-administered drugs could not be achieved.

### *6.3. Mobile phase*

The same type of column packings for reversed-phase LC differ from manufacturer to manufacturer. The quality of packing material depends upon starting silica, its treatment prior to bonding with the alkyl chain or other groups, binding process and treatment after binding, *i.e.* end-capping. Above all, the performance of the column also depends on the way it has been packed. The method development of a reversed-phase LC procedure for the determination of antidepressants involves the optimization of the mobile phase for a particular column. Antidepressant drugs are strong bases and produce tailing peaks by their interaction with the remaining silanols despite end-capping. There is still no concensus on the optimal way to eliminate the tailing of antidepressant peaks despite the publication of a large number of LC procedures for the determination of these drugs.

In some procedures, tailing of peaks has been avoided by using a phosphate buffer at a neutral pH of 7–7.5 [26,30,38,50,51]. This pH is quite

compatible with silica-based columns. At this pH, the strongly basic drugs may form ion pairs with $HPO_4$ ions. However, this hypothesis may not be quite satisfactory. Johnson *et al.* [62] have obtained sharp peaks of antidepressants using an acetate buffer of pH 7 and an Ultrasphere CN column. As compared with phosphate ions, acetate ions have a relatively weak capacity of making ion pairs with strong bases. Wong and McCauley [63] believe that tailing of peaks in their procedure has been eliminated because of use of a temperature of 50°C for their Bondapak $C_{18}$ column and a mobile phase containing phosphate buffer of pH 4.7. Johnson *et al.* [62] have also used a temperature of 45°C for their procedure for the separation of antidepressants with the use of a mobile phase containing acetate buffer. In a vast majority of procedures, LC separation of antidepressants has been carried out at ambient temperatures.

Another common approach to minimize tailing has been to use an alkaline mobile phase (pH > 8) [19,21,64,65]. One would have expected a reduction of column life because of dissolution of silica by this alkaline mobile phase. However, according to these reports there was no significant effect on column life by the use of such a mobile phase with silica columns. The mobile phase for these procedures is a mixture of methanol and ammonia [19,65] or acetonitrile, methanol and ammonia [64]. It is believed that methanolic ammonia dissolves silica to a much lesser extent than aqueous ammonia. Bidlingmeyer *et al.* [35] replaced ammonia by *n*-butylamine in their mobile phase which was used with a silica column.

It is common practice to use ion-pairing reagents such as heptanesulfonic acid to increase the retention of highly polar amines like catecholamines on reversed-phase LC columns. Such reagents have also been used to reduce peak tailing of antidepressants. Heptanesulfonic acid has been used by Suckow and Cooper [24] with a $C_3$ column and by Matsumoto *et al.* [54] with a $C_{18}$ column. Hung *et al.* [66] have used sodium laurylsulphate as the ion-pairing reagent with a $C_{18}$ column. Use of these reagents do give sharp peaks. However, antidepressant drugs become strongly retained and require a relatively high proportion of organic solvent in the mobile phase containing such ion-pairing reagents. Also, it takes much longer to equilibrate the column with such a mobile phase than it takes with a mobile phase without an ion-pairing reagent. Gupta and Steiner [39] and Preskorn and Glotzbach [67] have used perchlorate as the ion-pairing reagent. Perchlorate is only a mild ion-pairing reagent, as compared to long-chain alkylsulfonates or sulfates, resulting in only a small increase in the retention of polar amines. Columns are relatively easily equilibrated with perchlorate-based mobile phases. Preskorn and Glotzbach [67] have described sharp peaks of antidepressants with a $C_{18}$ column and a perchlorate-based mobile phase.

It appears that the most commonly used approach to minimize peak tailing for the determination of antidepressants is to add an excess of a competing base to the mobile phase to minimize the interaction of silanols with the amino groups of the analytes. Kiel [16], Lensmeyr and Evenson [41] and Sokolowski and Wahlund [68] have studied the influence of pH, the nature of amine, the amount of amine and the temperature on the separation of antidepressants. However, there is still no agreement about any of these parameters for optimal separation of antidepressants. A variety of amines, methylamine [16], triethylamine [24,36], *n*-butylamine [35,69], dimethyloctylamine [40] and nonylamine [61,70], have been used with apparently similar results. Some of these amines are toxic, and the mobile phase has to be prepared in a fume hood [61]. To avoid this toxicity, non-volatile quarternary salts such as tetramethylammonium salts have been used to minimize peak tailing [39]. Minder *et al.* [71] have suggested that the use of triethylamine is better than the use of heptanesulfonic acid for the separation of basic drugs.

In some cases both the ion-pair reagent and the competitive amine have been added to the mobile phase to minimize peak tailing [24,39,66]. However, the additional advantage of using two agents over one has not been clearly established.

Some manufacturers describe the percentage carbon loading of their bonded phases. However,

it is believed that description of a bonded phase in terms of percentage carbon load may not be an adequate parameter and the column packing is better described by the concentration of silyl groups per unit of surface area of silica. In any case, some manufacturers are promoting bonded silica with as high as 30% carbon loading for the separation of polar basic drugs without the addition of any competing amine or ion pair to the mobile phase. In our experience, such a column did not behave any differently from the column packed with the conventional bonded-phase silica of average (12–15%) carbon load, as far as the peak shape of antidepressant drugs was concerned. Columns packed with high-carbon-load bonded silica show significantly higher back-pressure than columns packed with conventional bonded silica.

### *6.4. Detection*

For the detection of antidepressants as a group after LC separation, absorbance detection is the only practical approach. The current popularity of LC methods for the determination of antidepressants over GC is partly due to the availability of sensitive absorbance detectors. Variable-wavelength absorbance detectors are now available which match fixed-wavelength detectors in sensitivity. A number of procedures described in the 1980s have monitored the absorbance of column eluate at 254 nm using fixed-wavelength detectors to detect antidepressants. For improved sensitivity, other wavelengths for detection of antidepressants, *e.g.* 210 nm [54], 215 nm [30,47,61], 220 nm [64], 240 nm [12], 242 nm [40] and 245 nm [62], have been used. In our experience, the use of 215 nm is most suitable for the therapeutic concentrations of all of the commonly prescribed antidepressants. Detection at 215 nm provides higher sensitivity and comparable selectivity than detection at 240 or 250 nm. Recently, Puopolo and Flood [72] have pointed out the usefulness of simultaneous detection at 214 and 254 nm. Comparison of the ratios of absorbance at two wavelengths helped to detect interference of fluoxetines with imipramines.

For specific drugs, other selective detectors have also been used. Thus fluorescence detection has been used for imipramines and analogues [62], fluoxetines [39] and trazodone [48]. Electrochemical detection is most suitable for the sensitive detection of imipramines and analogues (TRI) [28,73].

### *6.5. Commercial LC kits*

A number of commercial companies marketing LC products have developed procedures for the LC determination of antidepressants for easy adoption in routine clinical laboratories. It appears that Bio-Rad Labs. are marketing a complete system for the simultaneous determination of antidepressants and benzodiazepines. The system includes LC hardware, extraction cartridges for SPE, reagents for selective SPE of benzodiazepines and antidepressants, analytical column, pre-made mobile phase and pre-made plasma-based drug standards and controls. This reagent kit is based on the procedure published by Mazhar and Binder [50]. Supelco has adopted a procedure published by Carfagnini *et al.* [46] and has marketed the two required types of extraction cartridges and analytical columns. Consumers have to prepare their own reagents. It seems that most of the companies marketing SPE cartridges or analytical columns have published analytical notes for the determination of antidepressants and there are too many to be listed here. However, it may be appropriate to mention the procedure developed by Worldwide Monitoring Corporation (Horsham, PA, USA). In this procedure a mixed-phase silica containing a cation-exchange and a hydrophobic component of a reversed phase has been used for SPE of antidepressants from serum. The eluate has to be evaporated and is suitable for both GC and LC determinations. Use of similar extraction cartridges marketed by Varian has been described by Dixit *et al.* [58].

### *6.6. LC data*

Determination of antidepressants by LC is summarized in Table 2.

TABLE 2

COLUMN LIQUID CHROMATOGRAPHIC DETERMINATION OF ANTIDEPRESSANTS

| Drug[a] | Sample volume[b] (ml) | Extraction | Column[c] dimensions, packing and temperature | Mobile phase and flow-rate | Detector | Detection limit (μg/l) | Ref. |
|---|---|---|---|---|---|---|---|
| AT-N-oxide<br>NT<br>DMI★ | 1.5 | Dichloromethane, 3 × 3 ml → evaporation | 25 cm × 4 mm, Lichrosorb CN, 10 μm, 45°C | Acetonitrile–0.07% ammonia + 0.1% lauryl sulfate in water (85:15), 1 ml/min | Absorbance, 210 nm | 10 | 32 |
| *trans*-10-OH-NT<br>*cis*-10-OH-NT<br>*trans*-10-OH-AT<br>Desmethyl-NT<br>AT-N-oxide<br>IMI★<br>AT | 2 | Hexane–1-butanol (9:1) → 100 μl of 3 *M* phosphoric acid | 25 cm × 4.6 mm, Supelcosil C8, 5 μm 40°C | Methanol–water (2:1) containing 25 m*M* dibasic sodium phosphate + 250 m*M* methylamine–HCl, pH 7.25 with phosphoric acid, 2 ml/min | Absorbance, 214 nm | 1 | 16 |
| 10-OH-NT<br>10-OH-AT<br>DDOX★<br>NT<br>AT | 1 | Column switching | 10 cm × 4 mm, Techsphere, 3 μm | Acetonitrile–0.05 *M* acetate buffer (60:40), pH 7, 0.9 ml/min | Absorbance, 215 nm | 10 | 53 |
| NT<br>AT | 2 (dialysate) | Column switching | 10 cm × 4 mm, Spherisorb ODS, 3 μm | Water–85% phosporic acid–triethylamine–acetonitrile (50:0.225:0.225:49.55), 0.65 ml/min | Absorbance, 238 nm | N.A. | 10 |
| TRI<br>DOX<br>AT<br>CMI<br>IMI<br>*trans*-10-OH-NT<br>DOX<br>NT<br>DCMI<br>DMI<br>PT★ | 2 | Hexane–acetonitrile (98:2) → evaporation | 30 cm × 3.9 mm, μBondapak CN, 10 μm | Acetonitrile–methanol–5 m*M* phosphate buffer, pH 7 (625:155:220), 1.5 ml/min | Absorbance, 254 nm | 2 | 26 |
| CMI<br>AT,DOX<br>IMI<br>NT<br>DDOX | 0.5 | Hexane–isoamyl alcohol (95:5) → evaporation | 25 m × 4.6 mm, Zorbax Sil, 5 μm | Methanol–ammonia (998:2), 1.5 ml/min | Absorbance, 254 nm | 10 | 65 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DCMI★<br>DMI★ | | | | | | | |
| 7-OH-loxapine<br>DDOX<br>DOX<br>DMI<br>NT<br>IMI<br>AT<br>TRIM★ | 2 | BondElut CN (1 ml) column → wash 1 × with phosphate buffer, pH 7, 1 × with water → elute with 0.5 ml of 0.5 *M* acetic acid–acetonitrile-*n*-butylamine (30:70:0.08) and 0.35 ml of 0.5 *M* acetic acid → eluates mixed and injected | 25 cm × 4.6 mm, Zorbax-CN, 5 μm | Acetonitrile–0.5 *M* acetic acid-*n*-butylamine (41:59:0.02)[d], 1.5 ml/min | Absorbance, 254 nm | 10 | 41 |
| N-Propionyl-procainamide★<br>TRI<br>DOX<br>AT<br>IMI<br>DDOX<br>DMI<br>NT<br>PT | 0.5 | BondElut $C_{18}$ (1 ml) column → wash 2 × with water, 2 × with 0.1 *M* acetic acid, 1 × with 10% methanol in 0.25 m*M* HCl → elute 2 × with 0.2 ml of methanol containing 10 m*M* acetic acid + 5 m*M* diethylamine → eluates mixed and injected | 15 cm × 4.6 mm, Supelcosil LC-PCN, 5 μm | Acetonitrile–methanol–10 m*M* phosphate buffer, pH 7 (58:14:28), 1.2 ml/min | Absorbance, 254 nm | 25 | 38 |
| DDOX<br>DOX<br>DMI<br>IMI<br>NT<br>AT<br>TRI★ | 1 | Bio-Rad $C_{18}$ (1 ml) column → wash 3 × with 20% aqueous acetonitrile, 2 × with 0.4 ml of water–methanol–acetonitrile (2:3:3)[e] → elute 2 × with 0.3 ml of methanol–diethylamine (100:0.6) → eluates mixed, dilute with 0.3 ml of water and injected | 10 cm × 2.1 mm Bio-Rad $C_8$, 3 μm, 35°C | Acetonitrile–0.01 *M* phosphate buffer, pH 6.4 + 0.1 ml/l N,N-dimethyloctylamine (30:70), 0.6 ml/min | Absorbance, 242 nm | 25 | 40 |
| 8-OH-AMOX<br>7-OH-AMOX<br>AMOX<br>Procainamide★,[f]<br>DOX<br>AMI<br>IMI<br>DDOX<br>NT<br>DMI<br>MAP | 1 | Carbopack B (1 ml) column → wash with 1 ml of methanol → elute with 4 ml of methylene chloride–methanol (6:4) → pass eluate through SCX (200 mg) column → wash with 2 ml of 0.1 *M* KCl in methanol–water (15:85) → elute with 1.2 ml of acetonitrile–methanol–water (72:18:10) saturated with KCl → eluate diluted with an equal volume of water and injected | 25 cm × 4.6 mm, Supelsosil LC-PCN, 5 μm | Acetonitrile–methanol-0.01 *M* phosphate buffer, pH 7.8 (52:13:35), 2 ml/min | Absorbance, 215 nm | 4.5 | 46 |
| AMOX<br>DOX<br>DMI<br>IMI | 0.2 | TSK gel ODS precolumn, wash for 10 min at 1 ml/min with 50 m*M* phosphate buffer, pH 7.5 → column switching in a back-flush mode | 15 cm × 4 mm, TSK gel ODS, N.A. | Acetonitrile–0.1 *M* phosphate buffer + 0.2 g/l 1-heptane sulfonate, pH 2.7 (32.f:67.5)[g], 1 ml/min | Absorbance, 210 nm | 10 | 54 |

*(Continued on p. 206)*

TABLE 2 *(continued)*

| Drug[a] | Sample volume[b] (ml) | Extraction | Column[c] dimensions, packing and temperature | Mobile phase and flow-rate | Detector | Detection limit (μg/l) | Ref. |
|---|---|---|---|---|---|---|---|
| NT<br>MAP<br>AT<br>TRI<br>CMI | | | | | | | |
| Clobazam★<br>DMI<br>NT<br>IMI<br>AT<br>CMI | 1 | *n*-Hexane → evaporation | 15 cm × 4.6 mm, Supelco LC-8, 5 μm | Acetonitrile–0.01 *M* phosphate buffer + 1.2 ml/l butylamine, pH 3 (50:50), 1 ml/min | Absorbance, 254 nm | 25 | 69 |
| CMI<br>IMI★<br>DCMI | 0.5 | Varian AASP[h] octyl-silica cartridge, wash with 6 ml of 0.005 *M* phosphate buffer, pH 11.5, elution in a back-flush mode | 15 cm × 4.6 mm, Spherisorb CN, 5 μm | Acetonitrile–methanol–0.005 *M* phosphate buffer, pH 7.2 (50:25:30), 1.5 ml/min | Absorbance, 252 nm | 10 | 51 |
| AT★<br>2-OH-DMI<br>IMI | 2 | Ethyl acetate–hexane–isoamyl alcohol (50:49:1) → evaporation | 15 cm × 4.6 mm, Supelcosil LC-PCN, 5 μm | Acetonitrile–methanol–0.01 *M* phosphate buffer, pH 7.0 (600:180:220), 3 ml/min | Absorbance, 214 nm | 1 | 30 |
| Dihydrohaloperidol<br>NFLU<br>FLU | 1 | Hexane–isoamyl alcohol (97:3) → 1 ml of 0.1 *M* HCl → alkaline pH, extract with hexane–isoamyl alcohol (97:3) → evaporate with 0.3 ml of methanolic HCl | 15 cm × 3.0 mm, Nova-Pak Phenyl, 4 μm | Acetonitrile–water (400:600) containing 1 ml triethylamine adjusted to pH 5.5 with acetic acid, 1.7 ml/min | Absorbance, 226 nm | 15 | 95 |
| NFLU<br>FLU<br>CMI★ | 2 | *n*-Hexane–isoamyl alcohol (99:1) → 0.2 ml of 0.05% phosphoric acid → aqueous layer injected | 30 cm × 4.6 mm μBondapak $C_{18}$, 10 μm, 50°C | Acetonitrile–0.05 *M* phosphate buffer, pH 4.7 (4:6), 2 ml/min | Absorbance, 214 nm | 6 | 96 |
| PT★<br>NFLU<br>FLU | 0.5 | BondElut $C_{18}$ (1 ml) column → wash 2× with water, 1× with 50% aqueous methanol and 1× with acetonitrile → elute with 0.25 ml of 0.1 *M* perchloric acid–acetonitrile (1:3) | 15 cm × 4.6 mm Ultrasphere $C_8$, 5 μm | Acetonitrile–water (375:625) containing 1.5 g tetramethylammonium perchlorate + 0.1 ml of 70% perchloric acid, 2 ml/min | Fluorescence, ex = 235 nm, em = 310 nm | 20 | 39 |
| Clovocamine★<br>FLUV | 1 | Heptane–2-propanol (98:2) → 0.1 ml of 0.1 *M* HCl → aqueous layer injected | 12 cm × 4.6 mm, Nucleosil C8, 5 μm | Acetonitrile–0.016 *M* phosphate buffer, pH 2.5 (36.64), 1 ml/min | Absorbance, 215 nm | 25 | 97 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| FLUV<br>Metapramine* | 2 | Diethyl ether–hexane (1:1) → 2 ml of 0.25 *M* sulfuric acid → alkaline pH → diethyl ether–hexane (1:1) → evaporated → residue derivatized with dansyl chloride | 12.5 cm × 4.6 mm Hypersil ODS, 5 μm, 30°C | Acetonitrile–water, linear gradient from 45 to 65% acetonitrile, 1.5 ml/min | Fluorescence, ex = 7.54, 7.60, em = 3.71, 4.76, filter combinations | 1.5 | 98 |
| FLUV<br>Clovoxamine* | 1 | Hexane → evaporation | 15 cm × 3.9 mm, Resolve silica, 5 μm | Methanol–acetonitrile–tetrahydrofuran–water–diethylamine (98:59:1:0.2:0.01), 1 ml/min | Absorbance, 254 nm | 0.5 | 99 |
| FLUV<br>TRI* | 0.5 | BondElut $C_{18}$ (1 ml) column → wash 3× with water, 1× with 50% methanol and 1× with acetonitrile → elute 2× with 0.15-ml aliquot of methanol–20% perchloric acid (95:5) containing 0.2% tetramethyl ammonium perchlorate | 15 cm × 4.6 mm, Ultrasphere $C_8$, 5 μm | Acetonitrile–water (350:650) containing 2 g tetramethylammonium perchlorate + 0.1 ml of 70% perchloric acid, 1.8 ml/min | Absorbance, 245 nm | 25 | 100 |
| DMI<br>IMI<br>CMI* | 1 | Methanol (4 ml) → Sep-Pak $C_{18}$ cartridge → wash with 4 ml of 50% methanol → elute with 2 ml of acetonitrile–1% triethylamine–HCl → evaporation | 15 cm × 4.6 mm, Cosmosil $C_{18}$, 5 μm | Acetonitrile–1% triethylamine, pH 6.0 with phosphoric acid (38:62), 1.0 ml/min | Absorbance, 254 nm | 10 | 36 |
| MAP<br>DMAP<br>Oxaprotiline* | 1 | Toluene, 2 × 1.5 ml → 0.5 ml of 5 m*M* sulfuric acid → alkaline pH and derivatization with dansyl chloride → hexane, 2 × 1 ml → evaporation | 30 cm × 4.5 mm, Nucleosil silica, 7 μm | Hexane–ethanol (95:5), 1.8 ml/min | Fluorescence, ex = 365 nm, em = 420 nm | 10 | 101 |
| Harmine*<br>TRAZ | 0.5 | BondElut $C_{18}$ (1 ml) column → wash 2× with water, 2× with 10% methanol–water (1:9) → elute with 0.5 ml of methanol | 15 cm × 4.6 mm, Ultrasphere $C_8$, 5 μm | Acetonitrile–water (500:500) containing 0.5 ml of 20 g/l tetramethylammonium perchlorate in methanol and 0.5 ml of 70% perchloric acid, 1 ml/min | Fluorescence, ex = 320 nm, em = 440 nm | 1 | 48 |
| 2-OH-TRI<br>TRI<br>Chlorpromazine*<br>DTRI | 2 | Hexane–isoamyl alcohol (98:2) → evaporation | 25 cm × 4.6 mm, Spherisorb CN, 5 μm | Acetonitrile–methanol–phosphate buffer, pH 6.5, 0.8 ml/min | Electrochemical, + 0.85V | 1 | 73 |

[a] Drugs, metabolites and internal standards (indicated by an asterisk) are listed according to their elution order.
[b] Plasma or serum unless indicated otherwise.
[c] Column temperature other than ambient.
[d] The mobile phase is recycled for one month.
[e] This wash can be collected for the determination of benzodiazepine.
[f] Added after the extraction.
[g] Variable ratio of acetonitrile/phosphate buffer was used for the determination of different drugs.
[h] A semi-automatic solid-phase extraction system. Its sale has now been suspended.

## 7. PLANAR LIQUID CHROMATOGRAPHY (TLC)

### *7.1. Quantitative TLC*

Planar liquid chromatography differs from LC in that a number of samples can be separated simultaneously prior to quantitation. A TLC spectrophotometric or fluorometric scanning system, the instrument used for quantitation, is capable of measuring a number of pre-separated samples relatively rapidly. TLC is relatively faster than other chromatographic techniques where more than eight samples are processed. Furthermore, the compounds separated on a TLC plate can be chemically converted to coloured or fluorescent products, a feature not readily and efficiently possible with LC.

Antidepressant drugs do not have strong UV absorption and, with the exception of protriptyline (PT), do not have strong native fluorescence. It is certain that presently TLC is a rarely used technique for the quantitative determination of therapeutic concentrations of antidepressants in plasma. However, TLC has been applied in the 1980s for the determination of imipramines [74]. In this procedure the specificity and sensitivity of detection have been increased by exposing the plate to nitrous acid fumes. The resulting intense yellow spots are measured at 405 nm. We have observed that this approach can also be applied for the determination of TRI and its secondary amine metabolite. However, CMI, another drug with a similar structure to that of IMI, is only poorly stained with nitrous acid.

### *7.2. Qualitative TLC*

Antidepressant drugs are commonly ingested in overdose. An overdose of a member of this class of drugs is probably one of the most serious drug overdoses seen in emergency departments. The treatment of an antidepressant-overdose patient is essentially supportive therapy under intensive care and observation. However, emergency and intensive care physicians demand emergency identification or assay of these drugs to confirm the diagnosis and plan the degree of care. It appears that TLC is the most viable technique for the detection of antidepressant overdose in a majority of clinical laboratories. In general, TLC does not have the required sensitivity to determine therapeutic concentrations of these drugs in plasma or serum. However, TLC has been quite useful for screening urine for the detection of antidepressants. Besides, $R_F$ values, colours produced by these drugs with different reagents, *e.g.* Mandelin's reagent (acidified ammonium vanadate) or perchloric acid [75], help in the identification of the drug. Detection with general reagents for basic drugs, such as acidified iodoplatinate, produces similar colours with most of these drugs and is not as useful as Mandelin's reagent for the identification of an antidepressant. To reduce the variability inherent in most TLC procedures, a commercial TLC kit system "Toxi-Lab" (Irvine, CA, USA) has been specially designed for drug analysis. Jarvie and Simpson [76] report that this system was specially useful for the detection and identification of antidepressants.

## 8. IMMUNOASSAYS

Immunoassays are being routinely carried out in clinical laboratories for the measurement of both large and small molecules: peptide hormones, steroids and drugs. Technologists prefer them to chromatographic techniques as immunoassays are relatively fast, require minimal sample handling and are available as complete ready-to-use kits.

### *8.1. Radioimmunoassays (RIA)*

In the 1970s, a number of RIA procedures for the determination of antidepressant drugs were developed. These procedures have been summarized by Gupta and Molnar [77]. However, RIA procedures are no longer used for the determination of antidepressants. There is an increasing trend to avoid the use of radioisotopes in clinical laboratories. Furthermore, RIA procedures are not amenable to complete automation.

### 8.2. Homogeneous immunoassays

Assays for a large number of drugs is now routine in clinical laboratories due to the availability of fully automated non-isotopic immunoassay procedures. These procedures do not require the separation of antibody-bound and free drug labels and are referred to as homogeneous immunoassays. A large number of techniques for homogeneous immunoassays for the determination of commonly prescribed antiepileptic and antiarrhythmic drugs have been developed and marketed by a number of commercial companies. However, there are only two techniques. EMIT (Syva, Palo Alto, CA, USA) and fluorescence polarization immunoassay, commonly referred to as TDx procedure (Abbott Diagnostics, Dallas, TX, USA), which include reagent kits for the detection or determination of antidepressants.

#### 8.2.1. EMIT

In 1982, Syva introduced EMIT-tox for the screening of suspected overdoses with antidepressants. This assay provides the answer as positive or negative. A result is positive when the concentration of the drug plus that of its desmethyl metabolite is more than 300 ng/ml. The result does not allow the assessment of the degree of poisoning. Only a negative result indicates that the concentration of the antidepressant, even if present, is not in the toxic range. In 1983, the company introduced another reagent system, EMIT-st, which has a cut-off limit of 200 ng/ml of the drug plus its secondary amine metabolite. These reagent systems have been evaluated for their analytical reliability and clinical applications [78,79]. The EMIT-tox serum antidepressant assay was developed for use with serum or plasma. With slight modifications, these reagent kits have been used with urine samples also [80,81].

In 1986, Syva introduced another reagent kit for the quantitative determination of therapeutic concentrations of the major four antidepressant drugs. This EMIT assay differs from the previously described antidepressant immunoassays in that it involves a SPE step to remove polar metabolites and some other interfering compounds [49]. Two separate assays are performed with the same extract to measure either tertiary amines (AT or IMI) or secondary amines (NT or DMI). This reagent system has also been evaluated for its analytical performance and clinical application [82,83]. Dorey *et al.* [82] have pointed out some limitations of EMIT quantitative procedures for the assay of antidepressants. Detection limits of antidepressants by this procedure are relatively high and in a number of cases EMIT results were higher than those obtained by LC. In my opinion, the high cost of performing two separate assays for tertiary and secondary amines is unnecessary when in most cases the results of the two determinations are added for clinical interpretation. However, these assays are rapid and convenient despite their high cost and other limitations. Some laboratories have adopted EMIT assays on large clinical analyzers such as Hitachi 705 [84].

#### 8.2.2. Abbott TDx procedure

In 1985, Abbott Labs. also introduced direct toxicological assays for antidepressants. This assay differs from EMIT-tox in that it provides a quantitative result and has lower limits of detection. Thus it is capable of providing some information about the extent of poisoning. This assay has been developed for the analysis of serum or plasma. However, it has also been applied for the analysis of urine. Unlike EMIT reagents, Abbott assays can be performed only with the Abbott analyzer.

### 8.3. Specificity

The antibody used for both EMIT-tox and Abbott TDx procedures are similar. They cross-react with different antidepressants to different extents. Thus the reactivity of the antibody for maprotiline (MAP) or FLU, commonly prescribed antidepressants, is very low as compared to that for AT, IMI and their desmethyl analogues. Grossly erroneous or falsely negative results can be obtained if the identity of the antidepressant is not exactly known and if it is not one of the major tricyclic antidepressants [77,85]. Falsely positive results for the presence of anti-

depressants can also be obtained due to tricyclic phenothiazines or antihistamines which are relatively less toxic than antidepressants. Chromatographic procedures are also not absolutely specific. However, in many instances, the chromatogram helps to identify the problem. It appears that unlike anticonvulsants, immunoassays have not been universally accepted for therapeutic monitoring or toxicological screening of antidepressants. However, EMIT quantitative assays for antidepressants have been successfully used to monitor non-compliance in chronic depression in a controlled study [86].

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