HPLC as a Tool in Medicinal Chemistry for the Monitoring of Tricyclic Antidepressants in Biofluids

V.F. Samanidou*, M.K. Nika and I.N. Papadoyannis

Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 541 24 Greece

Abstract: HPLC is discussed as an essential tool in medicinal chemistry for the monitoring of tricyclic antidepressants in biofluids, providing clinicians with efficient fast and reliable methods to define individual optimum therapeutic concentrations in treatment of depressions. Additional information on mechanism of action, structure activity relationship and metabolism is provided.

Key Words: HPLC, tricyclic antidepressants, monitoring, medicinal chemistry, biological fluids.

1. INTRODUCTION

Tricyclic antidepressants (TCAs) namely amitryptiline, doxepin, imipramine, nortryptiline, trimipramine, etc. have been in use for many years in psychiatry for treatment of disorders. An "antidepressant" is a medication designed to treat or alleviate the symptoms of clinical depression. Many new advances in antidepressants have been made over the past half century. Generally speaking, there are three classes of antidepressant medications in use: (1) The tricyclics (or heterocyclics) (TCAs), (2) the monoamine oxidase inhibitors (MAOIs) and (3) the newer, so-called second-generation agents: Selective Serotonin Reuptake Inhibitors, (SSRIs) with first member fluoxetine, developed in the 1980's. Although there is a considerable overlap in their actions and uses, these different categories of antidepressants work by distinct mechanisms, have different side effect profiles, and may be preferred for varying indications. The first group of antidepressants, that were used, was MAOIs discovered in the early 1950s and their major member, iproniazid, was initially used as possible treatment for tuberculosis. Tricyclic antidepressants were the second group with imipramine as first member, discovered accidentally during a search for a new antipsychotic in the late 1950s. TCAs were as effective as MAOIs, safer than them, but dangerous in overdose. Tricyclic antidepressants being the principal pharmacological treatment for endogenous major depressions in the period between 1960 and 1980 consist in a homogeneous group of drugs, which differs mostly in their potency to inhibit presynaptic norepinephrine or serotonin uptake and in their propensity for causing variety of unwanted effects. Despite the introduction of newer and safer antidepressants the prescription of tricyclic antidepressants is still widespread as they are cheaper and are still considered to be the most effective group of antidepressants [1, 2].

Methods for the routine analysis of TCAs are required to provide clinicians with the individuals' serum levels. Analytical procedures that allow the rapid quantification of these psychotropic drugs are of paramount significance. HPLC has been proved an efficient, fast and reliable tool in medicinal chemistry for the multi-component analysis of TCAs in samples of biological origin.

Only a limited number of review articles can be found in literature concerning tricyclic antidepressants determination in biological matrices. These are briefly cited below.

Scoggins B. A., Maguire K.P., Norman T.R. and Burrows G.D., in their review on the measurement of tricyclic antidepressants, published in 1980, discuss the approaches used since 1967, with emphasis on specificity, sensitivity, accuracy, precision (reproducibility), expense, convenience, and ease of sample processing [3].

In 1985, Norman T.R., wrote a review on the analysis of TCAs in plasma and serum by chromatographic techniques, with emphasis on application to the clinical situation [4].

In 2005, Smyth W.F. presented a review on the electrospray ionisation mass spectrometric behaviour of selected nitrogen-containing drug molecules and the application of Liquid chromatography–electrospray ionisation mass spectrometry (LC–ESI-MS) to the detection and determination of TCAs in biomatrices, pharmaceutical formulations, etc. Analytical information on sample concentration techniques, chromatographic separation conditions, recoveries from biological media, degradation products and limits of detection (LODs) is provided, covering the period 2004–2005. Comparisons, where available, are also made with rival analytical techniques such as Gas Liquid Chromatography–Mass Spectrometry (GLC–MS), Capillary Electrophoresis–Electrospray Ionisation Mass Spectrometry (CE–ESI-MS) and Stripping Voltammetry (SV) [5].

Maurer H. H. in his review published in 2005, describes multi-analyte procedures for screening and quantification of drugs in blood, plasma, or serum by Liquid Chromatography-single stage or tandem mass spectrometry (LC-MS or LC-MS/MS) relevant to clinical and forensic toxicology. TCAs are discussed among other drugs such as amphetamines, cocaine, hallucinogens, opioids, anesthetics, hypnot-

*Address correspondence to this author at the Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 541 24 Greece; Tel:+302310997698; Fax: +302310997719; E-mail: samanidu@chem.auth.gr

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ics, benzodiazepines, neuroleptics, antihistamines, sulfonylurea-type antidiabetics, beta-blockers, and other cardiac drugs. Basic information on the procedures is given in two tables while multi-analyte screening, identification, and quantification are illustrated in three figures. A critical discussion on the pros and cons of such LC-MS procedures is also included [6].

Finally in 2006, Kerr G. W., McGuffie A. C. and Wilkie S., presented a review on tricyclic anti-depressant overdose, which is among the most common causes of drug poisoning seen in accident and emergency departments. This review discusses the pharmacokinetics, clinical presentation and treatment of tricyclic overdose [7].

The present review focuses on the recent advances on the application of HPLC in medicinal chemistry for the routine analysis of TCAs and metabolites in clinical samples. Sample preparation methods used in these methodologies attracted much attention, since sample pre-treatment is the most limiting and crucial step in analyses of biological fluids. Information on medicinal chemistry of TCAs in terms of mechanism of action, structure activity relationship (structures of antidepressants) etc is also provided.

2. CHEMISTRY AND ACTIVITY

2.1. Structure Chemical Characteristics

Tricyclic antidepressants bare their name due to their chemical structure, which consists of three rings of atoms. The middle ring is usually alicyclic and contains seven atoms, except for some members that possess a heterocyclic middle ring. Their side chain consists of N-alkylmethylamine or N-alkyldimethylamine. Typical TCAs are imipramine, desipramine, clomipramine, amitriptyline, nortriptyline, doxepin, trimipramine etc [8-11]. General characteristics of antidepressant drugs extracted from literature are shown in Table **1**. The structures of these TCAs are shown in (Fig. (**1**)).

2.2. Mechanism of Action

The exact mechanism of action of TCAs in the treatment of depression is unclear and not completely understood. It is believed that their action is based on blocking the reuptake of the monoamine neurotransmitters, serotonin (5-hydroxytryptamine; 5-HT) and nor-epinephrine (NE). According to this theory these neurotransmitters are increased through inhibition of their reuptake by the presynaptic neuronal membrane.

Mood disorders are associated with reduced levels of monoamines in the brain. Neurotransmitters adjust the mood in the central nervous system. TCAs binding to 5-HT and nor-epinephrine (other name noradrenaline) reuptake transporters prevents the reuptake of these monoamines from the synaptic cleft and their subsequent degradation. As their concentration increases in the space between the nerve cells (neural synapse), by blocking the reuptake, depression relief is achieved. This reuptake blockade leads to the accumulation of 5-HT and nor-epinephrine in the synaptic cleft and the concentration returns to within the normal range. The main effect of TCAs is to block the uptake of monoamines by nerve terminals, by competing for the binding site of the carrier protein [12, 13].

Most TCAs are non-selective and inhibit nor- epinephrine and 5-HT uptake to a similar degree. Tertiary amines, such as amitriptyline, imipramine, clomipramine, doxepin and trimipramine cause reuptake inhibition of serotonin. Amitriptyline and clomipramine appear to be more potent than other tricyclics in blocking serotonin, although, through

Table 1. General Characteristics of Antidepressant Drugs [11]

TCA	Metabolites	pK_a D pK_a M	Concentration Range (mg/mL)	Half Life (h)	Time of Steady State (days)	Doses (mg/day)
Amitriptyline	Nortriptyline, 10- Hydroxy , *N*-oxide	9.4	500-300 (ami+Nor) 500-200 Nor only	35-50 M 20-100	7-8 M 4-10	75-150
Clomipramine	Norclomipramine 8- Hydroxy, Nor, 2- and 8- Hydroxy Clomipramine	D: 9.4 M:10.2	D: 90-300 M: 150-350	17-28 M 35	7-10	50-200
Desipramine	2- and 8-Hydroxydesipramine	9.5	20-300	2-54	14	50-200
Doxepine	Nordoxepine	8	D: 70-400 M: 75-250	12-17 28	9	50-300
Imipramine	Desipramine, Hydroxyimipramine	9.5	50-500	6-40	1.5-3	50-150
Opipramol	Dehydroethylopipramol		D: 14-64 M: 42-445	6-23		10-300
Trimipramine	Hydroxy, nor, desamino, desalkyl		50-150	7-14		50-300

Abbreviations: D drugs, M major metabolite.

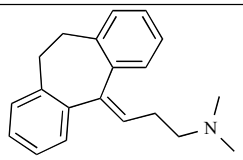
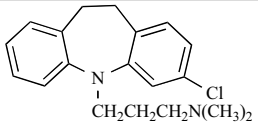
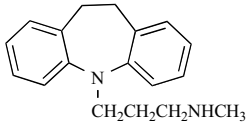
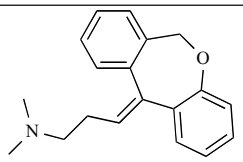
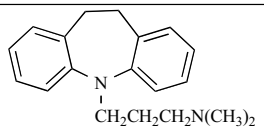
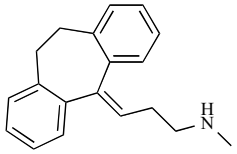
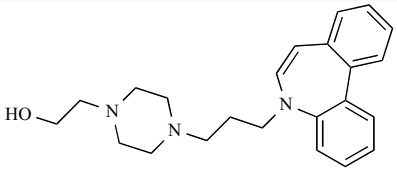
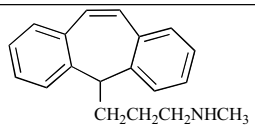
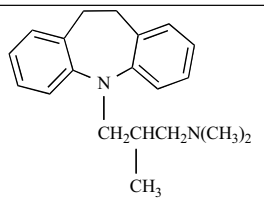
Amitriptyline 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5-ylidene)-*N*, *N*-dimethyl-1-propanamine	
Clomipramine [3-(3-chloro-10,11-dihydro-dibenzo[*b*,*f*] azepin-5-yl)-propyl]-dimethyl-amine	
Desipramine [3-(10,11-dihydro-dibenzo[*b*,*f*] azepin-5-yl)-propyl]-methyl-amine	
Doxepin 11-(3-(Dimethylamino) propylidene)-6H-dibenz(b,e)oxepine	
Imipramine [3-(10,11-dihydro-dibenzo[*b*,*f*] azepin-5-yl)-propyl]-dimethyl-amine	
Nortriptyline 3-(10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5-ylidene)- N-methyl-1-propanamine	
Opipramol 4-[3-(5H-Dibenz[*b*,*f*]-azepin-5-yl)-propyl]-1-piperazineethanol	
Protriptyline [3-(5H-Dibenzo[*a*,*d*] cyclohepten-5-yl)-propyl]-methyl-amine	
Trimipramine [3-(10,11-dihydro-dibenzo[*b*,*f*] azepin-5-yl)-2-methyl-propyl]-dimethyl-amine	

Fig. (1). Chemical structures of imipramine, desipramine, clomipramine, amitriptyline, nortriptyline, doxepin, trimipramine and opipramol.

their metabolites, they become powerful inhibitors of nor-epinephrine reuptake as well. Secondary amines, such as desipramine and nortriptyline, mainly inhibit the reuptake of norepinephrine. Imipramine inhibits reuptake of nor-epinephrine and serotonin equally. Doxepin is a moderate inhibitor of nor-epinephrine and a weak inhibitor of serotonin. Mechanism of action of selective serotonin reuptake inhibitors is shown in (Fig. (**2**)) [12, 14].

Additionally, recent research has shown that TCAs can cause beta-adrenergic downregulation as well. Where down-regulation is the phenomenon of a long-lasting increase in

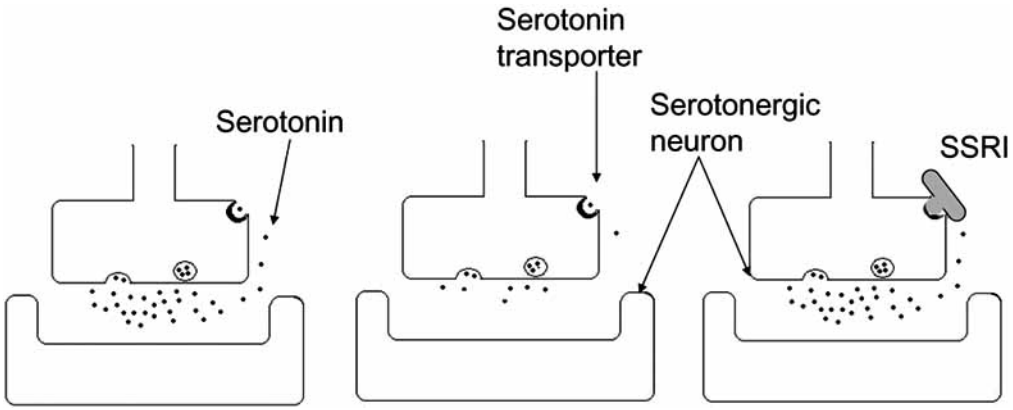


Fig. (2). Mechanism of action of selective serotonin reuptake inhibitors.(A) Serotonergic neuron, (B) Short-term administration of SSRI blocks reuptake of serotonin. (C) Long-term administration causes downregulation of $5HT_{1A}$ autoreceptors. In the presence of blockade of reuptake, more serotonin is available to act postsynaptically.

the availability of neurotransmitter at a synaptic receptor site resulting in a decrease in the number of receptors on the cell surface. Long-term treatment with antidepressants results in changes in postsynaptic beta-adrenergic receptor sensitivity and increased responsiveness of the adrenergic and serotonergic systems to physiologic and environmental stimuli thus contributing to the mechanism of action. Antidepressants may produce a downregulation (desensitization) of alpha 2 - or beta-adrenergic and serotonin receptors, equilibrating the noradrenergic system, and thus correcting the dysregulated monoamine output of depressed patients. Receptor changes resulting from chronic administration of TCAs appear to correlate better with antidepressant action than does the synaptic reuptake blockade of neurotransmitters, and may also account for the delay of two to four weeks in therapeutic response [15].

2.3. Pharmacokinetics and Metabolism

Pharmacokinetic studies on TCAs have shown that due to their lipophilic nature these drugs are absorbed very quickly from the small intestine and then they are metabolised in the liver (first path effect). Subsequently, the kidneys eliminate the products of the hepatic metabolism, some of which may have pharmacological action. The whole procedure is characterised as enterohepatic and enterogastric circulation. TCAs are also highly protein bound and have a long half-life, due to their large volume of distribution. Their half-life is about 24 hours, while there are some exceptions for some TCAs, which need more time to be excreted from the organism [16]. The onset of action is 3-4 weeks, because an antidepressant takes 2-4 weeks to build up its action and the maximum concentration of TCAs in plasma is reached in 2-8 hours [17].

Tertiary amines are metabolised more rapidly than secondary amines and for this reason they are preferred for therapeutic purposes [8]. Tertiary amines are metabolized in the hepatic cytochrome P450 system to secondary amines, which have pharmacological action. This metabolism is a result of a demethylation. Then, the enzyme CYP2D6 hydroxylates the secondary amines to pharmacologically inactive metabolites [18]. Although the activity of P450 system depends on genetic parameters, external factors like the use of other drugs can influence its action. Patient variabilities, such as ethnicity, age and gender also affect TCA metabolism [19]. Finally, tricyclic antidepressants (TCAs) have a narrow therapeutic index and for this reason the regulation of the dosing, the role of the metabolism and the pharmacokinetic parameters are very crucial in order to avoid toxic effects [20].

2.4. Side Effects and Toxicity

Beside their beneficial activity, TCAs cause a number of side effects as well. These are mainly due to interference with autonomic control. Some of these adverse effects relate to their anticholinergic properties. Except for atropine-like effects (muscarinic, mainly M1, cholinergic receptor block), they include postural hypotension (α1-adrenergic receptor block) and sedation (H1 histamine receptor block). Cardiotoxic side effects are also induced related to their action on cardiac Na^+ and Ca^{2+} channels. Poor dental health, due to effects of TCAs on salivary secretion, is a common problem among middle-aged and elderly patients [13].

Possible toxic effects of TCAs are the result of their properties, namely the inhibition of norepinephrine and other neurotransmitter receptors reuptake, the adrenergic blocking, the anticholinergic action and effects on the myocardium [16, 21]. These toxic effects include dry mouth, blurred vision, constipation, urinary retention, sinus tachycardia and memory dysfunction (anticholinergic effects) [21], seizures [22], mental status changes, such as coma, respiratory failure (central nervous system effects), tachycardia, cardiac arrhytmias, orthostatic hypotension and dizziness (inhibition of nor-epinephrine reuptake, anticholinergic action, a1-adrenoreceptor antagonist properties of the tricyclics and effects on the myocardium). Also, TCAs have quinidine-like antiarrhythmic actions, which cause cardiac conduction delays and arrhythmias. These cardiovascular effects are caused by the fast sodium channel blockade and the a-1 adrenergic receptor blockade, while the anticholinergic effects are caused due to the acetylcholine inhibition.

2.5. Therapeutic Drug Monitoring

The pharmacological action of TCAs and their therapeutic results depend on their structure. Therapeutic drug monitoring is based on the measurement of serum concentration

of medications to obtain optimal effective concentration. This is very crucial because many side effects of TCAs are concentration-dependent and potentially life-threatening, especially in case of drugs with narrow effective range or narrow therapeutic/toxic index such as TCAs. Obviously a possible toxic dose and a therapeutic dose are very close. Despite this, it is important to adjust the dosing according to the requirements of each patient, as indications for therapeutic drug monitoring include serious consequences for over/underdosing, narrow therapeutic/toxic index, poor relationship between drug dose and circulating concentration. Additionally good relationship between circulating concentration and therapeutic or toxic effects, alter physiologic state that may unpredictably affect circulating drug concentration, drug interactions and of course patient compliance. In order to control a possible intolerance the dosing is low at the first period of the therapy and increases gradually. It is important to understand that increasing the dosage does not normally shorten the therapy period and may increase the incidence of side effects and toxicity. Therapeutic concentrations for TCAs and their major metabolites are well established for the typical TCAs (amitriptyline, imipramine, nortriptyline, desipramine) and there is a logical correlation between drug concentration and clinical effects of these drugs. Usual dosage ranges for some antidepressant drugs (amitriptyline, clomipramine, desipramine, dothiepin, doxepin, imipramine, nortriptyline, trimipramine) from 75 to 300 mg/day [8, 12, 18, 21, 23].

3. ANALYTICAL METHODS

TCAs monitoring in biological fluids can be achieved by different analytical techniques, such as chromatographic techniques, capillary electrophoresis, voltammetry, etc. However HPLC is the prevalent technique in the analysis of TCAs as shown in (Fig. (**3**)). Both normal and reversed phase HPLC methods are applied to the analysis of TCAs. Chromatographic conditions used in the literature are extensively described below.

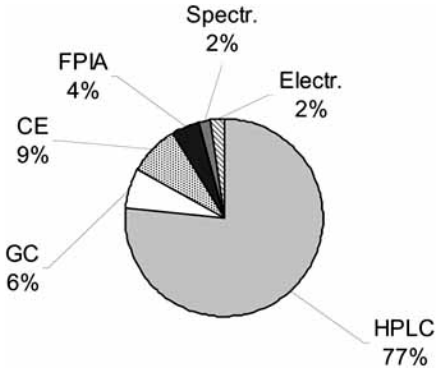


Fig. (3). Analytical techniques used in TCAs analysis since 1990.

3.1. Chromatographic Conditions

In the majority of the published methods the chromatographic separation of TCAs is performed at room temperature. However in some cases elevated column temperature is also used, such as 30°C [24] and 45°C [25, 26].

Detection of separated analytes is mainly performed using ultraviolet or diode array detectors where eluted TCAs are monitored at different wavelengths within the range 200-265 nm [24, 26-50, 53]. Other detection techniques were also used by some researchers: Electrochemical detection [43, 50-53], fluorescence detection after post-column photochemical reaction [37], Chemiluminescent detection using 0.75mM $Ru(bipy)_3Cl_2$ solution [54] and MS/MS [55-59].

In the following paragraphs chromatographic conditions used in methods published since 1990 are cited briefly. More information upon reviewed papers is given in Table **2**.

Amitriptyline, nortriptyline, imipramine, trimipramine, clomipramine and doxepine were determined in human blood and urine using a Waters Symmetry C_8 (250 x 4.6mm, 5μm) analytical column with a 20mm guard column (Waters Symmetry C_{18}). Mobile phase consisted of solvent A=phosphate buffer (pH = 3.8), solvent B=acetonitrile, used in a step gradient: 15% B for 6.5 min, then 35% until 25min and 80% B for 3min [24].

Amitriptyline, imipramine, trimipramine, nortriptyline, protriptyline and doxepin were determined in human serum and urine using a precolumn 10x2.1mm and a 150x4.6mm main column packed with Hypersil 5μm octadecylsilane (C_{18}). Mobile phase was a mixture of solvent A: 50 mL/L acetonitrile and solvent B: 500 mL/L acetonitrile, both in 50 mmol/L phosphate buffer (pH 3.0), with 375 mg/L sodium octyl sulfate and 3 mL/L triethylamine. Solvent gradient conditions changed linearly from 15% B to 90% B in 20min, stayed at 90% B for 5min, and returned to 15% B in 3min [25].

Amitriptyline, nortriptyline, E and Z-hydroxy-amitriptyline, E and Z-hydroxy-nortriptyline were determined in rabbit plasma using an analytical column Supelco 5μm C_{18}, 250 x 4.6 mm. The mobile phase was a mixture of A (acetonitrile-water 10/90, 900 μL of 85% phosphoric acid, 1.22g of potassium dihydrogen phosphate) and B (acetonitrile). A 20-50% mobile phase B gradient was applied in 21 min. Clomipramine (10μg/mL) was used as IS. Linearity was observed in the range 5 –1000 ng/mL [26].

A PE Pecosphere-5C silica column was used for the determination of desipramine in rabbit plasma and rabbit bone marrow, with a mixture of methanol:NH_4OH:l M NH_4NO_3 (190:6:4 v/v/v) as mobile phase and chlorpromazine (1000 pg/mL) as internal standard. Linearity extended from 0.076 to 0.112 μg/mL in rabbit plasma and from 1.196 to 3.764 μg/g in rabbit bone marrow [27].

Desipramine, nortriptyline, imipramine, amitriptyline and clomipramine in human serum were separated on a RP C_8 (5μm, 150 x 4.6mm) analytical column connected to a 2-cm long Pelliguard LC-8 guard column with 40 μm packing. Mobile phase of acetonitrile-phosphate buffer (pH 3 with phosphoric acid) in a 50:50 (v/v) mixture using clobazam (20ng/mL) as internal standard provided a linearity from 100 to 500 ng/mL [28].

A Spheri-5 RP-18 (5μm, 100 x 4.6mm) analytical column and an RP-18 Newguard cartridge (7μm, 15 x 3.2mm) were used for the determination of amitriptyline, nortriptyline, doxepin, imipramine, desipramine, nordoxepin, clomipramine, mianserin, trimipramine in human serum and liver. A

Table 2. Overview of HPLC Methods for the Determination of Tricyclic Antidepressants in Biofluids

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
AMI, NorTRP, IMI, TRI, CLO, DOX	Human blood and urine	Add.of urine or whole blood and deion. H_2O. Mix. Centr. Removal of the org. phase, evap. Rediss. by ACN-H_2O (50:50, v/v). Vortex and centr.	Waters Symmetry C_8 (250x4.6mm, 5µm) with a 20mm guard column (Waters Symmetry C_{18}). Column Temp.: 30°C. Solvent A=phosphate buff. (pH = 3.8), solvent B=ACN. Step gradient: 15% B for 6.5 min, then 35% until 25min, 80% B for 3min. FR: 1mL/min for 6.5min, then linear increase to 1.5mL/min from 6.5min to 25min. Hold for 3 min.		UV: 200-350 nm		[24]
AMI, IMI, TRI, NorTRP, protriptyline, DOX	Human serum, urine	Serum: Add.of phosphate buff. (pH 6.0). Vortex. SPE columns wash. with MeOH and phosphate buff. (pH 6.0). Sample application. Wash with MeOH in phosphate buff. (pH 6.0) and evap. El.with MeOH and 10% ammonia (5:1 v/v). Urine: inj. of the extract directly into the chromatograph.	Precolumn 10x2.1mm and a 150x4.6mm analytical column Hypersil 5µm (C_{18}). Temp.: 45°C. MPs: Solvent A: 50 mL/L ACN and solvent B: 500 mL/L ACN, both in 50 mmol/L phosphate buff. (pH 3.0), with 375 mg/L sodium octyl sulfate and 3 mL/L TEA. Gradient changed linearly from 15% B to 90% B in 20min, stayed at 90% B for 5min, and returned to 15% B in 3min. FR: 1.0 mL/min.		DAD: 210 nm		[25]
AMI, NorTRP, E and Z-OH-AMI, E and Z-OH-NorTRP.	Rabbit plasma	Homog. Add. of IS. Mix. samples with NaOH, heptane and ethyl acetate. After centr. add. of H_2SO_4 to the org. layer, mix.and centr. Org. layer discarded and an aliquot of the aq. phase mix. with K_2HPO_4.	Supelco 5µm C_{18} 250x4.6 mm. MP A: ACN-H_2O (10:90 v/v), 900µL of 85% H_3PO4, 1.22g of KH_2PO_4. MP B: ACN. A 20-50% MP B gradient in 21 min. IS: CLO (10µg/ml). FR: 1 mL/min.		UV: 205 nm	5 –1000	[26]
DES	Rabbit plasma, rabbit bone marrow	Plasma sample add. of IS and saturated aq. $Na_2B_4O_7$. Vortex. Add. Hexane:isoamyl alcohol 98:2, vortex. After centr. an aliquot of the solvent phase was transferred to another centrifuge tube, dried under stream of N_2 and the cooled residue diss. with MeOH.	PE Pecosphere - 5C Silica. MP: MeOH:NH_4OH:l M NH_4NO_3 (190:6:4 v/v/v) IS: chlorpromazine (1000pg/mL). FR: 1.3 mL/min.	Rabbit plasma: 24.2-30.2 Rabbit bone marrow: 24.5-34.2	UV: 265 nm	Rabbit plasma: 0.076-0.112µg/mL and rabbit bone marrow: 1.196-3.764µg/g	[27]
DES, NorTRP, IMI, AMI, CLO	Human serum	Serum sample mix. with IS, saturated $Na_2B_4O_7$ (pH 11 with 6M NaOH) and n-hexane. Centr. Separation of the org. phase, evap. Rediss. with MP.	RP C_8 (5µm, 150x4.6mm) with a 2cm long Pelliguard LC-8 guard column with 40µm packing.MP: ACN-phosphate buff. (pH 3 with H_3PO_4) in a 50:50 (v/v) mixture. IS: clobazam (20ng/mL).FR: 1mL/min.	93.2-110.6	UV: 254 nm	100-500	[28]

(Table 2. Contd....)

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
AMI, Nor-TRP, DOX, IMI, DES, NorDOX, CLO, TRP	Human serum, liver	Vortex mix. of samples, cyanopramine, deion. H_2O. Add.of Na_2CO_3 and vortex. Add.of hexane-butan-1-ol (95:5 v/v). Agitation. Centr. of the org. layer with H_3PO_4.	Spheri-5 RP-18 (5µm, 100x4.6mm) and RP-18 Newguard cartridge (7µm, 15x3.2mm). MP: ACN 0.1M-NaH_2PO_4-diethylamine (40:57.5:2.5 v/v/v) (pH 8.0). RT.IS: cyanopramine. FR: 2.0mL/min.	Human serum: >60 Liver: >40	UV: 220 and 254 nm	0.20-2.5mg/L.	[29]
DOX, DMDOX (E- and Z-isomers)	Human serum	Add. of MeOH containing IS. Direct inj. to HPLC.	Column 1: Hypersil MOS C_8 [10x4.6mm, 10µm]. RT.MP 1: H_2O:MeOH (950:50, v/v). Column 2: Nucleosil 100 CN (250x4.6mm, 5µm). MP: MeOH: ACN:0.01 moI/L potassium phosphate buff., pH 6.8 (188:5778:235, v/v). IS: TRP (140 µg/L, 489 nmol/L).		UV: 214 nm	36-500 nmol/L	[30]
AMI, CLO, demexiptili-ne, DES, DOX, IMI, MAPR, OPI, TRP	Human serum	Extr. with aq. NaOH sol.and a mixture of n-heptane, ethylacetate and isoamyl alcohol. The plasma or blank sample spiked of working sol.and add. of IS, NaOH, ethylace-tate and n-heptane. Isoamyl alcohol was also added to the extr. mixture when the CN column was used for analysis. Vortex 20 s. Centr. The org. layer into a glass tube with methanolic HCl sol.and evapor. Rediss. in MeOH.	Columns: Kromasil C_{18} (5µm, 150x2.1mm), Zorbax C_3 TMS (5µm, 150x4.6mm), Nucleosil CN (5µm, 150x4.6mm). RT. MP: ACN - 0.015 mol/L KH_2PO_4 buff. (adj. to the optimal pH with 0.1 mol/L H_3PO_4). MP:40/60, 30/70, 55/45 and pH of MP: 6.5, 5.8, 6.4 resp.. FR: 0.35, 1.5, 1.0mL/min resp.).		UV: OPI 220 nm, IMI: 254 nm	50-300	[31]
IMI, DES	Mice serum	Add.of IS. Vortex. Add. NaOH and hexane. Vortex and centr. Org. layer dried under N_2 gas at 20°C. Re-diss. with MP and inj.	Microsorb MV C_{18} column (15x0.46cm, 5 µm). MP: 60% ACN and 40% 0.01 M TEA in distilled H_2O (pH 3.0 by 85% H_3PO_4). IS: CLO.FR: 1.0 mL/min.	>89.6	UV: 260 nm	10-1000	[32]
IMI, DES, AMI, NorTRP	Human plasma	Add.of IS, NaCl, NaOH, and hexane/isoamyl alcohol (99:1, v/v) to plasma sam-ples. Shaking. Centr. Evapor. of the org. solvent at RT. Add. of MP to the residue. Vortex.	LiChrospher® 60 RP-select B (4x250mm, 5µm). MP: 50% ACN and 50% 0.25N CH_3COONa buff. (pH 5.5). IS: CLO in MeOH (5µg/mL). FR: 1.0mL/min.	89.6-103.2	UV: 254 nm	20-1500	[33]
Cis- and trans- DOX, cis- and trans-DMDOX	Human serum	Mix. with ACN, IS, 0.25N NaOH, isoamyl alco-hol.Vortex. Add. of heptane. Shaking and centr. Org. layer mix. with 0.1M glycylglycine buff. (pH 3). Shake. Centr.. Add.of 0.25N NaOH and n-heptane to the glycylglycine layer. Shake. Centr. Add.of n-heptane. Vortex. Evap. Re-diss. with MP.	Silica column (3µm, 6x100mm) with a guard column 40µm pellicular silica. MP: 0.025M Na_2HPO_4 (pH 3 with H_3PO_4)/ACN/ n-nonylamine (80/20/1, v/v/v). ISs: amoxapine and loxapine (4mg/mL). FR: 1.6mL/min.	65-75 (desme-tylDOXs), 75-85 (DOXs).	UV	DOXs: 25-250, DMDOXs: 10-175	[34]

(Table 2. Contd....)

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
DES, IMI, NorTRP, MAPR, AMI, DMCLO, CLO	Human plasma	Alkal. with 2M Na_2CO_3. Add.of IS and Extr. with n-hexane. Shaking. Centr.. The lower aq. layer was frozen (dry ice-acetone bath). Back Extr. of the org. layer with H_3PO_4. Shaking and centr. Inj. of acidic sol.to HPLC.	RP-C_{18} symmetry column (5µm, 250x4.6mm). MP: KH_2PO_4 0.067M (pH 3.0 with H_3PO_4)-ACN (65:35 v/v) IS: clovoxamine. FR: 1.2mL/min.	>80	UV: 200-450 nm. 226, 254, 400 nm.	10-3000	[35]
DOX, DMDOX	Human plasma, urine	Add of 3M ammonia (sol.A) and a mixture of n-pentane-IPA (95:5, v/v, sol.B) were added. Shaking and standing. Add. 0.1M HCl to the upper org. layer. Shaking. Aq. Layer wash. with pentane. Add sol. A and B to the wash. aq. residue, shake. Evap. org. phase and diss. with MP.	Spherisorb silica (3µm, 150x4.5mm). RT. MP:Hexane, MeOH and nonylamine (95:5:0.3 v/v/v). FR: 1.0 mL/min.	61-64 (plasma) and 63-68 (urine).	UV: 254 nm	Plasma: 1-200 Urine: 1-400	[36]
MIA, AMI, NorTRP, IMI, DES	Human plasma	Add. of Protein Releasing Reagent [PRR: an aq. sol.of 1M HCl and 25% (v:v) glycerol] and inj. into the donor ch. of the dialysis cell. Transport of acceptor sol. (0.001M ammonium phosphate buff., pH 7.0) to the acceptor ch. Enriched analytes eluted onto the analytical column by the HPLC MP. Both sides of dialyser wash. with the donor sol. (1mM dodecyltrimethyl ammonium bromide in H_2O) and acceptor solution, resp. The precolumn regenerated with the acceptor sol. Next sample inj. into donor ch. of the dialysis cell.	Supelcosil LC-PCN cyanopropyl (5µm, 150x4.6mm). MP: ACN–MeOH–0.005 M ammonium phosphate buff., pH 7.0 (70:15:15, v:v). FR: 1.5mL/min.		UV and Fl. After post-column photochemical reaction. UV: 254 nm connected to a Fl. λ_{exc} 270nm, λ_{em} 430nm	50–2000nmol/L	[37]
AMI, NorTRP, IMI, DES, CLO, DOX, TRP	Human plasma.	Mix. with IS. Alkal. by the add. of 1M NaOH. Extr. by shaking with hexane containing 1% isoamyl alcohol. Centr. Back extr. of org. phase with 0.05M HCl. Vortex. Centr. Acidic phase inj. onto the HPLC.	Ultrasphere C_8 (250x4.6mm). MP: 35% CH_3CN-aq. phase containing 4mM 1-octanesulphonic acid and 0.5mM N,N,N,N-tetramethylethylene diamine (pH 2.5 with H_3PO_4). FR: 2 mL/min.IS: 200ng AMI or 100ng DMDOX		UV: 230 nm.	20-1000	[38]

(Table 2. Contd....)

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
AMI, Nor-TRP, IMI, DES, CLO, NorCLO	Human serum and plasma	Single step LLE: Mix. with IS, 0.1M $Na_2B_4O_7$ sol. (pH 11 with 30% NaOH) and hexane in a Sovirel glass tube. Shaking. Centr. Evap. Rediss. in MeOH	Nova-Pak C_{18} 4μm, 4.6x150mm, Waters. MP: mixing 500 mL of 5mM aq. KH_2PO_4 buff., 500 mL ACN and 2mL diethylamine (pH 8 with H_3PO_4).RT. IS: econazole, 90 mg mL^{-1}. FR: 0.9mL/min.	92-105	UV: 242 nm	20-400 (60-1450nM)	[39]
CLO, DMCLO, 2-, 8-, and 10-OHCLO, 2-, and 8-OHDMCLO, diDMCLO	Human and rats serum and plasma	Sample clean up: Proteins and other interfering compounds wash. to waste by deion. H_2O containing 35% (v/v) ACN. Centr.	Lichrosher CN (5μm, 250x4.6mm) and a clean up column Hypersil CN (10μm CN, 10x4.6mm). MP: 38% ACN and 62% $NaClO_4$ sol.(0.02M) (pH 2.5 with $HClO_4$). FR 1.5mL/min (5-8min).	64-110	UV: 260 nm		[40]
DOX, DES, MAPR, IMI	Human plasma	C_{18} Bond-Elut cartridges and mixtures of MeOH–aq. buff. as washing and el.solvents. SPE cartridge activ. with MeOH. Blood samples collected using sodium citrate. Wash cartridge with aq. acetate buff., ACN and washing solvent and then with el.solvent. Evap. Rediss.with MP containing IS.	Nova Pack C_{18} column (4μm, 15cmx3.9mm) and a Nova Pack C_{18} guard column (4μm, 20x3.9mm). MP: ACN-0.02M TEA (pH 5.5 with H_3PO_4) (35:65 v/v) IS: p-HBA n-butyl aster (butyl paraben). FR: 1.0mL/min.	83.3	UV:215 nm	0.005-2μg/mL	[41]
NorTRP	Human serum	SPE on CN cartridges. LLE: add. 1M NaOH and IS containing 89 mg/mL of MAPR and 3mL heptane/isoamylalcohol (98.5:1.5, v/v). Shaking. Centr. Org. layer evap. Diss. in MP.	IS: TRP. SPE samples: a Luna C_{18} (3μm, 150x4.6mm) equipped with a C_{18} guard column. MP: ACN–0.01 M TEA pH 3.0 with H_3PO_4 (34:66 v/v) in MeOH–H_2O (50:50, v/v), 6.2mg ml^{-1}.FR: 0.85 mL/min. LLE samples: MP: ACN–MeOH–conc. ammonia H_2O, 950:50:7 (v/v).FR: 1.3mL/min.	SPE: 93-98 LLE:75 ±13	UV/VIS: 242 nm	0–1802	[42]
AMI, NorTRP	Human serum	Blood was collected and centrifuged. Inj. To the HPLC after dilution (1:10) in 0.15M SDS-6% pentanol at pH 7. Filtr. through 0.45μm nylon membranes.	Column: Kromasil 5 C_{18} (5μm, 250×4.6 mm).MP: SDS (0.15M)-6% pentanol (v/v) (pH 7).FR: 1.5 mL/min.	99.8-101.6 (AMI) and 98.5-99.7 (NorTRP).	UV: 240 nm and electr. 650mV.	AMI: 120–250 NorTRP: 50–150	[43]
CLO, N-DMCLO, 8-OHCLO, 2- and 8-OHDMCLO	Human plasma	Add. of phosphate buff. and IS. Vortex. Centr. SPE with an Isolute C_2. Wash columns prior sample application with MeOH/H_2O/phosphate buff. (pH 9.2) (2/1/2 v/v/v). Washing with phosphate buff./mixture of H_2O and ACN (80:20 v/v) (1/2 v/v). El.with MeOH.	Lichrospher CN, (5 μm, 250x4 mm) with a 2-cm precolumn of the same material. MP: 10mM K_2HPO_4–ACN–MeOH (35:25:40 v/v/v). IS: MAPR. FR: 1.5 mL/min.	99.1-100.3	UV: 214 nm	CLO:5-500, DMCMI: 5-500 , 8-HCMI: 5-100, 2-HDMCMI and 8-HDMCMI: 5-100	[44]

(Table 2. Contd....)

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
AMI, Nor-TRP, IMI, DES, DOX, NorDOX, CLO, NorCLO, TRP	Human serum	SPE: 3M-Empore Extr. disk cartridges. Centr. serum. Sorbent cond. with MeOH and H_2O. The supern., melperone and 0.1M KH_2PO_4 buff. (pH 6.0) mixed. Sample extr. through disk cartridge. El. with IPA–ammmonia sol.(25%)–CH_2Cl_2 (20:2:78). Evap. Diss. in ACN–H_2O (3:7).	Nucleosil 100-5-Protect 1 (250x4.6 mm, 5 µm). MP: 25mM KH_2PO_4 (pH 7.0)–ACN (60:40 v/v). IS: melperone (3000 ng/mL). FR: 1.0mL/min.	75-100.2	UV: 230 nm		[45]
AMI, CLO, DES, DOX, IMI, NOX, OPI	Human blood	Sample of whole blood was extr. with 0.6M NaOH and mix. Add.of hexane-isoamyl alcohol (99:1, v/v). Agitation. Centr. Evapor. of the org. layer. Reextr. of the drugs into 0.05% H_3PO_4 vortex. org. layer with diluted acid and centrif.	LiChroCART (125x4mm) packed with octasilica LichroSpher RP Select B (5µm). MP: H_2O with H_3PO_4 and ACN (in gradient mode). ISs: OPI and IMI.FR: 1mL/min.	75-90. Exceptions: AMI (67) and OPI (47)	DAD: 254 and 220 nm.	DOX, DES, IMI, CLO: 0.125-2.0µg/mL	[46]
AMI, OPI, NOX	Urine	Sodium phosphate buff. (pH 9.0), sample sol.containing TCAs pumped into a mix.coil. Mix. solutions passed over the liquid membrane in membrane separator made of two PTFE blocks with machined spiral grooves. Impregnation of membrane by soaking in n-undecane. The membrane separated 2 ch.: the donor ch. for extr. of analytes and IS and the acceptor ch. with acidic sol. for reextr. of analytes from the membrane solvent. Sample of acceptor sol. inj. to HPLC.	Column: Shiseido RP-18, (250x4.6mm, 5µm). MP: 0.01M Na_3PO_4 (buff. pH 3.2)–MeOH–ACN–dimethylamine (37:55.4:7.4:0.2 v/v).IS: diethazine.FR: 0.8 mL/min.		UV: 254 nm		[47]
DOX, Nor-DOX, DES, IMI, Nor-TRP, AMI	Human breast milk	Add. of IS. Saturation with NaCl and Add.of 2M NaOH and 2% butanol in hexane. Mix. by rotation. Centr. The aq. layer was frozen (dry ice/acetone bath). Org. layer to another tube with H_2SO_4. Rotation and centr. Aspiration of org. layer and Neutr. of the samples with 1% $KHCO_3$. SPE cartridges (C_{18}) cond. with diethylamine in MeOH, followed by 1% K_2CO_3 in 10% ACN in H_2O. Sample application. Dryness. Add.of 20% ACN in H_2O. Mixing. Add.of H_2O+MeOH+ACN. El.of TCAs with diethylamine in MeOH, followed by 1% $KHCO_3$ in 10% ACN in H_2O. Evap. Rediss. with MP.	Column: MOS-2 Hypersil (C_8) (3µm, 100×2mm).MP: 0.02M KH_2PO_4+85µL N,N-dimethyloctylamine/L (pH 6.5) and 34% ACN.IS: TRP (2.5 µg/mL).FR: 0.5 mL/min.	85.8-104.6 (50mg/mL) and 90-102.3 (200ng/mL)	UV: 242 nm	800	[48]

(Table 2. Contd....)

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
AMI, DOX, CLO, TRP and their N-demethyl metabolites	Human plasma	Mixed-mode SPE(IST Isolute HCX, 80 mg/1 mL)	Phenomenex Synergi Hydro-RP HPLC (250x4.6mm). MP: aq. CH_3COONH_4/MeOH/ACN. IS: butriptyline.FR: 1.0mL/min.		UV: 240 nm	10-550mg/L	[49]
IMI, DES	Human serum	Direct inj. into the HPLC, after filtr. (0.45μm nylon membranes).	Column: Kromasil 5 C_{18} (5μm, 250x4.6mm).RT. MP: 0.15M SDS–6% (v/v) pentanol–0.001M NaCl–0.01M NaH_2PO_4 (pH 7). FR: 1.5mL/min.		UV–Vis: 190–700 nm. Electrochemical: (−400–1400mV). Voltage: 0.650V.	50-1000	[50]
IMI, DES, 2- and 10-OH-IMI, 2- and 10-OH-DES	Human plasma, urine	Add. of IS and 1M sodium carbonate buff. (pH 9.6). Extr. by shaking with diethyl ether. Centr.. Add. of 0.1M H_3PO_4 to the org. layer. Shaking and centr. Aliquot of acidic layer inj. to the HPLC.	(Phenomenex Bondclone 10 C_{18} 300x3.90mm, with a pre-column (RP-18, 10μm, 40x4.6mm). RT. MP: 30% ACN in 0.1M K2HPO4 buff. (with conc. H_3PO_4, pH 6.0). FR: 2 mL /min. IS: pericyazine in MeOH (200ng).	78.6-94.3 (plasma) 10.24-28.80 (urine)	Electr.	IMI,DES: 15.63-500, 2- and 10-hydroxylated metabolites: 7.82-250	[51]
IMI	Human serum and plasma	One-step LLE with diethylether in presence of Na_2CO_3. Add. of IS.	Nucleosil 100 C_{18}, (5μm, 125x4mm) (IMI) MPs: MeOH–phosphate buff. (pH 3) (30:70, v/v), 15% MeOH in 0.01M CH_3COOH, MeOH–phosphate buff. (pH 3) (60:40, v/v), MeOH-0.008M H_3PO_4 (for, IMI resp.).IS: Methyl parahydroxybenzoate, sulfathiazole, chlorpromazine hydrochloride, naproxen (IMI). FR: 1 mL/min except IMI (1.2 mL/min).	Human serum: >85	Electr. at a glassy carbon electrode. Potential v.s. Ag/AgCl: 1.10, 1.30, 1.10, 1.15 (V).	5-2000	[52]
IMI, DES, CLO, AMI, NorTRP, DOX.	Human blood	Blood sample centr. Store at −20°C. Add. of $HClO_4$, vortex and centr. Neutr. of the supern. (NaOH). Filtr. Direct inj. into the HPLC.	(Inertsil ODS-3 150x4.6mm, 5μm, connected to the FIA system. RT. MP: mixture of ACN and phosphate buff. 0.05M (KH_2PO_4/K_2HPO_4, pH 6.9 ± 0.1) (375:625, v/v) for the detection of all TCAs, except for CLO (50:50).	DES 92.3 IMI 90.8,	Electr. Potential: 0.85V versus Ag/AgCl/1M LiCl for the IMI/DES couple, 0.93V versus Ag/AgCl for CLO and 1.3V versus Ag/AgCl for the AMI/NorTRP couple and DOX	0.05-100μM.	[53]

(Table 2. Contd....)

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
IMI, DES, AMI, Nor-TRP, CLO.	Human plasma	Add. of IS. Extr. by shaking with diethyl ether. Centr. Add. of H_3PO_4 to the upper org. layer. Shake. Centr. Aliquot of the acid layer inj. to HPLC.	Trimethylsilyl (TMS) (5 µm, 150 x 4.6mm). MP: 50mM sodium phosphate buff. (pH 7.0)-ACN (55:45, v/v).IS: CLO. FR: 1.0 mL/min.	83.0-93.8	CL: 0.75mM Ru(bipy)3Cl 2 solution	0.5-500	[54]
AMI, CLO, DES, DOX, IMI, MAPR, MIA, Nor-CLO, Nor-DOX, NorTRP, OPI, TRP.	Human serum	Add of IS. Vortex. Protein precip. Centr. Supern. dil. with the MP. Inj. onto the HPLC.	Chromolith Speed ROD C_{18} (5µm, 50×4.6mm). RT. MP: mix.of MeOH and 5mM acetic acid (pH 3.9). Starting at 20% MeOH and 80% buff. sol.with linear gradient to 70% MeOH in 4 min. IS: Mixture containing 50µg/L clonidine, 10µg/L dehydromethylrisperidon and 10µg/L methabenzthiazurone in ACN/MeOH (9/1 v/v). FR: 1.0 mL/min.	92-111 (average 101) except of olanzapin (185).	MS coupled turbo ion spray interface in positive MRM mode. Turbo spray, Temp.: 600°C, ionization voltage: 4500V, entrance potential: 10V.	1-10000	[55]
DOX, DMDOX.	Human plasma	Add.of IS, 2M NaOH and 2% sol.of isoamyl alcohol in hexane. Vortex. Centr. The aq. phase was frozen and the org. phase into an ampoule with 1% formic acid solution. Vortex and the aq. phase inj. on the HPLC.	Phenomenex Luna C_{18} (5µm, 150x2.1mm). RT. MP: MeOH-H_2O-0.05% formic acid (600:400:1, v/v/v). IS: Benzoctamine-HCl. FR: 0.25mL/min.	DOX: 90 DMDOX:75	MS-MS: the protonated molecular ions *m/z* 280.2, 266.2 to the product ions *m/z* 107.1	DOX:81.1–0.320 DMDOX: 45.1–0.178	[56]
CLO, Nor-CLO.	Blood, hair	Blood: LLE. Mix. with CH_3COONH_4 buff. (pH 5.0) Enzymatic hydrolysis by b-glucuronidase-aryl sulfatase. Neutr. with 0.1M NaOH, add. of tris buff. to pH 9.0. Mix. of the supern. with a mixture of $CHCl_3$ -IPA (3:1, v/v). Separation of the sol. on silicone treated filter paper. Org. phase, evapor. Diss. with MP. Hair: Division into three segments. Decontamination with H_2O and IPA in ultrasonic bath. Pulv. of the samples in mill-ball. Add.of diazepam-D5 and 0.1M HCl and incub. Neutr. with 0.1M NaOH. Add. $(NH_4)_2CO_3$ buff. (pH 9.3). Supern. collected, centr. Add. to the column SPE RP-18. Washing with $(NH_4)_2CO_3$ buff. and evap. El.with a mixture of 0.1% acetate acid-MeOH. Evap. Rediss. in MP.	LiChroCART column (125x3mm, 5µm) with Purospher RP 18 and a LiChroCART precolumn (4x4mm, 5µm) with LiChrospher 60 RP—select B. MP: [A] 0.1% HCOOH in H_2O and [B] 95% ACN + 5% of the phase [A]. Gradient: 95% [A] and 5% [B] for 2 min, a linear change to 30% [A] and 70% [B] in 30 min, then 30% [A] and 70% [B] for 2 min, change to 95% [A] and 5% [B] for 8 min. IS: prazepam (1.0 µg/g). FR: 0.4 mL/min.	Blood :88-90, Hair: 91-93	MS: 50–650 m/z (positive ions).	Blood 0.25-10µg/g Hair: 0-10µg/g.	[57]

(Table 2. Contd....)

Analytes	Sample Type	Sample Preparation	Chromatographic Conditions IS	Recovery (%)	Detection	Linear Range (ng/mL)	Ref.
AMI, NorTRP, DOX, OPI.	Human plasma	Plasma samples diluted IS sol.and 0.1% formic acid. Vortex. Online Extr. on an Oasis HLB Extr. column (30μm, 1x50mm).	Symmetry C_{18} (5μm, 3.0×150mm) with Sentry guard column Symmetry C18 (5μm, 3.9×20mm).RT. MP: ACN (A) and 0.1% formic acid (B) (gradient profile: 28% A for 4 min, 70% A in 1 min, isocratic at 70% A for 3 min, 28% A in 0.7 min). IS: lofepramine (10mg/L). FR: 0.6mL/min.	>90	MS-MS.	DOX, AMI, NorTRP: 10-800, OPI: 50-1500.	[58]
DOX, DES, IMI, AMI, TRI.	Human plasma.	LLE. Add. of 2 M Na_2CO_3. Extr. with hexane by rotation on a Roto-Rack mixer for 30 min. Centr. for 10 min at 3000 rpm. Org. layer evap. under N_2 at 30 °C. Rediss. in 60 μL of MP.	C_{18} column (15 x 2:1 mm i.d.). MP: 3 mM CH_3COONH_4 (pH 3.3)–ACN (66: 34). FR:1.4 ml min^{-1}. Deuterated IS, imipramine-*d*3.	75 for DESI >90 for other TCAs	Fast LC-API-TOF MS	DESI :2 -100, Other TCAs: 2 – 50.	[59]

mixture of acetonitrile 0.1M-sodium dihydrogen phosphate-diethylamine (40:57.5:2.5 v/v/v) (pH 8.0) was used as mobile phase with cyanopramine as internal standard. Analysis was completed in 40 min. Linearity was observed in the range of 0.20-2.5mg/L [29].

Doxepin, desmethyldoxepin (E-and Z-isomers) were determined in human serum using a Hypersil MOS C_8 [10 x 4.6mm, 10μm] column with a mixture of water:methanol (950:50, v/v) as mobile phase and a Nucleosil 100 CN analytical column (250 x 4.6mm, 5μm) with a mixture of methanol:acetonitrile:0.01 moI/L potassium phosphate buffer, pH 6.8 (188:5778:235, v/v) as mobile phase. Trimipramine was used as internal standard (140 μg/L). A linear range of 36-500 nmol/L was achieved [30].

Amitriptyline, clomipramine, desipramine, doxepine, imipramine, opipramol and trimipramine were determined in human serum using different analytical columns: Kromasil C_{18} (5μm, 150 x 2.1mm), Zorbax C_3 TMS (5μm, 150 x 4.6mm) and Nucleosil CN (5μm, 150 x 4.6mm). Mixtures of acetonitrile and 0.015 mol/L potassium dihydrogenphosphate buffer (adjusted to the optimal pH with 0.1 mol/L phosphoric acid) were used as mobile phases at different proportions: 40/60, 30/70, 55/45 and different pH values for each column: 6.5, 5.8, 6.4 respectively. Linearity extended from 50 to 300 ng/mL [31].

A Microsorb MV C_{18} column (15x 0.46 cm, 5 μm) was used for the determination of imipramine and desipramine in mice serum, with a mobile phase of 60% acetonitrile and 40% 0.01 M triethylamine in distilled water (pH 3.0 by addition of 85% phosphoric acid). Clomipramine was used as internal standard. Linearity was observed within the range of 10-1000 ng/mL [32].

Imipramine, desipramine, amitriptyline and nortriptyline in human plasma were separated on a LiChrospher 60 RP-select B (4 x 250mm, 5μm) analytical column. Mobile phase consisted of 50% acetonitrile and 50% 0.25N sodium acetate buffer (pH 5.5). Clomipramine in methanol (5μg/mL) was used as IS. Linear range extended from 20 to 1500 ng/mL [33].

Cis- and trans- doxepin, cis- and trans- desmethyldoxepin in human serum were separated on a Silica column (3μm, 6x100mm) with a guard column containing 40 μm pellicular silica. Mobile phase consisted of 0.025M dibasic sodium phosphate (pH 3 with phosphoric acid)/acetonitrile/ n-nonylamine (80/20/1, v/v/v). Amoxapine and loxapine (4mg/mL) were used as internal standards.

Linear ranges were 25-250ng/mL and 10-175ng/mL for doxepins and desmethyldoxepins respectively [34].

An RP-C_{18} symmetry analytical column (5 μm, 250 x 4.6mm) was used for the determination of desipramine, imipramine, nortriptyline, maprotiline, amitriptyline, desmethylclomipramine and clomipramine in human plasma. Mobile phase consisted of KH_2PO_4 0.067 M (pH 3.0 with H_3PO_4)-acetonitrile (65:35 v/v) with clovoxamine as IS. Linearity was observed within the range 10-3000 ng/mL [35].

Doxepin and desmethyldoxepin was determined in human plasma and urine using a normal phase Spherisorb silica (3μm, 150 x 4.5mm) analytical column, with a mobile phase of hexane, methanol and nonylamine (95:5:0.3 v/v/v). Linearity extended from 1 to 200ng/mL or 400ng/mL in plasma and urine respectively [36].

Amitriptyline, nortriptyline, imipramine and desimipramine were analysed in human plasma on a cyanopropyl column Supelcosil LC-PCN (5μm, 150 x 4.6mm). Mobile phase was formed by acetonitrile–methanol–0.005 M ammonium phosphate buffer, pH 7.0 (70:15:15 v:v). Linearity was observed in the range from 50 to 2000 nmol/L [37].

Amitriptyline, nortriptyline, imipramine, desipramine, clomipramine, doxepin and trimipramine were determined in human plasma using an Ultrasphere C_8 (250 x 4.6mm) analytical column. A mixture of 35 % CH_3CN mixed with an aqueous phase containing 4mM 1-octanesulphonic acid and 0.5 mM N,N,N,N-tetramethylethylene diamine (pH 2.5 with H_3PO_4) was used as mobile phase. Amitriptyline (200ng) was used as IS for samples from patients treated with doxepin, imipramine, or trimipramine and desmethyldoxepin (100ng) for samples from patients treated with amitriptyline, nortriptyline, dothiepin, or clomipramine. Linearity extended from 20 to 1000 μg/L [38].

Amitriptyline, nortriptyline, imipramine, desipramine, clomipramine and norclomipramine from human serum and plasma were separated within 34 min using a reversed-phase C_{18} analytical column (Waters, Nova-Pak C_{18}, 60A, 4μm, 4.6 x150mm). Mobile phase consisted of 500 mL of 5mM aqueous KH_2PO_4 buffer, 500mL acetonitrile and 2mL diethylamine (pH 8 with phosphoric acid). Methanolic solution of econazole, 90 mg mL^{-1} was used as IS. Linearity extended from 20-400 ng/mL [39].

Clomipramine, demethylclomipramine, 2-, 8-, and 10-hydroxyclomipramine, 2-, and 8-hydroxydemethylclomipramine and didemethylclomipramine were determined in human and rat serum and plasma using a Lichrosher CN (5μm, 250 x 4.6mm) analytical column and a clean up column Hypersil CN (10 μm CN, 10 x 4.6mm). Mobile phase consisted of 38% acetonitrile and 62% sodium perchlorate solution (0.02M) (pH 2.5 with $HClO_4$) [40].

Doxepin, desipramine and imipramine were determined in human plasma using an RP Nova Pack C_{18} column (4μm, 15cm x 3.9mm) and a Nova Pack C_{18} guard column (4μm, 20 x 3.9mm). Mobile phase consisted of acetonitrile-0.02M triethylamine (pH 5.5 with H_3PO_4) (35:65 v/v). p-hydroxybenzoic acid n-butyl ester (butyl paraben) was used as IS. Linearity was observed within the range 0.005-2μg/mL [41].

Nortriptyline was determined in human serum using trimipramine as IS. A Luna C_{18} (3μm, 150 x 4.6mm) analytical column equipped with a C_{18} guard column was used with a mobile phase consisted of acetonitrile–0.01 M triethylamine adjusted to pH 3.0 with H_3PO_4 (34:66 v/v). Linearity was observed up to1802 ng/mL [42].

Amitriptyline and nortriptyline were determined in human serum using a Kromasil 5 C_{18} (5μm, 250x4.6 mm) column. Mobile phase was a mixture of SDS (0.15M)-6% pentanol (v/v) (pH 7). The method was linear up to 1000 ng/mL [43].

Clomipramine, N-desmethylclomipramine, 8-hydroxyclomipramine, 2- and 8- hydroxyl-desmethylclomipramine were determined in human plasma using a Lichrospher CN, (5 μm, 250 x 4 mm) analytical column with a 2-cm precolumn filled with the same material. Mobile phase consisted of 10 mM K_2HPO_4–acetonitrile–methanol (35:25:40 v/v/v). Maprotiline was used as IS. Linearity was observed in the range 5-500ng/mL for clomipramine, 5-500 ng/mL for N-desmethylclomipramine, 5- 100ng/mL for 8-hydroxyclomipramine and 5-100 ng/mL for 2- and 8- hydroxydesmethylclomipramine [44].

Amitriptyline, nortriptyline, imipramine, desipramine, doxepin, nordoxepin, clomipramine, norclomipramine, trimipramine, mianserine, maprotiline and normaprotiline were determined in human serum using a Nucleosil 100-5-Protect 1 analytical column (250 x 4.6 mm, 5 μm). Mobile phase consisted of 25mM KH_2PO_4 (pH 7.0)–acetonitrile (60:40 v/v). Melperone (3000 ng/mL) was used as IS [45].

Amitriptyline, clomipramine, desipramine, doxepin, imipramine, noxiptyline and opipramol were analysed in human blood, using a LiChroCART (125x4mm) column packed with octadecylsilica LichroSpher RP Select B (5μm). Mobile phase consisted of water with orthophosphoric acid and acetonitrile. Opipramol and imipramine were used as IS. Linearity was observed in the range 0.125-2.0 μg/mL [46].

Amitriptyline, opipramol and noxiptyline were determined in urine using an analytical column Shiseido RP-18, (250 x 4.6mm, 5μm). Mobile phase consisted of 0.01M sodium phosphate (pH 3.2)–methanol–acetonitrile–dimethylamine (37: 55.4: 7.4: 0.2 v/v). Diethazine was used as IS [47].

Doxepin, nordoxepin, desipramine, imipramine, nortriptyline and amitriptyline were determined in human breast milk using a reversed-phase MOS-2 Hypersil (C_8) analytical column (3μm, 100 x 2mm). Mobile phase consisted of 0.02M monobasic potassium phosphate and 85μL N,N-dimethyloctylamine/L (pH 6.5) and 34% acetonitrile. Trimipramine (2.5 μg/mL) was used as IS. The method was linear up to 800 ng/mL [48].

Amitriptyline, doxepin, clomipramine, trimipramine and their N- demethyl metabolites were determined in human plasma using a Phenomenex Synergi Hydro-RP analytical column (250 x 4.6mm) with a mobile phase of aqueous ammonium acetate/methanol/acetonitrile. Butriptyline was used as IS. The method was linear from 10 to 550 mg/L [49].

Imipramine, desipramine in human serum were separated on a Kromasil 5 C_{18} analytical column (5μm, 250 x 4.6mm) using a mobile phase of 0.15M SDS–6% (v/v) pentanol–0.001M NaCl–0.01M NaH_2PO_4 (pH 7). No internal standard was used. Linearity extended from 50-1000 ng/mL [50].

Imipramine, desipramine, 2- and 10-hydroxyimipramine, as well as 2- and 10-hydroxydesipramine were determined in human plasma and urine using a reversed-phase C_{18} column (Phenomenex Bondclone 10 C_{18}, 300 x 3.90mm) coupled to a pre-column (RP-18, 10μm, 40 x 4.6mm). Mobile phase consisted of 30% acetonitrile in 0.1M K_2HPO_4 buffer (with concentrated orthophosphoric acid, pH 6.0). Pericyazine in methanol (200 ng) was used as IS. Linearity was observed in the range of 15.63-500 ng/mL for imipramine and desipramine and 7.82-250 ng/mL for 2- and 10-hydroxylated metabolites [51].

Imipramine was determined in human serum and plasma using a Nucleosil 100 C_{18} analytical column (5μm, 125 x 4mm) using methanol–phosphate buffer (pH 3) (30:70, v/v) as mobile phase. Linearity was observed in the range 2-100 ng/mL [52].

Imipramine, desipramine, clomipramine, amitriptyline, nortriptyline and doxepin were determined in human blood

using an Inertsil analytical column (ODS-3 150 x 4.6mm, 5μm). Mobile phase was a mixture of acetonitrile and phosphate buffer 0.05M (KH_2PO_4/K_2HPO_4, pH 6.9 ± 0.1) (375: 625, v/v) for the detection of all TCAs, except for clomipramine (50:50). Linearity was observed in the range 0.05-100 μM [53].

Imipramine, desipramine, amitriptyline, nortriptyline and clomipramine were determined in human plasma using a trimethylsilyl (TMS) analytical column (5 μm, 150 x 4.6mm) with a mobile phase of 50 mM sodium phosphate buffer (pH 7.0) and acetonitrile (55:45, v/v). Clomipramine was used as IS. Linearity was observed in the range 0.5-500ng/mL [54].

Amitriptyline, clomipramine, desipramine, doxepin, imipramine, norclomipramine, nordoxepin, nortriptyline, opipramol and trimipramine were separated in human serum using a Chromolith Speed ROD C_{18} (5μm, 50 x 4.6mm) analytical column. Mobile phase was prepared by mixing of methanol and 5mM acetic acid (pH 3.9). Starting condition was 20% methanol and 80% buffer solution with linear gradient to 70% methanol in 4 min. A mixture containing 50μg/L clonidine, 10μg/L dehydromethylrisperidon and 10 μg/L methabenzthiazurone in acetonitrile/methanol (9/1 v/v) was used as IS. Linearity extended from 1 to 10000 ng/mL [55].

Doxepin and desmethyldoxepin were determined in human plasma using a Phenomenex Luna C_{18} analytical column (5μm, 150 x 2.1mm) with a mobile phase of methanol-water-0.05% formic acid (600:400:1, v/v/v). Benzoctamine-HCl was used as IS. Linearity was observed in the range 81.1–0.320 ng/mL for doxepin and 45.1–0.178ng/mL for desmethyldoxepin [56].

Clomipramine and norclomipramine were determined in blood and hair using a LiChroCART column (125x3mm, 5μm) filled with Purospher RP 18 and a LiChroCART precolumn (4 x 4mm, 5μm) filled with LiChrospher 60 RP-select B. Mobile phase was a mixture of [A] 0.1% formic acid in water and [B] 95% acetonitrile + 5% of the phase [A]. The gradient was programmed as follows: 95% [A] and 5% [B] for 2 min, a linear change to 30% [A] and 70% [B] in 30 min, then 30% [A] and 70% [B] for 2 min and then changed to 95% [A] and 5% [B] for 8 min. Prazepam (1.0 μg/g) was used as IS. Linearity was observed in the range 0.25-10μg/g in blood and 0-10μg/g in hair [57].

The determination of amitriptyline, nortriptyline, doxepin and opipramol in human plasma was achieved on a Symmetry C_{18} (5μm, 3.0 x 150mm) analytical column with a Sentry guard column (Symmetry C_{18}, 5μm, 3.9 x 20mm). Mobile phase consisted of acetonitrile (A) and 0.1% formic acid (B) using a gradient profile: 28% A for 4 min, 70% A in 1 min, isocratic at 70% A for 3 min, 28% A in 0.7 min. Lofepramine (10mg/L) was used as IS. Linearity extended in the range 10-800μg/L for doxepin, amitriptyline and nortriptyline and 50-1500μg/L for opipramol [58].

Doxepin, desipramine, imipramine, amitriptyline and trimipramine were determined in human plasma by Fast LC-API-TOF MS. The relatively short HPLC separation (18 s) was achieved using a short C_{18} column (15 x 2:1 mm i.d.) SB-C_{18} Mac Mod 15 x 2.1 mm i.d. cartridge packed with 3 mm particles (Hewlett-Packard Analytical). A high aqueous mobile phase of 3 mM ammonium acetate (pH 3.3)–acetonitrile (66:34) was used as mobile phase [59].

A typical chromatographic separation of four TCAs on a Kromasil C_{18} (5 μm, 250 x 4.0 mm) analytical column is presented in (Fig. (**4**)).

3.3. Other Techniques

Other techniques used for the analysis of TCAs in biofluids include voltammetry with lipid - coated electrodes [60], derivative spectrometry using both first- and second-derivative spectra [61], Fluorescence Polarization Immunoassay [30, 62], Gas Chromatography [63], GC – MS [64, 65], Non Aqueous Capillary Electrophoresis [66-68], Capillary Electrophoresis With Electrochemiluminescence detection based on end-column reaction of tris-(2,2_-bipyridyl)ruthenium(II) with aliphatic tertiary amino moieties [69]. These methods are briefly presented in Table **3**.

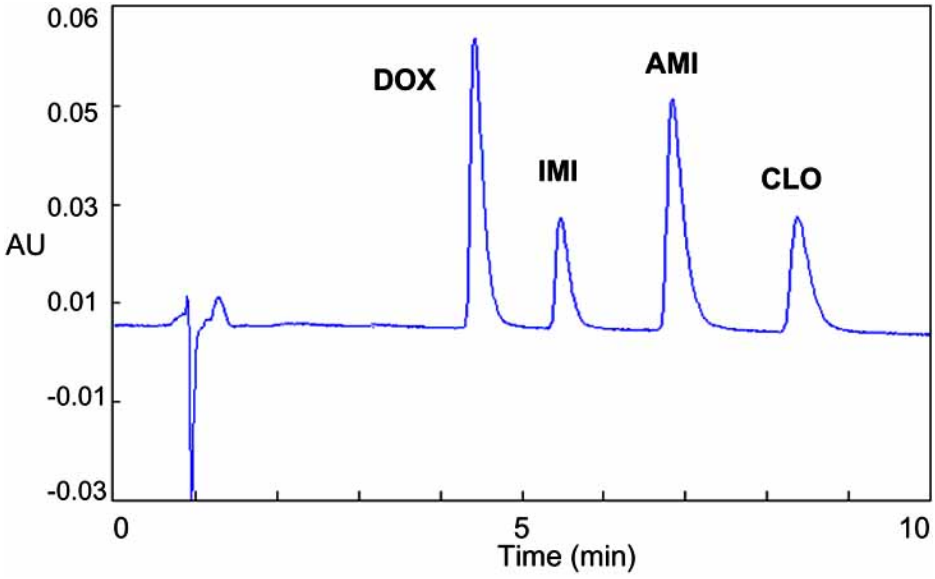


Fig. (4). HPLC separation of AMI, IMI, DOX, CLO. Data from authors' laboratory. Unpublished results.

Table 3. Overview of Analytical Methods for the Determination of Tricyclic Antidepressants in Biofluids

Analytes	Sample Type	Analytical Technique	Sample Preparation	Recovery (%)	Linear Range(LR)-LOD	Ref.
IMI, AMI, DES, NorTRP, CLO, DOX, OPI, TRI	Human serum	Fluorescence polarization immunoassay	Dilution		LR:50 - 1000μg/L (IMI, CLOMI, DOX, AMI), 50 - 500μg/L (DESI, NORTRP).	[30]
DOX, DES, IMI, AMI, TRI	Human plasma	Fast LC-API-TOF MS	LLE (hexane)		LR: 2 - 100ng/mL (DESI), 2 – 50ng/mL (the other four TCAs). LOD: 2 ng/mL (DESI) and 1ng/mL (the other four TCAs).	[59]
DES, IMI, TRI	Urine	Voltammetry with lipid - coated electrodes.	Dilution		LR:1-8x10^{-7}M. LOD: 1x10^{-8}M.	[60]
IMI, AMI	Blood serum	Derivative spectrophotometry	LLE (n-hexane)		LR: 0.62 - 10.14μg/mL (IMI) and 0.63-10.04μg/mL (AMI).	[61]
IMI	Human serum	Fluorescence polarization immunoassay.	Dilution	97.1-102.0	LR:0.20 - 3.0ng/mL	[62]
IMI, AMI, TRI, CLO	Urine, plasma, whole blood	GC - SID.	SPE (C_{18} cartridges).		LR: 10 - 80pg. LOD: 5 - 10pg.	[63]
IMI, DES	Human plasma	Capillary GC – MS with D4 - IMI and D4 - DES as IS. Mass selective detector at *m/z* 234 for IMI, *m/z* 238 for D4 - IMI, *m/z* 412 for DES and *m/z* 416 for D4 - DES.	LLE (*n*-heptane–isoamyl alcohol (99:1, v/v)	IMI:91-99 DES:94-103	IMI: LR:0.580-116ng/mL DES: LR :0.545- 109ng/mL	[64]
AMI, TRI, IMI, DOX	Hair	GC - MS.	Digestion and SPME.		AMI:LR:0.22 - 10.4 ng/mg. LOD: 0.05ng/mg, DOX, TRIMI:0.2ng/mg AMI: LOQ: 0.15ng/mg, DOX,TRIMI:0.7ng/mg	[65]
AMI, DES, norTRP, IMI	Urine and human serum	Non aqueous Capillary electrophoresis	Dialysis – SPE (20x2mm I.D. SPE cartridge, C_2 - bonded silica)	AMI, DES: 60-70, norTRP: 80 IMI:95	LODs: in the 40 – 80ng/mL range for urine, and the 60 – 100ng/mL range for serum.	[66]
IMI, AMI, nor-TRP	Human plasma	Non aqueous Capillary electrophoresis	LLE. (hexane/isoamyl alcohol 99:1 (v/v)	97.0-103.9.	LR:50 - 500ng/mL. LOD: AMI and NOR: 20ng/mL, IMI and DES: 30ng/mL. LOQ: AMI and NOR: 30ng/mL, IMI and DES: 50ng/mL.	[67]
IMI, DES	Urine	Non aqueous Capillary electrophoresis	SPE (C_{18})	DESI: 95.1 ± 4.2 - 99.6 ± 4.7. IMI: 96.2 ± 4.7 - 101.2 ± 5.0.	LR:0.05 - 1.0mg/ L. LOD: DES: 15.0μg/L, IMI: 11.0μg/L. LOQ: DES: 50.0μg/L, IMI: 35.0μg/L.	[68]
AMI, DOX	Urine	Capillary Electrophoresis with electrochemiluminescence detection.	LLE (heptane:ethyl acetate = 90:10,v/v)	83 – 93.	LR:5.0 - 800ng/mL. LODs: 0.8ng/mL (AMI), 1.0ng/mL (DOX)	[69]

3.4. Sample Preparation

Various sample preparation techniques have been used for the isolation of TCAs from biological matrices. These include Liquid-Liquid Extraction (LLE), Solid Phase Extraction (SPE) and Solid Phase Microextraction (SPME), however direct methods after dilution, protein precipitation or dialysis have been also proposed.

Doxepin and desmethyldoxepin (E-and Z-isomers) were determined in human serum samples directly after addition of methanol containing the IS [30].

Clomipramine, demethylclomipramine, 2-, 8-, and 10-hydroxyclomipramine, 2-, and 8-hydroxydemethylclomipramine, didemethylclomipramine were determined in human and rat serum and plasma after removal of proteins and other interfering compounds by washing to waste using deionized water containing 35% (v/v) acetonitrile and centrifugation. Recovery ranged from 64 to 110% [40].

Amitriptyline and nortriptyline were determined in human serum directly after dilution (1:10) in 0.15M SDS-6% pentanol at pH 7, and filtration through 0.45 μm nylon membranes. High recovery rates were obtained: 99.8-101.6% for amitriptyline and 98.5-99.7% for nortriptyline [43].

Imipramine, desipramine, clomipramine, amitriptyline, nortriptyline and doxepin were determined in human blood after protein precipitation with $HClO_4$. Average recoveries obtained after neutralization of the supernatant with NaOH, filtration and direct injection into the HPLC column were 92.3 and 90.8% for desipramine and imipramine, respectively [53].

Imipramine and desipramine were determined in human serum directly after filtration (0.45 μm nylon membranes) [50].

Amitriptyline, clomipramine, desipramine, doxepin, imipramine, norclomipramine, nordoxepin, nortriptyline, opipramol and trimipramine were determined in human serum after protein precipitation and dilution with mobile phase. Recoveries ranged from 92 to 111% [55].

Simple dilution was performed for the determination of desipramine, imipramine and trimipramine in urine [60], for determination of imipramine in human serum [62] and imipramine, amitriptyline, desipramine, nortriptyline, clomipramine, doxepin, opipramol and trimipramine in human serum [30].

The vast majority of the published methods involve LLE with different extraction media. Desipramine, nortriptyline, imipramine, amitriptyline and clomipramine were determined in human serum using n-hexane yielding recovery rates in the range from 93.2 to 110.6% [28]. Hexane was also used for imipramine and desipramine determination in mice serum (recovery >89.6%) [32], for desipramine, imipramine, nortriptyline, amitriptyline, desmethylclomipramine and clomipramine in human plasma (recovery >80%) [35], for amitriptyline, nortriptyline, imipramine, desipramine, clomipramine and norclomipramine in human serum and plasma (recovery 92-105%) [39], for imipramine and amitriptyline from blood serum [61], and for doxepin, desipramine, imipramine, amitriptyline and trimipramine in human plasma with recovery rates 75% for desipramine and >90% for the other four tricyclic amines [59].

Mixtures of hexane with isoamyl alcohol in different ratios (98:2 or 99:1) were used for the extraction of desipramine from rabbit plasma and rabbit bone marrow (with low recovery rates: 24.2-30.2% in rabbit plasma and 24.5-34.2% in rabbit bone marrow) [27], as well as for the extraction of different TCAs from human plasma: imipramine, desipramine, amitriptyline and nortriptyline (with recovery 89.6-103.2%) [33], amitriptyline, nortriptyline, imipramine, desipramine, clomipramine, doxepin and trimipramine [38], doxepin, and desmethyldoxepin using (with recovery of 90% (doxepin) and 75% (desmethyldoxepin) [56], amitriptyline, clomipramine, desipramine, doxepin, imipramine, noxiptyline and opipramol (recovery 75-90% except for amitriptyline (67%) and opipramol (47%) [46] and imipramine, amitriptyline and nortriptyline (recovery 97.0-103.9%) [67].

Amitriptyline, nortriptyline, doxepin, imipramine, desipramine, nordoxepin, clomipramine, and trimipramine were isolated from human serum and liver using hexane-butan-1-ol (95:5 v/v). More than 60% recovery was obtained for human serum and more than 40 % for liver [29].

A mixture of n-heptane, ethylacetate and isoamyl alcohol was used for the extraction of amitriptyline, clomipramine, demexiptiline, desipramine, doxepine, imipramine, opipramol and trimipramine from human serum [31], a mixture of isoamyl alcohol and heptane for cis- and trans- doxepin, cis- and trans- desmethyldoxepin isolation from human serum (65-75% recovery for desmetyldoxepins and 75-85% for doxepins) [34] and for imipramine and desipramine from human plasma after derivatization to their pentafluoropropionyl derivatives (recovery 91-99% for imipramine and 94-103% for desipramine) [64], and a mixture of heptane and ethyl acetate for doxepin, desmethyldoxepin extraction from human plasma (recovery 61-64%) and urine (recovery 63-68%) [36], for amitriptyline, nortriptyline, E and Z-hydroxy-amitriptyline, E and Z-hydroxy-nortriptyline isolation from rabbit plasma [26] and for amitriptyline and doxepin from urine (recovery 83 – 93%) [69].

Diethyl ether was used for extraction of imipramine, desipramine, 2- and 10-hydroxyimipramine, 2- and 10-hydroxydesipramine from human plasma and urine (recovery: 78.6-94.3% (plasma), 10.24-28.80% (urine) [51], for imipramine from human serum (recovery 87.6%) and plasma (recovery 86.2%) [52], and for imipramine, desipramine, amitriptyline, nortriptyline, clomipramine from human plasma with diethyl ether 83.0-93.8% [54].

Amitriptyline, nortriptyline, imipramine, trimipramine, clomipramine and doxepine were extracted from human blood and urine using Toxi-Tube A and Toxi-Tube B from Toxi-Lab (Irvine, CA, USA). These tubes are designed for use with 5-mL urine samples, containing sodium carbonate and bicarbonate to give a pH of 9.0 in a mixture of dichloromethane and dichloroethane [24].

Clomipramine and norclomipramine were determined in blood by LLE with a mixture of chloroform and isopropanol (3:1, v/v) and separation of the solution on silicone treated

filter paper Whatman 1PS. Recovery obtained was 88-90% [57].

Solid Phase Extraction was applied to the isolation of TCAs from biomatrices using various sorbents. Mixed-mode SPE (IST Isolute HCX, 80 mg/1 mL) cartridges were used for the extraction of amitriptyline, doxepin, clomipramine, trimipramine and their N- demethyl metabolites from human plasma [49], while C_{18} cartridges for extraction of imipramine, amitriptyline, trimipramine and chlorimipramine from urine, plasma, and whole blood [63].

Bond Elut Certify (Varian Sample Preparation Products, Harbor City, CA) SPE columns were used for the extraction of amitriptyline, imipramine, trimipramine, nortriptyline, protriptyline and doxepin from human serum and urine. SPE columns were washed with methanol and phosphate buffer (pH 6.0). Elution was performed with a mixture of 100% methanol and 10% ammonia (5:1 v/v). Enhancement of sensitivity was achieved by evaporation of the SPE extract after addition of a few drops of HCl [25].

C_{18} Bond-Elut cartridges were used for doxepin, desipramine, maprotiline and imipramine extraction from human plasma. Mixtures of methanol–aqueous acetate buffer were used as washing and elution solvents. The recoveries of the drugs using other sorbent materials (C_8, C_2, cyclohexyl, cyanopropyl and phenyl Bond Elut as well as copolymer HLB waters cartridges) were also examined. Mean absolute recoveries were 82-84% [41].

SPE on cyanopropyl cartridges Isolute SPE column from International Sorbent Technology LTD (Hengoed,Mid Glamorgan, UK), were used for the extraction of nortriptyline and trimipramine from human serum using ASPEC XL automatic SPE apparatus by Gilson with high recoveries (93-98 %). LLE was used in comparison using heptane/isoamyl-alcohol (98.5:1.5, v/v) with recovery 75 ± 13 [42].

SPE with an Isolute C_2 column was used for the extraction of clomipramine, N-desmethylclomipramine, 8-hydroxy-clomipramine, 2- and 8- hydroxydesmathylclomipramine from human plasma. Before applying the sample, the columns were washed with methanol/water/phosphate buffer (pH 9.2) (2/1/2 v/v/v). Interference were washed out with phosphate buffer/mixture of water and acetonitrile (80:20 v/v). Elution with methanol yielded high recovery rates 99.1-100.3% [44].

Amitriptyline, nortriptyline, doxepin and opipramol were determined in human plasma by on-line extraction on an Oasis® HLB extraction column (30μm, 1 x 50mm) with recovery >90% [58].

RP-18 SPE cartridges were used for the extraction of clomipramine and norclomipramine from hair. The samples after division into three segments were pulverized in mill-ball and incubated with diazepam-D5 and 0.1M hydrochloric acid. After neutralization with 0.1M NaOH and treatment with ammonium carbonate buffer (pH 9.3), the supernatants were collected, centrifuged and added to the column. Washing with ammonium carbonate buffer and elution with a mixture of 0.1% acetate acid-methanol provided recovery of 91-93% [57].

Imipramine and desipramine were determined in urine on C_{18} (Waters Sep-Pak Plus, Milford, MA, USA) cartridges yielding recovery rates 95-101% [68].

A combination of LLE and SPE was applied for the extraction of doxepin, nordoxepin, desipramine, imipramine, nortriptyline and amitriptyline from human breast milk. LLE was performed using 2% butanol in hexane. SPE C_{18} cartridges were conditioned with diethylamine in methanol, followed by 1% potassium bicarbonate in 10% acetonitrile in water. After sample application benzodiazepines were washed off by a mixture of water-methanol-acetonitrile, while elution of TCAs was performed with diethylamine in methanol yielding recovery in the range 85.8-104.6% [48].

3M-Empore high-performance extraction disk cartridges (Varian, Darmstadt, Germany) were used for the extraction of amitriptyline, nortriptyline, imipramine, desipramine, doxepin, nordoxepin, clomipramine, norclomipramine and trimipramine from human serum. The sorbent was conditioned with methanol followed by water. Elution with 2-propanol–ammmonia solution (25%)–dichloromethane (20: 2:78) yielded 75-100.2% recovery [45].

Membrane technologies were used for the determination of amitriptyline, nortriptyline, imipramine and desimipramine in human plasma after mixing with Protein Releasing Reagent [PRR: an aqueous solution consisting of 1M HCl and 25% (v:v) glycerol]. The mixture was injected into the donor channel of the dialysis cell while the acceptor solution (0.001M ammonium phosphate buffer, pH 7.0) was transported to the acceptor channel. The enriched analytes were eluted onto the analytical column by the HPLC mobile phase. The donor side and the acceptor side of the dialyser were simultaneously washed with the donor solution (1mM dodecyltrimethyl ammonium bromide in water) and acceptor solution, respectively. The precolumn was regenerated with the acceptor solution and the next sample was injected into donor channel of the dialysis cell [37].

A membrane separator was also used for the extraction of amitriptyline, opipramol and noxiptyline from urine. Sample solutions containing sodium phosphate buffer (pH 9.0) were passed over the liquid membrane in membrane separator, which was made of two PTFE blocks with machined spiral grooves into a rigid construction. The membrane was impregnated in n-undecane by soaking. The membrane separated two channels: the donor channel for extraction of analytes and IS and the acceptor channel with acidic solution for re-extraction of analytes from the membrane solvent. Sample of acceptor solution was injected into the HPLC column [47].

On-line combination of Dialysis and SPE (20 x 2mm I.D. SPE cartridge, C_2 -bonded silica was used for the determination of amitriptyline, desipramine, nortriptyline and imipramine in urine and human serum yielding recovery rates of 60-70% for amitriptylin, desipramine, 80% for nortriptyline and 95% for imipramine [66].

Solid phase microextraction was applied to the extraction of amitriptyline, trimipramine, imipramine and doxepin from hair after digestion [65].

CONCLUSIONS

Tricyclic antidepressants consist in a homogeneous group of drugs differing mostly in their potency to inhibit presynaptic norepinephrine or serotonin uptake and in their tendency for causing variety of unwanted effects. Between 1960 and 1980 tricyclic antidepressants (TCAs) represented the major pharmacological treatment for depression. Despite the introduction of newer and safer antidepressants the prescription of tricyclic antidepressants is still widespread as they are cheaper and are still considered as the most effective group of antidepressants. For this group of drugs, distinct ranges of optimal plasma concentration for therapy are required due to the wide range of inter-individual variability in metabolism and elimination. Lower concentrations are associated with sub-optimal effects, while toxicity can appear at high concentrations. They are frequently encountered in emergency toxicology screening, drug abuse testing and forensic medical examinations. Therefore, the analysis of these compounds is important not only for quality assurance in pharmaceutical preparations, but for obtaining optimum therapeutic concentrations in order to minimize the risk of toxicity as well.

Moreover continued progress in understanding the neurobiology of antidepressant drugs will lead to further identification of the phenomenon of how the drugs act and work and development of more effective and faster acting therapeutic agents. Analytical methods presented in this review prove that HPLC is an efficient fast and reliable tool in medicinal chemistry for the multi-component analysis of TCAs in clinical samples.

ABBREVIATIONS

ACN = Acetonitrile

Activ. = Activated

Add. = Addition

Adj. = Adjusted

Alkal. = Alkalisation

AMI = Amitriptyline

API = Atmospheric Pressure Ionization

buff. = Buffer Solution

CE = Capillary Electrophoresis

Centr. = Centrifugation

Ch. = Channel

CH_2Cl_2 = Methylene Chloride

CLO = Clomipramine

Conc. = Concentrated

Deion. = Deionized

Deprot. = Deproteinization

DES = Desipramine

Det. = Detection

Dil. = Dilution

Diss. = Dissolution

El. = Eluent/Elution

Electr. = Electrochemical

ESI = Electrospray Ionization

Evap. = Evaporation To Dryness

Extr. = Extraction

Filtr. = Filtration

FL = Fluorescence

FR = Flow Rate

Homog. = Homogenization

5-HT = 5-Hydroxytryptamine

IMI = Imipramine

Incub. = Incubation

Inj. = Injection

IPA = Isopropanol

IS = Internal Standard

LLE = Liquid Liquid Extraction

LR = Linear Range

MAOIs = Monoamine Oxidase Inhibitors

MAPR = Maprotiline

MeOH = Methanol

Mix. = Mixing

MP = Mobile Phase

Neutr. = Neutralization

NorCLO = Norclomipramine.

NACE = Non-Aqueous Capillary Electrophoresis

NE = Nor-Epinephrine

NorTRP = Nortriptyline

Org. = Organic

OPI = Opipramol

FPIA = Fluorescence Polarization Immunoassay.

Prec. = Precipitation

ProTRP = Protriptyline

Pulv. = Pulverised

Resp. = Respectively

RT = Room Temperature

SDS = Sodium Dodecyl Sulfate

Sol. = Solution

SPE = Solid Phase Extraction

SPME = Solid Phase Microextraction

Supern. = Supernatant

SID = Surface Ionization Detection

SSRIs = Selective Serotonin Reuptake Inhibitors

TCAs = Tricyclic Antidepressants

TEA = Triethylamine

TEA = Triethylamine

Temp. = Temperature

TRI = Trimipramine

TOF = Time-Of-Flight

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