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Review

Analytical methodologies for the determination of sertraline

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ABSTRACT

Sertraline is a widely used antidepressant belonging to the selective serotonin reuptake inhibitor class; its efficacy has been demonstrated not only in the treatment of major depression, obsessive compulsive and panic disorders, but also for eating, premenstrual dysphoric and post-traumatic stress disorders.

Several methods have been published for the determination of sertraline in pharmaceuticals, biological materials and environmental samples. The purpose of the current review is to provide a systematic survey of the latest analytical techniques for the determination of sertraline covering the period from 1987 until 2008.

Contents

1. Introduction

Depression, a common mental disorder, is a chronic or recurrent illness that affects both economic and social functions of patients and can eventually lead to suicidal behaviour. Antidepressant medications have been used to treat all forms of major

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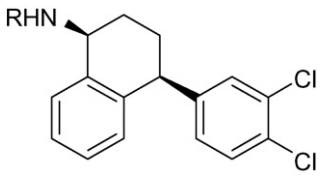

Fig. 1. Chemical structure of: sertraline, R = Me; desmethylsertraline or norsertraline, R = H.

depressive disorders. In the last years prescription of antidepressants has increased dramatically. Many cases of depression can be related to changes in the neurochemistry of three monoamine neurotransmitters that are derivatives of amino acids, i.e. serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (noradrenaline, NA) and dopamine.

Serotonin (5-HT) has been implicated in the aetiology of many disease states and may be particularly important in mental illnesses such as depression, anxiety, schizophrenia, eating disorders, obsessive compulsive disorder, migraine, panic disorders, bulimia, etc. Indeed, many currently used treatments of these disorders are thought to act by modulating the serotoninergic tone. During the last decade, multiple 5-HT receptor subtype has been characterised. This has led to the realization of many treatments acting via the serotoninergic system, such as selective serotonin reuptake inhibitors (SSRIs), antidepressants that increase presynaptic 5-HT function.

SSRIs are the most widespread class of second-generation antidepressant drugs and are in fact becoming the drugs of first choice for the treatment of depression. SSRIs block the reuptake of serotonin at central synapses selectively and powerfully.

SSRIs have a therapeutic efficacy similar to that of traditional, tricyclic antidepressants, but have a much more favourable side- and toxic-effect profile; furthermore, the former are also very useful in the treatment of depression-related disorders, such as anxiety, panic and obsessive–compulsive disorders. The members of this class are fluoxetine (FLU), citalopram (CIT), paroxetine (PAR), sertraline (SRT) and fluvoxamine (FLV). Analytical methods for therapeutic drug monitoring of SSRIs are useful in several instances. In 1996, Eap and Baumann [1] reviewed the analytical methods for the quantitative determination of these compounds for therapeutic drug monitoring (TDM) purposes. Over recent decades major advances have occurred in the pharmacological management of depression. These improvements have led to substantial increases in antidepressant prescribing rates in most developed countries. Despite this progress, high rates of poor compliance, considerable genetic variability in metabolism, and the clinical heterogeneity of depression have meant that the practical application of such treatments has often proven difficult. One means of minimising such problems has been the use of TDM of these agents. In general, there are four major clinical rationales for the use of TDM with antidepressants in the management of depression, i.e., achievement of therapeutic ranges, identification of potentially toxic blood concentrations, confirmation of subtherapeutic concentrations in treatment non-responsive patients, and monitoring after overdose. In this way, Mitchell [2] reviews studies of TDM for the SSRIs and other non-tricyclic antidepressants.

SRT, ((1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthyl(methyl)amine), is a potent inhibitor of serotonin reuptake in the central nervous system and is used clinically to treat depression and obsessive–compulsive behaviour. The chemical structure of this antidepressant is shown in Fig. 1. In this sense, Caruso et al. [3] report the results of a crystal structure determination of the *S*,*S* stereoisomer of sertraline (Zoloft) hydrochloride which is the active form used as an antidepressant in humans.

SRT daily doses range from 50 to 200 mg; the drug is slowly absorbed after oral administration, with steady state plasma concentrations between 55 and 250 ng mL^{-1}. Hepatic metabolism leads to the weakly active metabolite *N*-desmethylsertraline ((1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine (DMS)) (Fig. 1).

SRT shows the characteristic side effects of the SSRI antidepressants and most frequently gastrointestinal disturbances, sexual dysfunctions and anxiety; less frequent are anorexia, urinary retention and orthostatic hypotension.

The fundamental physico-chemical parameters of SRT were determined by Deak et al. [4]. These experimental data were used to interpret the excellent pharmacokinetic properties of the molecule.

A wide variety of analytical methods have been reported for the determination of SRT in pharmaceutical preparations and in biological fluids and also in some environmental samples. These methods include spectrophotometry, voltammetry, high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE), principally. In this work, we have recompiled the described methods in the literature for determination of SRT alone, with their metabolite DMS and in combination with other similar drugs.

2. Pharmaceutical preparations

For analytical determination of SRT in formulated products and in drug substance, the literature indicates ultraviolet and visible spectrophotometry, potentiometric titration and chromatography. It is not included in any pharmacopoeia.

2.1. Spectrophotometric methods

Searching the published methods for the determination of SRT shows that the colorimetric techniques have not been previously applied; consequently the work presented by Bebawy et al. [5] describes the first colorimetric methods. The methods are based mainly on charge transfer complexation reaction of this drug with both π acceptors chloranil and 2,3-dichloro-5,6-dicyanoquinone (DDQ) or σ acceptor iodine.

Latter, Singhvi and Chaturvedi developed a visible spectrophotometric method based on the formation of chloroform extractable coloured complex of drug with nitrosonapthol [6] and Aktas and Ertürk proposed a method based on the ion pair complex forming between SRT and methylorange [7]. Also, Onal et al. [8] developed diverse procedures based on the reactions between SRT and ion pair agents to produce yellow-coloured ion-pair complexes in acidic buffers. After extracting in chloroform, the ion-pair complexes are spectrophotometrically determined at the optimum wavelength.

Recently, Darwish develop three methods based on the reaction of the *N*-alkylvinylamine formed from the interaction of the free secondary amino grouping the investigated drug and acetaldehyde with each of three haloquinones, i.e., choranil, bromanil, and 2,3-dichloro-naphthoquinone, to give coloured vinylamino-substituted quinines [9].

The first derivative spectrophotometry was developed and validated for the assay of STR, and works without solving equations or separation steps [10]. The most striking feature of the derivative spectrophotometry is its simplicity and rapidity, no requiring time-consuming sample preparation such as filtration, degassing that are needed for example in HPLC procedure.

The analytical characteristics of the above cited methods and other procedures described in the literature are presented in Table 1.

Table 1
Spectrophotometric methods.

Reagent	λ (nm)	Linear range ($\mu g\,mL^{-1}$)	Reference
Chloranil	450.0	16–160	[5]
DDQ (2,3 dichloro-5,6-dicyanoquinone)	455.0	15–105	[5]
Iodine	290.0	6–48	[5]
–	228.0	1–20	[11]
Nitrosonapthol	441.5	20–100	[6]
Potassium permanganate	433.5	60–100	[12]
3-Methyl-2-benzothiazolinone hydrazone in presence of ferric chloride	391.5	20–100	[12]
Methylorange	423.0	1–11	[7]
–	271.6–275.5 (peak-to-peak amplitude)	8–46	[10]
Chloranil	665.0	4–120	[9]
Bromanil	655.0	4–120	[9]
2,3-Dichloro-naphtoquinone	580.0	4–120	[9]
Bromothymol blue, bromocresol green or bromophenol blue	–	1–15	[8]

2.2. High performance liquid chromatography (HPLC)

Several HPLC methods have been reported for the determination of SRT and their metabolites in pharmaceutical formulations.

During synthesis of SRT, it probably introduces *cis*-(1*R*,4*R*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenmine hydrochloride, *trans*-(1*S*,4*R*) and (1*R*,4*S*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenemine hydrochloride. Therefore, the stereoselective determination of SRT is important in order to assure therapeutic efficacy and safety. Cyclodextrins (CDs), cyclic oligosaccharides compound of six, seven, or eight α-D-glucopyranose units (α-, β-, γ-CD, respectively), form a family of excellent chiral selectors in HPLC. Chen et al. [13] reports the use of hydroxypropyl-β-cyclodextrin (HP-β-CD) as chiral mobile phase additive to separate SRT enantiomers and *trans* diastereoisomers. The method was easy to perform, precise and accurate. The whole procedure may be extended to the applications on quality control of commercial products.

A high-performance thin-layer chromatography method for the estimation of SRT and alprazolam in combination is developed using silica gel plates with fluorescent indicators [14]. The system is equipped with an automated sample applicator, and the detection was performed at 254 nm by using UV absorption densitometry.

In Table 2 are presented the principal HPLC conditions of the methods that appear in the literature for the determination of SRT in pharmaceutical preparations.

2.3. Electrodriven methods

A fundamental part of the quality control of pharmaceutical formulations is the determination of enantiomeric excess and enantiomeric purity; this is also important for TDM of depressed patients. For this purpose, efficient and reliable analytical methods are needed and electrodriven techniques, most of all capillary electrophoresis (CE), capillary electrochromatography (CEC) and micellar electrokinetic chromatography (MEKC), are very efficient and inexpensive candidates for the role. Ha et al. [18] summarizes developments and applications of chiral CE in the pharmaceutical field; in this work, developments are classified according to CE modes, namely capillary zone electrophoresis (CZE), MEKC, microemulsion electrokinetic chromatography (MEEKC).

Van Eeckhaut and Michotte provides an overview of the different classes of chiral selectors that are used in CE and pharmaceutical and biomedical applications are summarized [19]; the enantioselective electrodriven methods available for the analysis of second-generation antidepressant are presented and discussed by Mandrioli and Raggi [20]. Previously, Buzinkaiova and Medvedova [21] present a short review of analytical methods used in the determination of SRT and other antidepressant drugs; since these compounds are produced in ionic form to be used in pharmaceutical industry, the attention was paid to the use of capillary isotachophoresis and also, Scriba [22] summarizes applications of electromigration techniques with regard to the enantioseparation of chiral drugs.

The first paper on this subject [23] reports that the separation of SRT and isomers can be obtained by MEKC using a mixture of charged (sulphated) and neutral (hydroxypropyl) β-CD, dissolved in a background electrolyte composed of a pH 9.0 borate buffer and sodium cholate (as the surfactant). Subsequently, MEKC strategy for the simultaneous separation of the five SSRIs (SRT, CIT, FLU, FLV and PAR) was developed involving a sodium dodecylsulfate (SDS) MEKC system [24]. The electroosmotic flow and the migration of the analytes were determined for separation buffers of several surfactant concentrations and organic modifier percentages. The most favourable MEKC system consisted of 20 mmol L^{-1} SDS in a phosphate buffer (pH 7.5) with 30% methanol; the separation was carried out using an uncoated fused-silica capillary, a separation voltage of 25 kV with currents typically less than 40 μA, and spectrophotometric detection at 200 nm.

Capillary isotachophoresis (ITP) with coupled columns could provide both qualitative and quantitative analyses of SRT and similar drugs because of their ionic forms. Buzinkaiova and Polonsky determined an optimal electrolyte system for ITP with conductivity detection of SRT, CIT, FLU and FLV [25]. The pre-separation and analytical columns (90 mm × 0.8 mm ID and 160 mm × 0.3 mm ID, respectively) were made from fluorinated ethylenepropylene copolymer; the voltage varied between 1 and 15 kV.

Subsequently, another paper has reported the separation of all four diastereoisomers of SRT [26], the separation of SRT diastereoisomers is obtained by using either highly sulphated α-CD or highly sulphated γ-CD. The authors suggest that the separation obtained with γ-CD is more reliable for routine analyses.

Another paper has been published in 2004 [27], which uses an MEKC system quite similar to that of the first paper, namely a pH 11.5 borate buffer containing sodium deoxycholate and hydroxypropyl-β-CD. Optimum separation was achieved using a buffer (pH 11.5) of 35 mM sodium borate containing 30 mM sodium deoxycholate and 20 mM hydroxypropyl-β-cyclodextrin; the optimum voltage and temperature were 25 kV and 20 °C, respectively. A detection wavelength of 210 nm was used.

Table 2
HPLC-UV methods.

Other analytes	Stationary phase	Mobile phase	Internal standard	UV detection (nm)	Linear range ($\mu g\,mL^{-1}$)	Reference
–	Intertsil C18	Methanol:acetate buffer (pH 2.8) (80:20)	Caffeine	220	10–250	[6]
Alprazolam	Inertsil ODS-3	0.1 M phosphate buffer:acetonitrile	–	221	50–375	[15]
–	RP-8 LiChrospher®	Acetonitrile:sodium phosphate buffer (pH 5.5) (70:30)	–	270	500–2500	[16,17]
–	Supercosil RP-18	Methanol:phosphate buffer (pH 4.5) (20:80)	–	270	0.04–1.5	[10]
–	Alltima C18	Phosphate buffer (pH 3.0) with HP-β-CD:acetonitrile (68:32)	–	225	1–120	[13]

CE suffers from inherent low concentration sensitivity. Analyte detection limits can be improved by combining CE with SPE. An overview is given of different SPE–CE set-ups by Tempels et al. [28]. Fundamental aspects of coupling of SPE and CE, and interfaces for SPE–CE are discussed. Furthermore, inline and online SPE–CE systems are evaluated, and both approaches are illustrated with examples that including SRT separation and determination. In this way, the separation of SRT, FLU and FLV by CE with fully integrated solid-phase extraction (SPE) is described by Schaller et al. [29]. Polymeric monolithic SPE modules were prepared in situ within a fused silica capillary from either butyl methacrylate-*co*-ethylene dimethacrylate or 3-sulfopropyl methacrylate-*co*-butyl methacrylate-*co*-ethylene dimethacrylate. Using a 1 cm SPE module placed at the inlet of the capillary, the mixture was extracted from aqueous solution by applying a simple pressure rinse. Under pressure-driven conditions, efficient elution was possible from both SPE materials investigated using 50 mM phosphate buffer, pH 3.5 in acetonitrile (20:80).

2.4. Other methods

Berzas et al. [30] described a method by GC-FID which permits the simultaneous determination of SRT, FLU, FLV, CIT and PAR without a derivatization step, using clomipramine as internal standard. The reliability of the proposed chromatographic method has been evaluated by means of an extensive validation study and an exhaustive robustness test. This method is the first screening one that allows the determination of the five SSRIs by GC, permitting also to avoid prederivatization for the first time and it is even a pioneering work including an extensive analytical validation and robustness treatment on placebo pharmaceutical formulations too. Optimal conditions for the quantitative gas capillary separation were investigated: column head pressure (100 kPa), injector and detector temperatures (210 and 260 °C, respectively), time and temperature for the splitless step (0.80 min and 80 °C, respectively), volume injected (2 μL) and oven temperature program, providing analysis times shorter than 7 min. Later, the same research group presents the first GC–MS method that allows the simultaneous determination of these five SSRI antidepressants, with some advantages over other GC methods previously published, i.e., the lack of necessity for prederivatization, the sensitivity and selectivity levels reached in the determination, and, indeed, the proved reliability in terms of method validation and robustness [31]. Imipramine was used in this method as an internal standard for quantification. Optimum parameters for GC separation were investigated, i.e., flow rate, column head pressure, injector temperature, injection splitless conditions and oven temperature program. MS detection was performed in SIM mode to increase the sensitivity.

The electrochemical behaviour of SRT at a hanging mercury drop electrode was described by Vela et al. [32]. Different voltammetric techniques, such as cyclic, linear sweep, differential pulse and square wave voltammetry, were used. Voltammograms were obtained at different pH values with a Britton–Robinson buffer solution used as supporting electrolyte. The best results were found by square wave voltammetry with electrodeposition at alkaline pH using a borate buffer with a pH 8.2 for the samples, containing 12% (v/v) methanol. The possibility of combining a flow injection system with a voltammetric detector, applying adsorptive stripping square wave voltammetry, for the determination of STR in a pharmaceutical preparation was explored by Nouws et al. [33].

For routine analysis, a dedicated instrument is often required to decrease instrument setup time to ensure rapid turnaround of analytical results. Further chemical derivatization of the molecule may be necessary to observe the enantiomers by chromatographic detectors and/or to obtain separation. Salsbury and Isbester [34] presented a nuclear magnetic resonance (NMR) and chiral solvating agent (CSA, 1,1-bi-2-naphthyl) technique for the routine determination of enantiomeric purity. The technique was applied to three common active pharmaceutical ingredients (APIs: SRT, PAR and fenfluramine), to demonstrate that NMR is a useful and convenient technique to complement or provide an alternative to optical rotation and chiral HPLC for evaluation of enantiomeric purity. The techniques described herein provide comparable enantiomeric purity results with those obtained with traditional chiral HPLC and other published methods for these compounds. Enantiomeric purity determinations by NMR utilizing CSA do not require special instrumental techniques, chemical derivatization or standards and is therefore ideally suited for rapid routine analysis.

Reflectance near-infrared (NIR) spectroscopy has been investigated as a method to distinguish between the sites of manufacture of a number of proprietary tablets [35]. NIR spectroscopy is a rapid and non-destructive technique that is sensitive to both the chemical and physical properties of the sample. In combination with various chemometric procedures, it has been used to classify raw materials, clinical samples, solvents and herbal products. This investigation examines the feasibility of using the technique to identify and/or authenticate the source of manufacture of tablets containing SRT, nifedipine, enalapril maleate or diclofenac sodium produced at different sites. The principal component analysis (PCA) score plots showed that spectra of tablets originating from different sites of manufacture often gave rise to statistically different populations. PCA loadings indicated that the differences were related to moisture content and excipients.

3. Biological samples

Biological materials such as urine are very complex. They often contain proteins, salts, acids, bases, and numerous organic compounds which can interfere with the analytes of interest. Sample-preparation is usually performed by solid-phase extraction (SPE) or liquid–liquid extraction (LLE), sometimes with derivatization of the SRT and other SSRIs. There is, however, an increasing demand for simple, rapid, and cost-effective analytical methods

capable of achieving very low detection limits in real or almost real-time.

Methods for the measurement of biological SRT concentrations have included gas chromatography with mass spectrometric, electron capture or nitrogen-selective detection. HPLC, however, offers advantages of economy, simplified sample preparation and ease of measurement of SRT and also desmethylsertraline. Published HPLC methods for SRT and desmethylsertraline rely on the use of conventional alkyl-modified silica columns with aqueous acetronitrile and/or methanol eluents and UV detection (205–235 nm) [36–40]. Solid-phase sample preparation was used in the methods applied to human serum [36,37].

3.1. HPLC methods

3.1.1. UV detection

Wiener et al. [39] describes a rapid, isocratic reversed-phase HPLC method that requires minimal sample preparation and is the first HPLC method to quantify both SRT and desmethylsertraline simultaneously. The study also describes preliminary pharmacokinetic data for SRT and desmethylsertraline in mouse cerebral cortex following a single intraperitoneal injection of SRT.

Peterson et al. [41] introduces a flow-based extraction method where an aqueous sample and organic solvent are injected sequentially into an extraction coil, then mixed and separated due to the differential flow velocities of the aqueous and organic phases. A 500 μL aqueous sample is propelled through a 50 μL segment of organic solvent whose flow is impeded due to hydrophobic interactions with the walls of a Teflon extraction coil. This wall drag allows the faster moving aqueous sample to penetrate through and ultimately separate from the slower organic solvent. These steps are repeated with a back extraction into a second aqueous segment (100 μL) that is collected and analyzed with HPLC.

A method for the measurement of SRT and norsertraline in plasma or serum suitable for use in single-dose pharmacokinetic studies has been developed by Patel et al. [42] using a simple liquid–liquid extraction at an alkaline pH followed by injection onto a S5 SCX HPLC column.

Frahnert et al. [43] presents an isocratic HPLC method with UV detection preceded by SPE to cost- and time-effectively analyse 22 psychotropic drugs (including SRT), seven of them also including their active metabolites. This method was developed for therapeutic drug monitoring and validated by internal (recovery, linearity, accuracy, precision, interferences) and external quality control allowing an efficient and rapid analysis of serum concentrations within 24 h with a single system, thus reducing the time for apparatus preparation and system instabilities linked to this process.

The analytical methods described in the literature to analyze antidepressants in biological fluids usually use conventional sample pretreatment techniques that are laborious, time-consuming, and require large amounts of organic solvents [44]. Solid-phase micro-extraction (SPME) has been successfully applied to analyze drugs in biological fluids by chromatography techniques, mainly by coupling to gas chromatography. Most of the described methods showed low recoveries that became laborious to develop methods to evaluate drugs in very low plasma or serum levels for therapeutic drug monitoring. However, SPME–LC with UV detection method for simultaneous determination of sertraline, mirtazapine, citalopram, paroxetine, and fluoxetine, in human plasma was developed, validated, and further applied to analyze plasma samples obtained from patients with depression by Silva et al. [45]. The response of the SPME–LC method for most of the drugs was linear over a dynamic range of 50–500 $\mu g\,L^{-1}$ and the limit of quantitation of the nontricyclic antidepressants in plasma varied from 25 to 50 $\mu g\,L^{-1}$ with a coefficient of variation lower than 5%. Later, in 2008, the same investigation group developed, validated and further applied to the analysis of plasma samples from elderly patients undergoing therapy with antidepressants, a sensitive, selective, and reproducible in-tube solid-phase micro-extraction and liquid chromatographic (in-tube SPME/LC-UV) method for simultaneous determination of sertraline, mirtazapine, citalopram, paroxetine, duloxetine, and fluoxetine in human plasma [46].

Also recently, stir bar-sorptive extraction (SBSE), a sample-preparation technique based on the same principles as SPME, partitioning coefficient of the solutes between the silicone phase and the aqueous phase, has been evaluated for the enrichment of organic solutes from biological fluids. The aim of the study realized by Chaves et al. [47] was to evaluate SBSE, followed by liquid desorption and LC–UV analysis, for the determination of nine antidepressant drugs, including SRT, in plasma samples. SBSE consists of a 10 mm length glass-encapsulated magnetic stir bar, externally coated with 22 μg of PDMS. This layer is 0.5 mm thick, which corresponds to a volume of 24 μL of PDMS. Prior to the first use, the stir bars were placed into a vial containing an acetonitrile:methanol solution (80:20) and conditioned for 24 h, under agitation. Among the successive extractions, used stir bars were cleaned in methanol for 30 min at 50 °C, under magnetic stirring rate of 1200 rpm, followed by a drying step using a lint-free tissue.

Recently, the applicability of hollow fibber-based liquid phase micro-extraction (HF-LPME) was evaluated for the extraction and preconcentration of three antidepressant drugs (amitriptyline, imipramine and sertraline) prior to their determination by HPLC–UV [48]. The target drugs were extracted from 11.0 mL of aqueous solution with pH 12.0 (source phase) into an organic extracting solvent (*n*-dodecane) impregnated in the pores of a hollow fibber and finally back extracted into 24 μL of aqueous solution located inside the lumen of the hollow fibber and adjusted to pH 2.1 using 0.1 M of H_3PO_4 (receiving phase). The extraction was performed due to pH gradient between the inside and outside of the hollow fibber membrane.

In recent years, there has been increasing focus on the use of SPE principle in toxicological analysis. SPE allows for reduced solvent consumption compared to liquid–liquid extraction, which reduces the exposure on personnel and environment. Furthermore, the SPE approach can easily be automated using commercial robotics systems. Various types of SPE columns exist, and by using columns that combine several extraction principles a broad range of drugs can be extracted in one step. For these reasons, four different mixed-mode cation exchange SPE columns were compared for extraction of basic drugs (including SRT) from urine using HPLC–DAD analysis: Isolute HCX-3, ABN, Bond Elut Certify and Oasis MCX [49]. Using the automated extraction procedure described, the basic compounds performed reasonably well in all SPE mixed-mode columns. Factors such as availability and price may be decisive in regard to choice of column.

On the other hand, the high incidence of psychiatric illness in the postpartum period and increasing percentage of women who breastfeed has focused attention on the treatment of breastfeeding women with psychotropic medications and, additionally, the exposure of nursing infants to these medications. Consequently, there has been an increased effort to develop standardized methods for quantifying psychotropic medications in breast milk. In this way, Hostetter et al. [50] develop a method that consists of a common liquid/liquid and solid-phase extraction followed by HPLC separation on a common column and UV detection. Assay system 1 measures fluoxetine, norfluoxetine, fluvoxamine, and paroxetine; assay 2 measures sertraline and desmethylsertraline; and assay 3 measures the tricyclic antidepressants including doxepin, nordoxepin, desipramine, imipramine, nortriptyline, and amitriptyline.

The method is shown to be a highly accurate and precise technique for measuring 12 different antidepressants in human breast milk and to be free of the matrix effects often encountered in breast milk drug analyses.

Table 3 shows the optimal conditions of HPLC–UV methods described above and other methods which appear in the literature.

3.1.2. Fluorescence detection

Lacassie et al. [57] presents a rapid and sensitive method for the quantitation of eight SSRIs, including SRT, and three active metabolites using either GC–NPD (nitrogen phosphorus) or isocratic reverse phase HPLC combined with fluorescence detection (FL) after derivatization. The isocratic mobile phases consisted of different mixtures of sodium acetate (0.005 M, pH 4.5) and methanol. The flow-rate was 1.0 mL min^{-1}. The effluent was continuously monitored at 340 nm (excitation) and 520 nm (emission) wavelengths.

The simultaneous determination of plasma concentrations of four selective serotonin reuptake inhibitors (SSRIs) is presented by Lucca et al. [58]. Fluvoxamine, paroxetine, sertraline, and fluoxetine were extracted from plasma with ethyl acetate and then derivatized with dansyl chloride. The analytes were separated using Hypersyl ODS C18 (5 μm) 250 mm × 4.6 mm column. For continuous gradient separation, the mobile phase consists of two eluents, acetonitrile and potassium phosphate buffer (10 mM, pH 7.2) at total flow rate of 1.5 mL min^{-1}. Detection was carried out at λ_{exc} = 366 nm and λ_{em} = 490 nm. The assay was linear from 10 to 1500 ng mL^{-1} for SRT.

3.1.3. MS detection

Moraes et al. [59] compare the bioavailability of two SRT tablets formulations in 24 healthy volunteers who received a single 50 mg dose of each SRT formulation. Plasma samples were obtained over a 96 hour interval and SRT concentrations were analyzed by combined reversed phase liquid chromatography and tandem mass spectrometry (LC–MS–MS) with positive ion electrospray ionization using selected ion monitoring method. Koytchev et al. [60] designed a similar study to evaluate the bioavailability of two SRT formulations. Blood samples were taken up to 96 h post-dose, the plasma was separated and the concentrations of SRT were determined by HPLC–MS–MS. In the light of this study it can be concluded that SRT test capsules are bioequivalent to the reference formulation. Later, Tassaneeyakul et al. compare the bio-availability of two SRT tablet (50 mg) formulations in 24 healthy Thai male volunteers under fasting condition. Plasma concentrations of SRT were determined using validated LC–MS/MS method [61].

Nagy et al. [62] evaluated the safety and pharmacokinetics of donepezil HCl and sertraline HCl when administered separately and in combination. Plasma donepezil and SRT concentrations were determined by LC-MS. Safety was evaluated by physical and laboratory evaluations and the monitoring of adverse events.

Goeringer et al. [63] describes a LC method capable of being used with either DAD or MS detection for the identification and quantitation of 10 antidepressant and 2 antipsychotic drugs (including SRT), all of which have serotonergic activity. The organic buffers ammonia, glycine, and triethylamine were each used in a mobile phase made up of 32.5% buffer/67.5% methanol (v/v) at a pH of 10.0. This method has been used to satisfactorily analyze brain, blood, liver, urine, vitreous fluid, and stomach contents in subjects known to have used these drugs.

He et al. [64,65] develop a HPLC–ESI-MS assay for the determination of SRT in human plasma and to investigate the pharmacokinetics and bioequivalence of two SRT tablets in human. With zaleplon as the internal standard, SRT is extracted from the alkalized plasma with cyclohexane. The organic layer is evaporated and the residue is re-dissolved in the mobile phase of methanol–10 mM ammonium acetate solution–acetonitrile (62:28:10). An aliquot of 20 μL is chromatographically analyzed on a Shimadzu ODS C18 column by means of selected-ion monitoring mode of MS. The calibration curve of SRT in plasma exhibits a linear range from 0.5 to 25.0 μg L^{-1}.

Jia et al. [66] establish a LC–MS/MS method for the determination of SRT hydrochloride in human plasma, using paroxetine hydrochloride as IS. SRT hydrochloride was chromatographed by using a Discovery C18 column. The mobile phase consisted of 0.1% formic acid–acetonitrile (50:50). Electrospray ionization (ESI) source was applied and operated in the positive ion mode. Selected reaction monitoring (SRM) mode with the transitions of m/z 306.0 → 274.9 and m/z 330.1 → 191.9 was used to quantify SRT hydrochloride and the IS, respectively. Detection limit was 0.334 μg L^{-1}.

Jain et al. [67] employed a simple, rapid and sensitive isocratic reversed-phase LC–MS method equipped with Turbo Ion spray (TIS) source, operating in the positive ion and selective reaction monitoring (SRM) acquisition mode to quantify SRT in human plasma. A new and superior procedure of SPE (compared to liquid–liquid extraction) was followed to extract SRT and imipramine as internal standard (IS) from the human plasma. Sample preparation was performed using waters hydrophilic–lipophilic balance cartridge and this method yielded extremely clean extracts with very good recovery, 81.47 and 85.79% for SRT and IS, respectively. The response of the LC–MS/MS method for SRT was linear over the dynamic range of 0.5–60.0 μg L^{-1}.

After comprehensive investigations of the ionization behaviour of SRT in positive atmospheric pressure chemical ionization (APCI) mode and further fragmentation pattern during the collision-induced dissociation (CID) procedure, Chen et al. [68] developed a more sensitive LC/APCI-MS/MS method for the determination of SRT with an LLOQ of 0.10 μg L^{-1} using only 0.25 mL of plasma. Compared to the ESI method reported [64,65,67] APCI provided higher mass spectrometric response for SRT and less susceptibility to ion suppression. A simple and inexpensive one-step LLE instead of SPE [36,67] was used for plasma sample preparation. This method was validated and successfully applied to the pharmacokinetic study and bioequivalence evaluation of SRT in 18 healthy volunteers after a single oral dose of 50 mg SRT hydrochloride tablets.

Castaing et al. [69] proposed a method for quantification of SRT and other seven antidepressants and five of their active metabolites in whole blood by HPLC–MS. After a liquid–liquid extraction from blood, the compounds and the IS (methylrisperidone) were eluted on an XTerra® RP18 column with a gradient of acetonitrile/ammonium formate buffer 4 mM pH 3.2. They were then detected by electrospray ionization mass spectrometry with multiple reactions monitoring mode. The calibration curves were linear over the range 5–500 μg L^{-1}. Hattori et al. combined an LC–MS–MS instrument with a new polymer column, which enables direct injection of crude biological samples without complicated pretreatments and without a column switching system [70]. SRT, FLV, and PAR in human serum specimens were analyzed with this method. The polymer column functions as a column for both drug extraction and LC separation.

In other way, TDM of antidepressant and antipsychotic drugs is necessary for an optimal supervision of patients and their drug therapy to avoid medical complications, intoxication, nonresponsiveness or noncompliance. Multi-drug methods for screening or quantification have been generated for HPLC, GC(MS) or LC–MS(MS) approaches previously, but these methods are either used for screening purposes only or suffer from the disadvantage that not all common antidepressants and antipsychotics are included for quantification. The novel method presented by

Table 3
Optimal conditions of HPLC–UV methods for determination of SRT in biological samples.

Stationary phase	Mobile phase	Internal standard	Flow rate ($mL\,min^{-1}$)	UV detection (nm)	Linear range ($\mu g\,L^{-1}$)	Other analytes	Reference
Versapack Cl8	0.25M potassium phosphate buffer (pH 2.7) with 30% (v/v) acetonitrile	Tetracaine	2.0	235	–	Desmethylsertraline	[39]
Spherisorb S5 SCX	Methanol:water (19:1) with ammonium perchlorate, final pH 7.0	Fenethazine with Tris buffer (pH 10.6)	1.2	215	25–300	Norsertraline	[42]
Vydac C18	0.085 M phosphate buffer:acetonitrile (71:29), pH 3.5 using KOH	SRT internal standard	2.0	232	100–600 nM	Desmethylsertraline	[51]
Beckman C18	–	Clomipramine	–	200.4	0.05–5	6 SSRIs and some metabolites	[52]
Reversed-phase	0.01 M NaH_2PO_4 (pH 2.5):CH_3CN (64:36)	Amitriptyline	–	210	7.5 (DL)	PAR	[53]
Hypurity C18	Acetonitrile:phosphate buffer (pH 3.8)	–	1.0	DAD at 220, 240 and 290	0.025–1	Ten nontricyclic antidepressants and two metabolites	[44]
Symmetry C8	Gradient of acetonitrile:phosphate buffer 10 mM pH 3.8	–	–	DAD at 230 and 290	0.025–0.5	Seven antidepressants and five metabolites	[54]
Nucleosil 100-5-protect 1 (endcapped)	25 mM potassium dihydrogenphosphate (pH 7.0):acetonitrile (60:40)	Melperone	1.0	230	0.05–3	Twenty-eight psychotropic drugs and some active metabolites	[43]
MOS-2 Hypersil (C8) reverse-phase	0.02 M monobasic potassium phosphate + 120 μL *N*,*N*-dimethyloctylamine/L (pH 6.5) and 35% acetonitrile	Citalopram	0.6	215	0.00125 (DL)	Assay 2: desmethylsertraline	[50]
Genesis C8 reversed phase	Acetonitrile:12.3 mM (pH 3.0) phosphate buffer containing 0.1% triethylamine (35:65)	Clomipramine	1.2	220	0.0075–0.25	Desmethylsertraline	[55]
RP 18 LichroCART	Acetate buffer solution (0.25 M, pH 4.5):acetonitrile:methanol (60:37:3)	Clomipramine	1.0	230	Up to 1.0	Eight antidepressants	[47]
Zorbax Extend C18	0.02 M acetic acid solution (pH 4.0):methanol (54:46)	Chloropromazine	0.25	215	5–500	Amitriptyline and imipramine	[48]
LiChrospher 60 RP Select B C18	35% of a mixture of acetonitrile:methanol (92:8) and 65% of 0.25 M sodium acetate buffer (pH 4.5)	Etidocaine	1.0	230	10–2000	Nine antidepressants	[56]
LiChrospher60 RP Select B C18	Phosphate buffer 0.05 M (pH 3.8):acetonitrile (53:47)	Clomipramine	1.0	230	50–500	Five antidepressants	[46]

Kirchherr and Kühn-Velten incorporates all the antidepressants, antipsychotics and metabolites recommended for inclusion in TDM in psychiatry [71]. Drugs were assigned to subgroups covering low, medium and high concentrations (overall range of therapeutic levels to be considered: 0.5–2000 $\mu g\,L^{-1}$) by further dilution of the supernatant obtained after the first protein precipitation. Chromatographic separation was necessary for isobaric mass fragments and performed on a monolithic C18 column with methanol gradient and 5 mM acetate buffer at pH 3.9. The injection interval was 8 min. A set of three internal standards was used for quantification of drugs with widely varying hydrophobicity. After ESI, positive ion fragments were detected in the multiple reaction monitoring modes with a tandem mass spectrometer.

Smyth et al. [72] investigating the electrospray ionisation and ion trap mass spectrometry (ESI-MS^n) and ESI-QToF-MS/MS behaviour of SRT and other antidepressant drugs with the intention of establishing rules of fragmentation for these molecular types. No such study has appeared in the literature to date. In addition, the HPLC/ESI-MS^2 behaviour of these drugs has also been studied with a view to their simultaneous identification and quantisation in mixtures at $\mu g\,L^{-1}$ concentrations which can also be used for their analysis in hair samples. Following Soxhlet extraction, the presence of SRT in a hair sample was confirmed using HPLC/ESI-MS^2 analysis based on its retention time on the HPLC column (17.71 min) and its fragmentation characteristics using mass spectrometry (m/z $306 \rightarrow 275$). The concentration of SRT in this hair sample was calculated to be 1.90 $ng\,mg^{-1}$.

Youdim et al. reports a miniaturized high-throughput cocktail IC_{50} assay designed to simultaneously assess IC_{50}s for up to 16 compounds in duplicate, in conjunction with a robust, rapid resolution LC–MS/MS end-point offering increased sample throughput without compromising analytical sensitivity or analyte resolution. [73]. Eight compounds, including SRT, were studied as part of a cocktail assay.

In recent times particular concerns have been raised about suicide as a potential side effect of commonly prescribed antidepressants. As can be see above, a few studies have been reported regarding the simultaneous analysis by LC/ESI-MS of some of these antidepressants and their metabolites, but these studies have generally involved the use of the less specific selected ion monitoring of the protonated molecules and the analyses have been directed towards biological fluids. Drug determination in these samples only reflects recent usage as the SSRI drugs are quickly eliminated from the body. It is for this reason that hair has become the matrix of choice for long-term compliance analysis. A tandem mass spectrometric investigation of the collision-induced dissociation of five commonly prescribed psychoactive pharmaceuticals (SRT, PAR, risperidone, trimipramine, and mirtazapine, and their metabolites) has been carried out [74]. Quadrupole ion trap mass spectrometry was employed to generate tandem mass spectrometric (MS/MS) data of the compounds under investigation and structural assignments of product ions were supported by quadrupole time-of-flight MS. These fragmentation studies were then utilised in the development of a LC method to identify the drugs and their metabolites in human hair and saliva samples, thus providing relevant profiling information.

3.2. GC–MS methods

Many methods have been developed for the determination of SRT in biologic specimens. Almost all assays are based on the separation by GC and HPLC.

Martinez et al. [75] present the simultaneous determination of SRT and other five antidepressant drugs in whole blood and a comparison of two extraction/cleanup procedures for capillary gas chromatography with nitrogen-phosphorus detection. The first procedure consists of the employment of Chem Elut columns (diatomaceous earth) and is based on the principle of liquid–solid absorption extraction that is closely related to conventional liquid–liquid extraction. The second focuses on the use of Bond Elut Certify columns and a mixed SPE, reversed-phase and cation-exchange sorbent. Limits of detection (LOD) and quantitation (LOQ) ranged from 39 to 153 $ng\,mL^{-1}$ and from 128 to 504 $ng\,mL^{-1}$, respectively.

Lacassie et al. [76] described a specific and sensitive method for the analysis of 24 antidepressants in human serum. This method allowed the simultaneous determination of antidepressants belonging to different classes: tricyclic antidepressants (TADs), selective serotonin reuptake inhibitors (SSRIs), including SRT and selective inhibitors of monoamine oxidase A (IMAOs). Antidepressants were submitted to LLE at pH 9.5 using a mixture of heptane/isoamyl alcohol (98.5/1.5) without derivatization. Cyproheptadine was used as IS. Separation was obtained with a nonpolar PTE5 capillary column (30 m × 0.32 mm; film thickness 0.25 μm). Mass spectrometry consisted of electron impact ionisation (70 eV), and full scan acquisition. Limits of quantitation ranged from 20 to 100 $ng\,mL^{-1}$ for most of the antidepressants.

In order to remove the interference in biological fluid and separate SRT, re-extraction at a different pH using another organic solvent [77–79] or solid-phase extraction [37] were proposed in previous studies. In addition, derivatization techniques like perfluoroacylation with TFAA; trifluoroacetic anhydride [78] or MBTFA; *N*-methyl-bis(trifluoro-acetamide) [79] was used for the selective and sensitive determination of SRT in the analysis of GC–MS.

Kim et al. [80] developed and validated a rapid and sensitive determination method for SRT in human plasma with a $pg\,mL^{-1}$ level of detection limit (0.1 $ng\,mL^{-1}$). This method contains simple liquid–liquid extraction and highly sensitive HFBA-derivatization.

Salgado-Petinal et al. [81] report that SPME can be used for rapid quantification of SRT and other SSRIs in urine with minimum manipulation of the sample, achieving detection limits below 0.4 $ng\,mL^{-1}$.

Biological matrices alternative to urine and plasma have recently been introduced for assessing drug exposure. Oral fluid (saliva), sweat and hair are alternative biologic matrices, which have been extensively and successfully used to assess recent and past and/or acute and chronic exposure to drugs of abuse. Within the framework of the MACIUS project (designed to estimate the prevalence of psychoactive drugs among persons injured by any mechanism who attended an emergency room for medical care within the 6 h posterior to the injury), Pujadas et al. [82] developed and validated a simple and reliable assay to simultaneously identify 36 psychoactive drugs (including SRT) and quantify 30 of them, candidate to be present in oral fluid. After the addition of deuterated analogues of morphine, 3,4-methylenedioxymethamphetamine, (±)-11-nor-9-carboxy-delta-9-tetrahydrocannabinol and clonazepam as ISs, all the compounds were simultaneously extracted from oral fluid by solid-phase extraction procedure. Acid compounds were eluted with acetone while basic and neutral compounds with dichloromethane:isopropanol:ammonium (80:20:2).

Wille et al. [83] evaluates the performance of electron (EI) and chemical ionization (CI) [PICI (positive ionization) and NICI (negative ionization)] in a GC–MS method for the simultaneous determination of new generation antidepressants and their active metabolites, including SRT, in plasma. Although CI can offer advantages in selectivity and sensitivity, there has never been a GC–MS CI method published for monitoring these antidepressants. In this investigation, the different ionization techniques are compared during the validation of this simultaneous determination procedure by GC–MS. This method is of interest for therapeutic drug

monitoring (TDM) laboratories as it offers the analytical strategy for each of the individual antidepressants.

Table 4 shows the optimal conditions of GC–MS methods for STR determination in biological samples.

3.3. Other methods

Due to the side effects associated with the use of SRT reliable fast methods (such as bio-sensing) for determining SRT metabolic profile of patients are essential for adequate dosing. In this sense, nanobiosensor for the determination of SRT biotransformation was prepared with cytochrome P450-2D6 (CYP2D6) and poly(8-anilino-1-napthalene sulphonic acid) nanotubes (90 nm in diameter and 600–800 nm in length) potentiodynamically deposited on gold. The biosensor gave a linear response over the concentration range of 0.2 and 1.4 M of SRT [85].

The extractability of 58 different basic drugs, including SRT, by 3-phase liquid-phase microextraction (LPME) was studied by Pedersen-Bjergaard et al. [86]. Extraction recoveries were correlated to solubility data. The basic drugs were extracted from 1.5 mL water samples (pH 13) through approximately 15 μL of dodecyl acetate immobilized within the pores of a porous polypropylene hollow fibre (organic phase), and into 15 μL of 10 mM HCl (acceptor solution) present inside the lumen of the hollow fibre. Compounds with a calculated solubility below 1 mg mL^{-1} at pH 2 were poorly recovered and remained principally in the organic phase. For these drugs, 2-phase LPME may be used as an alternative technique, where the aqueous acceptor phase is replaced by an organic solvent. In the solubility range 1–5 mg mL^{-1}, most drugs were effectively extracted (recovery >30%), whereas drugs belonging to the solubility range 5–150 mg mL^{-1} were all extracted with recoveries above 30% by 3-phase LPME.

Labat et al. [87] described a new determination procedure for SRT and other compounds (milnacipran, venlafaxine, desmethylvenlafaxine, mirtazapine, desmethylmirtazapine, citalopram, desmethylcitalopram, fluvoxamine, paroxetine and fluoxetine) by micellar electrokinetic capillary chromatography (MEKC) with diode array detection (DAD). Separation and determination were optimised on an uncoated fused-silica capillary. The migration buffer consisted of 20 mM sodium borate, pH 8.5, with 20 mM SDS and 15% isopropanol, at an operating voltage of 25 kV. The column temperature was maintained at 40 °C. Injection in the capillary was performed in the hydrodynamic mode (0.5 psi, 15 s). In these conditions, the migration time of the antidepressants was less than 11 min. In most cases, calibration curves were established for 30–2000 ng mL^{-1}. The limit of detection and the limit of quantification were ranged between 10 and 20 and between 20 and 30 ng mL^{-1}, respectively, for all the molecules.

Recently, Musenga et al. [88] proposed the determination of SRT and *N*-desmethylsertraline in human plasma by CE with LIF detection (λ = 488 nm). A SPE procedure is employed for biological sample pre-treatment, followed by a derivatization step with FITC; reboxetine was the IS. The final BGE consisted of 20 mM carbonate buffer, pH 9.0, with 2.5 mM heptakis(2,6-di-*O*-methyl)-β-CD, 50 mM GLC and 20% (v/v) acetone. With 30 kV applied voltage, the electrophoretic run is completed in 7.5 min. Linearity was observed in the plasma concentration range from 3.0 to 500 ng mL^{-1} for SRT.

4. Environmental samples

In the last decade there has been a growing public concern about pharmaceuticals entering the environment through human and livestock usage and disposal, and the possible harmful effects this might have on non-target organisms. Production and consumption of pharmaceuticals are increasing throughout the world, both in number of different active compounds and in the amount of pharmaceuticals distributed for both human and livestock usage.

Residues of pharmaceuticals and their metabolites are ubiquitous in the aquatic environment. The excretion of pharmaceuticals by patients after therapy and the incomplete elimination in sewage treatment plants is considered to be the primary pathway of such compounds to surface waters. Although most pharmaceutical drugs are not highly persistent in the environment and do not show significant bioaccumulative properties, they present a permanent low concentration of bioactive compounds. The ecotoxicological aspects of this situation are still hardly understood, and it is quite obvious that regular monitoring of surface waters with respect to drug residues is required.

It is well established that the source, presence, and fate of pharmaceutically active compounds in the aquatic environment is of concern. This area of research has progressively received more attention as the ubiquity of many pharmaceuticals in surface water becomes evident. Currently more than 80 pharmaceutical compounds have been detected in sewage effluent, surface water, and groundwater in at least 12 different nations.

The toxicological effects of pharmaceuticals in the environment to humans and free living aquatic and terrestrial organisms are not well understood. Of particular concern are the potential impacts on aquatic organisms that may experience subtle, chronic life-cycle and multi-generational exposure to low doses of pharmaceuticals. In order to elucidate these environmental and ecological impacts, there is a need for analytical methods that allow quantitation of pharmaceuticals in aquatic matrices. Ideally these methods should be simple, rapid, sensitive, selective, broad spectrum, and able to overcome the negative effects of matrix components.

4.1. HPLC–MS

The objective of the study carried out by Conley et al. [89] was to develop a LC–MS/MS method for the determination of a broad spectrum of commonly used pharmaceuticals in surface water. To this end, a SPE sample pre-concentration coupled with a LC–MS/MS method for separation and detection of 13 different pharmaceuticals, including SRT, and 1 primary metabolite has been developed. Separation of compounds was carried out using an ultra performance liquid chromatograph fitted with a 50.0 mm × 2.1 mm, bridged-ethyl-siloxane/silica hybrid Shield RP-C18 column coupled with a 30.0 mm C18 guard column. Separation was achieved using a step-wise binary elution gradient consisting of 95:5 ultrapure water:acetonitrile with 0.1% formic acid as the aqueous phase (A) and acetonitrile with 0.1% formic acid as the organic phase (B). The gradient was as follows: 5% B held for 0.70 min, increased linearly to 30% B in 0.10 min, then a gradual increase to 35% B over 1.00 min, followed by an increase to 95% B in 0.1 min and held for 0.3 min, finally a return to initial conditions of 5% B over 0.25 min and held for 0.80 min to allow for equilibration before the next injection. All flow was directed into the mass spectrometer. Additional parameters were: flow rate, 0.4 mL min^{-1}; injection volume, 5 μL; column temperature, 40 °C; sample temperature, 25 °C. The mass analyzer operated in positive ionization mode for all analytes and ESI source conditions were: source temperature, 130 °C; desolvation temperature, 400 °C; cone gas, 25 L h^{-1}; desolvation gas, 750 L h^{-1}; capillary voltage, 0.7 kV; multiplier, 650 V. Nitrogen was used as the desolvation and nebulization gas, and ultra-pure argon was used as collision gas.

With a unique combination of HPLC–MS and hollow fibre supported liquid phase microextraction (HF-LPME), providing detection limits at the low pg L^{-1} level, Vasskog et al. [90] identify

Table 4
GC–MS methods for determination of SRT in biological samples.

Other analytes	Remarks	Linear range ($ng\,mL^{-1}$)	DL ($ng\,mL^{-1}$)	Reference
–	Equipped with an accelerating voltage alternator unit set to monitor the ions at *m/z* 274 and 236(IS); ionization energy 20 eV; glass column packed with 3% Silar 1OC; injector, column and separator temperatures at 200, 255 and 290 °C, respectively; flow-rate carrier gas, helium, 40 mL min^{-1}	1–40	–	[77]
Desmethylsertraline	Temperature parameters programmed for an initial temperature of 100 °C then ramped at 50 °C min^{-1} to a final temperature of 210 °C; injection temperature 250 °C and detector temperature 300 °C; also HPLC–UV detection	10–250	10	[37]
Five SSRIs	After derivatization with *N*-methyl-bis(trifluoroacetamide)	20–400	1	[79]
Twenty-three antidepressants	Liquid–liquid extraction at pH 9.5 using a mixture of heptane/isoamyl alcohol (98.5/1.5) without derivatization. Cyproheptadine was used as IS; a non-polar PTE5 capillary column; MS consisted of electron impact ionisation (70 eV), and full scan acquisition	Up to 1000	–	[76]
–	Samples injected into fused-silica capillary column coated with cross-linked methyl silicone in the split-injection mode; oven temperatures: initial temperature 160 °C, it was increased to 220 °C at a rate of 10 °C min^{-1} and held there for 10 min, it was finally increased to 320 °C at a rate of 50 °C min^{-1} and held there for 2 min; electron energy 70 eV, ion source temperature 200 °C and injector temperature 280 °C. Helium, a carrier gas, was set to a column head pressure of 25.5 kPa (column flow: 1 mL min^{-1} at 160 °C). The selected ion-monitoring (SIM) mode was used. The quantitation ions for SRT and ethylsertraline(IS) were *m/z* 501 and *m/z* 515, respectively	0.2–10	0.1	[80]
Five SSRIs	Column CP-SIL 8 CB; temperature program, 60 °C for 2 min, heated to 200 °C at 20 °C min^{-1} and then to 280 °C at 5 °C min^{-1} (total analysis time, 25 min). Helium, as carrier gas, at constant flow of 1.2 mL min^{-1}; injector programmed to return to the split mode 2 min after the beginning of a run. Injector temperature was held constant at 270 °C. Trap and transfer-line temperatures 220 and 290 °C, respectively; mass spectrometer used in positive electron-impact mode, electron energy 70 eV, with automatic gain control; mass range of 43–420 *m/z* scanned and detector turned off for the first 11 min of the run	1–100	0.4	[81]
Twenty-nine psychoactive drugs	Methylsilicone capillary column and analytes, derivatized with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, were determined in the selected-ion-monitoring (SIM) mode at 70 eV; oven temperature programmed at 70 °C (2 min), followed by a 30 °C min^{-1} ramp to 160 °C, 5 °C min^{-1} to 170 °C, 20 °C min^{-1} to 200 °C, 10 °C min^{-1} to 220 °C and finally increased 30 °C min^{-1} ramp to 300 °C; injector and the interface operated at 280 °C. Helium, as carrier gas at flow rate of 0.8 mL min^{-1}	–	6.2	[82]
Twelve antidepressants and nine active metabolites	Sample preparation consisted of a strong cation exchange mechanism and derivatisation with heptafluoro-butyrylimidazole; J&W-5 ms column; initial column temperature at 90 °C for 1 min, ramped at 50 °C min^{-1} to 180 °C where it was held for 10 min, where after the temperature was ramped again at 10 °C min^{-1} to 300 °C; pulsed splitless injection temperature was held at 300 °C, while purge time and injection pulse time were set at 1 and 1.5 min, respectively. Meanwhile, the injection pulse pressure was 170 kPa and 1 μL of the sample, resolved in 50 μL toluene, was injected. Helium with a constant flow of 1.3 mL min^{-1} was used as carrier gas	–	–	[83]
Eleven antidepressants	Drugs isolated from blood or serum by SPE; also by TLC	–	2	[84]

and quantify SSRIs, including SRT, and four of their metabolites in seawater and sewage influents and effluents. The method is based on a three-phase hollow-fibre supported liquid phase micro-extraction of 1.1 L samples, followed by HPLC–ESI-MS.

A quantitative method was developed for the determination of trace levels of antidepressants, including SRT, in environmental aquatic matrixes using SPE coupled with LC–ESI-MS [91]. The validated methodology was applied to two unique hydrologic sample sets: a suite of municipal wastewater-effluent samples collected from a metropolitan urban center and surface water samples collected from a waste-dominated stream.

Lajeunesse et al. [92] develop a method for the determination of six basic antidepressants (SRT, PAR, CIT, FLU, venlafaxine and amitriptyline) and four of their metabolites (*O*-desmethylvenlafaxine, desmethylsertraline, nortriptyline, and norfluoxetine) in raw sewage and roughly primary-treated wastewater. For analytical development purposes, two ion exchange SPE cartridges were compared. Extracts were analyzed using LC–MS/MS with positive-mode electrospray (+ESI) and selected reaction monitoring transitions. The choice of a basic mobile phase significantly improved the instrumental sensitivity relative to common +ESI acidic mobile phases.

Recently, to determine spatial and temporal variations in concentrations of pharmaceuticals in the Tennessee River, water samples were collected from multiple points along the river and at the inflow of major tributaries. Sampling structure was designed to investigate trends between surface and subsurface samples, seasonal trends, the direct influence of sewage treatment plants (upstream versus downstream), and the effect of downstream distance on pharmaceutical concentrations [93]. All samples were quantified via SPE followed by LC–MS/MS. This method yielded reproducible quantitation at low parts per trillion levels for all 14 analytes (including SRT).

4.2. GC–MS

A rapid and sensitive method for quantification of five SSRIs (venlafaxine, fluvoxamine, fluoxetine, citalopram and sertraline) using solid-phase microextraction coupled to gas chromatography–mass spectrometry (GC–MS) is proposed by Lamas et al. [94]. The optimization of the method is fully discussed and the validation parameters are presented. The optimized method has been applied to different real water samples (river water, and influent and effluent waters of sewage treatment plants), and results demonstrate that these compounds occur in urban sewage waters. Water samples previously filtered, were placed in 22 mL headspace vials. To improve the extraction of some of the target compounds, a derivatization process was carried out. The vial was sealed with an aluminium cap and a Teflon-faced septum after addition of NaCl and reagents required for the acetylation process (potassium hydrogen carbonate and acetic anhydride). In the experiments run at 50 and 100 °C, the vial was immersed in a water bath and let to equilibrate for 5–15 min before SPME. The fibber was exposed to the sample magnetically stirred during 30 min. The fiber was then immediately inserted into the GC injection port and analysis was carried out. Desorption time was set at 3 min. Experimental parameters for GC–MS were: column, CP-SIL 8 CB 30 m, 0.25 mm i.d., 0.25 μm film; temperature program, 60 °C for 2 min, heated to 250 °C at 25 °C min^{-1}, heated to 280 °C at 10 °C min^{-1}, and finally heated to 292 °C at 1.5 °C min^{-1} (total analysis time, 20.6 min). Helium was employed as carrier gas at an initial head column pressure of 8 psi. Injector was programmed to return to the split mode after 2 min from the beginning of a run. Injector temperature was held constant at 270 °C. Trap and transfer line temperatures were 220 and 292 °C, respectively. The mass spectrometer was used in the positive electron impact mode at 70 eV with automatic gain control. A mass range of m/z 43–420 was scanned, and the detector was turned off for the first 11 min of the run.

Studies that characterize the fate and effects of pharmaceuticals in aquatic systems are limited, and data regarding pharmaceutical accumulation in fish of effluent-dominated ecosystems have not been previously reported until the study developed by Brooks et al. [95], in which fish populations were sampled from a reference stream and an effluent-dominated stream. *Lepomis macrochirus*, *Ictalurus punctatus*, *Cyprinus carpio*, and *Pomoxis nigromaculatus* were killed; the liver, brain, and lateral filet tissues dissected; and the tissues stored at −80 °C until analysis. Fish tissues were extracted using solid-phase extraction and then analyzed by GC–MS in the negative chemical ionization mode. SRT and FLU and the metabolites norfluoxetine and desmethylsertraline were detected at levels greater than 0.1 ng g^{-1} in all tissues examined from fish residing in a municipal effluent-dominated stream.

4.3. CE–MS

The investigation group of Himmelsbach development recently two analytical methods for the determination of SRT and other antidepressants in water samples by CE–MS [96,97].

Major aim of the first work was the optimization of a CE separation for antidepressants in environmental samples using aqueous as well as non-aqueous carrier electrolytes. Detection was carried out by ESI-MS in the positive-ion mode, whereby it was intended to do a critical comparison of the performances of a quadrupole MS and a TOF MS. Optimum results were obtained with a system consisting of 1.5 M formic acid and 50 mM ammonium formate in acetonitrile:water (85:15). A separation voltage between +20 and +30 kV was used for all CE experiments. Sample injection was performed by application of 50 mbar for 10 s, with UV detection and a sequence of 50 mbar 10 s of the sample followed by 50 mbar 10 s of the BGE for MS detection. The capillary temperature was thermostated to 228 °C. Direct UV detection of the analytes was performed at 200 nm. The CE was coupled to the MS instruments in both cases via a coaxial sheath flow interface.

In the second work, a method for the quantitative determination of seven major antidepressants, including SRT, in surface waters and sewage treatment plant effluents by CE using ESI-MS is proposed. For sample preparation eight different SPE materials were investigated. Best results were obtained for a resin based on hydrophilic divinylbenzene. Water samples (500 mL) were adjusted to pH 11 using NaOH, filtered using a 0.8 mm glass fibre filter and passed through the SPE cartridge at a flow rate of 10 mL min^{-1}. Consequently, the solutes were eluted employing 4 mL of 100 mM H_3PO_4 in methanol. Finally, the extract was evaporated to dryness using a stream of dry N_2 gas and redissolved in 500 mL of acetonitrile:water (8:2). The following electrolyte systems were employed: 1.5 M H_3PO_4 in acetonitrile for experiments using UV detection at 200 nm and 1.5 M formic acid, 50 mM ammonium formate in acetonitrile:water (85:15) for CE–MS experiments. A separation voltage between 120 kV (CE–MS) and 130 kV (UV detection) was used. Sample injection was performed by application of 50 mbar for 10 s, with UV detection and a sequence of 10 s 50 mbar of the sample followed by 10 s 50 mbar of the BGE for MS detection. The capillary temperature was thermostated to 227 °C. ESI-MS was carried out in the positive ion mode. The CE was coupled to the MS instrument (operated in the positive ion modes throughout this work) via a coaxial sheath flow interface employing a sheath liquid consisting of 5 mM ammonium formate in isopropanol:water (8:2) at a flow rate of 2 mL min^{-1}.

5. Conclusions

The separation of enantiomers is a very important topic to the pharmaceutical industry. It is well recognized that the biological activities and bioavailabilities of enantiomers often differ. Although chromatographic methods have proven to be the most reliable and versatile analytical techniques for measurement of stereochemical composition, CE has become a viable alternative for certain applications because of its high efficiency, short analysis time, and economy of sample and reagent consumption. In this way, SRT hydrochloride is a relatively novel drug substance belonging to the group of SSRIs in the brain. It has some characteristics that offer advantages over the other members of this class of antidepressants in the treatment of elderly patients with major depression. SRT has two chiral carbons and forms four stereoisomers. Therefore, the stereoselective separation of SRT is important in order to assure therapeutic efficacy and safety.

Almost all recent assays developed for the quantitative determination of SRT (and other SSRIs) and their metabolites in blood are based either on the separation by HPLC or GC.

The analytical techniques employed in recent years for the estimation of SRT in human plasma include HPLC, GC or in combination with MS i.e. liquid chromatography and tandem mass spectrometry (LC–MS/MS) and GC–MS/MS. All these reported methods require either a lengthy extraction and/or derivatization procedure, yet the desired sensitivity is not achieved.

Recently, it was demonstrated that in-tube SPME in combination with LC–UV/DAD, offers high sensitivity, accuracy, and enough reproducibility for quantification of SRT and other nontricyclic antidepressants in human plasma after the oral administration of the antidepressant. The in-tube SPME compared with other extraction techniques (on fibre SPME, LLE, and SPE) allows automation analysis, presents minor exposition of the analyst to the biological samples and organic solvent, and provides short analysis time.

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