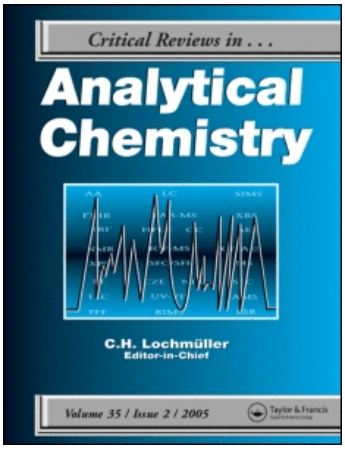

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Review of Analytical Methods for Identification and Determination of PHEs and Tricyclic Antidepressants

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Review of Analytical Methods for Identification and Determination of PHEs and Tricyclic Antidepressants

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A critical review of analytical methods for identification and determination of some phenothiazine derivatives and tricyclic antidepressants in a variety of materials is presented. The methods have been divided into five groups accordingly to the applied analytical techniques: spectrometric, chromatographic, immunoassay, electrochemical, and capillary electrophoretic. The review is based on a survey of chemical and toxicological literature covering the articles mainly from 2000 until now. A brief description of physicochemical properties of the compounds of interest has been also presented.

Keywords PHEs, tricyclic antidepressants, identification and determination methods, biological samples, pharmaceutical preparations

INTRODUCTION

Phenothiazine (PHEs) derivatives and tricyclic antidepressants (TCADs) belong to classic psychotropic drugs, which are still frequently used. PHEs are prescribed mainly for treatment of patients with psychotic or other serious psychiatric disorders marked by agitation and impaired reasoning. These drugs also have other properties that are clinically useful, including antiemetic and antihistamic effects, and the ability to potentate analgesic sedatives (1). TCADs belong to the first generation of the antidepressants with the precursor imipramine and have been used for depression treatment. PHEs and TCADs are also often used together in psychiatric pharmacotherapy. The mentioned drugs, especially TCADs, due to their relatively narrow therapeutic window are dangerous in overdosages and they are notorious for many drug-related deaths. The blood levels of the selected neuroleptic PHEs and TCADs, covering therapeutic, toxic and lethal concentrations (2–4) have been placed in Table 1.

The mechanism of action of tricyclic psychotropic drugs is not exactly known. However, it is assumed that TCADs potentate the action of biogenic amines (norepinephrine, serotonin) by blocking their physiological inactivation and the antagonism of D2 receptor activity of neuroleptic PHEs is emphasized (1). Moreover, some PHEs also interact with D1 dopaminergic, 5-HT_2 serotonergic, and α-adrenergic receptors (1).

TCADs and PHEs, due to their pharmacological properties and potent toxicity, have become groups with increasing interest in such fields as clinical monitoring, clinical and forensic toxicology, and pharmacokinetical study. The worldwide interest in tricyclic psychotropic drugs has resulted in generation of extensive literature concerning identification and determination of these classes of drugs.

In this paper a critical review of the analytical methods and techniques for determination of tricyclic psychotropic drugs is presented. The methods and techniques were divided into five groups: spectrometric, chromatographic, immunoassay, electrochemical, and capillary electrophoretic. The review is preceded by description of some basic properties of these compounds

PHYSICOCHEMICAL PROPERTIES OF PHEs AND TCADs

Similarity of the structure of PHEs and TCADs entail similarities in their properties. These drugs are usually administrated as salts of basic compounds, mainly as hydrochlorides and more rarely as maleates, dimalonates, mesilates, decanoates and others, and they are known all over the world as various pharmaceutical preparations (Table 1). Some basic physicochemical properties of the commonly used psychoactive compounds such as

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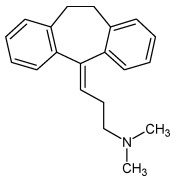
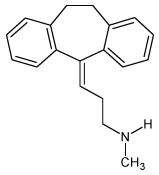
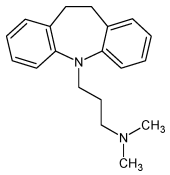
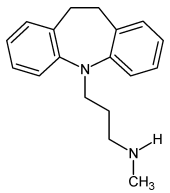
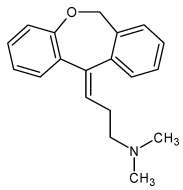
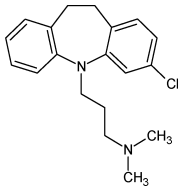
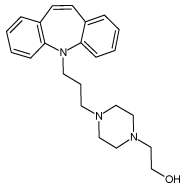
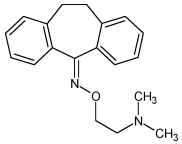
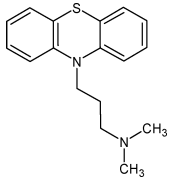
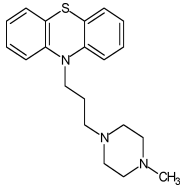
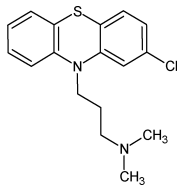
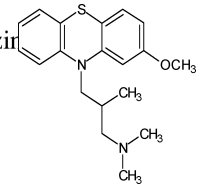
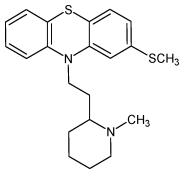
TABLE 1
Structures, physicochemical properties (5, 6), blood or serum levels (2-4) and pharmaceuticals of the commonly used tricyclic psychotropic drugs.

Drug	Structure	Molecular weight	pK_a	Log P	Salt of drug[c]	Melting point of salt	Drug blood level (μg/mL)		Dosage formulas
							Therapeutic	Toxic (lethal)	
Amitriptyline		277.4	9.4 (25°)	4.94	Colorless crystals or white powder	~196–197°	0.12–0.25	> 0.5 (2–20)	Adepril Elavil Laroxyl Sarotex Triptil
Nortriptyline		263.4	9.7	1.7[b]	White powder	213–215°	0.05–0.375	0.5 (13)	Aventyl Martimil Motival Norfenazin Norterol Psychostyl
Imipramine		280.4	9.5 (24°)	2.5[b]	White or slightly yellow crystalline powder	174–175°	0.15-0.25	0.5–1[d]	Antidep Impril Melipramine Sermonil Tofranil
Desipramine		266.4	10.2 (24°)	1.4[b]	White crystalline powder	215–216°	0.05–0.684	>0.5 (10–20)	Deprexan Norpramin Nortimil Pertofran Petylyl
Doxepin		315.8	9.0 (25°)	2.4[b]	White crystalline powder	184–186° (188–189°)	0.10–0.25	0.12–4.3 (2–26)	Aponal Doxal Gilex Mareen Sinequan Xepin

(Continued on next page)

TABLE 1
Structures, physicochemical properties (5, 6), blood or serum levels (2-4) and pharmaceuticals of the commonly used tricyclic psychotropic drugs. *(Continued)*

Drug	Structure	Molecular weight	pK_a	Log P	Salt of drug[c]	Melting point of salt	Drug blood level (μg/mL)		Dosage formulas
							Therapeutic	Toxic (lethal)	
Clomipramine		314.9	-[a]	5.2	White or slightly yellow crystalline powder	~192°	0.1–0.45	0.4–0.6[d]	Anafranil
Opipramol		363.5	3.8	3.4	Light yellow, crystalline powder	~210°, with decomposition	0.1–0.5[d]	2–3[d]	Indison
Noxiptiline		294.4	—[a]	4.3	white crystalline powder	189°-191°	-[a]	-[a]	Agedal Nogédal
Promazine		284.4	9.4 (25°)	2.5[b]	White or slightly yellow, crystalline powder	181°, with decomposition	0.1–0.4[e]	> 1 (> 5)	Liranol Prazine Promazin Protactyl Sparine Talofen
Perazine		339.5	8.0	2.9[b]	White crystalline powder	114–116°	0.02–0.35[d]	0.5[d]	Pernazinum Taxilan

Chlorpromazine		318.0	9.3 (20°)	3.4[b]	White or creamy-white crystalline powder	179–180° (94–196°)	0.01–0.5	1–2 (3–12)	Chlorazin Chlorpromed Fenactil Hebanil Largactil
Levomepromazie (Methotrimeprazir		328.5	9.2	4.7	White crystalline powder	~190°, with decomposition	0.02–0.271	0.4[d]	Levium Levoprome Methozane Nozinan Immobilon
Thioridazine		446.6	—[a]	2.9	Fine, white or pale cream powder	~227°	0.1–2.6	>5 (1–18)	Aldazine Mellaril Novoridazine Thioril

[a]Date have not been found.

[b]Partition coefficient was determined versus octanol/buffer pH 7.4 (or pH 7.0), in the rest cases versus octanol/water.

[c]The drugs are usually administered as hydrochlorides with some exceptions such as for example: perazine (dimalonate), levomepromazine (maleate) and thioridazine (mesilate).

[d]Data from [3].

[e]Date from [4].

molecular weight, dissociation constant (pKa), partition coefficient (log P), physical forms and melting points of the salts have been given in Table 1. It should be mentioned that none of these drugs are stable in the basic form, especially in daylight. PHEs are particulary sensitive to light and easy undergoing decomposition. PHEs are easily oxidized by means of many oxidizing reagents [e.g. $Ce(SO_4)_2$, $K_2Cr_2O_7$, $KBrO_3$, NH_4VO_3, KIO_4, $NaNO_2$, H_2O_2, etc.]. In the first step PHEs are reversibly oxidized to color free radicals or semiquinones, which are further oxidized irreversibly to colorless sulphoxides. The free radicals are stable under certain conditions and their color depends on the type of the substituents. Reducing properties of PHEs also permit their easy transformation into free radicals at relatively low redox potentials. Salts of tricyclic psychotropic drugs dissociate in aqueous solution creating large cations which are able to form colored ion-associates, change-transfer complexes or crystalline precipitates. Although the generated compounds are insoluble in water, a quantitative extraction to organic solvents is possible. The extracts are intensely colored and stable from some hours to a few days. PHEs and TCADs display absorption spectra with characteristic maxims in the UV region. In addition, PHEs exhibit slight fluorescent spectral properties. Stronger fluorescence emission of PHEs may be invoked by UV light, as well as by complexometric reactions with some organic compounds. Both classes of drugs undergo metabolism in living organisms; from the pharmacological point of view the metabolites of TCADs are more potent than those of PHEs, and in some cases (e.g. nortriptyline, desipramine) constitute individual psychotropic drugs. Metabolites of the drugs are more hydrophilic and their recoveries from organic solvents during extraction are often lower than parent compounds. Some of the described properties of PHEs and TCADs are the base of the reported analytical methods concerning determination of these drugs in a biological material and in pharmacological preparations as well.

ANALYTICAL METHODS AND TECHNIQUES

The review of the papers showed that a large variety of analytical techniques have been applied for determination of tricyclic psychotropic drugs. From among them the following techniques may be mentioned: colorimetry, fluorimetry, volumetry (7), chromatography, voltammetry, immunology, capillary electrophoresis, gravimetry (8) and electron spin resonance (9). However, according to the authors the most important methods used for determination of PHEs and TCADs may be divided into five groups, which are described below.

Spectrometric Methods

Photometry, fluorimetry and chemiluminometry belong to the spectrometric techniques which are widely used in tricyclic psychotropic drug analysis. These methods are applied for determination of PHEs and TCADs in pure forms, pharmaceutical formulations and more rarely in biological fluids. Flow injection analysis (FIA) is among the most important automatic technique which has found wide application in many fields of routine analysis including pharmaceutical ones. This methodology was also successfully employed for spectrometric methods developed for PHEs and TCADs analysis, improving their effectiveness considerably.

Many photometric methods are based on generation of colored oxidation products. Misiuk (10) determined imipramine and desipramine in pharmaceutical preparations using the oxidation of the drugs in a sulphuric acid medium by ammonium metavanadate. The blue-colored products for imipramine and desipramine were measured at 620 and 618 nm, respectively. Maximum intensity of the color remained constant for 2 hours at room temperature. The lower limit of the method was found to be 0.07 and 0.11 μg/mL for imipramine and desipramine, respectively. Reproducibility of the measurements varied to 0.5%. Nagaraja et al. (11) described a spectrophotometric method for five tricyclic antidepressants, belonging to the dibenzazepine class, i.e. imipramine hydrochloride, desipramine hydrochloride, clomipramine hydrochloride, trimipramine maleate and opipramol. This method was based on the reaction of the drugs with iron (III) and the subsequent reaction with ferricyanide in an acetic acid medium. The yielded blue products were measured at 720–730 nm. The colored products were stable over 3–4 hours at room temperature. The method was applicated to assay commercial tablets containing the examined drugs. Precision of drug recoveries was from 0.23 to 1.14%. Syeda et al. (12) proposed 2,2′-bipyridine as a reagent for determination of the above-mentioned tricyclic antidepressants in pharmaceuticals. The method was based on reduction of iron (III) solution by the drugs and subsequent complexation with 2-2' bipyridine in an acetic acid medium. The absorption of the formed pink complex was measured at 530 nm. The product was stable over 3–4 hours at 27°C. Interference studies indicated that among commonly used additives and excipients only vitamin C was found to interfere seriously. According to the authors the method is simple, sensitive, selective and cost-effective and, thus, may be addresed to the routine quality control of the drugs in preformulation and dosage forms. Nascentes et al. (13) presented the FIA system for the screening of five PHEs in human urine. The PHEs were oxidized with hexacyanoferrate (III) ions in a nitric acid medium to colorless sulphoxides via orange or purple intermediates which were monitored at 520 nm. The configuration of the proposed system permited selective retention of PHEs on a LiChrolut®-EN sorbent column before their oxidation. Detection limits (DLs) were 1 μM for methotrimeprazine, promethazine and chlorpromazine, and 2 μM for fluphenazine and thioridazine. The precision and accuracy of the method for each drug tested was on the average $\sim$4 and 3%, respectively. The realiability of the proposed method was checked by analysis of 50 control urine samples spiked with chlorpromazine; 6% of false positive and 0% false negative results were obtained, however no possible interferences with PHE metabolites present in real urine samples have been reported. A bead injection spectroscopy-flow injection analysis (BIS-FIA) system for the spectrophotometric detection of promethazine and trifluoperazine has been also

described (14). The PHEs were oxidated by Fe (III) which was later determined by formation of a complex between Fe (II) and ferrozine. Absorbance of the complex was measured at 567 nm. The complex was retained on beads placed in the flow-cell, and after a single measurement, beads were automatically discarded from the cell and removed from the system. The authors justified the use of this bead injection (BI) reusable flow-through sensor by the fact that the complex is very strongly retained on the solid support and the regeneration of the sensor surface would be extremelly difficult. DLs were 0.09 and 0.14 μg/mLfor promethazine and trifluoperazine, respectively, and precision was lower than 2% for both drugs.

Basavaiah et al. (15) developed an indirect spectrophotometric method for determination of six PHEs in pharmaceutical preparations, using potassium dichromate as an oxidant and iron-thiocyanate system for measuring the unreacted dichromate. DLs for the studied drugs ranged from 0.083 to 0.157 μg/mL, and precision was on the average 1.03%.

Extractive spectrophotometric determinations are another group of spectrophotometric methods. The formed colored ion-association complexes or sediments are insoluble in water but may be quantitatively extracted into organic solvents. Starczewska (16) determined imipramine in its pharmaceutical preparations using eriochrome cyanine R to form a reddish compound, which was quantitatively extracted into n-butane. The extraction was quantitative after 1 minute at room temperature and the extracts were stable for about 1 hour. The method worked at the concentration range 10–80 μg/mLand precision of measurements was on the average $\sim$ 1.6%. Regulska et al. (17) used dipicrylamine and picric acid for determination of promethazine and perphenazine in pharmaceutical preparations. The drugs precipitated from a neutral solution in the form of orange-brown sediment, which was quantitatively extracted with chloroform or benzene. DLs were 1.00 and 1.75 μg/mLfor promethazine and 1.80 and 1.34 μg/mLfor perphenazine using picric acid and dipicrylamine, respectively. The reproducibility of the method ranged from 0.4 to 0.8%. The yellow-colored extracts were stable from two to six days. According to the authors the proposed methods are superior to pharmacopoeia methods and other extractive spectrometric methods in regards to simplicity and stability of the formed complexes.

Derivative UV-spectrophotometry belongs to the methods which may be used for simultaneous analysis of two or more compounds. The derivative UV-spectrometric method was used for simultaneous determination of promazine hydrochloride and its decomposion product-sulphoxide (18). Promazine was determined using the first-order derivative spectrum at 268 nm, and the quantification of its sulphoxide was achieved by application of third-derivative spectrum based on measurements of the amplitude at 342–344 nm. The DLs were 1.56×10^{-6} and 5.94×10^{-6} M for promazine and promazine sulphoxide, respectively. Precision of the method was 2×10^{-3}% for promazine and 2.6% for its sulphoxide. This method was successfuly applied for analysis of two outdated (decomposed) pharmaceutical preparations. According to the author a single measurement run (measurement and mathematical treatment of the spectrum) took about 10 minutes.

The procedures based on measurement of transient emission light make another group of spectrometric methods, which are frequently used for determination of tricyclic psychotropic drugs. According to the applied kind of source-inducing light, spectrofluorimetric and chemiluminmetric methods may be distinguished, where the latter have attracted special interest in the past few years. The DLs obtained by using chemiluminometry are generally lower than those provided by photometry. Marques et al. (19) recommended a multi-commutated flow system for chemiluminometric determination of clomipramine based on sulphite/Ce (IV) reaction. Clomipramine acted as a sensitizer in chemiluminescent oxidation of sulphite by Ce (IV). The method was characterized for two sampling times of 5 and 10 seconds and its parameters were: working ranges 30–60 mg/mL and 2.5–10 mg/mL, precision 4.6 and 3.7%, the DLs 0.65 and 0.7 μg/mL, respectively. The developed methodology was applied in the analysis of pharmaceutical preparations. Huang and Chen (20) quantitated chlorpromazine hydrochloride in tablets as well as in biological fluids (plasma and urine) by the method, which was based on the reaction between the drug and Ce (IV) in an acid medim, and measurements of chemiluminescency produced by rhodamine 6G used as a sensitizer. The detection limit was 6.5 ng/mLand precision was 4.1%. Before analyzing, the urine samples required only dilution with water and plasma was deproteinated using trichloroacetic acid. Two chemiluminescence FIA assays of thioridazine hydrochloride (21) and promethazine hydrochloride (22) using acidic permangate have been also presented. Both methods were based on chemiluminescence emission intensity produced as a result of oxidation reaction of a drug with permangate in a sulfuric acid medium. The limit of detection for thioridazine was 1.2×10^{-6} mol/L and the working concentration range of promethazine hydrochloride was between 1.56×10^{-5} and 1.87×10^{-3}mol/L. Both methods are recommended for analysis of drug formulations. A mixture of luminol with $KMnO_4$has been used as a reaction mixture in the FIA chemiluminescence method for determination of four hydrochloride PHEs: chlorpromazine, perphenazine, fluphenazine and thioridazine (23). The DLs were 0.4 ng/mL (chlorpromazine hydrochloride), 0.7 ng/mL (perphenazine hydrochloride), 2ng/mL (fluphenazine hydrochloride) and 0.7 ng/mL (thioridazine hydrochloride). The proposed method was applied for determination of chlorpromazine hydrochloride in injection and in mental patient's urine samples. Mohamed et al. (24) elaborated a spectrofluorimetric method for determination of five psychoactive drugs: two PHEs (chlorpromazine and thioridazine) and three tricyclic antidepressants (clomipramine, imipramine and desipramine). The method was based on oxidation of the studied drugs using Ce (IV) in presence of sulphuric acid and monitoring the fluorescence of the formed Ce (III) at $\lambda_{ex.} = 254$ nm and $\lambda_{em.} = 355$ nm. A complex validation study of the proposed method was performed. The DLs and quantification ranged from 0.035 to

0.038 μg/mL and from 0.116 to 0.125 μg/mL, respectively. This method was recommended for routine quality control of the studied drugs either in bulk or in their corresponding dosage forms. The authors mentioned advantages of the proposed method such as sensitivity, simplicity, rapidity and low costs, but we can not agree with the final conclusion that: ' ... the possibility of oxidation of readily oxidizable drugs by Ce (IV) does not exist since the studied drugs are not prescribed in combinations.' A flow injection (FI) system based on the extraction-fluorimetric method for determination of the six most used tricyclic antidepressants (imipramine, desipramine, amitriptyline, nortriptyline, clomipramine and doxepine) was presented (25). The studied drugs formed ion pairs with 9,10–dimethoxyantracene-2-sulphonate (DMAS) and then were quantitatively extracted into dichloromethane where the fluorescence ($\lambda_{ex.} = 265$ nm and $\lambda_{em.} = 448$ nm) was measured. For optimization of the proposed system, the FI parameter and the chemical variables were investigated separately using experimental design approaches. The obtained DLs were lower than 0.30 mg/L and calibration curves were linear over the working range 0.25–3.00 mg/L. The method was applied for determination of the studied antidepressants in pharmaceutical preparations.

Chromatographic Methods

Chromatographic techniques still belong to the most often employed group of analytical methods for identification and determination of the tricyclic psychotropic drugs in biological material. Liquid chromatography (LC) and gas chromatography (GC) with a variety of detection systems are particularly employed in this area. Thin layer chromatography (TLC) is not a technique of choice due to its lack of sensitivity and selectivity; however the short time and cost of analysis (especially when numerous samples are run simultaneously) also make this technique useful in some cases of laboratory practice.

LC TECHNIQUES

High performance liquid chromatrography (HPLC) with UV absorption detection (HPLC-UV) seems to be still most widely used for TCADs and PHEs determinations, especially in body fluids. In most cases the reversed-phase chromatography has been applied. The advantages of reversed-phase chromatography are the possibility to inject aqueous extracts, the use of less toxic and cheaper mobile phases, and the possibility of using diode-array detectors (DADs). Other detection methods such as fluorescence, chemiluminescence or electrochemical are often more sensitive but are also less universal than photometric detection. LC with mass spectrometry (MS) or tandem MS (MS-MS) detection belongS to the modern and highly advanced analytical techniques; their contribution to toxicological and pharmacokinetical investigations is growing. These techniques are especially powerful tools in the examination of metabolites.

The HPLC-UV method was applied for simultaneous determination of clomipramine and its desmethyl and hydroxy metabolites in plasma of patients with obsessive-compulsive disorder (26). After a solid phase extraction (SPE) on Isolute C2 columns, the separation of the compounds was performed on Lichrospher CN column using an eluent consisting of 10 mM K_2HPO_4-acetonitrile-methanol (35:25:40, v/v/v). The limit of quantification for all the analytes was 5 ng/mL. According to the authors the proposed method, compared to other methods, had the advantage of efficient and rapid extraction which allowed determination of clomipramine with its metabolites in several plasma samples within a few hours with higher sensitivity, better resolution of the peaks, and may be realistically applied in clinical examinations. Olesen et al. (27) developed a fully automated on-line SPE-HPLC-UV method for determination of nortriptyline in human serum. SPE was performed on cyanopropyl cartridges and HPLC was carried out using the C18 column with acetonitrile-0.01 M triethylamine (34:66; v/v) buffer (pH 3.0) as the mobile phase. The lower level of quantification was 2.0 ng/mL. The inter-day variation was less than 5%. Studies of analytical interferences from 38 coadministrated psychoactive drugs, including their metabolites, revealed that only imipramine and a methotrimeprazine metabolite interfered. The time used for a single on-line analysis of nortriptyline in human serum was ~30 minutes. Song and Putcha (28) presented a chromatographic method for quantification of promethazine and its three metabolites (promethazine sulfoxide, monodesmethyl promethazine sulfoxide and monodesmethyl promethazine) in urine employing on-line SPE and column-switching. Two different columns, an extraction column Oasis HLB and an analytical column Zorbax SB-CN, were used in the column-switching system. The matrix interference was removed on the extraction column using a gradient of water-methanol-30 mM ammonium acetate containing 2% ammonium hydroxide and further chromatographic separation was performed on the analytical column by mixture of acetonitrile and 30 mM ammonium acetate buffer (60:40, v/v, pH 5.5). The UV detection of the analytes was made at 236 nm. The lower limits of quantification were 3.75 ng/mL for promethazine and 2.50 ng/mL for its metabolites. The time of the on-line SPE chromatographic process was 25 minutes. The full validation data, including the method rubustness evaluation based on fractional factoral experimental design were provided. The authors suggested that the proposed method would be useful for pharmacokinetical and pharmacodynamical study in normal human subjects as well as in astronauts during space flight. Trocewicz (29) used the supported liquid membrane (SLM) technique for urine sample preparation and enrichment for determination of three tricyclic antidepressants by HPLC-UV. The highest extration efficiencies (43% for opipramol, 56% for noxiptyline and 43% for amitriptyline) were achieved when 0.05 M phosphate buffers (pH 4.0 and 9.5) were used as donor and acceptor solutions, respectively, and n-undecane with 5% tri-n-octylphosphine oxide was used as a liquid membrane. Limit of quantification for all tested compounds after enrichment of 100 mL urine was about 1 ng/mL. The sensitive HPLC-UV method for determination of opipramol in human plasma was described (30). Opipramol was extracted into

tert-butylmethyl ether, then separated on a cyanopropyl silica column and detected at 254 nm. A mixture of 20 mM NH_4OAc-20 mM HOAc-acetonitrile (240:60:700, v/v/v) was used as the mobile phase. The limit of quantification was 250 pg/mL using 1.5 mL plasma. The precision was better than 9% and inaccuracy less than 8%. The method was applied in the pharmacokinetic study. Madej et al. (31) proposed the HPLC/ DAD screening method for 13 psychotropic drugs from two pharmaceutical groups: PHEs and tricyclic antidepressants. The examined drugs were extracted from blood samples using two-step L-L extraction with n-hexan + isoamyl alcohol (99:1, v/v) and re-extraction into 0.05% phosphoric acid. The influence of experimental conditions on gradient chromatographic separation of the drugs was studied according to the 2^3factorial design. The LichroSpher RP Select B column and the mobile phase, water with phosphoric acetonitrile used in optimized gradient mode were selected as optimal chromatographic conditions. The analytes were detected at two wavelengths (254 and 220 nm) and the extra identification parameter, absorbance ratios A_{254}/A_{220} (apart from relative retention times), was calculated. It was concluded that the elaborated method might be a suitable tool for identification of tested psychotropic drugs for forensic purposes.

HPLC-UV methods are also employed for analysis of pharmaceutical preparations. Ruiz-Angel et al. (32) developed two reversed-phase LC procedures for control of seven TCADs in pharmaceutical preparations using UV detection. Two optimal chromatographic systems were selected: XTerra C18 column/35% (v/v) acetonitrile (pH 3) and C8 column/0.075 M sodium dodecylsulfate-6% (v/v) pentanol. In both cases preparation of samples was simple because no extraction of these drugs was needed. The repeatability of drug recoveries were below 0.6 and 2.0% for intra- and inter-day assays. Karpinska and Starczewska (33) also presented a reversed-phase HPLC-UV for simultaneous determination of some TCADs (amitriptyline, imipramine) and neuroleptics (chlorprotixene, thioridazine) in their quaternary mixtures. The chromatographic analysis was performed on the C-18 column using acetonitrile and 0.01 M aqueous solution of triethylamine (1:1), pH 2.7, as the mobile phase. The precision of the method for the drugs at 3 ppm levels ranged from 0.34 to 2.1%. The proposed method was also applied for determination of tested drugs individually in their pharmaceutical preaparations.

HPLC-UV methods are also employed in separation and determination of racemic drugs, which attracted a great attention in pharmaceutical analysis during the last decade. The HPLC-UV method was applied in resolution of racemic thioridazine into its enantiomers using the β-cyclodextrin-bonded stationary phase (34). The active substance was extracted from commercial formulations, purified by preparative TLC, and then resoluted into two enantiomers using the chiral column and mobile phase of 0.05 M phosphate buffer (pH 6.5)-acetonitrile (50:50). The detection limit was found to be 5 μg for each enantiomer.

In order to improve the reversed-phase HPLC separation of basic tricyclic antidepressants, covering a wide range of polarity, buffered and fluoroform (CHF_3)-enhanced fluidity liquid mobile phases were used (35). The chromatographic behavior of seven structurally-related tricyclic antidepressants was studied on the C18 stationary phase in methanol-phosphate buffer and methanol/phosphate buffer/CHF_3, with and without triethylamine (TEA) addition. The best separation results were obtained using methanol/20 mM phosphate buffer (pH 7)/ CHF_3(53.4/30.0/16.6 mole%) with 12 mM TEA as the mobile phase. The advantages of using $CHF_3$and buffered mobile phases were shown in a much shortened analysis time, increased efficiency, lower pressure drop and improved selectivity.

Diehl and Karst (36) presented a post-column chemical derivatization method for HPLC determination of PHEs. Peroxyacetic acid was used as a derivatizing agent for thirteen PHEs to yield colored radical cations or fluorescent sulfoxides. Both reaction products were employed for detection of the examined PHEs after their chromatographic separation. Separation of the compounds was performed on the C18 column in a binary gradient with the mobile phase consisting of 575 mg ammonium formate and 1.3 mL of formic acid in 500 mL of deionized water (Eluent One), and acetonitrile (Eluent Two). The fluorescence detection of the sulfoxides proved to be a more sensitive and robust method. The DLs ranged from 4 to 300 nM for the fluorescence detection of the sulfoxide and from 0.3 to 2 μM for the UV-Vis detection of the radical cation.

A sensitive method for determination of five tricyclic antidepressants (imipramine, desipramine, amitriptyline, nortriptyline and clomipramine) was presented using HPLC with chemiluminescence detection (37). The method was based on detection of aliphatic secondary and tertiary amino moietes in the tricyclic antidepressants with post-column tris(2,2'-bipyridyl) ruthenium (III) chemiluminescence. Five antidepressants were separated on a trimethylsilyl column using 50 mM phosphate buffer (pH 7.0)/acetonitrile (55:45,v/v) as the mobile phase. The DLs for the examined drugs were 3.2–33.1 pg for injection volume of 20 μl. The method was applied for drug monitoring of human plasma obtained after a signal oral dose of imipramine hydrochloride and amitriptyline hydrochloride. The plasma samples were pepared for analysis using two-step liquid-liquid (L-L) extraction. The proposed method was recommended for therapeutic and pharmaceutical investigations of tri- or tetracyclic antidepressants.

Bose et al. (38) developed a micellar liquid chromatographic method with electrochemical detection to determine amitriptyline and nortriptyline in serum samples for use in clinical monitoring. The drugs were separated on a C18 column using 0.15 M SDS-6% (v/v) pentanol buffered at pH 7 as the mobile phase. Only filtration was required before injection of serum samples into the HPLC system. The DLs were 0.25 and 0.31 ng/mLfor amitriptyline and nortriptyline, respectively. The analysis time was 14 minutes.

Ivandini et al. (39) used boron-doped diamond (BDD) electrodes for electrochemical detection of six tricyclic

antidepressants: imipramine, desipramine, clomipramine, amitriptyline, nortriptyline and doxepin. Separation of the drugs was performed by high performance chromatography in the FIA-mode. In this work the authors stressed the superiority of diamond over glassy carbon (GC) electrodes which are widely used electrodes for electrochemical detection. Using BDD electrodes, the sensitivity was about 2 to 4 times greater than those at glassy carbon electrodes. The DLs were 3 nM for imipramine and desipramine, 0.5 nM for clomipramine, 163 nM for amitriptyline, 1,080 μM for nortriptyline and 92 nM for doxepin. HPLC with a diamond electrode as an amperometric detector was applied for determination of imipramine and desipramine in plasma samples. During plasma analysis, the surface of BDD was reproducible with no adsorption of plasma components, however the reproducibility data were provided only for the above-mentioned (imipramine and desipramine) two drugs.

Seventeen PHEs were identified and characterized by high-performance LC/fast atom bombardment mass spectrometry (HPLC/FAB-MS) (40). All tested PHEs were separated on the chromatogram and their mass spectra were obtained by positive and negative modes. Approximate DLs for PHEs were less than 5 ng/mL in the positive mode and 5 μg/mL in the negative mode. The method was applied for analysis of serum samples of two psychiatric patients to detect four PHEs and their metabolites. The serum samples were purified by SPE on a C2 column and the drugs were detected without derivatization.

Badenhorst et al. (41) presented a sensitive method for simultaneous determination of doxepin and its metabolite desmethyldoxepin in plasma by (LC-MS-MS). The plasma samples were prepared by L-L extraction with hexane-isoamyl alcohol and separated on a C18 column with the mobile phase consisting of methanol-water-formic acid (600:400:0.5, v/v/v). The limits of quantification were 0.320 and 0.178 ng/mL (at relative standard deriavation (RSD) $< 20\%$) for doxepin and desmethyldoxepin, respectively, using 0.5 mL plasma for analysis.

GC METHODS

GC with universal and commonly used MS detection is rather rarley used for determination of psychotropic drugs in biological samples due to the necessity of derivatization. However, GC with nitrogen-phosphorus detection (GC-NPD) has proved to be a useful analytical technique in the area of underivatized drug analysis.

GC with mass detection was used for quantification of perphenazine in otter urine (42). The procedure involved enzymatic hydrolysis with β-glucuronidase-arylsulftase, followed by a SPE with Bond Elut Certify cartridges and futher derivatization of the dry extract with N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA). The derivatized extracts were analyzed by GC-MS using single ion monitoring (SIM) acquisition mode. Extraction recovery was $87.6 \pm 8.2\%$ and the limit of quantification was 1.2 ng/mL.

Shen et al. (43) examined the possibility of detection of nine antidepressant and antipsychotic drugs present at therapeutic levels in hair samples taken from 35 subjects. The drugs and their metabolites were identified using GC-MS at two ionization conditions: electron ionization (EI) and chemical ionization (CI). The quantification of the compounds was performed using GC-NPD. In order to select an optimal pretreatment of hair samples for analysis, three methods were studied: alkaline digestion, acid hydrolysis and methanol extraction. In all cases a 10-20 mg hair sample was treated. From these methods, ether extraction after alkaline digestion was selected, and the recoveries of extraction of nine drugs ranged from 64.6 to 88.1% with repeatability of 2.9–10.8%. Maximal concentrations in a hair sample for the tricyclic psychotropic drugs tested were 57.7 ng/mg for amitriptyline, 183.3 ng/mg for doxepin, 68.2 ng/mg for chlorpromazine, and 36.8 ng/mg for trifluoroperazine. Segmental analysis established that there was a correlation between the history of exposure of the drug (chlorpromazine) administered to the patient and distribution of drug along the hair shaft (in the Chinese black hair). According to the authors these psychotropic drugs can be detected in normally kept hair for at least 16 months after intake.

Two SPE procedures were studied for simultaneous determination of seven antidepressants including amitriptyline, nortriptyline, trimipramine and clomipramine in the whole blood by capillary gas-liquid chromatography with NPD (44). Two columns were compared: the Chem Elut (a diatomaceous earth) and the Bond Elut Certify (a mixed reversed-phase and cation exchange sorbent) with advantage to the latter. Recoveries of the componds using Bond Elut Certify columns at 500 ng/mL were 69% for amitriptyline, 62% for nortriptyline, 65% for trimipramine and 63% for clomipramine with intra- and interassay precisions of less than 9 and 17%, respectively. Limits of quantification for amitriptyline, nortriptyline, trimipramine and clomipramine were 25, 51, 37 and 223 ng/mL, respectively.

TLC METHODS

TLC is a simple and efficient method in which numerous samples may be analyzed simultaneously using small amounts of reagents, but it is not very selective and sensitive. Therefore, TLC is usually used for analysis of components present in relatively high concentrations, for instance in pharmaceutical preparations.

Wiater et al. (45) developed the optimum TLC system for separation and identification of 12 psychotropic drugs from three pharmaceutical groups: PHEs, tricyclic and tetracyclic antidepressants. Three kinds of eluents and two types of solid phases (RP 18 and Silica gel 60) were used. The composition of mobile phases was optimized by the Simplex method. The experimental data obtained during optimization were interpreted using a matrix presentation. In optimal conditions, ten of the examined drugs were separated and identified, but two compounds remained unresolved. This method was presented on standard drug solutions and no practical application was shown.

A high performance thin layer chromatography (HPTLC) method combined with densitometry for determination of PHE

derivatives was described (46). Optimal conditions were examined, and LiChrospher Si 60 plates and the mobile phase composed of methanol and water with addition of 25% ammonia (90:10:0.1, v/v/v) were chosen. The method was validated and compared with HPLC. The applicability of the method was also verified by determination of promethazine hydrochloride in pharmaceutical preparations. According to the authors the proposed method is quick, precise and sensitive (LODs for the examined PHEs ranged from 2.4 to 5.8 μg/mL), and may be used alternatively to traditionally used HPLC in drug routine control.

Immunoassay Methods

Optical immunoassays such as the enzyme immunoassay ($EMIT_{tox}$ TCA, ADx TCA) and the fluorescence polarization immunoassay (FPIA TDx TCA) are used frequently as the first screening for tricyclic antidepressants and PHEs. These commercially available assays are usually designed to detect TCADs in serum or plasma. However, in order to extend the $EMIT_{tox}$ serum tricyclic antidepressant assay over other toxicologically significant specimens such as urine and whole blood, some slight modifications in the assay (47) and sample preparation (48) were performed. Asselin and Leslie (47) proposed a modification of $EMIT_{tox}$ TCA assay enabling its use directly for urine. The proposed method allowed twice as many assays (100) and utilizes a lower cutoff value (25 ng/mL for nortriptyline) to detect TCADs at low concentrations. The same authors applied simple methanolic extraction of whole blood samples to extend the Syva $EMIT_{tox}$ serum tricyclic antidepressant assay for analysis of this material (48). Using 200 μL of whole blood the minimum detectable concentration of most of the tricyclics was in the range of 25 to 50 ng/mL The cross-reactivity of 12 TCADs and the relationship between drug structure and relative assay response were also examined. This relationship calculated for nine separate molecular features allowed quantitative prediction of EMIT assay response to compounds of similar structures.

The main advantages of immunoassay methods are fastness, simplicity and a wide range of measured concentrations but they suffer from lack of distinguishing ability of parent drug and their metabolites or other structure-related compounds, and therefore they are not suitable for quantitative analysis.The capability of the Syva EMIT and Abbott ADx assays for the quantification of tricyclic antidepressants and PHEs in plasma and serum was evaluated by testing cross-reactivities using chromatography as the reference technique (49). Cross-reactivity tests of the assays were performed with nine compounds including their active metabolites. In conclusion, the authors stated that immunoassays for TCADs are valuable screening techniques but not acceptable to obtain quantitative results.

The cross-reactivities were also investigated for the TDx serum TCA assay used with FPIA technique (50). Significant cross-reactivities (from 20 to 113%) were found for a variety of tricyclic antidepressants, tetracyclic antidepressants, PHEs, and several metabolites from these compounds. Lappenberg-Pelzer and Tenczer (51) studied and determined the cross-reactivity of opipramol and its metabolites in immunoassays. The authors stated that one of the major metabolites of opipramol–acetic acid metabolite (I) contributed to arbitrarily high concentrations in commercially available immunoassays. The cross-reactivities of the metabolite (I) were determined as 64 and 66% using EMIT and ADx, respectively.

Banger et al. (52) compared FPIA with high pressure chromatography HPLC from the point of view of applicability to therapeutic drug monitoring of patients treated with clomipramine alone. Total TCAD concentrations measured by FPIA and the sum of clomipramine and desmethylclomipramine measured by HPLC correlated significantly ($r = 0.70$ and $p < 0.01$), however 40% of individual FPIA determinations yielded results that differed by more than 50% from HPLC concentrations. The authors concluded that the FPIA is unsuitable for therapeutic drug monitoring in patients under clomipramine treatment.

The method for simultaneous measurement of two psychoactive compounds (a psychotropic drug and a stimulant) in rabbit serum, by timed-resolved fluoroimmunoassay (TR-FIA) has also been presented (53). In this assay chlorpromazine and methamphetamine or desipramine and methamphetamine were measured by a combined use of europium chelate and samarium chelate, as labels. Detection limits of both chlorpromazine and desipramine were 10 ng/mL.

Apart from the immunoassays, which are performed on large automated random access analyzers, small "pocket" devices for easy and quick urine drug screening have also been developed. Recently, another kind of such device, Signify® ER Drug Screen Test (Signify ER), has been displayed and evaluated in comparison to the Triage DOA Panel (54). Both assay systems are emergency testing devices, measuring nine single drugs or classes of components, including tricyclic antidepressants. The cutoff concentration for TCAD (including metabolites) of these two testing devices was 1000 ng/mL.

Electrochemical Methods

Since the second half of the 1990s application of new electrochemical sensors in analysis of PHEs and TCADs exerted a significant impact on the development of electrochemical methods for determination of these groups of drugs.

The DNA-drug association was utilized for designing sensitive electrochemical biosensors for tricyclic psychotropic drugs (55, 56). The DNA-modified carbon paste electrodes permitted measurement of nanomolar concentrations of azepine (imipramine, clomipramine and trimipramine) and PHE (chlorpromazine, thioridazine, prochlorpromazine, promethazine and PHE) drugs after a short time accumulation.

Accumulation ability of various cyclodextrins (CD) or cyclodextrin condensation polymers (CDPs) such as β-CD(β-CDP) (57, 58), carboxymethylated β-CD(β-CDPA) (58) and alkylated α-, β-, and γ-cyclodextrins (59) as modifies of carbon composite electrodes was examined and they were used for determination of selected azepine-type TCADs and PHEs.

Ferancová et al. (57) used carbon paste electrodes modified with β-CDP for different pulse (DP) voltametric determination of three tricyclic psychotropic drugs: imipramine, trimipramine and thioridazine. Accumulation ability of β-CDP led to DLs down to nanomolar concentrations for a 2 minutes accumulation. The electrode was applied for the determination of imipramine and thioridazine in pharmaceuticals. Ferancová et al. (58) also investigated screen-printed carbon-based electrodes with surfaces modified by use of β-CDP or β-CDPA polymer films, as electrochemical sensors for determination of azepine and PHE drugs. After 2 minutes preconcentration the drugs were quantified by DP voltametry with DLs from 4×10^{-8} to 5×10^{-7} M and $1 \times 10^{-8}$to 1.1×10^{-7} M for β-CDP and β-CDPA modified electrodes, respectively. The β-CDPA modified electrodes were successfully applied to a model serum spiked with levomepromazine, but appropriate preparation of this fluid was necessary. Kataky et al. (59) used alkylated α-, β- and γ-CDs in potentiometric and amperometric sensors for detection of hydrochloride salts of imipramine, desipramine and trimipramine. 2,6-Didodecyl α-, β- and γ-CDs appeared to be suitable as neutral ionophoresis for incorporation in ion-selective electrodes for measurement of the tested antidepressants. Potentiometric ion-selective electrodes exhibited micromolar levels of detection, combined with selectivity over "serum" levels of Na^+, K^+ and Ca^+. Sensors suitable for amperometric measurements were prepared by depositing alkylated CDs on screen printed carbon electrodes. These electrodes allowed measurement of subnanomolecular levels of the analytes in phosphate buffer using oxidative square wave voltametry. However, the studied sensors were not applied for analysis of any real or model biological serum samples.

A horseradish peroxidase immobilized in solid carbon paste electrode was introduced for the amperometric study of five PHEs (60). Analyses were performed in acetate buffer in the presence of hydrogen peroxide with amperometric detection capabilities down to 10^{-8} M. The biosensor response resulted from enzymatic generation of a stable PHE cation radical with subsequent reduction at remote graphite particles. The biosensor was tested on determination of promazine and promethazine hydrochloride (HCl) salts in drug formulations. The authors suggested that technological improvements unable to exploit this biosensor in bioanalytical analyses.

BDD electrodes have been examined for the electro-oxidation of six TCADs (39). At diamond electrodes well-defined and highly reproducible voltammograms were obtained. Diamond appeared to be the first electrode material showing well-defined voltammograms for nortriptyline due to its wide potential window. The LODs for the analyzed drugs ranged from 3 to 1080 nM. The method was applied for determination of imipramine and desipramine in plasma samples. According to the authors diamond is a promising electrode material for amperometric detection of TCADs.

El-Ragehy et al. (61) described the construction and electrochemical response characteristics of four poly (vinyl chloride) membrane sensors for determination of fluphenazine HCl and nortriptyline HCl. The sensors were based on formation of water insoluble ion-pair complexes between the drug cations and sodium tetraphenylborate or tetrakis (4-chlorophenyl) borate in a slightly acidic medium. The sensors were used for determination of both drugs in their pharmaceutical dosage forms and in presence of their degradated products. The sensor response to nortriptyline HCl was suitable for direct determination of this drug in motival tablets containing nortriptyline HCl and fluphenazine HCl but the fluphenazine sensor suffered from interference during application without prior treatment.

An electrochemiluminescence sensor based on tris (2,2′–bipyridyl)ruthenium (II) immobilized in Eastman-AQ55-silica composite thin films on a glassy carbon electrode was also developed (62). The sensor was used for the measurement of chlorpromazine in a FIA system with DL of 0.1 μmol/L and precision of 2.4%. The sensor was applied for determination of chlorpromazine in commercial injections after dilution with phosphate buffer.

Ni et al. (63) proposed a method for simultaneous determination of chlorpromazine HCl and promethazine HCl by differential pulse stripping voltametry (DPSV) using a glassy carbon electrode in Britton-Robinson buffer of pH 9. Both drugs had well-formed oxidation voltametric waves but their voltametric peaks overlapped. In order to determine the drugs individually from a response of a drug mixture, appropriate chemometric methods (multi-variate calibration) were used. The proposed approach was applied for determination of these two drugs in rabbit blood samples taken after injection of the PHE mixture into a rabbit muscle.

Polarographic activity of sulphoxides, obtained from chlorpromazine, promazine and promethazine with the use of nitrous acid as an oxidant, was employed for differential pulse polarographic (DPP) determination of these PHEs (64). A dropping mercury electrode (DME) was used as the working electrode. The DLs were 3×10^{-7}M for chlorpromazine and promazine, and 4×10^{-7} for promethazine, respectively. The proposed method was applied for determination of all tested PHEs in dosage forms, as well as for determination of promazine in spiked urine.

Capillary Electrophoretic Methods

Since the last decade of 20th century capillary electrophoresis (CE) has been playing a significant role in the drug analysis area. A number of papers concerning the determination of tricyclic psychotropic drugs by CE have also appeared. In this area two separation modes of CE, capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC), are mostly used.

Five tricyclic antidepressants (desipramine, nortriptyline, imipramine, doxepin and amitriptyline) were separated by CZE using N,N,N′,N′-tetramethyl-1,3-butanediamine (TMBD) as an additive in the background electrolyte (65). TMBD was used as a dynamic coating agent for supression of drug interactions

with silanol groups of a capillary wall giving reduction of the electro-osmotic flow (EOF), and in consequence separation efficiency and selectivity were improved. The drugs were detected at 214 nm. Only optimal separation conditions were examined and no statistical data were given.

Maier et al. (66) synthesized and used a new type of quaternary ammomium salt [S-(−)-2-hydroxymethyl-1,1-dimethylpyrrolidinium tetrafluoroborate] as an electrolyte additive for separation of a model mixture of five tricyclic antidepressants (amitriptyline, nortriptyline, imipramine, desipramine and clomipramine). Five antidepressants were baseline separated in a counterbalance mode, i.e. direction of the EOF was opposite to the migration direction of the tested drugs. Application of the above-mentioned additive in an acidic background electrolyte was beneficial because it reduced and reversed the EOF and generated relatively smaller anodic EOF related to cationic surfactants that are mostly used in CE for this aim.

Lara et al. (67) presented a validated method for quantitative analysis of three PHEs (thiazinamum methylsulphate, promazine HCl and promethazine HCl) in pharmaceutical formulations by the CZE method. In order to obtain the highest efficiency the influence of various parameters affecting separation was investigated by means of multivariate optimization using experimental designs. The time of the analysis was five minutes at optimized conditions: 100 mM tris(hydroxymethyl)-aminomethane (Tris) buffer at pH 8.0, 15% acetonitrile, capillary with 58.5 (effective length 50 cm) at 25°C and voltage of 30 kV. The PHEs were detected at 254 nm with the following DLs: 2.8 μg/mL for thiazinamium and 3.3 μg/mL for promazine and promethazine. The precision of measurements was 5.3%. According to the authors this procedure may be used in pharmaceutical quality control due to low cost of reagents, simplicity, quickness, accuracy and efficiency.

Two separation modes, CZE and MECC, were used for the study of electrophoretic separation of 12 PHEs and N-demethyl derivatives (68). The separation results obtained in CZE mode using 3×10^{-2} mol/L H_3PO_4 (pH 2.5) were not satisfactory; not all examined PHEs and PHE-N-demethyl derivative pairs were resolved. Application of MECC mode with the use of 3×10^{-2} mol/L H_3PO_4 (pH 2.5) with the addition of octaethylene glycol monododecyl ether as a neutral surfactant resulted in considerable improvement of separation efficiency, and in one run 11 PHEs or three PHEs and their N-demethyl derivatives were baseline separated. However, from the given results it may be seen that improvement of separation efficiency using MECC mode was at the cost of baseline disturbance and peak shape worsening (especially for compounds with longer migration times).

Chen et al. (69) proposed a sensitive CE method for the determination of amitriptyline and its metabolite nortriptyline in human plasma using field-amplified sample stacking (FASS). Plasma samples, after L-L extraction and evaporation, were reconstituted with water and than electrokinetically injected (3 kV, 99.9 s) into a capillary after insertion of a 6 second water plug at 10 psi. The background buffer was Tris (1.4 M, pH 4.50) containing β-CD (1.0 mM) and 50% (v/v) ethylene glycol. Detection of the drugs occurred at 200 nm with the DLs of 2.0 ng/mL for both compounds. Ethylene glycol increasing the viscosity of the separation buffer slowed down the migration of stacked samples, which were trapped between the interference of water plug and running buffer. The method was verified by the analysis of a plasma sample of a healthy volunteer after a single dose (125.0 mg) of amitriptyline.

CE methods, due to their separation efficiency, are employed for enantioseparations of racemic drugs.

The enantiomeric separation of promethazine and trimeprazine enantiomers by affinity electrokinetic chromatography (AEKC)-partial filling technique using human serum albumin (HSA) as chiral selector has been reported lately (70). 50 mM Tris has been used as the electrphoretic buffer. After multivariate optimization of the selected experimental variables (running pH, HSA concentration and plug length), the experimental conditions were applied: pH 7.5, 170 μM HSA and plug length 190 seconds for trimeprazine and pH 7.6, 170 μM HAS and plug length 170 seconds for promethazine. The developed methodologies were applied for enantiomeric quality control of the mentioned drugs in pharmaceutical formulations.

A fast non-aqueous capillary electrophoresis (NACE) method for simultaneous determination of four tricyclic antidepressants (imipramine, desipramine, amitriptyline and nortriptyline) in pharmaceutical preparations and plasma samples was described (71). Four antidepressant drugs were separated in $\sim$ 4.3 minutes at optimized conditions: 50 mM ammonium acetate, 1 M acetic acid in acetonitrile, capillary with 48 cm (effective length 40 cm) and voltage 30 kV. The limits of quantification of the drugs determined in plasma after L-L extraction were between 30 and 50 ng/mL and precision (RSD) was 14.1%. The presented method may be suitable for use in clinical practice.

Veraart and Brinkman (72) presented a combination of dialysis SPE extraction with NACE with UV detection for improved detectability of tricyclic antidepressants in body fluids. An automated on-line sample preparation procedure enabled removal of proteins, salts and water, and consequently led to a constant composition of an analyte-containing matrix that might be inserted into a capillary by electrokinetic injection. The proposed method was examplified with determination of five tricyclic antidepressants in serum and urine. The DLs were in the 0.02–0.1 μg range and the inter-day repeatabilities were between 2.5 and 9.5%.

Jinno et al. (73) developed fiber-in-tube solid-phase microextraction (SPME) combined on line with CE for determination of four tricyclic antidepressants (desipramine, nortriptyline, imipramine and amitriptyline) in urine. The extraction medium was Zylon fiber packed into DB-5 capillary of the same length. The analytes interacted with the fiber and the DB-5 polymer coating and adsorbed in the fiber-packed capillary. Acetonitrile was used as a desorption solvent. In order to find the most efficient extraction conditions, various factors (type of Zylon fiber

and capillary coating, fiber packing density, sample volume and e.t.c.) were examined. In optimal conditions fiber-in-tube SPME-CE established preconcentration power more than 100 times better than direct CE analysis without SPME, and demonstrated the best performance compared to other SPME methods, such as conventional SPME or wire-in-tube SPME. DLs for the antidepressants ranged from 44 to 153 ng/mL with reproducibility (RSD) from 3.0 to 5.1%.

Application of CE methods in forensic toxicological analysis was also reported (74, 75). Madej et al. (74) presented the NACE method for screening and quantification of seven PHE derivatives (levomepromazine, promazine, thioridazine, chlorpromazine, promethazine, perazine and trifluoperazine) in whole blood samples. All tested PHEs were separated in less than eight minutes. The method was validated using control blood samples spiked with the tested PHEs and imipramine which was used as the internal standard. Precision of qualification and quantification parameters ranged from 0.29 to 1.38% and from 1.21 to 6.63% (at drug concentration 0.5 μg/mL), respectively. The determined DLs were 0.08 μg/mL for promazine and 0.15 μg/mL for the rest of the examined drugs (for trifluoperazine LD was not determined). The method was verified by analysis of two forensic blood samples containing promazine alone and promazine and perazine together. The quantitative results obtained by the CE method and HPLC were comparable. The same authors (75) performed a comparison study of two analytical techniques—non-aqueous electrophoresis (NACE) and reversed-phase high pressure liquid chromatography (RP-HPLC), both with UV detection, referring to application for determination of PHEs in autopsy samples. Nine autopsy samples (six blood samples and three urine samples) from six forensic cases, containing one or two PHE drugs, were subjected to analysis by these two methods. The correlation coefficient (r^2) of the obtained resuts by two methods was 0.9869. The screening NACE-UV method for 14 tricyclic psychotropic drugs from PHEs and tricyclic antidepressants present in whole blood samples has also been elaborated (76). The authors conclude that estimated parameters (DLs and repeatability of identification parameters) allowed to conclude that the proposed method may be useful for screening analysis in forensic and clinical toxicology studies.

Although up to date UV detection is the most universal and commonly used method in CE drug analyses, application of other detection techniques such as MS and electrochemistry (electrochemiluminescence ECL, amperometric) may be indicated.

Peri-Okonny et al. (77) compared HPLC-UV and NACE-UV (and MS) as to separation of nine basic drugs (seven tricyclic antidepressants and two bronchodilator drugs). A significant improvement in separation efficiency was achieved by employing NACE compared to use of HPLC. In optimal separation conditions, 80 mM ammonium formate dissolved in methanol-acetonitrile (80:20, v/v), having an apparent pH of 8.7, nine tested drugs were baseline separated by NACE-UV in less than 30 minutes. The drugs were also characterized by CE-MS, but using optimal conditions for this analytical system increased the time of analysis considerably (to about 90 minutes).

Li et al. (78) developed the method for simultaneous monitoring of amitriptyline, doxepin and chrorpromazine in human urine using CE coupled with electrochemiluminescence (ECL) detection based on end-column ECL reaction of tris-(2,2′-bipyridyl)ruthenium (II) with aliphatic tertiary amino moieties. In order to eliminate the influence of ionic strength, a urine sample (200 μL) was subjected to two step L-L extraction with heptane/ethyl acetate, 9:1 (v/v). A sample was introduced into the capillary using electrokinetic injection for 10 seconds at 10 kV. The running buffer consisted of 20 mM phosphate–buffered saline (pH 7.2) and 60% (v/v) acetone. The DLs were 0.8, 1.0 and 1.5 ng/mL for amitriptyline, doxepin and chlorpromazine.

CE with electrochemical (amperometric) detection of PHEs in urine samples was also reported (79, 80). Perphenazie alone (79) and simultaneously promethazine and thioridazine (80) were determined by CZE with end-column amperometric detection at a carbon fiber micro-disc electrode. The DLs of both methods were at a concentration level of 10^{-8}mol/L. The methods were verified by direct analysis of diluted control urine samples spiked with appropriate PHEs.

CONCLUSIONS

TCADs and PHEs belong to psychotropic drugs, which are frequently prescribed for treatment of depression and other psychotic disorders. Some PHEs are also used as antihistamic and antiemetic drugs.

The critical review of chemical and toxicological literature from the last eight years (since 1990 in the case of immunoassay methods) has showed that a variety of analytical procedures for identification and determination of PHEs and TCADs have been developed and described. At present direct spectrometry, especially photometric detection, due to its low selectivity and sensitivity, is mainly applied for analysis of PHEs and TCADs in pharmaceutical formulations. Many spectrometric methods are connected with FIA which is an important automatic technique in routine drug analysis.

Chromatographic techniques, especially HPLC and GC with various detection systems, constitute the most extensive group of methods for determination of tricyclic psychotropic drugs in biological samples. HPLC and GC exhibit high separation potential, selectivity and sensitivity, and thereby they are useful for analysis of samples containing complex organic matrix and relatively low concentrations of analytes. Numerous methods for determination of PHEs and TCADs were developed employing HPLC with UV absorbance detection. This technique is widely used considering its universality and low cost of detectors. However, when the detection of low therapeutic concentrations of psychotropic drugs is required, more sensitive methods such as HPLC-FL, HPLC-EC, GC-NPD or LC-MS-MS should be applied.

Application of new electrochemical sensors such as DNA- and CD-modified biosensors, and BDD electrodes may

considerably influence the development of direct electrochemical as well as HPLC-EC methods for determination of tricyclic psychotropic drugs in biosamples.

Coupling HPLC with single MS or MS-MS detection is a highly advanced technique with increasing importance in toxicological analysis. This technique fulfills most requirements of modern analysis—it is very sensitive, selective and specific, and gives the most reliable results, but it needs expensive apparatus and has a high operating cost.

GC-MS is the method of choice for identification and confirmation of drugs, but in the case of analysis of psychotropic drugs and particularly their relatively polar metabolites, a derivatization step is necessary. GC methods with specific detectors such as NPD enable sensitive determinations of TCADs and PHEs but they are not commonly used in laboratory practice.

Immunoassays play an important role in routine screening analysis of TCADs in biological fluids for diagnosing intoxications. Immunoassay techniques are simple due to their full automation and no requirement for a preliminary extraction stage. These techniques may be successfully applied for simultaneous assays of a large number of samples allowing quick emergency analyses and therapeutic drug monitoring. However, immunoassay methods are only semi-quantitative and selective for the whole group of TCADs, and therefore the positive results should be confirmed by another analytical technique, for example HPLC.

Since the last decade of the 20th century dynamic development of the CE methods for analysis of drugs in biological and non-biological material has been observed. A number of papers concerning determination of tricyclic psychotropic drugs in dosage forms and body fluids have also been presented. However, the position of CE methods in psychotropic drug analysis has not been settled yet. The basic limitation of the CE techniques with most commonly used UV absorbance detection lies in a relatively low sensitivity. The high cost of good quality apparatus and lack of suitable procedures for clinical and toxicological analyses do not make this technique a method of choice for determination of PHEs and TCADs in biological material. However, a small sample volume required for CE analysis might be an advantage in the cases of analysis of very small specimens. In the future, if the price of CE systems decreases, the combination of CE and MS-MS may gain considerable importance in clinical and forensic toxicology.

Taking into consideration the dynamic development of various methods of determination of tricyclic psychotropic drugs as well as equipment, skills and individual preferences of each laboratory, it seems that in the future more than one analytical technique will be the objects of interest. The fields such as new electrochemical sensors, enatiomeric analysis and use of analytical potential of LC-MS (MS-MS) and CE techniques may be a challenge to further exploration in the area of drug analysis.

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