Rev Anal Chem 30 (2011): 87–122 © 2011 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/REVAC.2011.018

Analytical methods for determination of selective serotonin reuptake inhibitor antidepressants

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) are commonly used to treat depression. SSRIs are classified as fluoxetine, paroxetine, escitalopram, citalopram, sertraline, and fluvoxamine. Several methods have been published for the determination of SSRI drugs in pharmaceuticals, biological materials and environmental samples. This review covers the analytical methods described in the literature for the determination of SSRIs and their main metabolites or some compounds belonging to the same SSRI group. The analytical methods are generally chromatography based methods coupled to different detectors, electroanalytical methods, capillary zone electrophoretic methods, and spectrometric methods.

Keywords: analytical methods; antidepressant; pharmaceutical and biological samples; selective serotonin reuptake inhibitors.

Introduction

Depression is a chronic or recurrent mood disorder that affects both economic and social functions of approximately 121 million people worldwide. According to the World Health Organization, depression will be the second leading contributor to the global burden of disease. Depression can lead to suicide. Analyses of the risks of taking selective serotonin reuptake inhibitors (SSRIs) have resulted in warnings about suicidality and aggression when these medications are used with children and adolescents. The US Food and Drug Administration (FDA) indicated that there is a 1.5-fold increase of suicidality in the 18–24 age group. This resulted in a black box warning on SSRIs and other antidepressant medications regarding the increased risk of suicidality in patients younger than 24 years (Levenson and Holland 2007, Stone and Jones 2007, FDA 2008, Wille et al. 2008). Antidepressants are commonly used for the treatment of depression and obsessive compulsive disorders. It is thought that one reason for depression is an inadequate amount of serotonin, by preventing the reuptake of serotonin with the presynaptic neuron. Serotonin plays a part in the treatment of various disorders such as anxiety, depression, schizophrenia, pain, hypertension, vascular disorders, and migraine (Asberg et al. 1976).

SSRIs specifically prevent the reuptake of serotonin by increasing the level of active serotonin in synapses. They have varying degrees of selectivity for the other monoamine transporters, with pure SSRIs having only weak affinity for the noradrenaline and dopamine transporter. Therefore, determination of SSRI pharmaceutical or biological samples are very important in pharmacokinetic, in therapeutic drug monitoring, and in bioequivalence studies. For these reasons, reliable analytical methods are needed which reliably determine plasma levels of SSRI drugs in order to detect changes (either desired or otherwise) as early as possible in the plasma concentrations of the drugs themselves. SSRI drugs have side effects including sexual dysfunction, gastrointestinal effects, and disruption of the central nervous system (Khawam et al. 2006, Barlow and Durand 2009).

In recent years, advanced analytical methods have been developed and optimized in the field of pharmaceutical analysis, with the aim of improving precision and sensitivity, in order to accurately quantify trace concentrations of pharmaceuticals present in pharmaceutical formulations and biological fluids.

In this article, a review of the analytical methods for the determination of SSRI drugs is presented. SSRIs are classified as fluoxetine (FL), paroxetine (PXT), escitalopram (ESCIT), citalopram (CIT), sertraline (SER), and fluvoxamine (FLU). Analytical methods described in the literature for the determination of SSRIs and its main metabolites or some compounds belonging to the same SSRI group are generally chromatographic based methods coupled to different detectors, electroanalytical methods, capillary zone electrophoretic methods, and spectrometric methods. Additionally, analytical methods such as the immunoassay and titrimetry methods have also been described in the literature for the determination of SSRIs. However, these methods are not covered in the review. Figure 1 shows the chemical structures of SSRI drugs.

Sample preparation

Several methods for sample preparation of SSRIs in pharmaceutical formulations and biological fluids such as human plasma, human urine samples, rat plasma, rat brain samples, fish tissue, hair samples, and human serum have been described in the literature. The most common are liquid-

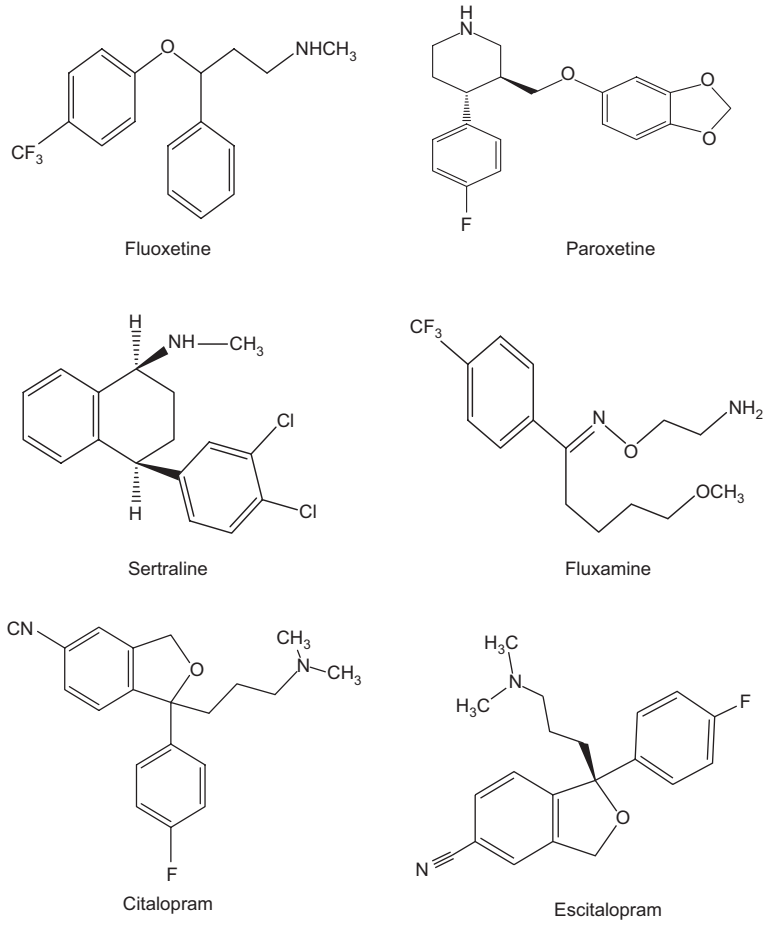

Figure 1 Chemical structures of SSRIs.

liquid extraction (LLE) and solid phase extraction (SPE) with C18 and C8 columns. However, these methods present some disadvantages and are laboriously time-consuming and use expensive solvents. Solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), protein precipitation and direct injection of biological samples without sample preparation supported liquid membrane extraction (SLM), liquid-solid extraction (LSE), liquid phase microextraction (LPME), pressurized liquid extraction (PLE) have also been employed for SSRI determination. These methods show some disadvantages being laborious and time-consuming.

Protein precipitation

Protein precipitation is the simplest means of bioanalytical sample pretreatment. It only involves the addition of a precipitating solvent, subsequent homogenizing and centrifugation. Deproteinization is rarely used in the extraction of SSRIs from biological matrices. Reubsaet and Bjergaard (2004) used a protein precipitation method for screening of more than 70 central nervous system-stimulating drugs in human plasma, including CIT, PXT, and SER. Acetonitrile was used to precipitate the proteins. Kirchherr and Kuhn-Velten (2006) developed another high-performance liquid chromatography-mass spectrometry (HPLC-MS) method in the multiple reaction monitoring (MRM) for the simultaneous determination of some drugs, including CIT, FL, FLU norfluoxetine, and PXT in serum of 0.1 ml, which requires protein precipitation using acetonitrile/methanol. Acids such as trichloroacetic acid can also be used for protein precipitation prior to analysis of SSRI samples. Deproteinization using trichloroacetic acid 10% (w/v) for the determination of FLU in plasma with recovery levels of 57.54% has been reported (Fernandes et al. 2006). This method demonstrated the effectiveness of simple precipitation of proteinaceous material using an organic solvent.

Liquid-liquid extraction (LLE)

LLE has been exploited as an extraction procedure for SSRIs from complex matrices. In a method published on the determination of SSRIs, human plasma or serum samples were prepared using a three-step LLE (Ulrich 2003). Massaroti et al. (2005) presented a high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method for PXT quantification in human EDTA plasma based on LLE using a mixture of ethyl acetate/hexane (50:50, v/v) with retention times of 1.6 and 1.7 for PXT and FL, respectively. Several papers have reported extraction with acetonitrile methanol or prior to clean-up of the extracts by

LLE with hexane. In some cases, this procedure was followed by SPE. SLM extraction and/or enrichment for selective separation and concentration of various metal ions from aqueous dilute solutions is similar to LLE. SLM uses the relatively small volume of organic components in the membrane and simultaneous extraction and re-extraction. An automated sample pretreatment of human blood plasma for liquid chromatographic determination of three antidepressant drugs (dibenzepine, reboxetine, fluvoxamine), based on SLM for unsurpassed sample clean-up and analyte enrichment has been developed by Barri and Jonssön (2004). The entire analytical procedure revealed good linearity and low detection limits of 5, 15, and 20 ng/ml for dibenzepine, reboxetine, and fluvoxamine, respectively.

Solid-phase extraction (SPE)

SPE is a separation process that is dissolved or suspended in a liquid mixture. SPE uses the affinity of solutes dissolved or suspended in a liquid for a solid through which the sample is passed to separate a mixture into desired and undesired components. SPE involves liquid-solid partition. This technique has been used extensively to extract trace organic materials from samples. Sample pretreatment procedures adopted in most analytical methodologies for determining SSRIs in pharmaceutical formulations and biological fluids are based on solvent extraction, followed by clean-up by SPE. Currently, the analyst can choose different SPE formats, such as cartridges, disks, and in-tube capillary columns. The SPEs with either off-line or on-line preconcentration procedures, coupled with various single element or multi-element analytical techniques are frequently used in the modern analytical laboratory. The main sample pretreatment methods and techniques currently being utilized for SSRIs are summarized in Tables 1–4. For SPE, many commercial cartridges are available. The sorbents used include the apolar C8, extraction disk or C18 and the mixed modes Bond Elut Certify or Oasis MCX. Oasis HLB, a hydrophilic-lipophilic balanced column, is also used in sample preparation before HPLC analysis of SSRIs.

Wille et al. (2005) presented gas chromatography-mass spectrometry (GC-MS) and HPLC-diode array detection (DAD) methods for 13 antidepressants, including FL, FLU, CIT, SER, and PXT, together with eight of their metabolites from plasma by using the SPE procedure. This procedure consisted of a conditioning step with 3 ml of eluent, 2 ml of methanol, and 3 ml of the eluting phosphate buffer. The SPE sorbents applied in this study can be divided into four different categories: namely, apolar sorbents (Bond Elut C18, Empore HD C8, and RPselect B Lichrolut), polymeric sorbents (Focus, Strata X, and Oasis HLB), ion-exchange sorbents (strong and weak cation exchangers), two mixed modes combining ion-exchange properties with C8 or a styrene-divinylbenzene polymer (Bond Elut Certify and Strata XC). Recoveries ranged between 70% and 109% for all antidepressants.

Saracino et al. (2006) reported a high-performance liquid chromatography-ultraviolet detection (HPLC-UV) method for the simultaneous determination of FLU and quetiapine in 300 µl human plasma by using a SPE procedure with mixed-mode cation exchange cartridges. The SPE cartridges were activated by passing 1 ml of methanol through the cartridge three times, and then conditioned by passing 1 ml of a pH 6.0, 25 mM phosphate buffer three times. CIT was used as the internal standard. Linearity ranged in the plasma over the 5.0–160.0 ng/ml for each FLU isomer. Precision was better than 4.0%.

In recent years, much attention has been focused on the development of on-line techniques. For such on-line systems, SPE is generally preferred over LLE as the isolation technique, because it is less laborious, uses smaller amounts of organic solvent, yields better analyte enrichment and is easier to couple on-line to the chromatographic technique to be used. As compared to off-line SPE, on-line SPE offers a series of advantages such as analysis of the total amount of analytes extracted, small sample volumes are sufficient to obtain enough sensitivity, matrix effects, ionic suppression or enhancement in MS spectrometry, less flexibility, and most systems do not allow the combined use of different cartridges, automatization and minimal sample handling which translates into better precision and accuracy, direct and fast elution of the sample after preconcentration, minimal consumption of organic solvents and reduced analysis time and high throughput. The use of on-line SPE techniques has made possible the development of faster methods by reducing the sample preparation time and thus increasing the sample throughput (Bones et al. 2006, Rodriguez-Mozas et al. 2007).

Saber (2009) presented a capillary liquid chromatography-electrospray ionization-mass spectrometry method (LC-ESI-MS) including on-line SPE for quantification of FL hydrochloride in human plasma with metronidazole (internal standard). The within assay and between assay precisions were between 8.5% and 11% and 6.6% and 7.5%, respectively. For spiked plasma samples, the lower limit of quantification (LLOQ) value was ~5.0 ng/ml. The limit of detection (LOD) value of the method was ~3.0 ng/ml.

The column-switching system presents major advantages over earlier off-line methods because of elimination tedious manual extraction and minimizes manipulation of the biological samples. Liu et al. (2008) reported a fully automated column-switching ion-pair HPLC-UV (254 nm) method for the analysis of FLU in rat plasma. The linearity for FLU ranged between 5 and 5000 ng/ml ($r>0.999$). LOQ and LOD values were 5 and 1.5 ng/ml. This method applies the integrated sample clean-up configuration using a RAM SPE pre-column connected via the electrically driven six-port switching column valve from a programmable autosampler to a reversed-phase analytical column. The plasma sample was injected onto a pre-column packed with Shim-pack MAYI-ODS (50 µm), where the drug was automatically purified and enriched by on-line SPE. Also, Souverain et al. (2003) reported a LC-ESI-MS method for the simultaneous determination of FL and its primary metabolite (norfluoxetine) in plasma based on a column-switching approach with a pre-column packed with large size particles in < 4 min. Flow rate was 4 ml/min. Linearity ranged between 25 and 1000 ng/ml

Table 1 GC methods.

Analyte	Matrix	Sample preparation	Column	Separation mode and detector	Sensitivity	Other parameters	References
FL and major metabolites	Human plasma or serum	LLE	Rtx-1 fused silica capillary (first capillary) (15 m×0.25 mm i.d., 1.0 mm film thickness, Restek, Sulzbach, Germany)	GC-nitrogen-phosphorus detection	LOD values: 1.5 and 6 ng/ml	Intra-day precision: between 5.4% and 12.7% at plasma levels of 25, 100, and 200 ng/ml for the four enantiomers. Inter-day precision: between 5.3% and 9.1% at 100 ng/ml	(Ulrich 2003)
FL, FLU, CIT, SER, PXT and other drugs	Human plasma	SPE	30 m×0.25 mm i.d., 0.25 µm Varian factor Four VF-5 ms column (Varian, Middelburg, The Netherlands)	GC-MS and HPLC-DAD			(Wille et al. 2005)
FLU, FL, CIT, SER	Environmental water	SPME	CP-SIL 8 CB 30 m, 0.25 mm i.d., 0.25 µm film	GC-MS	Sub-ng/ml		(Lamas et al. 2004)
CIT and its metabolites	Human plasma	LLE		GC-MS	1 ng/ml		(Reymond et al. 1993)
FLU and norfluoxetine	Plasma	LLE	Fused silica Optima 5 capillary column (15 m×0.25 mm i.d., 0.25 µm Macherey-Nagel, Oensingen, Switzerland)	GC-MS electron impact (EI) mode SIM	2 ng/ml	Recoveries: ranged from 50% to 66%. Inter-day coefficients of variation: ranged from 4% to 10% for FLU and from 4% to 13% for FL	(Eap et al. 1996)
FL		SPE	25 m×0.2 mm i.d. ×0.33 mm film thickness HP-5 capillary column preceded by a 0.5 m×0.53 mm i.d. deactivated methyl silica guard column	GC-MS SIM with an electron impact	1.0 ng/ml	Linearity: 1–100 ng/ml. Mean recoveries: 91% (FL) and 87% (norfluoxetine)	(Addison et al. 1998)
SER	Human plasma	LLE	Fused silica capillary column coated with crosslinked methyl silicone (Ultra-1, 17 m×0.2 mm i.d., 0.11 mm film thickness)	GC-MS SIM	0.1 ng/ml	Recovery: 80–85%. The linear response was obtained in the range of 0.2–10.0 ng/ml with a correlation coefficient of 0.999	(Kim et al. 2002)
PXT	Human plasma	LLE	Fused silica capillary column (15 m ×0.25 mm i.d.) coated with a 0.25 mm film thickness of 5% phenylmethyl silicone (J&W Scientific, Folsom, CA, USA)	GC-electron capture detector	LOQ: 28.4 ng/ml, LOD: 8.5 ng/ml		(Lai et al. 2000)
PXT	Human plasma	LLE	DB5-MS fused silica capillary column (15 m×0.25 mm i.d., 0.25 mm film thickness, ThermoQuest, Vienna, Austria)	GC-MS SIM	LOD: 0.2 ng/ml, LLOQ: 0.469 ng/ml	Linearity: 0.469–120 ng/ml	(Leis et al. 2002)
FL	Human plasma	SBSE	HP-5MS fused silica column (30 m, 0.25 mm, 0.25 µm, Agilent Technologies)	GC-EI-MS SIM	LOQ and LOD with liquid desorption: 30.0 and 10.0 pg/ml LOQ and LOD with thermal desorption: 37 and 0.46 pg/ml	Linearity of r^2>0.99 and precision of RSD<15%	(Fernandes et al. 2008)

(Table 1 continued)

Analyte	Matrix	Sample preparation	Column	Separation mode and detector	Sensitivity	Other parameters	References
PXT, SER	Oral fluid	SPE	Methylsilicone capillary column (Ultra-1, 16.5 m×0.2 mm i.d., 0.11 µm film thickness, Agilent Technologies)	GC-MS SIM	LOQ ranged from 0.9 to 44.2 ng/ml	Recovery ranged between 44.5% and 97.7%	(Pujadas et al. 2007)
FL, FLU	Pharmaceutical formulations		HP-5 (5% phenylmethyl silicone, 15 m×0.25 mm i.d., 0.25 µm film thickness) column	GC-flame ionization detector	LOQ values: 33.5, 300.0 µg/l for FL, FLU	Recovery: 100.37±0.45 for FL, 98.59±0.58 for FLU	(Nevado et al. 2005)
FL and major metabolites	Human plasma	LLE	OV-1 fused silica capillary column, 25 m×0.32 mm i.d., with a film thickness of 0.25 µm (Lara-Spiral, Couternon, France)	GC-nitrogen-phosphorus detection	LOD values: 0.3 and 2 ng/ml		(Fontanille et al. 1997)
CIT, FL	Urine samples	SPE	Equity-5 (5% phenylmethyl silicone, 15 m×0.25 mm i.d., and 0.25 µm, Supelco, Barcelona, Spain)	GC-MS	0.7 ng/l for CIT, 5.7 ng/l for FL		(Nevado et al. 2006a)
CIT, FL, FLU, SER, PXT, and other drugs	Human plasma	SPE	30 m×0.25 mm i.d., 0.25 µm J&W-5 ms column from Agilent Technologies (Avondale, PA, USA)	GC-MS	LOQ ranged from 5 to 12.5 ng/ml		(Wille et al. 2007)
FL, FLU, CIT, SER and PXT	Pharmaceutical formulations			GC-MS	LOD, LOQ: 3.6–41.5 mg/l	Recoveries ranged between 98.1% and 102.7%	(Nevado et al. 2006b)
FL, FLU, CIT, SER and PXT and other drugs	Postmortem blood, brain tissue, and hair	SPE		GC-MS chemical ionization mode	Brain to plasma ratios ranged from 0.8 to 17. Hair concentrations ranged from 0.4 to 2.5 ng/mg		(Wille et al. 2009)
FL	Discrete brain regions, blood, liver and hair of male rats	LLE	Varian Saturn II Ion-trap GC-MS apparatus equipped with an on-column injection and a 30 m, 0.25 mm i.d., DB-5 column	GC-MS	LOD: blood (ranging from 0.56 to 1.22 mg/l) and liver (ranging from 0.04 to 3.58 ng/mg)		(Lefebvre et al. 1999)
FL and other drugs	Liver and brain tissue	LLE	Fused silica capillary column (25 m×0.32 mm i.d.) coated with a 0.52 pm film thickness of 5% phenylmethyl silicone	GC-nitrogen-phosphorus detection	LOD: 1.32 and 1.36 µg/g liver and brain tissue		(Goodnough et al. 1995)

Table 2 HPLC methods with UV and DAD detection.

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
FLU	Human plasma	SLM	Nucleosil C18 column (250×2 mm, packed with 3 µm)	35% ACN and 65% 10 mM triethylamine aqueous solution (adjusted to pH 2.90 by phosphoric acid)	Ld-UV (230 nm)	LOD: 20 ng/ml	0.18	(Barri and Jonssön 2004)
FLU and quetiapine	Human plasma	SPE	Varian ResElut C8 column (150 mm×4.6 mm i.d., 5 µm)	ACN (30%) and phosphate buffer (10.5 mM, pH 3.5) containing 0.12% triethylamine (70%)	HPLC-UV (245 nm)	LOQ and LOD: 15.0 ng/ml	1.2	(Saracino et al. 2006)
FLU	Rat plasma	SPE	L-column octadecylsilane (ODS) analytical column (4.6×50 mm, Tokyo, Japan)	ACN-0.1% H_3PO_4 containing 2 mM sodium 1-octanesulfonate (36:64, v/v)	HPLC-UV (254 nm)	LOQ: 5 ng/ml and LOD: 1.5 ng/ml	1	(Liu et al. 2008)
FL and its metabolite	Plasma samples	SPME	C18 Phase Sep column (150 mm×4.6 mm, 3 µm)	ACN:acetate buffer 25 mmol/l with triethylamine 25 mmol/l pH 4.6 (70:30)	HPLC-UV (227 nm)	LOQ: 25 ng/ml	1.0	(Fernandes et al. 2007)
CIT, PXT, FL, SER and other drugs	Human plasma	SPME	LiChrospher 60 RP-select B (C18) column (250 mm× 4 mm, 5 µm; Merck, Darmstadt, Germany)	Phosphate buffer solution (0.05 mol/l, pH 3.8)-ACN (53:47, v/v)	LC-UV (230 nm)	LOQ values ranged from 20 to 50 ng/ml	1.0	(Silva et al. 2008)
CIT, PXT, FL, SER and other drugs	Plasma samples	SBSE	RP 18 LichroCART (125 mm×4 mm×5 µm particle size; Merck, Darmstadt, Germany)	Phosphate buffer solution 0.05 mol/l of pH 3.8, and ACN (53:47, v/v)	LC-UV (230 nm)	LOQ values ranged from 20 ng/ml to 50 ng/ml	1.0	(Melo et al. 2009)
SER and other drugs	Urine, plasma and tap water samples	LPME	Zorbax Extend C18 column (100 mm×2.1 mm, 3.5 µl; Agilent, Wilmington, DE, USA)	0.02 M AcOH solution of pH 4.0 and MeOH (54:46, v/v)	HPLC-UV (215 nm)	LOD values ranged from 0.5 to 0.7 µg/l	0.25	(Esrafili et al. 2007)
FLU and PXT	Human serum	LSE	Ultrasep ES 100 CN-column	ACN-MeOH-phosphate buffer (58:19:23, v/v/v)	HPLC-UV	LLOD values of 5 and 2 µg/l		(Bagli et al. 1997)
FL	Drug substances and formulated products		Zorbax SB-C8 column 0 (4.6 mm×250 mm, 5 g particle size; USA)	Gradient mobile phase (water-ACN-TFA)	HPLC-UV (260 nm) GC-flame ionization detection NMR		1.0	(Wirth et al. 1997)
FL and metabolites		LLE	LiChrospher 100 CN (150×4 mm, 5 µm particle size)	0.01 M phosphate buffer of pH 6.0 adjusted triethylamine, ACN, MeOH (13:65:22, v/v/v)	HPLC-UV (225 nm)	LOD: 3 ng/ml		(Meineke et al. 1998)
FL and major metabolites	Serum	SPE	Waters Symmetry C8 (150 mm×2.1 mm i.d., 5 µm; Waters, Australia)	67 mmol/l potassium phosphate buffer (pH 3.0) and ACN (67:33, v/v)	HPLC-UV (226 nm)	LLOQ: 10 nmol/l	0.3	(Li et al. 2004a)
FL	Human plasma	LLE	LiChrospher 60 RP-Select B column (125×3 mm i.d., 5 µm; Merck)		HPLC-UV (226 nm)	LOQ: 5 ng/ml		(Maya et al. 2000)

(Table 2 continued)

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
FLU	Human plasma and urine		Phenomenex C18 column (250 mm×4.6 mm i.d., 5 µm; Thermo Separation, USA)	ACN/water (80:20, v/v)	HPLC-UV (450 nm)	LOD: 1.4 and 1 ng/ml and LOQ: 5 and 2 ng/ml for plasma and urine	1	(Ulu 2007)
FLU, major metabolites	Human plasma	SPE	Grand Pack C_4–5 column (150×4.6 mm i.d., 5 µm; MASIS, Owani, Japan)	0.5% Potassium dihydrogen phosphate (pH 2.5)-ACN (75:25, v/v)	HPLC-UV (254 nm)	LOD: 10.0 ng/ml	1	(Zainaghi et al. 2003)
PXT	Human plasma	LLE	LiChrospher 60 RP-Select B column (250 mm×4 mm, 5 µm; Merck)	0.05 mol/l sodium phosphate buffer of pH 5.0 and ACN (50:50, v/v)	HPLC-UV (205 nm)	LLOQ: 5 ng/ml	1.0	(Zainaghi et al. 2003)
PXT	Human plasma	LLE	Beckman, Ultrasphere C column (150×2 mm i.d., 5 µm)	0.02 M Potassium dihydrogen phosphate buffer 85% H_3PO_4 of pH 2.5, ACN and 125 µl/l octylamine (62:38, v/v)	HPLC-UV (205 nm)	LOQ: 5 ng/ml	0.35	(Foglia et al. 1997)
PXT	Human plasma	LLE	Phenomenex C18 column (250×4.6 mm i.d., 5 µm; Thermo Separation, USA)	ACN-water (70:30)	HPLC-UV (567 nm)	LOD: 2 ng/ml	1.0	(Önal and Öztunç 2006)
CIT, PXT, FL, SER and other drugs	Plasma samples	SPME	LiChrospher 60 RP-Select B column (250 mm×4 mm, 5 µm particle size; Merck)	Phosphate buffer (0.05 mol/l, pH 3.8) and ACN (57:43, v/v)	LC-UV (230 nm)	LOQ ranged between 16 and 25 ng/ml	1.0	(Chaves et al. 2009)
Trazodone and FL	Urine samples	SPE	Eclipse X-DB-C8 column (4.6×150 mm)	Sodium dihydrogen phosphate buffer (pH 3.0) ACN-MeOH (70:25:5, v/v/v)	HPLC-UV (254 nm)	LOD: 90.1 ng/ml for FL	1.2	(Cruz-Vera et al. 2008)
SER, FL, CIT, PXT, and other drugs	Plasma samples	SBSE	RP 18 LichroCART (125 mm× 4 mm, 5 µm particle size; Merck, Darmstadt, Germany)	Two different mobile phases including acetate buffer solution: ACN:MeOH	LC-UV	LOQ: between 10.0 ng/ml and 40.0 ng/ml	1.0	(Chaves et al. 2007)
PXT, FLU, FL, SER, and CIT	Serum	SPE	Nucleosil 100-Protect 1 column (250×4.6 mm i.d., 5 µm, Macherey and Nagel, Duren, Germany)	25 mM potassium dihydrogen phosphate of pH 7.0-ACN (60:40, v/v)	HPLC-UV (230 nm)		1	(Frahnert et al. 2003)
PXT	Human plasma	LLE	RP-Select B column (250×4 mm; Merck, Darmstadt, Germany)	65% 0.05 M glacial AcOH (adjusted to pH 4.5 with 1 N NaOH) containing 2 g tetrabutylammonium hydrogen sulfate and 35% ACN-ethanol (3:2, v/v)	HPLC-UV (295 nm)	LLQ: 6 ng/ml	1	(Knoeller et al. 1995)
FL, SER, PXT, CIT and other drugs		LLE	LiChrospher 60 RP-Select B column (250 mm×4 mm, 5 µm particle size; Merck)	35% of a mixture of ACN/MeOH (92:8, v/v) and 65% of 0.25 mol/l sodium acetate buffer	HPLC-UV (230 nm)	LOQ: 5 ng/ml for CIT, 10 ng/ml for PXT, FL, SER	1.0	(Malfara et al. 2007)

(Table 2 continued)

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
SER and its main metabolite	Human plasma	SPE	Genesis C8 RP column (150×4.6 mm i.d., 4 μm, Mid Glamorgan, UK)	ACN and a 12.3 mM, pH 3.0 phosphate buffer containing 0.1% triethylamine (35:65, v/v)	HPLC-UV (220 nm)	LOQ and LOD: 7.5 ng/ml and 2.5 ng/ml	1.2	(Mandrioli et al. 2006)
CIT, FL, PXT and their metabolites	Plasma	SPE	Kromasil C18 column (0.32×300 mm, 3.5 μm; Waters, Milford, MA, USA)	ACN-45 mM ammonium formate of pH 4 (25:75, v/v)	LC-UV	LOQ ranged from 0.05 to 0.26 μM	5 μl min^{-1}	(Molander et al. 2001)
FL and olanzapine	Pure powder and tablet formulations		LiChrospher 100 RP-180, C18 column (250 mm, 4.0 mm, i.d., 5 μm)	0.05 M potassium dihydrogen phosphate buffer (pH 5.6 adjusted with *o*-phosphoric acid)-ACN (50:50, v/v)	LC-UV (233 nm)	LOD: 13.37 μg/ml and LOQ: 40.53 μg/ml		(Shah et al. 2007)
CIT enantiomers			Shim-pack (five micron particle size) cyanopropyl column with β-cyclodextrin	0.1% triethylammonium acetate buffer, pH 4.0 (adjusted with acetic acid), and ACN, containing 12 mM β-cyclodextrin (90+10, v/v)	LC-UV (240 nm)	LOD values: 5.51×10^{-3} and 4.35×10^{-3} pg/ml, LOQ values: 1.84×10^{-2} and 1.45×10^{-2} μg/ml for *S*-(+)citalopram and *R*-(-)citalopram	0.8	(El-Gindy et al. 2006)
FL enantiomers		LLE	Chiralcel ODR column	Potassium hexafluorophosphate/ acetonitrile	LC-UV (227 nm)	LOQ: 10 ng/ml for each enantiomer		(Gatti et al. 2003)
FL	Serum	LLE	Shimadzu, Microsorb MV (Rainin, Woburn, MA, USA) octa decyl column (15×0.46 cm i.d., 5 μm)	(55% ACN-45% distilled water containing 10 mM aqueous triethylamine)	LC-UV (226 nm)		1.0	(Holladay et al. 1997)
FLX and norfluoxetine (N-FLX)	Rat serum	SPE	Waters Symmetry C8 (150 mm×2.1 mm i.d., 5 μm) reversed-phase narrow bore column	67 mmol/l potassium phosphate buffer (pH 3.0) and ACN (67:33, v/v)	LC-UV (226 nm)	LLOQ for serum FLX and N-FLX was 10 nmol/l (on-column amount of 200 fmol)	0.3	(Li et al. 2004b)
FLX and N-FLX	Serum or homogenate from brain areas		Varian, C_8 end-capped column (125×4.0 mm i.d.; particle size, 4 μm) from Merck	Acidic aqueous solution (containing 0.1 ml of perchloric acid and 1.5 g of tetramethylammonium perchlorate per liter) and ACN (58:42, v/v)	HPLC-UV (227 nm)	LOD: 5.0 ng/ml	1.2	(Alvarez et al. 1998)
FLX and N-FLX	Human plasma	LLE	BDS Hypersil C column (100×4.6 mm i.d., 3 μm)	ACN (30%), water (67%), acetate buffer (3%), and 400 ml of dimethyloctylamine	HPLC-UV (226 nm)	LOQ: 14 nmol/l	0.6	(Llerena et al. 2003)
FLU, PXT	Human plasma	SPE	Nova-Pak, LiChrolut RP-18 column	MeOH-tetrahydrofuran-phosphate buffer at pH 2.65 (0.0657 mol/l) (53:5:42, v/v/v)	HPLC-UV (293 and 253 nm)	LLOQ for PXT and FLU: 10 ng/ml		(Skibinski et al. 2000)
CIT, FL, FLU and other drugs	Pharmaceutical formulations		Nova Pack C18 column (3.9 mm i.d.×150 mm, 5 μm particle; Waters Millipore, Milford, MA, USA)	ACN-pH 2.5 phosphate buffer (40:60, v/v)	HPLC-PAD (230–250 nm)	LOD and LOQ ranged from 1.0 and 10 μg/ml and 3.3 and 33.3 μg/ml	1.5	(Berzas et al. 2002)

(Table 2 continued)

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
FL and olanzapine	Pharmaceutical formulations		Inertsil C18 RP column (150 mm×4.6 mm, 5 µm; Shimadzu, Kyoto, Japan)	9.5 mM sodium dihydrogen phosphate (pH adjusted to 6.8±0.1 with triethylamine), ACN and MeOH (40:30:30, v/v/v)	HPLC-PAD (225 nm)	LOQ: 0.001 µg/ml, LOD: 0.003 µg/ml for FL	1.2	(Reddy et al. 2007)
FLU, PXT, SER, FL, CIT, other drugs	Serum	LLE	Beckman ODS C18 column (25 cm×4.6 mm, 5 µm; Beckman, Gagny, France)	(50%, v/v) ACN in a sodium phosphate buffer (0.05 M, pH 3.80)	HPLC-PAD (200.4 nm)	LOQ values ranged from 15 ng/ml and 50 ng/ml	1	(Tournel et al. 2001)
FL and its metabolite	Human plasma	SPE	Microsorb MV C18 RP column (150 mm×4.6 mm i.d., 5 µm)	ACN and 17 mmol/l tetramethylammonium perchlorate pH 3.0 (50:50, v/v)	HPLC-PAD (230 nm)	LOQ and LOD: 30 and 15 ng/ml	1.0	(Sabbioni et al. 2004)
CIT and FL	Human urine samples	SPME	Spherisorb ODS2 (15 cm×0.4 cm, 5 µm; Tracer, Barcelona, Spain)	TMACl (pH 4; 0.15%):ACN (50:50, v/v)	HPLC-PAD (230 nm)	LOD values close to 0.01 mg/l	1	(Unceta et al. 2008)
PXT	Raw material and pharmaceutical formulations		Carbamate derivative-based column (Chiralpak AD)	*n*-Hexane-ethanol-diethylamine (94:6:0.5, v/v/v)	HPLC-PAD (296 nm)	LOD and LOQ: 2 and 6 ng/ml		(Ferretti et al. 1998)
PXT		LLE	Chiralcel OD (250×4.6 mm, 10 µm), Chiralpak AD (250×4.6 mm, 10 µm) and Chiralcel OJ (250×4.6 mm, 10 µm)	Hexane, isopropanol, and diethylamine (96:0.4:0.3, v/v/v)	LC-PAD (265 nm)	LOQ and LOD: 2 and 7.5 µg/ml	1.0	(Vivekanand et al. 2003)
CIT, PXT, FL, SER, FLU and other drugs		LLE	5-microm Hypurity C18 (ThermoHypersil) analytic column (250×4.6 mm i.d.)	Acetonitrile-phosphate buffer pH of 3.8	LC-UV (220, 240, and 290 nm)	LOD values ranged from 2.5 to 5 ng/ml	1.0	(Duverneuil et al. 2003)

Table 3 HPLC methods with FLD detection.

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
FL	Rat plasma		TSKgel ODS-80Ts (250×4.6 mm i.d., 5 µm) from Tosoh Co. (Tokyo, Japan)	ACN-water (55:45, v/v)	HPLC-FLD λ_{ex}: 478 nm λ_{em}: 537 nm	LOQ: 10 nM in 100 µl of plasma	1.2	(Guo et al. 2003)
FLU, PXT, SER, and FL	Plasma		Hypersyl ODS C18 column (250×4.6 mm, 5 µm, ThermoQuest, Runcorn, UK)	Gradient mobile phase consisting of ACN and potassium phosphate buffer (10 mmol/l, pH 7.2)	HPLC-FLD λ_{ex}: 366 nm λ_{em}: 490 nm	LOD: 5 ng/ml for FLU, PXT, and FL, and 10 ng/ml for SER	1.5	(Lucca et al. 2000)
FLU	Human serum	LLE	Shimpack CLC-C18 (150 mm×4.6 mm i.d., 5 µm, Shimadzu, Japan)	MeOH and sodium phosphate buffer (0.05 M; pH 2.8) containing 1 ml/l triethylamine (72:28)	HPLC-FLD λ_{ex}: 470 nm λ_{em}: 537 nm	LOQ: 0.5 ng/ml		(Bahrami and Mohammadi 2007)
FL	Rat brain		C18 column	Gradient mobile phases (CH_3CN and TFA in H_2O)	HPLC-FLD λ_{ex}: 540 nm λ_{em}: 470 nm	LOD: 17 nM (23 fmol)		(Fukushima et al. 2004)
FLU	Rat plasma		C18 packing material column (150×4.6 mm i.d. with 5 µm particles; Kanto Chemical, Tokyo, Japan)	ACN (600 ml)-trifluoro AcOH (400 ml) (0.1% v/v) in water	HPLC-FLD λ_{ex}: 470 nm λ_{em}: 540 nm	LLOD and LLOQ: 0.008 and 0.015 µg/ml		(Higashi et al. 2005)
FL	Human plasma	LLE	Jones Apes Silica column (4.6×15 cm i.d., 5 µm; Littleton, CO, USA)	Isooctane:THF (70:30, v/v)	HPLC-FLD λ_{ex}: 285 nm λ_{em}: 313 nm			(Peyton et al. 1991)
CIT and metabolites	Rat plasma and brain tissue	LLE	Apex Silica column (25×0.46 cm, 5 µm, Littleton, CO, USA)	Hexane-isopropanol (82:18, v/v)	HPLC-FLD λ_{ex}: 224 nm λ_{em}: 336 nm	LOD values lower than 2.1 ng/ml and 42.8 ng/g	1	(Millan et al. 2008)
CIT	Human plasma	LLE	Cyano column (45×4.6 mm, 5 µm particles)	ACN-phosphate buffer, pH 6.0 (50:50, v/v)	LC-FLD λ_{ex}: 206 nm λ_{em}: 306 nm	LOQ: 0.96 ng/ml		(Macek et al. 2001)
PXT	Human plasma	LLE	LiChrosorb RP-8 column (250×4 mm, 10 µm particle size; Merck)	10 mM potassium phosphate buffer-ACN (40:60, v/v) adjusted to pH 3.2 with 80% phosphoric acid	HPLC-FLD λ_{ex}: 295 nm λ_{em}: 350 nm	LOD: 0.2 ng/ml	1.2	(Shin et al. 1998)
CIT	Human plasma	SPE	Phenomenex Luna C18 column (250×3.0 mm i.d., 5 µm; Torrance, CA, USA)	ACN:aqueous tetramethyl ammonium perchlorate of pH 1.9 (40:60, v/v)	HPLC-FLD λ_{ex}: 300 nm λ_{em}: 238 nm	LOQ: 1.5 ng/ml	0.5	(Raggi et al. 2003)
FL and its main metabolite	Human plasma	LLE	ResElut C8 RP column (150 mm×4.6 mm i.d., 5 µm; Varian, Walnut Creek, CA, USA)	17 mM tetramethylammonium perchlorate of pH 1.9 with 7% perchloric acid and ACN (45:55, v/v)	HPLC-FLD λ_{ex}: 230 nm λ_{em}: 294 nm	LOD: 1 ng/ml LOQ: 2.5 ng/ml	1.2	(Raggi et al. 1999)
CIT	Human plasma	LLE	Chirobiotic V, 5 µm, 150×4.6 mm; Astec, Basel, Switzerland)	MeOH-AcOH-triethylamine (99.9:0.055:0.060, v/v/v)	LC-FLD λ_{ex}: 240 nm λ_{em}: 296 nm	LOQ: 5 ng/ml	1	(Kosel et al. 1998)
CIT	Plasma	Direct	RP-18 (4.0 mm×4 mm; Merck, Darmstadt, Germany)	20 mM phosphate buffer of pH 4.6-ACN (70:30, v/v) containing 0.1% diethylamine	HPLC-FLD λ_{ex}: 249 nm λ_{em}: 302 nm	LOQ: 2.0 ng/ml		(Matsui et al. 1995)

(Table 3 continued)

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
FL	Plasma and brain tissue	SPE	Extrasil CN (15 cm×0.40 mm, 5 µm particle size; Tracer Anal., Barcelona, Spain)	75% tetramethyl ammonium chloride in CAN	LC-FLD λ_{ex}: 227 nm λ_{em}: 305 nm	LOD values: 3.2 and 2.1 ng/ml in plasma, and 31.5 and 26.1 ng/g in brain tissue	1	(Unceta et al. 2007)
CIT	Deproteinized plasma	SPE	Ultrasphere C18 RP silica column packed with 5 µm bonded silica (Beckman, USA)	10 mM KH_2PO_4-ACN (2:1, v/v) (pH=4)	HPLC-FLD λ_{ex}: 250 nm λ_{em}: 325 nm	LOQ: 12 ng/ml	0.7	(Meng and Gauthier 2005)
FL and its active metabolite	Human plasma	LLE	Zorbax SB-C18 column (150 mm×3.0 mm i.d., 3.5 µm; Agilent)	ACN and 40 mM potassium dihydrogen phosphate buffer of pH 2.3 (31:69, v/v)	HPLC-FLD λ_{ex}: 230 nm λ_{em}: 312 nm	LOQ and LOD: 1 and 0.9 ng/ml		(Vlase et al. 2005)
PXT	Human plasma	LLE	Zorbax Eclipse XDB-C18 5 µm column	ACN-phosphate buffer (KH_2PO_4 0.04 M; pH=3.5) (30:70, v/v)	HPLC-FLD λ_{ex}: 295 nm λ_{em}: 350 nm	LOQ: 7 ng/ml	1.0	(Vergi-Athanasiou et al. 2007)
PXT	Human plasma	SPE	NovaPak C column (4 µm, 150×3.9 mm i.d.)	ACN-10 mM phosphate buffer of pH 3.2 adjusted with ortho H_3PO_4 (45:55, v/v)	HPLC-FLD λ_{ex}: 295 nm λ_{em}: 350 nm	LOD: < 5 ng/ml LLOQ: 10 ng/ml	1.0	(Lopez-Calull and Dominguez 1999)
PXT and its three main metabolites	Human plasma	SPE	Luna C18 (250 mm×3.0 mm i.d., 5 µm; Phenomenex, USA)	66.7% aqueous phosphate at pH 2.5 and 33.3% ACN	HPLC-FLD λ_{ex}: 294 nm λ_{em}: 330 nm	LOD: 1.2 ng/ml	0.5	(Mandrioli et al. 2007)
CIT, FL, and other drugs	Human serum	LLE	C18 column	ACN-buffer (30:70, v/v)	HPLC-FLD λ_{ex}: 260 nm λ_{em}: 227/300 nm		1.5	(Waschgler et al. 2002)
CIT, FL, PXT, and metabolites	Whole blood and plasma	SPE	Symmetry C column (150×3.9 mm, 5 µm; Waters, Milford, MA, USA)	45 mM ammonium formate (pH 4.0)-ACN (70:30, v/v)	HPLC-FLD and UV	LOQ: 0.025 mmol/l for CIT, PXT with FLD LOQ: 0.10 mmol/l for FL with UV	1.2	(Kristoffersen et al. 1999)
FLU, PXT, SER, FL, and other drugs	Human serum	LLE	Hypersil ODS, 15034.6 mm i.d. (3 mm dp) column	Different mixtures of sodium acetate solution (0.005 M, pH 4.5) and MeOH	HPLC-FLD and GC-nitrogen-phosphorus	LOD values and LOQ values ranged from 2.5 to 5 mg/l and from 10 to 20 mg/l		(Lacassie et al. 2000)

Table 4 HPLC methods with MS detection.

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
CIT, PXT, SER and other drugs	Human plasma	Protein precipitation	Intersil ODS-3 column (50 mm×2.0 mm 100 Å, 3 µm; Varian Holger, Oslo, Norway)	Gradient mobile phases (mobile phase A: 5% ACN in 10 mM ammonium acetate of pH 5.0 with AcOH; mobile phase B: 90% acetonitrile in 10 mM ammonium acetate of pH 5.0)	LC-ESI/MS		0.2	(Reubsaet and Bjergaard 2004)
CIT, FL, FLU, PXT and other drugs	Serum	Protein precipitation	Chromolith Speed ROD C18 (50 mm×4.6 mm; VWR/Merck, Darmstadt, Germany)	MeOH gradient and 5 mM acetate buffer at pH 3.9	HPLC-ESI-MS MRM		1.0	(Kirchherr and Kuhn-Velten 2006)
FL	Human plasma samples	SBSE	Phenomenex Luna C18 (100×2 mm, 3 µm) column	MeOH-acetate buffer 5 mmol/l of pH 4.8 (60:40, v/v)	LC-ESI-MS MIM	LOQ and LOD: 10 and 3 ng/ml	0.2	(Fernandes et al. 2006)
PXT	Human EDTA plasma	LLE	Polaris C18 column (50×2.0 mm, 5 µm particle size; Varian)	HCOOH 0.1% in ACN: water (6:4, v/v)	LC-ESI-MS/MS MRM		0.15	(Massaroti et al. 2005)
FL	Human plasma	SPE	Zorbax C18 columns 0.3 mm i.d.×50 mm; Agilent, Palo Alto, CA, USA	ACN-0.05 mol/l ammonium formate buffer (25:75, v/v)	LC-ESI/MS	LOD: ~3.0 ng/ml and LLOQ: ~5.0 ng/ml	0.05	(Saber 2009)
FL and its metabolite	Human plasma	Column-switching	HS C18 column (50×2.1 mm i.d., 3 µm; Supelco, Bellefonte, PA, USA)	Water-ACN (95:5, v/v)	LC-ESI/MS	LOQ: 25 ng/ml	4	(Souverain et al. 2003)
FL	Serum samples	SPME	C18 column (150 mm×3.9 mm, 5 µm particle size; Waters, Dublin, Ireland)	1% Glacial AcOH in ACN (60%) and 20 mM ammonium acetate in water (40%)	LC-ESI/MS SIM	LOQ: 5.00 ng/ml	0.8	(Queiroz et al. 2007)
PXT, FL and its active metabolite	Fish tissue	SPE	Genesis C18 column (150 mm×2.1 mm i.d., 4 µm; Brockville, ON, Canada)	Gradient of mobile phases A (water containing 10 mM ammonium acetate) and B (95% ACN and 5% water containing 10 mM ammonium acetate)	LC-APCI-MS/MS	LOQ values: 0.24, 0.07, and 0.14 ng/g wet weight for PXT, FL and its active metabolite	0.2	(Chu and Metcalfe 2007)
CIT, FLU, PXT	Human plasma		Symmetry C18 (Waters, USA) 5 µm, 3.0×150 mm i.d., HPLC column	Formic acid (0.1%) mobile phase	HPLC-APCI-MS-M SRM	LLOQ for CIT, FLU, and PXT: 20, 20, and 10 µg/ml LOD: 5 µg/ml for all analytes	4	(Kollroser and Schober 2003)
FL	Human plasma	LLE	CHIROBIOTIC V, 250×4.6 mm	MeOH containing 0.075% (by weight) ammonium trifluoroacetate	LC-APCI-MS/MS MRM	LOD: 2 ng/ml	1.2	(Shen et al. 2002)
SER	Human plasma	LLE	Zorbax Eclipse XDB C18 column	MeOH/water/formic acid (75:25:0.1, v/v/v)	LC/APCI-MS/MS	LLOQ: 0.10 ng/ml		(Chen et al. 2006)
FL	Serum samples	LLE	Beckman C18 column (ODS-150 mm×2.1 mm, 5 µm)	ACN-water-HCOOH with 2 mM ammonium acetate (68:32:0.1, v/v/v)	HPLC-ESI-MS/MS MRM	LOD and LOQ: 0.06 and 0.17 ng/ml	0.2	(Franceschi et al. 2009)

(Table 4 continued)

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
PXT	Human plasma	LLE	Genesis C18 column (50×2.1 mm, 4 µm)	CAN-5 mM ammonium formate (4:3, v/v)	LC-MS/MS MRM	LLOQ: 0.2 ng/ml	0.22	(Zhu and Neirinck 2002)
CIT	Human plasma	LLE	Hypersil BDS C8 micro-bore column (250 mm×2.1 mm i.d., 3.5 µm particle size; Thermo-hypersil, USA)	10 mM Ammonium formate–HCOOH (pH 4.5) and ACN (30:70, v/v)	LC-ESI-MS SIM	LOQ: 0.50 ng/ml	0.15	(Pistos et al. 2004)
FL and its metabolite	Human plasma	LLE	Zorbax SB-C18 column (5 cm×2.1 mm i.d., 5 µm; Chadds Ford, PA, USA)	CH_3CN-water (274:276, v/v)	LC-TIS-MS/MS	LOD: 0.1 ng/ml	0.2	(Li et al. 2002)
FL and its metabolite	Human plasma	LLE	Phenomenex Luna C 18(2) (150×2 mm, 5 µm, column; Phenomenex, Torrance, CA, USA)	CAN-0.02% HCOOH (340:660, v/v)	LC-TIS-MS/MS MRM	LLOQ: 0.15 ng/ml	0.35	(Sutherland et al. 2001)
FL and its metabolite	Human plasma	SPE	Xterra MS C18 column (50×2.1 mm, 3.5 µm)	Solvent A: 0.05% HCOOH in MeOH Solvent B: 0.05% formic acid	LC-TIS-MS/MS MRM	LLOQ: 0.5 ng/ml	0.6	(Green et al. 2002)
SER and its metabolite	Human plasma	LLE	Betasil C8 column (100 mm×2.1 mm, 5 µm)	750 ml MeOH–250 ml deionized water–2.5 ml, 1.0 M ammonium trifluoroacetate	LC-TIS-MS/MS MRM	LLOQ: 0.5 ng/ml	0.35	(Patel et al. 2009)
FL and other drugs	Plasma		YMC ODS-AQ packing material column (pore size 120A°, 5 µm; YMC Europe, Schermbeck/ Weselerwald, Germany)	20 mmol/l Ammonium acetate-AcOH buffer of pH 5.4 with 70% ACN at 5 µl/min	LC-ESI-MS/MS MRM	LOQ: 1 ng/ml	0.05	(Santos-Neto et al. 2008)
FL, CIT, PXT	Human plasma	SPE	Macherey-Nagel C18 column (250 mm×4.6 mm, 5 µm; Germany)	Water (HCOOH 0.6%, ammonium acetate: 30 mmol/l)-ACN (35:65, v/v)	HPLC-ESI/MS SIR	LOD values: 0.5, 0.3, and 0.1 ng/ml	0.85	(Juan et al. 2005)
SER	Human plasma		Shimadzu ODS C18 column (5 µm, 150×4.6 mm i.d.)	Methanol-10 mmol/l ammonium acetate solution-acetonitrile (62:28:10, v/v/v)	HPLC-ESI-MS SIM	LOQ: 0.5 ng/ml		(He et al. 2005)
SER	Human plasma		Discovery C18 column	0.1% Formic acid-acetonitrile (50:50)	LC-ESI-MS/MS SRM	LOD: 0.334 ng/ml		(Jia et al. 2007)
PXT and its metabolite	Human plasma	LLE	Synergi 4µ MAX-RP 80A (150×2 mm, 4 µm; Phenomenex, Aschaffenburg, Germany)	ACN-0.02% HCOOH (66:34, v/v)	HPLC-ESI-MS MRM	LOD and LOQ: 0.20 and 0.70 g/l	0.25	(Segura et al. 2003)
PXT	Human plasma	LLE	Betasil silica column (5 µM, 50×3.0 mm i.d., Keystone Scientific, Bellefonte, PA, USA)	ACN-40 mM ammonium acetate in water-TFA (94:6:0.05, v/v/v)	LC-ESI-MS/MS MRM		0.5	(Weng and Eerkes 2003)
FL	Human plasma	LLE	Eclipse XDB C8 (150×4.6 mm, 5 µm; Zorbax)	ACN-water-triethylamine (35:65:0.4, v/v/v)	LC-MS-SIM and LC-FLD	LOQ for LC-MS: 2.5 µg/l; LOQ for LC-FLD: 20 µg/l	1.0	(Kovacevic et al. 2006)

(Table 4 continued)

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
FL	Human plasma samples	LLE	LiChrospher 100 RP-8 E 250-4, 5 µm column (Merck)	Acetate buffer and ACN (40:60, v/v)	LC-ESI/MS SIM	LOQ: 2.5 ng/ml	0.8	(Djordjevic et al. 2005)
FL	Rat liver		Purosphere STAR RP-18, LiChroCART (55×4 mm 3 µ particles; Merck)	Gradient mobile phases (0.01% trifluoroAcOH in water, and 0.01% TFA in ACN)	HPLC, MS PAD (227 nm)			(Jawecki 2007)
SER	Human plasma	SPE	Type Beta Basic C8 column (100 mm×4.6 mm, 5 µm)	5 mM ammonium acetate (pH 3):ACN (20:80, v/v)	LC-TIS-MS SRM		0.5	(Jain et al. 2005)
Escitalopram	Human plasma	LLE	ODS YMCTM AQ column (150 mm×4.6 mm, 5.0 µ)	2.0 mM ammonium acetate (pH 5.0)-ACN (54:46, v/v)	LC-ESI/MS	LLOQ: 1.0 ng/ml	1.0	(Singh et al. 2004)
SER, FLU, PXT	Human serum	Direct	Shim-pack MAYI-ODS (10×4.6 mm i.d.; Shimadzu, Kyoto, Japan)	Solvent A: 7.5 mM ammonium acetate aqueous solution; solvent B: 0.05% HCOOH in ACN	LC-MS/MS	LOD: 1–3 ng/ml		(Hattori et al. 2007)
FL, CIT, PXT, SER, and other drugs	Hair samples		C18 column (150×4.6 mm)	Gradient mobile phases (solvent:MeOH/water/HCOOH/ trifluoroAcOH)	HPLC-ESI/MS*n* ESI-QToF-MS/ MS MRM	LOD values: 6.25×10^{-7}, 9×10^{-9}, 1.56×10^{-7}, and 7.8×10^{-8} mol/l	0.5	(Smyth et al. 2006)
CIT and other drugs	Hair samples	LLE	Luna column C18 (150×1 mm, 5 µm; Phenomenex, Torrance, CA, USA)	Gradient mobile phases (solvent: water, 0.1% HCOOH, ammonium formate 2 mmol/l of pH 3 and solvent B: ACN, 0.1% HCOOH, ammonium formate 2 mmol/l)	LC-ESI-MS/MS	LLOQ: 25 pg/mg LOD: 10 pg/mg	0.05	(Frison et al. 2008)
FLU, PXT	Human plasma	SPE	Inertsil C8 column	Methanol:10 mM ammonium acetate (pH 5.0):ACN (70:20:10, v/v/v)	LC-SSI-MS	LOD between 0.03 and 0.63 mg/ml		(Shinozuka et al. 2006)
CIT, FL, PXT, SER and other drugs	Blank serum sample		XTerra MS C18 column (5 µm, 50×2.1 mm i.d.; Waters, Milford, MA, USA)	Gradient of mobile phase A (0.1% HCOOH in water) and mobile phase B (0.1% HCOOH in ACN)	Turbulent-flow LC-MS		0.2	(Sauvage et al. 2006)
PXT, SER, FLU	Human serum or plasma	Direct			LC-MS/MS	LOD values: 5 ng/ml serum and 10 ng/ml plasma		(Hattori et al. 2005)
CIT, FL, and other drugs	Pharmaceuticals in wastewater	SPE	Chirobiotic V (250 mm×4.6 mm i.d., 5 µm particle size; Whippany, NJ, USA)	MeOH:nanopure water with 20 mM NH_4OAc, 0.1% HCOOH, pH 4 (90:10)	HPLC-ESI-MS/ MS MRM	LOD values influent of 1.6 and 1.7 ng/l and 0.1 LOQ values influent of 6 ng/l	0.5	(MacLeod et al. 2007)
FL, PXT, SER, FLU, CIT, and other drugs	Human plasma	LLE	XTerra RP18 column	Gradient of ACN/ammonium formate buffer (4 mmol/l, pH 3.2)	LC-ESI-MS MRM	LOQ: 5 ng/ml		(Castaing et al. 2007)
FL, PXT, SER, FLU, and CIT	Oral fluid and plasma	SPE	Sunfire C18 IS column (20 mm×2.1 mm, 3.5 µm)	Gradient of ACN and ammonium formate (pH 3, 2 mM)	LC-ESI-MS/MS MRM	LLOQ: 2 ng/ml in oral fluid, and 2, 4 or 10 ng/ml	0.4	(de Castro et al. 2008)

(Table 4 continued)

Analyte	Matrix	Sample preparation	Stationary phase	Mobile phase	Separation mode and detector	Sensitivity	Flow rate (ml/min)	References
SER, FL, CIT, PXT, FLU, and other drugs	Human plasma	SPE	Gemini C18 guard column (4 mm×2.0 mm, 5 µm; Phenomenex, Torrance, CA, USA)	Gradient mobile phases including 10 mM ammonium hydrogen carbonate (pH 10) and ACN	LC-ESI-MS/MS MRM	LOQ: 10 µg/l		(de Castro et al. 2007)
Psychoactive drugs	Hair samples		Luna C18 column (150 mm×4.6 mm, 5 µ)	Gradient mobile phase including solvent A [MeOH-water (20:80, v/v)+0.1% HCOOH] and solvent B [MeOH-water (90:10, v/v)+0.1% HCOOH]	HPLC-ESI/MS ESI–MS*n* GC-flame ionization detection		0.5	(Doherty et al. 2007)
CIT, FLU, and other drugs	Serum	LLE	Reversed-phase C18 column	ACN-ammonium acetate buffer pH 4	HPLC-ESI-MS MRM	LLOQ values: between 1.2 and 54 nmol/l for the different drugs		(Gutteck and Rentsch 2003)
FLX and norfluoxetine	Human plasma				LC-ESI-MS SIR	LOQ: 0.15 ng/ml and 0.50 ng/ml		(Moraes et al. 1999)

with a determination coefficient higher than 0.99. LOQ values were 25 ng/ml for FL and norfluoxetine.

Solid-phase microextraction (SPME)

SPME can be thought of as a very short GC column turned inside out. SPME involves the use of a fiber coated with an extracting phase that can be a liquid (polymer) or a solid (sorbent), which extracts different types of analytes (including both volatile and non-volatile) from different types of media that can be in liquid or gas phase (Somenath 2003). The attraction of SPME is that the extraction is fast and simple and can be done without solvents, and detection limits can reach parts per trillion (ppt) levels for certain compounds.

Lamas et al. (2004) developed a SPME-GC-MS method for the analysis of venlafaxine, FLU, FL, CIT, and SER on direct SPME at 100°C by using polydimethylsiloxane (PDMS)-divinylbenzene fibers in environmental water with LOD values in the sub-ng/ml level. In these experiments, the sample mode [direct extraction SPME and headspace (HS) mode (HS-SPME)], the introduction of an *in situ* acetylation step to transform the analytes in less polar compounds, and the extraction temperature (25°C and 100°C) were studied. Fernandes et al. (2007) reported another HPLC-UV method by using SPME for determination of FL and its metabolite norfluoxetine in plasma samples with a heated liquid flow through the interface. SPME conditions were optimized employing a factorial design. The sampling step was performed using a PDMS-DVB fiber and desorption was carried out in a novel homemade heated interface. LOD was 10 ng/ml for FLU and 5 ng/ml for nor-FLU. The range was evaluated from 25 ng/ml to 500 ng/ml. Recovery values were different for concentrations of 25, 100, and 500 ng/ml because plasma proteins progressively adsorb on the fiber coating, decreasing the extracted amount. Because the assessment had started with the lowest concentration (25 ng/ml) the recovery was lower for the highest concentration (500 ng/ml).

In-tube solid-phase microextraction (in-tube SPME) has been successfully applied to the analysis of drugs in biological fluids. In-tube SPME has been used in HPLC as an efficient and simple preparation method, and it offers several advantages over the fiber SPME syringe-LC approach. In-tube SPME is similar to fiber SPME, but the extraction device has a piece of fused silica GC capillary column in place of a fiber. An inner surface coated capillary is commonly used as the extraction phase, such as the commercial GC capillaries and the newly developed ones, such as polypyrrole coatings. Organic compounds in aqueous samples are directly extracted and concentrated into the capillary columns stationary phase by repeated draw/eject cycles of the sample solution, being further transferred to the liquid chromatographic column (Wang et al. 2004, Queiroz et al. 2007).

Queiroz et al. (2007) reported a LC-MS method at ion monitoring mode with immunoaffinity in-tube SPME for analysis of FL in serum samples with LOQ of 5.00 ng/ml. Linearity ranged between 5.00 and 50.00 ng/ml with correlation coefficients better than 0.998. Silva et al. (2008) presented an in-tube SPME-LC-UV (230 nm for most of the drugs) method

for simultaneous determination of mirtazapine, CIT, PXT, duloxetine, FL, and SER in human plasma with quantification limits ranging between 20 and 50 ng/ml. Linearity for most of the drugs ranged from 50 to 500 ng/ml with correlation coefficients higher than 0.9985.

Stir bar sorptive extraction (SBSE)

SBSE is a new solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. The method is based on the same principles as SPME. Compared with SPME, a relatively large amount of extracting phase is coated on a stir bar. It uses a small amount of solvent and is based on the use of a stir bar incorporated in a glass tube coated with a PDMS layer. The stir bar is placed in the aqueous sample and the analytes distribute between this matrix and the PDMS phase during the stirring. The amount of PDMS can vary depending on the bar length and film thickness. The technique has been successfully applied to trace analysis in environmental, biomedical, and food applications. This technique is a very sensitive tool for the determination of volatile and semi-volatile compounds. However, the combination of SBSE with liquid desorption and HPLC is also possible, providing an attractive approach for the analysis of thermolabile solutes as well as higher molecular mass compounds (Baltussen et al. 1999, Tienpont et al. 2002, David et al. 2003). A SBSE/LC-UV method for the determination of antidepressants (mirtazapine, CIT, PXT, duloxetine, FL, and SER) in plasma samples with extractions based on both adsorption polypyrrole and sorption PDMS mechanisms have been developed by Melo et al. (2009). The LOQ values ranged from 20 ng/ml to 50 ng/ml.

Liquid phase microextraction (LPME)

LPME has been combined with LC and CE, in addition to the general way used by coupling to GC, and has been applied to various matrices, including biological, environmental, and food samples. In recent years, a porous-walled polypropylene hollow fiber has been used to support the organic phase in the pores of the wall while holding the second aqueous phase in the lumen. According to the hollow fiber-LPME method, a porous polypropylene hollow fiber acts as the interface between the donor contaminated sample and micro-volumes of the acceptor extractant phase solution. The advantages of using this microporous membrane include protection of the acceptor phase as well as efficient sample microfiltration through the pores of the hollow fiber (Zhu et al. 2001, Psillakis and Kalogerakis 2003, Pedersen-Bjergaard et al. 2005, Ratola et al. 2008).

Pedersen-Bjergaard et al. (2005) presented the extractability of 58 different basic drugs, including SSRIs, by three-phase LPME. 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 fiber (organic phase), and into 15 µl of 10 mM HCl (acceptor solution) present inside the lumen of the hollow fiber. Compounds with a calculated solubility below 1 mg/ml at pH 2 were poorly recovered and remained principally in the organic phase. For these drugs, two-phase LPME could be used as an alternative technique, where the aqueous acceptor phase is replaced by an organic solvent. In the solubility range of 1–5 mg/ml, most drugs were effectively extracted (recovery >30%), whereas drugs belonging to the solubility range of 5–150 mg/ml were all extracted with recoveries above 30% by three-phase LPME.

Esrafili et al. (2007) presented a hollow fiber-based LPME combined with a HPLC-UV method for extraction and determination of three antidepressant drugs (amitriptyline, imipramine, and SER) in urine, plasma, and tap water samples. The extraction was performed owing to pH gradient between the inside and outside of the hollow fiber membrane with calibration curves obtained in the range of 5–500 µg/l.

Pressurized liquid extraction (PLE)

PLE involves the use of liquid solvents at elevated temperatures (40–200°C) and pressures (1000–2500 psi). Under these conditions, solvents have enhanced solvation power and have increased the extraction rates. A LC-atmospheric pressure chemical ionization (APCI)-MS/MS method for the determination of residues of PXT and FL, and its active metabolite, norfluoxetine, in fish tissue including extraction of tissue by PLE is presented by Chu and Metcalfe (2007). The LOQ values were 0.24, 0.07, and 0.14 ng/g wet weight for PXT, FL, and norfluoxetine, respectively. The procedure for sample preparation includes extraction of tissue by PLE, followed by clean-up on a mixed-mode SPE cartridge, Oasis MCX. With the optimized method, matrix interferences were reduced and recoveries >85% were obtained.

Liquid-solid extraction (LSE)

In LSE, a solvent is added to a solid. Insoluble material can be separated by gravity or vacuum filtration, and soluble material is extracted into the solvent. A sequence of solvents, of varying polarity or pH, can be used to separate complex mixtures into groups.

Reymond et al. (1993) presented methods based on GC and GC-MS for the determination of levels of CIT, desmethylcitalopram, and didesmethylcitalopram in plasma using LSE. The demethylated amines are derivatized with trifluoroacetic anhydride, and the acid metabolite with methyl iodide. The LOQ values were 1 ng/ml for CIT and desmethylcitalopram and 2 ng/ml for the other metabolites. Also, an isocratic reversed-phase HPLC for simultaneous determination of FLU and PXT in human serum involving a pre-column technique for the on-line LSE was reported by Bagli et al. (1997) with direct injection of serum samples and for their preconcentration.

In summary, there are many different ways to extract SSRIs from pharmaceutical or biological sample matrices. However, sometimes compromising issues such as screening methods, time and cost issues are required so that a simple extraction system might be more suitable than a more complex extraction with higher recoveries.

Analytical methods

Many analytical methods for the detection and quantitation of SSRIs and their metabolites in pharmaceutical formulations and biological fluids have been reported. When classified upon separation methodology, the analytical methods can be categorized into chromatographic and non-chromatographic. Included within the chromatographic methods are GC, HPLC coupled with various detections by UV, fluorescence detection (FLD), mass MS, DAD, and capillary electrophoresis (CE). Included within the non-chromatographic methods are electroanalytical methods and spectrometric methods.

Chromatographic methods

Several analytical methods have been developed for the analysis of SSRIs and in some cases also their metabolites in biological fluids. Most of these methods are based on reversed-phase HPLC coupled to UV, MS detection and on FLD. GC is also applied in SSRI quantification coupled to nitrogen-phosphorous, flame ionization detector, and MS.

Gas chromatographic (GC) methods GC provides high separation efficiency, fast analysis, automation capabilities and generally requires a small sample injection volume with commercially available specific or universal detectors. In typical GC analysis, a defined sample volume is injected into the preheated inlet and almost instantaneously volatilized. The volatilized compounds are moved by the carrier gas towards the column for separation. The separation process is based on their relative affinity to the stationary phase of the column (capillary or packed) under a thermal gradient program (Korytar et al. 2002, Santos and Galceran 2002, Rompa et al. 2003, Marriott 2005). GC is a technique frequently used for the determination of SSRIs. Determination of low volatile compounds can be improved by derivatization. For derivatization, acid anhydrides, benzyl halides and alkyl chloroformates are preferred. Reagents most often used for derivatization of SSRIs are trifluoroacetic anhydride (Reymond et al. 1993), (*S*)-(-)-*N*-trifluoroacetylprolyl chloride (Eap et al. 1996), trifluoroacetic anhydride (Addison et al. 1998), heptafluorobutyric anhydride (Lai et al. 2000, Kim et al. 2002), pentafluorobenzyl carbamate (Leis et al. 2002), ethyl chloroformate (Fernandes et al. 2008), *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide trimethylsilyl (Pujadas et al. 2007), and heptafluorobutyrylimidazole derivatives (Wirth et al. 1997).

Prior to the application of LC-MS, GC-MS had been the most commonly used technique for the analysis of SSRIs and their metabolites in biological fluid. Even now, high-resolution GC directly combined with the wide range of low-cost bench-top MS instruments remains the most attractive technique. There are GC methods which include several detection systems available for the determination of SSRIs. They can be divided into four different groups according to their use: nitrogen-phosphorous detection, flame ionization detector, electron capture detector, GC-MS or GC-MS/MS.

Nevado et al. (2005) reported a capillary gas chromatographic using flame ionization detector method for the determination of FL, FLU, and clomipramine without derivatization steps in several pharmaceutical formulations. LOD and LOQ values were 10.1, 105.3, and 40.0 μg/l and 33.5, 300.0, and 80.0 μg/l for FL, FLU, and clomipramine, respectively. Recoveries were 100.37±0.45 for FL, 98.59±0.58 for FLU, and 100.13±0.32 for clomipramine.

Ulrich (2003) developed direct enantioselective assay of FL and norfluoxetine in human plasma or serum by two-dimensional capillary gas-liquid chromatography by using three-step LLE for sample preparation. LOD values ranged between 1.5 and 6 ng/ml, respectively. Fluvoxamine and nisoxetine were used as internal standards. Linearity ranged between 5 and 250 ng/ml for (*R*)- and (*S*)-FL as well as 15 and 250 ng/ml for (*R*)- and (*S*)-norfluoxetine. Intra-day precision ranged between 5.4% and 12.7% at plasma levels of 25, 100, and 200 ng/ml for the four enantiomers, and inter-day precision ranged between 5.3% and 9.1% at 100 ng/ml.

A method for determination of PXT levels in human plasma by using GC-electron capture detection is presented by Lai et al. (2000). LOD and LOQ values for PXT were 8.5 ng/ml and 28.4 ng/ml. Fontanille et al. (1997) reported a GC method with nitrogen-phosphorus detection for direct analysis of FL and norfluoxetine in plasma including an extraction with a mixed organic solvent and injection into a capillary GC with an OV-1 fused-silica column coupled to a nitrogen-phosphorus detector. The calibration curves were linear over the range 5–3000 ng/ml with detection limits of 0.3 and 2 ng/ml for FL and norfluoxetine, respectively.

GC-based methods provide high resolution and low LOD values, but they are labor-intensive and costly. A typical approach to overcome this limitation is the application of derivatization protocols using various reagents in organic medium. In this way, the analytes volatility and thermal stability are increased and the mass fragmentation pattern is improved when MS detectors are employed.

A capillary GC-MS in selected-ion monitoring (SIM) mode for the analysis of CIT, FL, and all of their metabolites in urine samples including an optimized SPE with LOD values between 0.7 ng/l for CIT and 5.7 ng/l for FL was reported by Nevado et al. (2006a). The GC-MS method with SIM shows significantly lower detection limits than all of the methods previously discussed, by approximately 1000. Imipramine hydrochloride was used as internal standard. The biological samples were treated to achieve the extraction of FL, norfluoxetine (NFL), CIT, and its metabolites by a SPE procedure using a reversed-phase cartridge C18. The best recoveries were obtained with phosphate buffer, methanol:water (30:70, v/v) and subsequent elution with methanol. An enrichment factor of 10 was provided by this extraction-preconcentration procedure for the analytes in the biological samples (5 ml of urine samples/0.5 ml of final extract).

Eap et al. (1996) developed a GC-MS for the simultaneous determination of the plasma concentrations of FLU and of the enantiomers of FL and norfluoxetine after derivatization with the chiral reagent, (*S*)-(-)-*N*-trifluoroacetylprolyl chloride.

LOQ values were 2 ng/ml for FLU and 1 ng/ml for the (*R*)- and (*S*)-enantiomers of FL and norfluoxetine.

A GC-MS method for the determination of FL, and its major metabolite, norfluoxetine involving SPE followed by acetylation with trifluoroacetic anhydride and analysis of the derivatives using SIM with LLOQ of 1.0 ng/ml is presented by Addison et al. (1998).

Leis et al. (2002) presented an improved sample work-up and derivatization procedure to the pentafluorobenzyl carbamate derivative in one step for the quantitative determination of PXT negative ion chemical ionization in human plasma by GC-MS subsequently analyzed without any further purification with the lower limit of detection (LLOD) of 0.2 ng/ml plasma. LLOQ was 0.469 ng/ml plasma for pharmacokinetic measurements. In addition, Kim et al. (2002) described a method for the determination of SER in human plasma by using GC-MS with the SIM mode using single LLE at alkaline pH after deproteinization of plasma protein and perfluoroacylation with heptafluorobutyric anhydride. The LOD value was 0.1 ng/ml and recovery was 80–85%.

A method obtained in the order of pg/ml of LOD and LOQ was presented by Fernandes et al. (2008) with *in situ* derivatization by using ethyl chloroformate on-line coupled to GC-MS for the analysis of FL by using SBSE in plasma samples. The developed method using liquid desorption showed linearity of r^2>0.99 and precision of RSD<15%. The LOQ and LOD values with liquid desorption were 30.0 and 10.0 pg/ml, respectively. LOQ and LOD values with thermal desorption were 0.37 and 0.46 pg/ml, respectively. Thermal desorption also demonstrated precision (RSD<12%).

Pujadas et al. (2007) developed a GC-MS method for identifying and quantifying psychoactive drugs in oral fluid including antidepressant drugs (amitryptiline, PXT, and SER) using the SPE procedure and derivatization with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide. LOQ was between 0.9 and 44.2 ng/ml oral fluid for the different analytes. Wille et al. (2007) reported a GC-MS method for the simultaneous determination of antidepressants (mirtazapine, viloxazine, venlafaxine, trazodone, CIT, mianserin, reboxetine, FL, FLU, SER, maprotiline, melitracen, and PXT) and their active metabolites in plasma by using different ionization modes with sample preparation consisting of a strong cation exchange mechanism and derivatization with heptafluorobutyrylimidazole. LOQ ranged between 5 and 12.5 ng/ml in electron impact and positive ionization and 1 and 6.25 ng/ml in negative ionization.

Table 1 shows the distribution of the principal GC determination (Reymond et al. 1993, Goodnough et al. 1995, Eap et al. 1996, Fontanille et al. 1997, Wirth et al. 1997, Addison et al. 1998, Lefebvre et al. 1999, Lai et al. 2000, Kim et al. 2002, Leis et al. 2002, Ulrich 2003, Lamas et al. 2004, Nevado et al. 2005, 2006a,b, Wille et al. 2005, 2007, 2009, Pujadas et al. 2007, Fernandes et al. 2008) strategies established for the quantification of SSRI drugs.

HPLC methods Different stationary phases have been employed for the bioanalysis of SSRIs. In contrast to "normal phase", the "reversed phase" HPLC mode of separation is performed on moderated as nonpolar stationary phases using almost exclusively packed columns with silica gel chemically bonded with OS or ODS groups. Most platforms use reversed-phased columns with either C8 or C18 material. Additionally, a CN column, a phenyl-hexyl column and a polar reversed-phase are described (Tables 2–4).

Currently, separation and analyses of SSRIs by HPLC are carried out with the mobile phase of methanol (MeOH) or acetonitrile (CH_3CN, ACN), phosphate buffer solution and water containing a small amount of different additives (acid, base, neutral salts), such as formic acid (HCOOH), trifluoroacetic acid (TFA), acetic acid (AcOH), diethylamine (DEA), triethylamine (TEA), phosphoric acid (H_3PO_4), ammonium acetate (NH_4AcO), and ammonium formate. The pH range covers (close to) neutral (pH 6–7) and acidic (in the pH range of 1.9–5) conditions depending on the detection method and on the SSRI drugs of interest (Tables 2–4).

For preparative purification of SSRIs, a column size of 150×4.6 mm i.d. can be a good compromise between cost and sample-loading capacity. HPLC columns with i.d. ranging from conventional (4.6 mm), narrow-bore (2.1 mm) are used for SSRIs analyses. Tables 2–4 show representative HPLC chromatograms of SSRIs. The flow of the eluting solvents is usually 0.2–1.5 ml/min, either in isocratic or in gradient modes, but flows up to 4.0 ml/min have also been used.

HPLC methods with UV and DAD A growing number of publications describe the use of GC-MS, HPLC-MS and HPLC-MS/MS for the determination of SSRIs and their metabolites, showing that these are techniques of choice. However, satisfactory results can also be obtained using HPLC-UV, which is cheaper and less complicated. HPLC-UV methods are very popular for the determination of SSRIs in biological samples. UV detection (Knoeller et al. 1995, Foglia et al. 1997, Holladay et al. 1997, Alvarez et al. 1998, Meineke et al. 1998, Maya et al. 2000, Skibinski et al. 2000, Molander et al. 2001, Frahnert et al. 2003, Gatti et al. 2003, Llerena et al. 2003, Ohkubo et al. 2003, Zainaghi et al. 2003, Barri and Jonssön 2004, Li et al. 2004a,b, El-Gindy et al. 2006, Mandrioli et al. 2006, Önal and Öztunç 2006, Saracino et al. 2006, Chaves et al. 2007, Esrafili et al. 2007, Fernandes et al. 2007, Malfara et al. 2007, Shah et al. 2007, Ulu 2007, Cruz-Vera et al. 2008, Liu et al. 2008, Silva et al. 2008, Chaves et al. 2009, Melo et al. 2009) or DAD (Ferretti et al. 1998, Tournel et al. 2001, Berzas et al. 2002, Duverneuil et al. 2003, Vivekanand et al. 2003, Sabbioni et al. 2004, Reddy et al. 2007, Unceta et al. 2008) has been used in most analytical methods for the determination of SSRIs. SSRIs have a commonly absorption maximum in the range between 220 nm and 300 nm, depending on the composition of mobile phase, especially in the presence of buffers of various pH values. The wavelength applied most often has been 230 nm, then 205 nm or 254 nm (Table 2). Generally, HPLC-UV assays for the determination of SSRIs in biological materials were developed with LOQ values ranging between 2 and 10 ng/ml. Mobile phases generally include acetonitrile, methanol, water, and acetate tampons. One limitation of UV detection is the relatively poor sensitivity and the requirement that good

detection limits require a high-absorbing UV complex for good sensitivity in the direct detection mode.

Applications of HPLC-UV and HPLC-DAD for the determination of SSRI drugs over recent years are listed in Table 2.

HPLC methods with FLD The necessity of determining very low levels of the SSRIs and their native fluorescence led us to the choice of HPLC with FLD as a sensitive and selective method for this purpose. The possibility of using HPLC-FLD for SSRI determination therefore depends on the availability of suitable derivatizing reagents. The amino group in the structure of SSRIs such as FL facilitates the derivatization with acyl chloride reagents such as dansyl chloride. The nonchiral fluorogenic reagents having a 2,1,3-benzoxadiazole (benzofurazan) moiety possess long excitation and emission wavelengths, which minimize interference by native fluorescence from endogenous substances so that highly selective and sensitive detection can be achieved (Guo et al. 2003). Pre-column derivatization with dansyl chloride (Lucca et al. 2000), 4-chloro-7-nitrobenzofurazan (Bahrami and Mohammadi 2007), 4-fluoro-7-nitro-2,1,3-benzoxadiazole (Fukushima et al. 2004, Higashi et al. 2005), 4-(*N*-chloroformylmethyl-*N*-methyl)amino-7-nitro-2,1,3-benzoxadiazole (Guo et al. 2003) as fluorescence label has been reported for HPLC-FLD analysis. FLD has also been applied to the chiral separation and analysis of the enantiomers of SSRIs. The pre-column chiral derivatization with (-)-(*R*)-1-(1-naphthyl)ethyl isocyanate has been reported for HPLC-FD analysis (Peyton et al. 1991, Millan et al. 2008).

When the pre-column derivatization of SSRIs was performed using dansyl chloride as a fluorescent reagent a large sample volume (1 ml) was required. 4-(*N*-Chloroformyl-methyl-*N*-methyl)-amino-7-nitro-2,1,3-benzoxadiazole as the reagent was adopted to measure levels of SSRIs in a volume of rat plasma as small as 100 µl (Guo et al. 2003). However, a disadvantage of this method is the reaction time, which takes approximately 2 h at 60°C derivatization and was time-consuming. Also, 4-fluoro-7-nitro-2,1,3-benzoxadiazole as the reagent was adopted to measure levels of SSRIs in rat plasma in 100 µl of plasma at 60°C for 5 min and injected into HPLC. Retention times of FLU and an internal standard (propafenone) derivative were 15.5 and 13.5 min, respectively (Guo et al. 2003). The detection wavelengths are commonly carried out by FD using nm in the range excitation of 224–470 nm and emission of 300–500 nm. The HPLC-FLD method using 4-chloro-7-nitrobenzofurazan as a pre-column derivatization agent and an internal standard (FL) could be very convenient for bio-analytical purposes with its LOQ value of 0.5 ng/ml.

A HPLC-FLD method for the quantitation of citalopram in human plasma was reported by Macek et al. (2001) involving LLE of CIT with hexane-isoamyl alcohol (98:2, v/v) and back-extraction of the drug to 0.02 M hydrochloric acid with LOQ of 0.96 ng/ml using 1 ml of plasma.

Shin et al. (1998) developed a reversed-phase HPLC method for the measurement of PXT in human plasma by using only one-step extraction of PXT and internal standard (dibucaine) with chloroform. Intra- and inter-day variation coefficients were in the range of 1.9–9.4% and 2.3–13.3%, respectively. LOQ and LOD values were 0.5 and 0.2 ng/ml. Another HPLC method for the determination of CIT and two metabolites was reported by Raggi et al. (2003) in human plasma by using SPE with 1 ml MeOH. LOQ was 1.5 ng/ml for CIT and desmethylcitalopram and 2.0 ng/ml for didesmethylcitalopram. Also, Raggi et al. (1999) presented a HPLC method for the determination of FL and its main metabolite norfluoxetine in human plasma with LLE and the internal standard (PXT). The LOD value was 1 ng/ml for both fluoxetines.

A chiral LC method for separation of the enantiomers of CIT and its two *N*-demethylated metabolites by using alprenolol as internal standard in human plasma was reported by Kosel et al. (1998). LOQ values were 5 ng/ml for each enantiomer of CIT and demethylcitalopram. Matsui et al. (1995) presented a HPLC method with a column-switching technique for simultaneous determination of CIT and its four metabolites in plasma by direct plasma injection. Unceta et al. (2007) developed a reversed-phase LC method for determination of FL and norfluoxetine racemic mixtures. LOD values were 3.2 and 2.1 ng/ml in plasma, and 31.5 and 26.1 ng/g in brain tissue for FL and NFL, respectively. Also, a HPLC method for the simultaneous determination FL and the *n*-desmethyl metabolite, norfluoxetine in rat brain microdialysis samples by using pre-column derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole was reported by Fukushima et al. (2004). The LOD values for FL and norfluoxetine were approximately 17 and 5 nM (23 and 7 fmol).

Applications of HPLC combined with FLD including chromatographic conditions and validation data for analysis of SSRI drugs reported in recent years are summarized in Table 3 (Peyton et al. 1991, Matsui et al. 1995, Kosel et al. 1998, Shin et al. 1998, Kristoffersen et al. 1999, Lopez-Calull and Dominguez 1999, Raggi et al. 1999, Lacassie et al. 2000, Lucca et al. 2000, Macek et al. 2001, Waschgler et al. 2002, Guo et al. 2003, Raggi et al. 2003, Fukushima et al. 2004, Higashi et al. 2005, Meng and Gauthier 2005, Vlase et al. 2005, Bahrami and Mohammadi 2007, Mandrioli et al. 2007, Unceta et al. 2007, Vergi-Athanasiou et al. 2007, Millan et al. 2008).

HPLC methods with mass spectrometric detection HPLC-MS working with SIM acquisition mode in positive electrospray ionization (ESI) and MRM are currently preferred for detection of SSRIs in biological materials. The more reliable identification can be obtained by LC coupled to tandem MS with a triple quadrupole. For mass spectrometric detection, the analytes have to be ionized first. A variant of ESI using a heated gas for desolvation of the eluent is called turbo ion spray (TIS) or heated electrospray ionization. APCI ionization is based on a principle generating ions via chemical ionization in the gas phase. APCI-MS, including electrospray (ESI) and APCI in selected reaction monitoring (SRM) or MRM mode, has been established as a sensitive and selective analytical technique for SSRIs.

Different ionization techniques were used for MS analyses of SSRIs in combination with reversed-phase HPLC, including APCI (Shen et al. 2002, Kollroser and Schober 2003, Chen et al. 2006, Chu and Metcalfe 2007), ESI (Moraes et al. 1999, Zhu and Neirinck 2002, Gutteck and Rentsch 2003, Segura et al. 2003, Souverain et al. 2003, Weng and Eerkes 2003, Pistos et al. 2004, Reubsaet and Bjergaard 2004, Singh et al. 2004, Djordjevic et al. 2005, He et al. 2005, Juan et al. 2005, Massaroti et al. 2005, Fernandes et al. 2006, Kirchherr and Kuhn-Velten 2006, Smyth et al. 2006, Castaing et al. 2007, de Castro et al. 2007, 2008, Doherty et al. 2007, Jia et al. 2007, MacLeod et al. 2007, Queiroz et al. 2007, Frison et al. 2008, Santos-Neto et al. 2008, Franceschi et al. 2009, Saber 2009), and thermo ion spray (Sutherland et al. 2001, Green et al. 2002, Li et al. 2002, Jain et al. 2005, Patel et al. 2009).

APCI is described for PXT, FL and its active metabolite, norfluoxetine in fish tissue including extraction of tissue by PLE by Chu and Metcalfe (2007). A specific and sensitive HPLC-APCI-MS/MS method in SRM mode was developed by Kollroser and Schober (2003) for the rapid identification and quantitative determination of CIT, FLU, and PXT in human plasma. After dilution with 0.1% formic acid, plasma samples were injected into the LC-MS/MS system. The inter- and intra-assay coefficients of variation for all compounds were <11%. The total analysis time was 6 min per sample. LLOQ values for CIT, FLU, and PXT were 20, 20, and 10 µg/ml. LOD values were 5 µg/ml for all analytes.

Shen et al. (2002) reported a high-throughput sample preparation procedure using LLE in a 96-well plate format in conjunction with LC-APCI-MS/MS in the MRM mode for quantification of FL enantiomers in human plasma. After addition of internal standard and ammonium hydroxide, samples were extracted with ethyl acetate. The organic extract was evaporated to dryness and reconstituted in methanol. Adequate separation of FL enantiomeric pairs (resolution of 1.17) was achieved on a vancomycin column eluted with methanol containing 0.075% (by weight) ammonium trifluoroacetate.

Chen et al. (2006) developed a more sensitive LC-APCI-MS/MS method for the determination of SER with a LLOQ value of 0.10 ng/ml using only 0.25 ml of plasma. The analyte and internal standard (diphenhydramine) were extracted with 3 ml of diethyl ether/dichloromethane (2:1, v/v) from 0.25 ml plasma, then separated on a Zorbax Eclipse XDB C18 column using methanol/water/formic acid (75:25:0.1, v/v/v) as the mobile phase. The triple quadrupole mass spectrometry was applied via an APCI source for detection. The method was linear over the concentration range of 0.10–100 ng/ml.

Once the ions have entered the mass spectrometer, detection takes place. This can be accomplished by means of selecting the molecular ion in SIM, but preferably a typical fragment ion is selected in MRM. The methods using SIM describe a lower LOQ than the methods using MRM (Table 4). Frison et al. (2008) developed a LC-MS method SIM with positive ion ESI for the determination of CIT, escitalopram, and their demethylated metabolites in 10 mg hair samples including LLE with diethyl ether/dichloromethane using clomipramine-d3 as an internal standard with a LOQ value of 25 pg/mg for all drugs. Linearity ranged between 25 and 2000 pg/mg. Flow rate was 50 µl/min and the injection volume was 8 µl. The acceptance range for recovery was set at 70–120% for both the internal standard and the analyte.

ESI-MS is currently the most common LC-MS method in SSRI analyses. Compared to APCI-LC-MS, ESI-MS has a fairly high sensitivity and is associated with lower background. Table 4 presents a summary of some representative LC-MS methods for SSRI analyses, and the general thought is that for MS, acidic eluents are most appropriate as they are able to donate a proton. Therefore, acidic eluents with ammonium acetate, ammonium formate, formic acid, acetic acid, and even TFA are often described for the bioanalysis of SSRIs.

Franceschi et al. (2009) reported an isocratic reversed-phase HPLC-ESI-MS/MS method in MRM for simultaneous quantification of FL and its major active metabolite in serum samples by using extraction in a simple three-step LLE ml/min with run time of less than 5 min for each injection. Linearity for FL and its major active metabolite ranged between 0.3 and 50 ng/ml. The LOQ value was 0.17 and 0.18 ng/ml for FLU and N-FLU, respectively, and the LOD value was 0.06 ng/ml for both analytes.

Zhu and Neirinck (2002) presented a LC-MS/MS method for the determination of PXT in human plasma involving a LLE with cyclohexane-ethyl acetate at a flow rate of 0.22 ml/min with total run time of 2.2 min. The LLOQ value was 0.2 ng/ml. The standard curve was linear over a working range of 0.2–50 ng/ml and absolute recovery was 70.8% for PXT and 84.1% for the internal standard with accuracy of inter-assay and intra-assay accuracy ranging between 24.8% and 20.5% and 23.4% and 4.8%, respectively. LC-MS/MS equipped with an ion spray source was selected and used as Q3 ion to be monitored.

A narrow-bore LC-MS method for the quantification of CIT in human plasma using internal standard (imipramine) and LLE with a mixture of hexane-heptane-isopropanol (88:10:2, v/v/v) was developed by Pistos et al. (2004) with run time of 10.0 min. Linearity ranged between 0.50 and 250 ng/ml (r^2>0.996) with LOQ of 0.50 ng/ml.

Kirchherr and Kuhn-Velten (2006) developed another HPLC-MS method in the MRM mode for the simultaneous determination of some drugs including CIT, FL, FLU norfluoxetine, PXT, and SER in a small sample volume of 0.1 ml of serum which requires only protein precipitation and stepwise dilution for sample preparation. LOQ values for CIT, FL, FLU, PXT, and SER were 1.00, 2.17, 1.17, 1.07, and 0.70 ng/ml.

Li et al. (2002) also developed a LC-TIS-MS/MS method in MRM mode (*m/z* 314–44) for the determination of FL and its main active metabolite norfluoxetine in human plasma using deuterated FL as internal standard with a run time 5 min. The method was validated and concentration ranged from 0.27 to 22 ng/ml with coefficients of correlation >0.999. The LOD value was 0.1 ng/ml for plasma FL and norfluoxetine.

Sutherland et al. (2001) presented another LC-MS/MS method in the MRM mode for the simultaneous determination of FL and its major active metabolite norfluoxetine in plasma

with extraction from alkalized plasma with hexane-isoamyl alcohol (98:2, v/v) and back-extraction into formic acid (2%). TIS ionization was used for ion production with run time of 2.60 min and retention times of 2.35 and 1.97 min for FL and norfluoxetine, respectively. The mean recoveries for FL and norfluoxetine were 98% and 97%, respectively, with LLOQ set at 0.15 ng/ml for the analyte and its metabolite.

Green et al. (2002) developed a LC-MS method with MRM using the TIS interface operating in positive ion mode. For the determination of FL and norfluoxetine in human plasma, automated SPE on Oasis HLB cartridges with run time of 4 min was used. The method was linear over the range of 0.5–50 ng/ml (using a sample volume of 0.5 ml) with a LLOQ value of 0.5 ng/ml.

Patel et al. (2009) developed a LC-MS/MS method in positive ion and MRM mode with TIS for simultaneous determination of SER its primary metabolite (*N*-desmethyl SER) in human plasma using FL as internal standard by applying LLE in methyl tert-butyl ether. Run time was 2.5 min. Linearity ranged from 0.5 to 150 ng/ml for SER and *N*-desmethyl sertraline with mean correlation coefficient of 0.9993 and 0.9980, respectively. LLOQ was 0.5 ng/ml.

Santos-Neto et al. (2008) reported a capillary restricted access media LC-MS/MS in MRM mode with positive ion ESI method for simultaneous analysis of five antidepressant drugs (FL, imipramine, desipramine, amitriptyline, and nortriptyline) by using direct injection of biofluids with a total run time of 8 min.

Juan et al. (2005) presented a HPLC-ESI/MS in the selected ion recording (SIR) mode method for simultaneous determination in human plasma of FL, CIT, PXT, and venlafaxine, and intra- and inter-day variation coefficients were less than 15.0%. The LOD values were 0.5, 0.3, 0.3, and 0.1 ng/ml for FL, CIT, PXT, and venlafaxine, respectively.

He et al. (2005) developed a HPLC-ESI/MS assay for the determination of SER in human plasma using zaleplon as the internal standard, SER 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 SIM mode of MS. The calibration curve of SER in plasma exhibits a linear range from 0.5 to 25.0 µg/l. The LOQ value was 0.5 ng/ml.

To obtain high selectivity and sensitivity, MS/MS techniques employ specific SRM conditions, which are convenient, especially in bioanalytical applications. Jia et al. (2007) reported a LC-MS/MS method for the determination of SER hydrochloride in human plasma, using PRX as internal standard. SER hydrochloride was chromatographed by using a Discovery C18 column. The mobile phase consisted of 0.1% formic acid-acetonitrile (50:50). ESI source was applied and operated in the positive ion mode. SRM mode with the transitions of *m*/*z* 306.0–274.9 and *m*/*z* 330.1–191.9 was used to quantify SER hydrochloride and the internal standard, respectively. Detection limit was 0.334 ng/ml.

Smyth et al. (2006) reported the electrospray ionization and ion trap mass spectrometry (ESI-MS*n*) and electrospray ionization quadrupole-time-of-flight tandem mass spectrometry (ESI-QToF-MS/MS) behavior of antidepressant drugs (CIT, FL, mirtazapine, PRX, SER, and venlafaxine). Following Soxhlet extraction, the presence of SER 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* 306–275). The concentration of SER in this hair sample was calculated to be 1.90 ng/mg. This procedure was repeated for the identification and quantification of paroxetine in a hair sample and yielded a result of 0.25 ng/mg.

Doherty et al. (2007) developed methods on HPLC-ESI/MS, ESI–MS*n*, GC-flame ionization detection and polarographic behavior in the determination of selected drug compounds in hair samples. GC-flame ionization detection was carried out on a Zebron ZB-5 (Phenomenex, Macclesfield, Cheshire, UK) column (30 m×0.25 µm). In polarographic analysis, the current axis was set from 0 to -160 nA and cathodic scans were initiated from -0.4 V to -1.6 V using 0.1 M acetate buffer (pH 4.47) as background electrolyte and a standard Ag/AgCl 3 M KCl electrode system. Applications of HPLC combined with various MS detectors including subjected sample, analyte, chromatographic conditions and validation data for analysis of SSRIs reported in recent years are summarized in Table 4 (Moraes et al. 1999, Sutherland et al. 2001, Green et al. 2002, Li et al. 2002, Shen et al. 2002, Zhu and Neirinck 2002, Gutteck and Rentsch 2003, Kollroser and Schober 2003, Segura et al. 2003, Souverain et al. 2003, Weng and Eerkes 2003, Pistos et al. 2004, Reubsaet and Bjergaard 2004, Singh et al. 2004, Djordjevic et al. 2005, Hattori et al. 2005, He et al. 2005, Jain et al. 2005, Juan et al. 2005, Massaroti et al. 2005, Chen et al. 2006, Fernandes et al. 2006, Kirchherr and Kuhn-Velten 2006, Kovacevic et al. 2006, Sauvage et al. 2006, Shinozuka et al. 2006, Smyth et al. 2006, Castaing et al. 2007, Chu and Metcalfe 2007, de Castro et al. 2007, 2008, Doherty et al. 2007, Hattori et al. 2007, Jawecki 2007, Jia et al. 2007, MacLeod et al. 2007, Queiroz et al. 2007, Frison et al. 2008, Santos-Neto et al. 2008, Franceschi et al. 2009, Patel et al. 2009, Saber 2009).

HPLC methods with other detection Greiner et al. (2007) developed a method for the determination of citalopram and escitalopram together with their active main metabolites desmethyl (es-)citalopram in human serum by column-switching HPLC and spectrophotometric detection (210 nm) with haloperidol as internal standard. Sample clean-up was carried on a LiChrospher CN 20 µm pre-column using 8% acetonitrile in deionized water. Then, separation was performed on the analytical column (LiChrospher CN 5 µm) using phosphate buffer (8 mmol/l, pH 6.4)-acetonitrile (50:50, v/v) as mobile phase at a flow rate of 1.5 ml/min. LOD for (es-)-citalopram was 6 ng/ml with inter-day variation of <9.05% for citalopram and <14.88% for desmethylcitalopram.

Schatz and Saria (2000) reported a HPLC method with coulometric detection for the simultaneous determination of PXT, risperidone, and its main metabolite 9-hydroxyrisperidone in human plasma involving a multistep SPLE. LLOD was 1 ng/ml in the range of 5–500 ng/ml. The drugs were separated on a cyano column.

Thin-layer chromatography (TLC) method TLC was an earlier chromatographic method. It is simple, cheap, and allows the screening of a larger number of samples; however, it has lower sensitivity, precision, accuracy, and reproducibility than other methods.

LC and high-performance thin-layer chromatography (TLC) in the absorption mode methods for the simultaneous estimation of FL and olanzapine in pure powder and tablet formulations was reported by Shah et al. (2007). Linearity ranged between 100 and 800 and 400 and 3200 ng/spot, respectively. LOD values for olanzapine and FL were 3.429 and 13.37 μg/ml, and LOQ values were 10.392 and 40.53 μg/ml with LC, respectively. LOD values for olanzapine and FL were 33.13 and 132.08 ng/spot, and LOQ values were 100.42 and 400.25 ng/spot with TLC, respectively.

Gondova et al. (2008) presented a TLC method using densitometric detection in the reflectance mode at 240 nm for the simultaneous analysis of CIT, SER, FL, and FLU. The separation was achieved on silica gel 60 F_{254s} TLC plates using acetone-benzene-ammonia (50:45:5, v/v/v) as mobile phase. Linearity was in the range of 500–5000 ng/spot for all compounds. The LOD value for FL, FLU, and SER was 40 ng/spot for CIT and 50 ng/spot, respectively. Sharma et al. (2007) developed a HPTLC densitometric method for the determination of PXT hydrochloride in tablet dosage forms with LOD and LOQ for PXT hydrochloride of 60 ng/spot and 160 ng/spot, respectively. Chromatographic separation was carried out using precoated silica gel 60 F_{254} aluminum sheets (10×10 cm, E. Merck, Cat. No. 1.05554.0007) with mobile phase consisting of ethyl acetate-acetic acid-water (7.5:1.5:1, v/v). Quantification was carried out at 296 nm. Linearity for PXT hydrochloride ranged between 160 and 960 ng/spot with a correlation coefficient of 0.995.

Non-chromatographic methods

Electrophoretic methods CE is an attractive approach for the separation of SSRIs, because of its high efficiency and rapid separation. The main advantages of capillary electrophoretic techniques include high separation efficiencies, minute amounts of sample are required; it is easily automated and consumes limited amounts of reagents, generating low volumes of waste. In recent years, capillary zone electrophoresis (CZE) has gained popularity as an analytical tool for SSRIs. Although some successful examples have been reported, most CE applications in SSRI analyses require sensitivity to be enhanced by using more specific detection systems (e.g., MS) and on-line or off-line sample preconcentration to increase sample solute concentration. Table 5 contains a summary of the applications of electrophoretic techniques to the analyses of SSRIs.

The different CE modes used for the separation of SSRIs are CZE and micellar electrokinetic chromatography (MEKC). Table 5 outlines the optimal electrophoretic conditions for the analyses of SSRIs in various biological samples. For ionic SSRIs, MEKC separations are based on both degree of ionization and hydrophobicity. MEKC results are consistent with results obtained using HPLC or LC-MS for the determination of SSRIs.

Capillary electrophoresis method Andersen et al. (2003) presented a chiral CE system for simultaneous enantiomer determination of CIT and its pharmacologically active metabolite desmethylcitalopram with LPME based on a rod-like porous polypropylene hollow fiber. The LOQ value was <11.2 ng/ml.

A CE method for separation of SER FLU and FL with fully integrated SPE *in situ* within a fused silica capillary from either butyl methacrylate-*co*-ethylene dimethacrylate or 3-sulfopropyl methacrylate-*co*-butyl methacrylate-*co*-ethylene dimethacrylate was developed by Halvorsen et al. (2001) developed a LPME and capillary electrophoresis of CIT and its main metabolite *N*-desmethylcitalopramin human plasma with extraction from 1 ml plasma samples through hexyl ether. Prior to extraction, the samples were made strongly alkaline and CIT and its main metabolite were enriched by a factor of 25–30. LOQ values for CIT and its main metabolite in plasma were 16.5 ng/ml and 18 ng/ml, respectively.

A CE method with a laser-induced fluorescence detection (λ=488 nm) for the analysis of SER together with its main metabolite *N*-desmethylsertraline in human plasma was developed by Musenga et al. (2007). It is based on a SPE procedure employed for biological sample pretreatment, followed by a derivatization step with fluorescein isothiocyanate isomer I (FITC). In addition, similar CE assays for the separation of SSRIs have been studied by other authors (Halvorsen et al. 2001, Nevado and Salcedo 2002, Andersen et al. 2003, Flores et al. 2004a,b, Schaller et al. 2006, Musenga et al. 2007).

Micellar electrokinetic chromatographic method (MEKC) CE based techniques – employing MEKC, a buffer containing sodium dodecyl sulfate and an organic modifier – have been developed for the successful separation of SSRIs (Lucangioli et al. 2000, Labat et al. 2002, Pucci et al. 2002, Chen et al. 2004, Flores et al. 2008, Su and Hsieh 2008).

Su and Hsieh (2008) presented a cation-selective exhaustive injection and sweeping MEKC method for the analysis of SER, FL, PXT, FLU, and CIT with LOD values ranging from 0.056 to 0.22 ng/ml using an on-line preconcentration method.

Chen et al. (2004) reported another MEKC system using a pH 11.5 borate buffer containing sodium deoxycholate and hydroxypropyl-β-CD with a detection wavelength of 210 nm was used. 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.

Pucci et al. (2002) developed a MEKC method involving a sodium dodecyl sulfate for the determination of CIT, FL, FLU, PXT, and SER with the most favorable MEKC system consisting of 20 mmol/l sodium dodecyl sulfate in a phosphate buffer (pH 7.5) with 30% methanol. Separation was achieved on an uncoated fused silica capillary with spectrophotometric detection at 200 nm.

Electroanalytical methods The main problems encountered in using HPLC, GC methods are either the need for derivatization or the need for time-consuming extraction procedures. Because these techniques have slightly expensive

Table 5 Electrophoretic methods.

Analyte	Methods	Experimental conditions	Results	Applications	References
CIT and desmethylcitalopram	Chiral CE system LPME based on a rod-like porous polypropylene hollow fiber	1% Sulfated-β-cyclodextrin as chiral selector in combination with 12% ACN in 25 mM phosphate pH 2.5	LOQ: </11.2 ng/ml. Intra-day precision: <12.8% RSD, and inter-day precision: <14.5% RSD, for all enantiomers		(Andersen et al. 2003)
CIT and *N*-desmethylcitalopramin	CE-LPME with extraction from 1 ml plasma samples through hexyl ether	A 30-cm effective length capillary was utilized (40.2 cm total length) with 75 mM Tris-acetic acid pH 4.6, 3% (w/v) Tween-20 and 75 mg/l FC-135 as the separation buffer	LOQ values for CIT and its main metabolite in plasma were 16.5 ng/ml and 18 ng/ml, respectively. The LOD values were 5 ng/ml and 5.5 ng/ml, respectively	Human plasma	(Halvorsen et al. 2001)
SER and its main metabolite *N*-desmethylsertraline	CE with a laser-induced fluorescence detection (λ=488 nm)	A SPE procedure, followed by a derivatization step with FITC. The final BGE consisted of 20 mM carbonate buffer, pH 9.0, with 2.5 mM heptakis (2,6-di-*O*-methyl)-β-cyclodextrin, 50 mM GLC and 20% v/v acetone	Linearity: 3.0–500 ng/ml Extraction: >97.1%, precision was <3.7, accuracy (recovery) was >95.6%	Human plasma	(Musenga et al. 2007)
PXT and three metabolites	Non-aqueous CE method	57 cm×75 m capillary using a non-aqueous buffer system of 9:1 methanol-ACN containing 25 mM ammonium acetate and 1% acetic acid	LOD values between 9.3 and 23.1 µg/l for PXT and its metabolites	Human urine	(Flores et al. 2004a)
PXT, tamoxifen, and their main metabolites	Non-aqueous CE method	Extraction-preconcentration step with a preconditioned C18 cartridge, a 57 cm×75 µm capillary using a non-aqueous buffer consisting of 18 mM ammonium acetate and 1.1% acetic acid in 80:20 (v/v) methanol-ACN	LOD values obtained between 3.0 and 7.1 µg/l		(Flores et al. 2004b)
FL, FLU, CIT, trazodone, and nefazodone	Capillary zone electrophoresis method	40 mM phosphate buffer adjusted to pH 2.5	LOD values for the five antidepressants ranged from 0.3 to 0.7 mg/l Recoveries obtained between 98% and 103% of the nominal content	Pharmaceutical formulations	(Nevado and Salcedo 2002)
SER, FLU, and FL	CE method with fully integrated SPE *in situ* capillary (50 µm i.d.×64.5 cm total length)	50 mM Phosphate buffer of pH 3.5 in ACN (20:80, v/v) with UV detection of 200 nm and sample concentration of 20 µg/ml each analyte			(Schaller et al. 2006)
SER, FL, PXT, FLU, and CIT	Cation-selective exhaustive injection and sweeping MEKC method	Fused silica capillary tube (60 cm×50 µm i.d.; Polymicro Technologies, Phoenix, AZ, USA)	The linearity for SER, 0.9962 for FL, 0.9977 for PRX, 0.9964 for FLU, and 0.9914 for CIT; the LOD values were 0.71, 0.90, 0.88, 1.5, and 0.35 ng/l, respectively		(Su and Hsieh 2008)
cis-trans Isomers and enantiomers of SER	MEKC system with a detection wavelength of 210 nm	A buffer (pH 11.5) of 35 mM sodium borate containing 30 mM sodium deoxycholate and 20 mM hydroxypropyl-β-cyclodextrin		Bulk drug, tablets and capsules	(Chen et al. 2004)
CIT, FL, FLU, PXT, and SER	MEKC method capillary with spectrophotometric detection at 200 nm	20 mmol/l sodium dodecyl sulfate in a phosphate buffer (pH 7.5) with 30% methanol on an uncoated fused silica			(Pucci et al. 2002)
Breast cancer (letrozole), an antidepressant (CIT)	MEKC method with a DAD (240 nm) after an extraction-preconcentration step with a preconditioned C18 cartridge (Waters, Milford, MA, USA)	Mobile phase consisting of 15 mM borate buffer (pH 9.2) containing 20 mM sodium dodecyl sulfate and 12% (v/v) 2-propanol through a fused silica capillary (57 cm total length×75 µm i.d.×375 µm o.d.)	Linearity ranges were 0.4–5.0 µg/l for all the compounds. LOD values were 12.5 and 25 ng/l	Human urine	(Flores et al. 2008)

(Table 5 continued)

Analyte	Methods	Experimental conditions	Results	Applications	References
CIT, FLU, PXT, SER, FL, and other drugs	MEKC methods with DAD with extraction including diethyl ether (5 ml) at pH 9.6	Uncoated fused silica capillary (600 mm, 75 mm i.d.) with migration buffer consisting of 20 mM sodium borate of pH 8.55 with 20 mM sodium dodecyl sulfate and 15% isopropanol at an operating voltage of 25 kV	LOD values and LOQ values ranged between 10 and 20 and between 20 and 30 ng/ml, respectively, for all the molecules Calibration curves were established for 30–2000 ng/ml (r=0.995)	Biological fluids (blood, urine)	(Labat et al. 2002)
SER hydrochloride and synthesis-related substances	Cyclodextrin-modified MEKC	Background electrolyte buffer consisting of 20 mM sodium borate (pH 9.0 with 50 mM sodium cholate) 15 mM sulfated β-cyclodextrin and 5 mM hydroxypropyl-β-cyclodextrin	LOD and LOQ were 0.2 mg/ml and 0.7 mg/ml for SER, respectively	Bulk drug	(Lucangioli et al. 2000)

instrumentation and running costs, the use of simpler, faster, and less expensive, but still sensitive, electrochemical techniques can be an interesting alternative. Stripping voltammetry comprises a variety of electrochemical approaches, having a step of preconcentration onto the electrode surface prior to the voltammetric measurement. In adsorptive stripping voltammetry, the analyte is adsorbed on the working electrode by means of a non-electrolytic process prior to the voltammetric scan. The high sensitivity of adsorptive stripping voltammetric methods makes it possible to work with very diluted samples with a corresponding decrease in possible interferences in the analysis. Adsorptive-stripping voltammetry (AdSV) is a technique mainly used for the analysis of organic compounds, which can be accumulated at, for example, the hanging mercury drop electrode surface and afterwards stripped-off by applying a potential scan. The introduction of high scan rate voltammetric techniques, for example, square-wave voltammetry (SWV), increases the sensitivity of AdSV even further (Wang 1985, Barros et al. 1999).

Nouws et al. (2008) presented two methods regarding the electrooxidative behavior of CIT with cyclic voltammetry and SWV at a glassy carbon electrode with a LOD value of 9.5×10^{-6} mol/l in a phosphate buffer of pH 8.2 and a flow-injection analysis system using amperometric detection with LOD of 1.9×10^{-6} mol/l. In addition, Nouws et al. (2007) described an electroanalytical method based on square-wave adsorptive-stripping voltammetry and flow-injection analysis with square-wave adsorptive-stripping voltammetric detection for the determination of FL with the reduction of FL at a mercury drop electrode in a phosphate buffer of pH 12.0. Furthermore, Nouws et al. (2006a) developed electroanalytical methods (square-wave adsorptive-stripping voltammetry) and flow-injection analysis with square-wave adsorptive-stripping voltammetry detection for the determination of PXT in a borate buffer of pH 8.8 at a mercury drop electrode with LOD and LOQ of 4.8×10^{-7} and 1.6×10^{-6} mol/l, respectively. Nouws et al. (2006b) also developed an electroanalytical method for the determination of CIT in pharmaceutical preparations with square-wave and square-wave adsorptive-stripping voltammetry using a mercury drop electrode at a potential of approximately -1.25 V vs. AgCl/Ag in an aqueous electrolyte solution of pH 12. Linearity for the proposed method ranged between 1.0×10^{-7} and 2.0×10^{-6} mol/l with a LOD value of 5×10^{-8} mol/l. Nouws et al. (2005) developed a flow-injection square-wave cathodic-stripping voltammetric method for the determination of SER in a pharmaceutical preparation with LOD and LOQ values of 1.5×10^{-7} and 5.0×10^{-7} mol/l, respectively. Linearity was obtained in the range of 0.20×10^{-6} and 1.20×10^{-6} mol/l. The proposed method was applied to the determination of SER in a commercial product.

Medyantseva et al. (2008) reported an amperometric biosensor based on a platinum screen-printed electrode and immobilized monoamine oxidase for the determination of antidepressants including petylyl, pyrazidol, and FL with determination limits of 8×10^{-9}, 8×10^{-7}, and 8×10^{-10} M, respectively.

Lencastre et al. (2006) presented an assay with regard to the oxidative behavior of FL at a glassy carbon electrode in various buffer systems and at different pH values using cyclic, differential pulse and SWV. SWV used a borate pH 9 buffer solution as supporting electrolyte with a LOD value of 1.0 μM.

Fast Fourier transform continuous cyclic voltammetric techniques for the monitoring of ultra trace amounts of CIT in a flow-injection system was reported by Norouzi et al. (2007) with LOD and LOQ values of 2.3 and 7 pg/ml, respectively.

Nevado et al. (2000) described electroanalytical methods for the determination of FLU in aqueous samples (pH 2.0 and 4.7) and pharmaceutical formulations with square-wave techniques (the hanging mercury drop electrode at -0.76 V, using an accumulation potential of -0.50 V) with the determination of FLU between 2×10^{-8} and 3×10^{-6} mol/l. Assay cyclic voltammetry, differential pulse voltammetry and Osteryoung SWV for the determination of PXT hydrochloride were developed by Erk and Biryol (2003) in the concentration range of 2×10^{-5} to 8×10^{-4} M in pure form and in human plasma. Erk and Biryol also developed a comparative HPLC assay using DAD with linearity between 2×10^{-7} and 6×10^{-5} M for PXT hydrochloride.

Roque da Silva et al. (1999) investigated the electrochemical reduction of FL with cyclic, linear sweep, differential pulse and SWV by using a hanging mercury drop electrode in alkaline buffer solution in water and in a water:ACN mixed solvent.

Vela et al. (2001) presented an assay with electrochemical behavior of SER at a hanging mercury drop electrode with a LOD value of 1.98×10^{-7} using different voltammetric techniques such as cyclic, linear sweep, differential pulse and SWV. Britton-Robinson buffer solution was used as supporting electrolyte at different pH values. Best results were found by SWV with electrodeposition at alkaline pH using a borate buffer of pH 8.2 containing 12% (v/v) methanol. Table 6 contains a summary of the applications of electroanalytical techniques to the analyses of SSRIs.

Spectrometric methods Spectrophotometric methods have several advantages such as low interference level, good analytical selectivity, easy-to-use and less expensive, and less time-consuming compared with most of the other methods. Spectrophotometric methods are simple and rapid and thus these methods can be successfully used for pharmaceutical analysis, involving quality control of commercialized product and pharmacodynamic studies. These methods are mostly based on the formation of colored complexes between SSRI drug and the reagent which can be determined by visible spectrophotometry. UV spectrophotometric and derivative spectrophotometric methods have also been used for SSRIs.

A spectrophotometric procedure for the determination of SER and/or clidinium bromide in bulk sample and in dosage forms were developed by Amin et al. (2009). The procedure is based on the formation of an ion pair complex by their reaction with bromocresol green, bromophenol blue, and bromothymol blue in buffered aqueous solution at pH 3.

Parham et al. (2008) developed an extraction-spectrophotometric method for the determination of FL in pharmaceuticals with a LOD value of 0.17 μg/ml. FL and an ion pair with Orange II quantitatively extracted into dichloromethane solvent was measured at 482 nm with linearity over concentration range of 0.2–9.0 μg/ml and a regression coefficient of 0.9995.

Darwish (2005) presented three spectrophotometric methods based on the reaction of the *N*-alkylvinylamine formed from the interaction of the free secondary amino group in the investigated drugs and acetaldehyde with each of three haloquinones to give colored vinyl amino-substituted quinones for determination of the hydrochloride salts of FL, SER, and PXT in their pharmaceutical dosage forms. Absorption maxima for colored products obtained with chloranil, bromanil, and 2,3-dichloronaphthoquinone appeared at 665, 655, and 580 nm, respectively. The LOD values for the assays ranged from 1.19 to 2.98 mg/ml.

Two methods with derivative spectrophotometry and HPLC with UV detector (265.0 nm for SER hydrochloride) for the determination of nefazodone hydrochloride and SER hydrochloride in pharmaceutical formulations were presented by Erk (2003). In the first derivative spectrophotometry, signals for nefazodone hydrochloride and SER hydrochloride were at 241.8–/256.7 nm and 271.6–/275.5 nm, respectively. In the HPLC-UV method, separation was performed on a Supercosil RP-18 column using mobile phases consisting of methanol:ACN:phosphate buffer at pH 5.5 (10:50:40, v/v/v) (nefazodone hydrochloride) and methanol:phosphate buffer at pH 4.5 (20:80, v/v) (SER hydrochloride). The LOD values for nefazodone and SER were 0.58 and 0.31 μg/ml with the spectrophotometric method. The LOD values for nefazodone and SER were 15.8 and 24.0 μg/ml with the HPLC method. The LOQ values for nefazodone and SER were 1.88 and 0.96 μg/ml with the spectrophotometric method. The LOQ values for nefazodone and SER were 26.4 and 45.3 μg/ml with the HPLC method.

Methods for determining SSRI drugs have also been based on nuclear magnetic resonance (NMR) detection which needs no previous treatment steps nor derivatization. Trefi et al. (2008) developed a simple and selective ^{19}F NMR method for the quantitation of FL and FLU in methanol solutions and in human plasma and urine with a good linearity in the range of 1.4–620 μg/ml with a LOD value of approximately 0.5 μg/ml and a LOQ value of approximately 2 μg/ml (4.6×10^{-6} mol/l). Determining the enantiomeric purity of chiral therapeutic agents is important in the development of active pharmaceutical ingredients. Shamsipur et al. (2007) described a ^{19}F NMR spectroscopy method for the quantitative determination of FL enantiomers using different chiral recognition agents in pharmaceutical formulations with LOD values of 5.9 and 7.5 μg/ml for the pure solutions of (*R*)- and (*S*)-FL, respectively. Also, a NMR and chiral solvating agent (CSA, 1,1-bi-2-naphthyl) technique for the routine determination of enantiomeric purity (SER, PRX, and fenfluramine) was reported by Salsbury and Isbester (2005). Similar spectroscopic assays for the separation of SSRIs have been studied by other authors (Erk 2003, Darwish 2005, Salsbury and Isbester 2005, Alarfaj and Razeq 2006, Onal et al. 2006, Raza 2006, Serebruany et al. 2007, Shamsipur et al. 2007, Darwish et al. 2008, Parham et al. 2008, Trefi et al. 2008, Amin et al. 2009, Darwish et al. 2009) (Table 7).

Table 6 Electroanalytical methods.

Analyte	Methods	Experimental conditions separation	Results	Applications	References
CIT	Phosphate buffer of pH 8.2	Cyclic voltammetry and square-wave voltammetry at a glassy-carbon electrode	LOD: 9.5×10^{-6} mol/l LOQ: 32×10^{-6} mol/l Recovery assays were 96–99%	Pharmaceutical products	(Nouws et al. 2008)
CIT		Flow-injection analysis system using amperometric detection	LOD: 1.9×10^{-6} mol/l LOQ: 6.3×10^{-6} mol/l Recovery assays were 94–106%	Pharmaceutical products	(Nouws et al. 2008)
FL	Reduction of FL at a mercury drop electrode in a phosphate buffer of pH 12.0	Square-wave adsorptive-stripping voltammetry	LOD: 6.5×10^{-8} mol/l LOQ: 2.2×10^{-7} mol/l Pharmaceutical products Recovery values between 98% and 102% Serum samples recovery values between 81.1% and 90.4%	Pharmaceutical products, human serum samples, and in drug dissolution studies	(Nouws et al. 2007)
PXT	Borate buffer of pH 8.8 at a mercury drop electrode	Square-wave adsorptive-stripping voltammetry	LOD and LOQ of 4.8×10^{-7} and 1.6×10^{-6} mol/l Recovery assays were performed at three concentration levels (3.0×10^{-7}, 6.0×10^{-7}, and 9.0×10^{-7} mol/l) and the results obtained 98–102%	Pharmaceutical products	(Nouws et al. 2006a)
CIT	A mercury drop electrode at a potential of -1.25 V vs. AgCl/Ag, in an aqueous electrolyte solution of pH 12	Square-wave and square-wave adsorptive-stripping voltammetry	Linearity for proposed method ranged between 1.0×10^{-7} and 2.0×10^{-6} mol/l LOD of 5×10^{-8} mol/l	Pharmaceutical preparations	(Nouws et al. 2006b)
SER		Flow-injection square wave cathodic stripping voltammetric method	LOD and LOQ of 1.5×10^{-7} and 5.0×10^{-7} mol/l, respectively Linearity was obtained in the range of 0.20×10^{-6} and 1.20×10^{-6} mol/l	Pharmaceutical preparations	(Nouws et al. 2005)
Petylyl, pyrazidol, and FL	Platinum screen-printed electrode and immobilized monoamine oxidase	Amperometric biosensor	LOD: 8×10^{-10}		(Medyantseva et al. 2008)
FL	Glassy carbon electrode in various buffer systems (borate pH 9 buffer solution as supporting electrolyte)	Cyclic, differential pulse and square-wave voltammetry	LOD: 1.0 μM		(Lencastre et al. 2006)
CIT		Fast Fourier transform continuous cyclic voltammetric technique flow-injection system	LOD and LOQ: 2.3 and 7 pg/ml The method was linear over the concentration range of 7–116 pg/ml		(Norouzi et al. 2007)
FLU	Hanging mercury drop electrode at -0.76 V, using an accumulation potential of -0.50 V)	Square-wave techniques	2×10^{-8} and 3×10^{-6} mol/l linear in the range 5×10^{-9} and 1×10^{-6} mol/l by stripping mode. The relative standard deviations obtained for concentration levels of FVX were as low as 1.5×10^{-6} mol/l, with square-wave 3.54% (n=10) and for 6.0×10^{-7} mol/l with stripping square-wave 2.39% (n=10) in the same day	Aqueous samples (pH 2.0 and 4.7) and pharmaceutical formulations	(Nevado et al. 2000)

(Table 6 continued)

Analyte	Methods	Experimental conditions separation	Results	Applications	References
PXT		Cyclic voltammetry, differential pulse voltammetry and Osteryoung square-wave voltammetry	The concentration range of 2×10^{-5} to 8×10^{-4} M	In pure form and in human plasma	(Erk and Biryol 2003)
FL	A hanging mercury drop electrode in alkaline buffer solution in water and in a water-ACN mixed solvent	Cyclic, linear sweep, differential pulse and square-wave voltammetry	Linearity ranged between 0.52 and 5.2 M		(Roque da Silva et al. 1999)
SER	Hanging mercury drop electrode Britton-Robinson buffer solution was used as supporting electrolyte	Cyclic, linear sweep, differential pulse and square-wave voltammetry	LOD of 1.98×10^{-7} Linearity ranged between 2.33×10^{-7} and 3.15×10^{-6} M with recoveries close to 100%	Pharmaceutical formulations	(Vela et al. 2001)

Flow injection is a low-pressure, low-cost continuous technique, the versatility and availability of which enable the desired level of automation to be implemented in enological laboratories.

Altıokka and Kırcalı (2003) developed a flow-injection analysis of PXT hydrochloride with 0.1 mol/dm^3 acetate buffer at pH 3.07 and LOD and LOQ values of 3.2×10^{-7} and 9.5×10^{-7} mol/dm, respectively. The analyte was detected at 293 nm and linearity ranged between 1.07×10^{-6} and 5.35×10^{-6} mol/dm^3.

Shah et al. (2008) developed a flow-injection spectrophotometric method for the determination of FL in a pharmaceutical preparation based on base hydrolysis with sodium ethoxide of the drug with LOD and LOQ values of 0.15±0.01 mg/l and 0.29±0.03 mg/l, respectively.

Conclusions

A review of the literature revealed that a variety of analytical procedures for the determination of SSRIs have been developed and described. The analytical methods used for the determination of SSRIs are generally based on chromatographic methods coupled to different detectors, electroanalytical methods, capillary zone electrophoretic methods, and spectrometric methods. Biological fluid sample preparation is usually performed by LLE or SPE. These methods usually consume organic solvents, are laborious and time-consuming. However, modern trends in analytical chemistry are near simplification, in miniaturization of sample preparation, and minimization of organic solvent used, and sample volumes. SPME, stir bar SBSE, protein precipitation, direct injection of biological samples without sample preparation SLM, LSE, LPME, PLE have also been employed for the SSRI determination. On-line extraction and analysis of SSRIs and its metabolite by column-switching HPLC coupled to mass spectrometry has recently been published. A promising approach to HPLC with integrated fully automated sample extraction is high speed on-line SPE which enables direct injection of plasma samples without prior extraction by using large particle size stationary phases with an extremely high linear flow velocity of the mobile phase.

In HPLC, analyses have commonly used fluorescence, ultraviolet (UV) and mass spectrometry (MS) detectors. The use of MS with HPLC provides high sensitivity and selectivity compared to traditional HPLC-UV and GC methods. In most HPLC-MS systems, ESI is employed as an interface between a HPLC instrument and an MS detector. APCI is also a useful technique coupling HPLC with MS. The column-switching technique has been found in the literature for SSRI analysis involving more expensive and complex instrumentation. Typical quantification limits are in ng/ml, µg/ml, and pg/ml range.

GC with nitrogen-phosphorus, electron-capture, mass spectrometric, flame ionization detection methods have been used to a lesser extent in SSRIs and analysis of their metabolites. GC-MS is a very good separation method for compounds that are volatile. In recent years, the electrochemical techniques

Table 7 Spectrometric methods.

Analyte	Reagent	Experimental conditions	Results	Applications	References
SER and clidinium bromide	Bromocresol green, bromophenol blue, and bromothymol blue	Spectrophotometric procedure based on formation of an ion-pair complex by their reaction with bromocresol green (BCG), bromophenol blue (BPB), and bromothymol blue (BTB) in buffered aqueous solution at pH 3	Concentration range: 1–30 µg/ml. For more accurate analysis, Ringbom optimum concentration range of 2–27 µg/ml was used	Bulk sample and in dosage forms	(Amin et al. 2009)
FL	FL and an ion-pair with Orange II extracted into dichloromethane solvent	Extraction-spectrophotometric method (482 nm)	LOD of the method of 0.17 µg/ml. Linearity over concentration range of 0.2–9.0 µg/ml and the regression coefficient of 0.9995	Pharmaceutical	(Parham et al. 2008)
SER and PXT	*N*-alkylvinylamine formed from the interaction of the free secondary amino group	Acetaldehyde with each of three haloquinones to give colored vinylamino-substituted quinones	LOD values for the assays ranged from 1.19 to 2.98 mg/ml	Pharmaceutical dosage forms	(Darwish 2005)
SER		Two methods with derivative spectrophotometry and HPLC with UV detector 241.8–/256.7 nm and 271.6–/275.5 nm	LOD: 0.31 µg/ml LOQ: 0.96 mg/ml	Pharmaceutical formulations	(Erk 2003)
FL and FLU		^{19}F NMR method	Linearity in the range of 1.4–620 µg/ml LOD: 0.5 µg/ml and LOQ: 2 µg/ml (4.6×10^{-6} mol/l). The accuracy ranged from approximately 96% to 103%, 93% to 104% in plasma, with standard deviations <7.5%.	In human plasma and urine	(Trefi et al. 2008)
FL enantiomers	Different chiral recognition agents	^{19}F NMR method	LOD values: 5.9 and 7.5 µg/ml for the pure solutions of (*R*)- and (*S*)-FL, respectively. The linearity for (*R*)- and (*S*)-FL: 0.10–1.35 mg/ml with recovery ranged from approximately 90% to 110% with relative standard deviations of <8%	Pharmaceutical formulations	(Shamsipur et al. 2007)
SER, PRX, and fenfluramine	Chiral solvating agent (CSA, 1,1-bi-2-naphthyl)	NMR method		Pharmaceutical ingredients	(Salsbury and Isbester 2005)
SER, FL, and venlafaxine	Ion-pair agents (bromothymol blue, bromocresol green, or bromophenol blue)			Pharmaceutical preparations	(Onal et al. 2006)
FLU	1,2-naphthoquinone-4-FLU sulfonate reagent	Alkaline medium of pH 9 with an orange-colored product exhibiting maximum absorption peak at 470 nm	LOD and LOQ: 0.2 and 0.6 µg/ml		(Darwish et al. 2009)
CIT	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone	590 nm	Concentration limit of 10–250 µg/ml with molar absorptivity 3.3×103 l/mol/cm	Tablet formulations	(Raza 2006)
PXT		Fluorescence intensity obtained in methanol at 340 nm using 290 nm for excitation	LOD of 0.015 mg/ml. Human plasma	Pharmaceutical formulations	(Alarfaj and Razeq 2006)

(Table 7 continued)

Analyte	Reagent	Experimental conditions	Results	Applications	References
PXT	4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole	545 nm after excitation at 490 nm in an alkaline medium of pH 8 to form a highly fluorescent derivative	Linearity: between fluorescence intensity and PXT concentration in the range of 80–800 ng/ml with correlation coefficient (0.9993) and LOD and LOQ of 25 and 77 ng/ml, respectively	Dosage forms and plasma	(Darwish et al. 2008)
CIT and escitalopram		Fluorimetry methods measured in the range 270–450 nm with excitation at 240 nm on a FluoroMax 3 spectrofluorimeter		Human plasma	(Serebruany et al. 2007)
PXT		Flow-injection analysis with 0.1 mol/dm acetate buffer at pH 3.07 detected at 293 nm	LOD and LOQ of 3.2×10^{-7} and 9.5×10^{-7} mol/dm^3, linearity ranged between 1.07×10^{-6} and 5.35×10^{-6} mol/dm^3		(Altıokka and Kırcalı 2003)
FL	Based on base hydrolysis with sodium ethoxide of the drug	Flow-injection spectrophotometric method	LOD and LOQ of 0.15±0.01 mg/l and 0.29±0.03 mg/l, respectively Absorbance was measured at 510 nm with a linearity between 0.5 and 25 mg/l and a molar absorptivity of 2.19×10^4	Pharmaceutical preparation	(Shah et al. 2008)

have led to the advancement in the field of analysis because of their sensitivity, low cost, and relatively short analysis time when compared to other techniques such as chromatographic techniques. Although SSRIs are being electrochemically active, there are limited assays with regard to determination of SSRIs with electrochemical techniques. However, owing to the importance of these compounds it is very likely to be widely studied and developed in the future with electrochemical techniques as well as HPLC with electrochemical detection methods for determination of SSRI drugs.

In general, spectrophotometric methods have several advantages such as low interference level, good analytical selectivity, easy-to-use and less expensive and less time-consuming compared with most of the other methods. However, the spectrophotometric methods are less sensitive due to measurement in the ultraviolet region compared with most of the other methods.

Very few methods are reported in the literature for SSRIs with CE due to the low sensitivity of this technique which limits its applicability to drug analysis in biological samples. MEKC is one of the most popular techniques in CE and is capable of separating neutral compounds as well as charged solutes. However, the combination of CE and MS-MS and MEKC-MS methods such as partial filling, high-molecular mass surfactan, adsorption, sorption, ESI, APCI could gain considerable importance in SSRI drugs analysis.

In summary, different pretreatment and detection procedures are usually applied for SSRI drugs analysis. Chromatographic methods potentially meet these requirements but at present are focused on SSRI drugs analysis and often have insufficiently low detection limits. Therefore, the development of more sensitive methods that are still simple to perform and methods is desirable.

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