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Analytical procedures for the determination of the selective serotonin reuptake inhibitor antidepressant citalopram and its metabolites

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ABSTRACT: The antidepressant citalopram (CIT) is a potent and highly selective serotonin reuptake inhibitor (SSRI) which has been introduced in therapy as a racemic drug. CIT has been used to treat central nervous system affective disorders such as depression, anxiety, obsessive-compulsive disorders, various phobias, borderline personality disorders, bipolar disorders as well as indications wherein inhibition of serotonin reuptake is desired. CIT is demethylated to demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT), which retain considerable activity as SSRIs. Therefore, in recent years, the monitoring of the levels of these analytes in biological fluids for toxicological and therapeutic purposes has been a target worthy of interest. In addition, the differences in activity between CIT enantiomers established the need to assess its behaviour in the field of pharmacological research. It is also necessary to develop analytical methodologies that make it possible to determine the levels of enantiomer concentrations. This review includes most of the published analytical methods for achiral assay of racemic CIT and its metabolites based on high-performance liquid chromatography coupled with UV, fluorescence and mass spectrometry detectors, capillary electrophoresis and gas chromatography with mass spectrometry detectors among others. With regard to the monitoring of enantiomers of CIT and of its metabolites, stereoselective methods based on chiral chromatographic columns, chiral additives in mobile phases and on the derivatization with a chiral reagent are also collected. In addition, different procedures of extraction are mentioned as well as liquid–liquid extraction, solid-phase extraction, solid-phase microextraction, automated online extraction or liquid-phase microextraction in different biological and environmental samples. **Copyright © 2010 John Wiley & Sons, Ltd.**

Keywords: citalopram; metabolites; enantiomers; analytical method; sample pre-treatment; biological samples; environmental samples

Introduction

The antidepressant citalopram (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile; CIT) is a bicyclic phthalate compound approved in 1998 by the US Food and Drug Administration for the treatment of depression. It is also indicated for other central nervous system (CNS) diseases such as anxiety, obsessive–compulsive disorders, various phobias (agoraphobia, social phobia), borderline personality disorders, bipolar disorders as well as as in cases in which inhibition of serotonin reuptake is desired (Baumann and Rochat, 1995). CIT, which has been marketed as a racemic compound, (–)-(*R*)-CIT and (+)-(*S*)-CIT, is a potent and highly selective serotonin reuptake inhibitor (SSRI). The SSRIs act by inhibiting the reuptake of serotonin (or 5-hydroxytryptamine, 5-HT) into the presynaptic nerve terminal, enhancing synaptic concentrations of 5-HT and facilitating serotonergic neurotransmission. CIT appears to have a little effect on noradrenaline or dopamine reuptake, thus improving the tolerability and safety in overdose when compared with tricyclic antidepressants and monoamine oxidase inhibitors (Willetts *et al*., 1999).

Orally administered CIT is well absorbed from the gastrointestinal tract and is cleared from the body primarily by hepatic metabolism, where CIT is stereoselectively metabolized (Fig. 1) by partial *N*-demethylation to demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT), as well as by oxidative deamination to a propanoic acid metabolite (CIT-PROP) and by N-oxidation to CIT-N-oxide (Van Harten, 1993). CIT is biotransformed by the specific human hepatic cytochrome P450 enzymes (CYP3A4, CYP2C19, and to a minimal extent CYP2D6) while the inhibition of these enzymes by CIT and DCIT is negligible. The bioavailability of the oral formulations of CIT is about 80%. CIT displays linear kinetics over the therapeutic dosage range of 10–60 mg/day and it achieves maximum plasma concentrations between 2 and 4 h after dosing (Gutierrez and Abramowitz, 2000). The elimination half-life of CIT and DCIT was found to vary between 23 and 45 h (De Vane, 1999) and 66 and 92 h respectively while the half-life of the DDCIT metabolite has

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Abbreviations used: ARS, arylsulfataseCIT, citalopram; CNS, central nervous system; DCIT, demethylcitalopram; DDCIT, didemethylcitalopram; FLV, fluvoxamine; FLX, fluoxetine; GRD, β-glucuronidase; PDMS, polydimethylsiloxane; PPY, polypyrrole; SBSE, sorpive stir bar extraction; SER, sertraline; SSRI, selective serotonin reuptake inhibitor.

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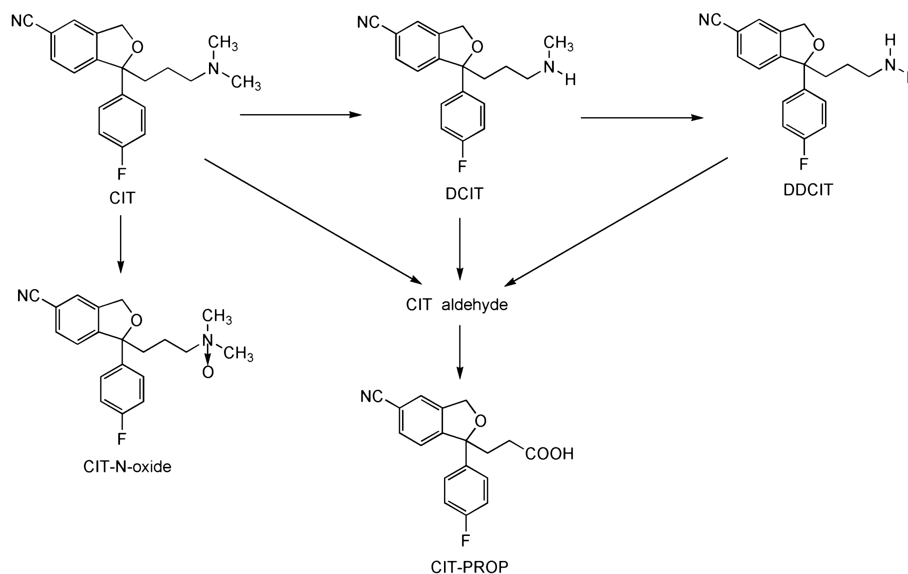

Figure 1. Hepatic metabolism of citalopram.

not been reported (Gutierrez and Abramowitz, 2000). Steady-state levels of DCIT and DDCIT are about 30 and 10% of those of CIT, respectively (Baumann and Larsen, 1995). CIT and its metabolites are cleared primarily by the kidneys and about 35% of the dose is excreted in urine (Dalgaard and Larsen, 1999).

The insignificant affinity of CIT for receptors of different neurotransmitters, enzymes and other reuptake sites is thought to account for its relative tolerability and safety of the drug in view of drug–drug interactions with other substrates (Willetts *et al*., 1999). This aspect increases the usefulness of CIT in drug combination therapy.

CIT possesses one stereogenic center and exists in (–)-(*R*) and (+)-(*S*) enantiomers. As shown in different *in vitro* studies in rat brain, the pharmacological effect of CIT resides mainly in the (*S*)-CIT form, and to a lesser degree in (+)-(*S*)-DCIT (Hyttel *et al*., 1992). These studies considered (*R*)-CIT as pharmacologically inactive and as being able to inhibit the activity of the (*S*)-enantiomer (Mork *et al*., 2003). This provoked the launch of pharmaceutical formulations which only include (*S*)-CIT or escitalopram as a new single-enantiomer drug. Enantioselective analysis of CIT and its metabolites DCIT and DDCIT performed in femoral blood from 53 autopsy cases by a chiral HPLC method revealed that the mean ($\pm$ SD) S/R ratio for CIT was 0.67 $\pm$ 0.25 and that for DCIT 0.68 $\pm$ 0.20. In addition, increasing S/R ratios with increasing concentrations of CIT were found (Holmgren *et al*., 2004).

In clinical practice, in the case of antidepressant drugs, the definition of an efficient and safe dose and the detection of the adherence and compliance to the treatment require the establishment of a therapeutic drug monitoring method. In addition, in recent years, interest in the detection of drugs in environmental samples has increased. This review summarizes the methods developed for the determination of CIT and/or its metabolites. These assays consist of three components: sample pre-treatment, chromatography and detection. All of them influence the accuracy, precision, selectivity and sensitivity of the analytical method. In the following paragraphs, the three steps of a bioanalytical method will be discussed. The advantages and disadvantages of the different approaches for the analysis of CIT will be commented upon and supplemented with our own experience in this field.

Sample Pre-treatment

The sample preparation is a fundamental part of the quantitative bioanalysis and is usually the most critical and time-consuming step when using chromatography or affinity techniques for drug analysis in biological matrices. During sample pre-treatment endogenous compounds such as proteins, lipids and in some cases salts are removed from the sample. The presence of these compounds is not suitable when both chromatographic interferences and column maintenance are taken into account. Sometimes, these compounds can influence the ionization efficiency of the mass spectrometers and therefore the sensitivity of the method. The sample pre-treatment depends on the matrix to be analyzed. When CIT is analyzed in plasma or serum samples, the most widely employed methodology is the precipitation of proteins, followed by a solid-phase extraction (SPE) step. In general, the samples are diluted with 4 ml of phosphate buffer (pH 2.5; 25 mM), vortexed and submitted to the SPE procedure (Wille *et al*., 2007). The protein precipitation has also been carried out using acetonitrile (Cao *et al*., 2007).

Liquid–liquid extraction (LLE) has been investigated extensively for CIT in plasma as shown in Tables 1–3. Generally, the process consists of the adjustment of the ionic strength and pH and the extraction with hexane–isoamyl alcohol (99:1, v/v). The mixture is stirred for 30 min and centrifuged. The organic phase is then collected and evaporated under a gentle nitrogen flow. The extract is dissolved in acetonitrile for analysis (Catai *et al*., 2009). A similar procedure was employed for the extraction of (–)-(*R*) and (+)-(*S*) enantiomers of DCIT and DDCIT in rat plasma and brain tissue (Millan *et al*., 2008). Usually, LLE procedures require long analysis times; hence they are gradually being replaced by other extraction techniques.

In order to reduce the analyte enrichment problems of LLE from small biological samples and eliminating the compatibility

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Table 1. Analytical methods based on liquid chromatography for the analysis of CIT

Analyte	Matrix	Sample pre-treatment	Separation mode	Detector	Sensitivity	Reference
CIT, DCIT	Plasma	LLE	TLC	FLD	LOQ: 20–50 ng/mL	Overo (1978)
CIT, DCIT, DDCIT	Plasma	LLE	TLC	FLD	LOQ: 5 ng/mL	Overø (1981)
CIT, DCIT, DDCIT	Plasma	LLE	HPLC	FLD	LOD: 0.5–2 ng/mL	Oeyehaug *et al.* (1982)
CIT, DCIT, DDCIT, CIT-N-oxide, CIT-PROP	Urine	LLE	HPLC	FLD	LOQ: 5 ng/mL in plasma, 10–20 ng/mL in urine	Oeyehaug *et al.* (1984)
CIT, AMIP, CLP and desmethyl-metabolites	Plasma	LLE	HPLC	UV	LOD: 5 ng/mL	Rop *et al.* (1985)
CIT	Plasma, brain tissue		HPLC	UV	LOD: <25 ng/mL	Wang and Lemmer (1989)
CIT; DCIT; DDCIT	Plasma	SPE	HPLC	UV	LOQ: 0.7–0.8 ng/mL	Rop *et al.* (1990)
CIT, DCIT, DDCIT, CIT-N-oxide, CIT-PROP	Plasma	Direct injection, retention in a precolumn and back-flush	HPLC	FLD	LOQ: 2 ng/mL	Matsui *et al.* (1995)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT, *R*- and *S*-CIT-PROP	Plasma	LLE	HPLC (Chiralcel OD column)	FLD	LOQ: 2–15 ng/mL	Rochat *et al.* (1995a)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT	Plasma	LLE	HPLC (acetylated β-cyclobond column)	FLD	LOQ: 3 ng/mL	Rochat *et al.* (1995b)
CIT, DCIT	Serum	LLE	HPLC	UV	LOQ: 2–3 ng/mL	Olesen and Linnet (1996)
R- and *S*-CIT	Plasma	LLE	HPLC (Chiral-AGP)	UV	LOD: 1 ng/mL	Haupt (1996)
CIT and other drugs	Urine, serum	SPE	HPLC	DAD	—	Lai *et al.* (1997)
CIT, DCIT, DDCIT	Plasma	SPE	HPLC	FLD	LOD: 0.8 nmol/L	Carlsson and Norlander (1997)
CIT, DCIT	Serum	SPE	HPLC	DAD	LOD: 15 nmol/L	Akerman *et al.* (1998)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT	Plasma	LLE	HPLC (Chirobiotic V column)	FLD	LOQ: 5–7.5 ng/mL	Kosel *et al.* (1998)
CIT, DCIT, DDCIT, CIT-N-oxide, PRX, FLX, NFLX, PRX metabolites	Plasma	SPE	HPLC	FLD (CIT, DCIT, DDCIT, CIT-N-oxide, PRX, PRX metab) UV (FLX, NFLX)	LOQ: 0.025–0.12 µmol/L	Kristoffersen *et al.* (1999)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT	Plasma	LLE	HPLC (Chirobiotic V column)	UV	LOD > 2 ng/mL	Zheng *et al.* (2000)
CIT, DCIT, MPT, DMPT, PPM	Hair	Ultrasonication and SPE	HPLC	ESI-CID/MS	LOD < 0.1 µg/L	Muller *et al.* (2000)

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Table 1. *Continued*

Analyte	Matrix	Sample pre-treatment	Separation mode	Detector	Sensitivity	Reference
CIT, DCIT	Plasma, human milk	LLE	HPLC	UV	LOD: 1 µg/L	Rampono *et al.* (2000)
CIT, DCIT	Plasma, urine	LPME	HPLC	FLD	LOD: 700 pg/mL (CIT)	Rasmussen *et al.* (2000)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT	—	—	HPLC (acetylated beta-cyclodextrin column)	FLD	—	Carlsson and Norlander (2001)
CIT, DCIT, DDCIT	Biological samples	On-line extraction	HPLC	FLD	—	Ohman *et al.* (2001)
CIT	Plasma	LLE	HPLC	FLD	LOQ: 0.96 ng/mL	Macek *et al.* (2001)
CIT, DCIT, DDCIT, FLV, PRX, SER, VLF, MCP, FLX, NFLX,	Serum	LLE	HPLC	UV	LOQ: 25 ng/mL	Tournel *et al.* (2001)
CIT, FLX, PRX	Plasma	SPE	Capillaty LC	UV	LOQ: 0.05–0.26 µM	Molander *et al.* (2002)
CIT, DCIT, FLV, FLX, NFLX, SER, PRX, VLF, DVLF, MCP, MTZ, DMTZ	—	—	HPLC	UV	—	Dallet *et al.* (2002)
CIT, FLX, NFLX, FLV, TRZ, CLP	Pharmaceutical formulations	—	HPLC	DAD	LOQ: 3.3–33.3 µg/L	Berzas *et al.* (2002)
CIT, CLZ, FLX, NFLX, MPT, DMPT, TRZ	Serum	LLE	HPLC	FLD	LOQ: 50 µg/L	Waschgler *et al.* (2002)
CIT, FLV, PRX	Plasma	On-line SPE	HPLC	APCI-MS/MS	LOQ < 20 µg/L	Kollroser and Schober (2003)
CIT, DCIT, DDCIT	Plasma	SPE	HPLC	FLC	LOQ: 1.5–2 ng/mL	Raggi *et al.* (2003)
CIT and 27 further antidepressants, atypical antipsychotics and metabolites	Plasma, serum	SPME	HPLC	UV	LOQ: 5 ng/mL	Frahnert *et al.* (2003)
CIT, DCIT, DDCIT, FLV, PRX, SER, FLX, NFLX, MTZ, MCP, DMCP, VLF, o-DVLF	Plasma	LLE	HPLC	UV	LOQ: 25 ng/mL	Titier *et al.* (2003)
FLX, NFLX, SER, PRX, CIT, FLV, VLF, o-DVLF, MCP, MTZ, MLB, TLX, VLX	Plasma	LLE	HPLC	DAD	LOD: 2.5–5 ng/mL	Duverneuil *et al.* (2003)
CIT, *S*-CIT and 11 further antidepressants and 5 neuroleptic drugs	Serum	LLE	HPLC	ESI-MS	LOQ: 1.2–54 nmol/L	Gutteck *et al.* (2003)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT	Blood	SPE	HPLC (acetylated β-cyclodextrin column)	FLD	LOQ: 0.002 µg/g	Holmgren *et al.* (2004)
CIT and other drugs	Blood, urine	LLE	HPLC	UV SERS	LOD: 1 µg/sample domain	Trachta *et al.* (2004)
CIT	Plasma	LLE	HPLC	ESI-MS	LOQ: 0.5 ng/mL	Pistos *et al.* (2004)
S-CIT	Plasma	LLE	HPLC	ESI-MS	LOQ: 1 ng/mL	Singh *et al.* (2004)

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Table 1. *Continued*

Analyte	Matrix	Sample pre-treatment	Separation mode	Detector	Sensitivity	Reference
CIT	Tablets	—	HPLC, HPTLC	UV Densitometric	LOD: 0.08 µg/mL LOD: 0.09 µg per spot	Skibinski *et al.* (2005)
CIT, DCIT	Plasma	SPE	HPLC	FLD	LOQ: 6 and 12 ng/mL	Meng and Gauthier (2005)
CIT; FLX, PRX, VLF	Plasma	SPE	HPLC	ESI-MS	LOD: 0.1–0.5 ng/mL	He *et al.* (2005)
CIT; DCIT; CIT-N-oxide	Water	—	HPLC	ESI-MS	—	Kwon and Armbrust (2005)
CIT	Plasma	LLE	HPTLC	ESI-MS	LOD: 0.5 ng/mL	Mendes *et al.* (2005)
CIT and 12 further antidepressants	Plasma	SPE	HPLC	DAD	—	Wille *et al.* (2005)
CIT and 12 further antidepresants	Serum	SPE	TFC	MS/MS	LOQ: 10 ng/mL	Sauvage *et al.* (2006a)
CIT, DCIT and other drugs	Serum, plasma, urine, gastric content	SPE	HPLC	ESI-IT-MS	—	Sauvage *et al.* (2006b)
R- and *S*-CIT	—	—	HPLC (*β*-CD)	UV	LOQ: 18.4–14.5 ng/mL	El-Gindy *et al.* (2006)
CIT, SER, PRX, FLX, FLV	Sewage influents and effluents	SPE and LLE	HPLC	MS	LOS: 120–290 pg/L	Vasskog *et al.* (2006)
CIT, FLX, MTZ, PRX, SER, VLF	Hair	Soxhlet	HPLC	ESI-IT-MS/MS ESI-QToF-MS/MS	LOD: 9×10^{-9} mol/L	Smyth *et al.* (2006)
CIT and 47 further antidepressants and antipsychotics	Serum	Dilution of first protein precipitation	HPLC	MS	LOQ: 1 ng/mL (CIT)	Kirchherr and Kuhn-Velten (2006)
CIT and other drugs	Blood, urine and tissue	SBSE	GC	MS	XX	Crifasi *et al.* (2006)
CIT, *S*-CIT, DCIT, *S*-DCIT	Serum	Column-switching procedure	HPLC	UV	LOD: 6 ng/mL	Greiner *et al.* (2007)
CIT, SER, MTZ, FLX, PRX, IMIP, NTL, AMIP, DSP	Plasma	SBSE	HPLC	UV	LOQ: 10 ng/mL	Chaves *et al.* (2007)
CIT	Plasma	LLE	HPLC	ECD	LOQ: 1.493 ng/mL	Al-Ghazawi *et al.* (2007)
CIT and 13 further antidepressants and metabolites	Plasma	On-line SPE	HPLC	MS/MS	LOQ: 10 µg/L	De Castro *et al.* (2007)
CIT and impurities	Bulk drug		HPLC	UV MSn	—	Sun *et al.* (2007)
CIT, IMIP, AMIP, CLP, FLX, SER, PRX, MTZ, MCB, DLX	Plasma	LLE	HPLC	UV	LOQ: 5 ng/mL	Malfara *et al.* (2007)
CIT	Pharmaceutical tablets, serum	On line SPE	HPLC	FLD	LOQ: 5.2×10^{-8} to 5.5×10^{-8} M	Satana *et al.* (2007)

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Table 1. *Continued*

Analyte	Matrix	Sample pre-treatment	Separation mode	Detector	Sensitivity	Reference
R- and *S*-CIT, *R*- and *S*-DCIT	Plasma	LLE	Chiral HPLC (Chiralcel® OD-R column)	MS/MS	LOQ: 0.1 ng/mL	Rocha *et al.* (2007)
R- and *S*-CIT, *R*- and *S* FLX, enantiomers of 6 further beta-blockers	Wastewater	—	HPLC (Chirobiotic V column)	MS/MS	LOD: 0.2–7.5 ng/L	MacLeod *et al.* (2007)
CIT	Tablets	—	HPLC	UV	LOQ: 10 µg/mL	Menegola *et al.* (2008)
CIT, MTZ, PRX, DLX, FLX, SER	Plasma	In-tube SPME	HPLC	UV	LOQ: 50 ng/mL	Silva *et al.* (2008)
CIT, CLN	Pharmaceutical formulations	—	HPTLC	UV	LOQ: 10 µg/mL	Gandhi *et al.* (2008)
CIT	Plasma	SME/BE	HPLC	FLD	LOQ: 0.8 ng/mL	Bagheri *et al.* (2008)
CIT; DCIT; DDCIT, FLX, NFLX	Urine	SPME	HPLC	DAD	LOD: 0.01 mg/L	Unceta *et al.* (2008)
R- and *S*-DCIT, *R*- and *S*-DDCIT	Plasma. Brain tissue	LLE and chiral derivatization	HPLC	FLD	LOD: < 2.1 ng/mL and 42.8 ng/g	Millan *et al.* (2008)
CIT, DCIT, DDCIT, SER, DSER, FLX, NFLX, FLV, PRX	Sewage influents and effluents	LPME	HPLC	ESI-MS	LOQ: 57 pg/L (CIT), 762 pg/L (DCIT), 781 pg/L (DDCIT)	Vasskog *et al.* (2008)
CIT and impurities	Bulk drugs and pharmaceutical formulations	—	HPLC	DAD ESI-MS/MS	LOQ: 0.174 µg/mL	Rao *et al.* (2008)
CIT; VLF, SER, PRX, AMIT, FLX, O-DVLF, DSER, NFLX, NTL	Sewage and wastewater	SPE	HPLC	MS/MS	LOD: 0.048–0.10 ng/mL	Lajeunesse *et al.* (2008)
R- and *S*-CIT,*R*- and *S*-DCIT	Hair	SLE and LLE	HPLC	ESI-MS	LOQ: 25 pg/mg	Frison *et al.* (2008)
CIT; SER; FLX, FLV	Pharmaceutical formulation	—	TLC	Densitometric detection	LOD: 40–50 ng per spot	Gondova *et al.* (2008)
CIT, AMIT, IMIP, CLP, FLX, PRX, SER, FLV, VLF, NFLX, NTL, DSP, NCLP	Oral fluid, plasma	On-line SPE	HPLC	MS/MS	LOQ: 2 ng/mL (CIT)	De Castro *et al.* (2008)
CIT, FLX, NFLX, SER, DSER, PRX, FLV, DLX, VLF, BUP	Wastewater effluent	SPE	HPLC	ESI-MS/MS	LOD: 0.9 ng/L	Schultz and Furlong (2008)
CIT, MTZ, DLX, PRX, FLX, SER	Plasma	Dual-phase SBSE (PDMS, PPY)	HPLC	UV	LOQ: 20–50 ng/mL	Melo *et al.* (2009)
CIT, MTZ, DLX, PRX, FLX, SER	Plasma	SPME (PPY coating)	HPLC	UV	LOQ: 20 ng/mL (CIT)	Chaves *et al.* (2009)
CIT and 11 further pharmaceuticals	Surface, ground and drinking water	SPE	HPLC	MS	LOQ: 10 ng/L	Fick *et al.* (2009)
CIT, IMIP, RBX, TRZ and metabolites	Brain microdyalisate	C_{18} ZipTips	Direct infusion	MS/MS	—	Erve *et al.* (2009)

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Table 1. *Continued*

Analyte	Matrix	Sample pre-treatment	Separation mode	Detector	Sensitivity	Reference
CIT and impurities	Bulk drug	—	HPLC	ESI-MS	—	Raman *et al.* (2009)
CIT, DCIT; DDCIT; FLX, NFLX; VLF, o-DVLF	Plasma, urine, brain tissue	SBSE	HPLC	FLD	LOQ: 0.2–2 μg/L plasma; 2–20 ng/g brain tissue; 1–10 μg/L urine	Unceta *et al.* (2010b)
CIT and other drugs	Plasma	Deproteinization	UPLC	MS/MS	LOQ: 5 nM	Smalley *et al.* (2009)
CIT and other drugs	River water, sewage influent and effluent	Molecularly imprinted polymer-based SPE	UPLC	MS	LOD: 0.5 ng/L	Demeestere *et al.* (2010)
CIT, DCIT	Plasma	Deproteinization	HPLC	MS/MS	LOQ: 0.25–0.5 ng/mL	Jiang *et al.* (2010)
CIT, FLX, NFLX, SER, NSER, PRX, FLV, DLX, VLF, BUP	Water, sediment, Fish neural tissue	SPE	HPLC	MS/MS	LOQ: 0.015 ng/g tissue; 0.25–2.5 ng/g sediment; 0.5 ng/L water	Schultz *et al.* (2010)
CIT, VLF, SER, BUP, FLX, PRX and metabolites	Wastewater, fish, surface water	PLE (fish)	HPLC	MS/MS	LOQ: 4–13 ng/L (wastewater); 3–9 ng/L (surface water) 0.5 μg/kg (fish)	Metcalfe *et al.* (2010)
CIT; FLX; NFLX; HLP	Plasma	SPE	HPLC	ESI-IT-MS	LOQ: 5 ng/mL	El-Rjoob *et al.* (2010)
CIT and 15 pharmaceuticals more	Wastewaters	Dual-SPME	HPLC	ESI-IT-MS	LOQ: 0.01 ng/mL	Unceta *et al.* (2010a)
CIT and other 51 drugs	Hair	LLE	UPLC	ESI-TOF	LOQ: 0.05 ng/mg	Nielsen *et al.* (2010)

Abbreviations of compounds: β-CD, beta-cyclodextrin; AMIP, amitriptyne; BUP, buproppion; CIT-PROP, citalopram propionic acid; CLN, clonazepam; CLP, clomipramine; CLZ, clozapine; DLX, duloxetine; DMPT, desmethylmaprotiline; DMTZ, desmethylmirtazapine; DSER, desmethylsertraline; DSP, desipramine; DVLF, desmethylvenlafaxine; *o*-DVLF, *o*-desmethylvenlafaxine; DXP, doxepin; FLV, fluvoxamine; FLX, fluoxetine; HLP, haloperidol; IMIP, imipramine; LTZ, letrozole; MCB, moclobemide; MCP, milnacipam; MLB, moclobemide; MPT, maprotiline; MNS, mianserine; MTZ, mirtazapine; NCLP, norclomipramine; NFLX, norfluoxetine; NSER, norsertraline; NTL, nortriptyline; SER, sertraline; PDMS, polydimethylsiloxane; PMP, pipamperone; PPY, polypyrrole; PRX, paroxetine; TLX, toloxatone; TRZ, trazodone; VLX, viloxazine; VLF, venlafaxine. Abbreviations of techniques: CID, in-source collision induced dissociation; ECD, electrochemical detection; ESI, electrospray ionization; HPTLC, high performance thin layer chromatography; LPME, liquid phase microextraction; CC, capillary chromatography; MSN, tandem multistage mass spectrometry; SERS, surface-enhanced Raman scattering; SLE, solid–liquid extraction; SME/BE, solvent microextraction with back extraction; SPE, solid-phase extraction; SPME, solid-phase microextraction; TLC, thin-layer chromatography; TFC, turbulent-flow liquid chromatography; UPLC, ultrahight-performance liquid chromatography.

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Table 2. Analytical methods based on gas chromatography for the analysis of CIT

Analyte	Matrix	Sample pre-treatment	Separation mode	Detector	Sensitivity	Reference
CIT, DCIT, DDCIT, CIT-PROP	Plasma	LLE LLE + SPE (CIT-PROP	GC	MS	LOQ: 1–2 ng/mL	Reymond *et al.* (1993)
CIT; DCIT, DDCIT; SER, DSER, PRX	Plasma	LLE	GC	MS	LOQ: 2 ng/mL (CIT), 0.5 ng/mL (DCIT and DDCIT)	Eap *et al.* (1998)
CIT, DCIT	Serum	LLE	GC	NPD	LOQ: 10–20 μg/L	Lacassie *et al.* (2000)
CIT and other drugs	Urine	SPE	GC	MS	—	Paterson *et al.* (2000)
CIT, FLV, PRX, MNS, DXP, ETP	Blood	SPE	GC	NPD	LOQ: 70 ng/mL	Martinez *et al.* (2004)
CIT, FLX, FLV, SER, PRX	Pharmaceutical formulations, urine	SPE (urine)	GC	FID	LOQ: 91.7 μg/L	Berzas *et al.* (2004)
CIT and 13 further drugs	Blood	LLE	GC	MS	LOQ: 0.05 μg/mL	Paterson *et al.* (2004)
CIT and other drugs	Methanolic solution	—	GC	MS	—	Song *et al.* (2004)
CIT and other drugs	Serum, plasma, whole blood	LLE	GC	MS	LOQ: 25 ng/mL (CIT)	Gunnar *et al.* (2004)
CIT, VLF, FLV, MTZ, FLX, SER	Urine	SPME	GC	MS	LOD < 0.4 ng/mL	Salgado-Petinal *et al.* (2005)
CIT; FLX, FLV, SER, PRX	Pharmaceutical formulations	—	GC	MS	LOQ: 3.6–41.5 mg/L	Nevado *et al.* (2006a)
CIT, DCIT, DDCIT, CIT-N-oxide, CIT-PROP, FLX, NFLX	Urine	SPE	GC	MS	LOD: 0.7 ng/L (CIT), 33.6 μg/L (CIT-PROP)	Nevado *et al.* (2006b)
CIT, DCIT, DDCIT and 12 further antidepressants and 7 metabolites	Plasma	SPE	GC	MS	LOQ: 5–12.5 ng/mL (electron ionization), 1–6.25 ng/mL (chemical ionization)	Wille *et al.* (2007)

Abbreviations of compounds: CIT-PROP, citalopram propionic acid; DSER, desmethylsertraline; DXP, doxepin; ETP, etoperidone; FLV, fluvoxamine; FLX, fluoxetine; MNS, mianserine; MTZ, mirtazapine; SER, sertraline; PRX, paroxetine; VLF, venlafaxine.

Table 3. Analytical methods based on electrodriven methods for the analysis of CIT

Analyte	Matrix	Sample pre-treatment	Separation mode	Detector	Sensitivity	Reference
CIT, FLX, FLV, SER	Pharmaceutical formulation	—	Capillary ITP	CD	LOQ: 2 μg/mL)	Buzinkaiova *et al.* (2000)
CIT, DCIT	Plasma	LPME	CE	UV	LOQ: 16,5–18 ng/mL	Halvorsen *et al.* (2001)
CIT, DCIT, FLV, FLX, SER, PRX, VLF, DVLF, MCP, MTZ, DMTZ	Blood, urine	LLE	MEKC	DAD	LOQ: 20–30 ng/mL	Labat *et al.* (2002)
CIT, FLX, FLV, PRX, SER	—	—	MECK	DAD	—	Pucci *et al.* (2002)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT	Pharmaceutical formulations	—	CE (β-CD)	DAD	LOQ: 0.5 μg/L	Mandrioli *et al.* (2003)
R- and *S*-CIT, *R*- and *S*-DCIT	Plasma	LPME	CE (sulfated-β-CD)	UV	LOQ: <11.2 ng/mL	Andersen *et al.* (2003)
CIT; FLX, FLV, PRX, CLP, TRZ	Pharmaceutical formulations	—	CZE	UV	LOQ: 0.11–0.38 mg/L	Flores *et al.* (2004)
R- and *S*-CIT	Pharmaceutical formulations	—	CE	DAD	LOQ: 0.2 mg/L	Nevado *et al.* (2005)
R- and *S*-CIT, *R*- and *S*-DCIT, *R*- and *S*-DDCIT, *R*- and *S*-CIT-N-oxide CIT-PROP	Urine	SPE	CE (carboxymethyl-γ-CD)	UV	LOQ: 80 340 μg/L	Berzas-Nevado *et al.* (2006)
CIT	Pharmaceutical tablets, serum	Protein precipitation (serum)	CE	UV	LOQ: 1.72×10^{-5} M	Satana *et al.* (2006)
CIT, LTZ	Urine	SPE	MEKC	UV	LOD: 12.5–25 ng/mL	Flores *et al.* (2008)
R- and *S*-citalopram, *S*-citadiol	Bulk drug and tablets	—	CE (β-CD)	DAD	LOQ: 5 mg/mL	Sungthong *et al.* (2008)
CIT, SER, FLX, PRX, FLV	Plasma	On-line preconcentration, cation-selective exhaustive injection	Sweeping-MECK	DAD	LOD: 0.35 ng/mL in plasma	Su and Hsieh (2008)
CIT, FLX, SER, PRX	Plasma	LLE	Nonaqueous CE	DAD	LOQ: 15–30 ng/mL	Catai *et al.* (2009)

Abbreviations of compounds: β-CD, beta-cyclodextrin; CLP, clomipramine; DMTZ, desmethylmirtazapine; DVLF, desmethylvenlafaxine; FLV, fluvoxamine; FLX, fluoxetine; γ-CD, gamma-cyclodextrin; LTZ, letrozole; MCP, milnacipam; MTZ, mirtazapine; PRX, paroxetine; PRX, paroxetine, SER, sertraline; TRZ, trazodone; VLF, venlafaxine/ Abbreviations of analytical techniques: CD, conductivity detector; CZE, capillary zone electrophoresis; ITP, isotachophoresis; LPME, liquid-phase microextraction; MECK, micellar electro-kinetic.

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problems of LLE with CE, a simple and disposable device for liquid-phase microextraction (LPME) was developed (Andersen *et al.*, 2003; Halvorsen *et al.*, 2001; Vasskog *et al.*, 2008). LPME was accomplished in conventional 4 mL vials utilizing a porous polypropylene hollow fiber as compartment for the final extract; each sample was transferred to a vial, the pH was adjusted to deionize the analyte within the sample, and the analytes were extracted through an organic solvent in the pores of the hollow fiber and into a new aqueous phase (acceptor phase) inside the hollow fiber. With a high volume ratio between the samples (1–2 mL) and the acceptor phase (10–25 µL), high enrichment factors were reported. In the procedure developed by Halvorsen *et al.*, (2001), prior to extraction, the samples were made strongly alkaline in order to promote LPME of the basic drugs. Owing to the high ratio between the volumes of sample and acceptor phase and to high partition coefficients, CIT and DCIT were enriched by a factor of 25–30. In addition, sample clean-up occurred during LPME since salts, proteins and the majority of endogenic substances were unable to penetrate the hexyl ether layer. Furthermore, the extracts were aqueous and they were injected directly into the CE instrument. In order to proceed with the current evaluation of LPME, repeatability, linearity, limit of detection and limit of quantification (16.5 and 18 ng/mL) were determined for CIT and DCIT respectively. High preconcentration enabled quantification in the therapeutic range and sample clean-up was highly efficient with no interfering peaks from matrix components. Some years later, Andersen *et al.* (2003) proposed a LPME method based on a rodlike porous polypropylene hollow fiber. The analytes were extracted from 1 mL plasma made alkaline with NaOH, into dodecyl acetate impregnated in the pores of a hollow fiber, and into 20 mM phosphate pH 2.75, inside the hollow fiber. The recoveries were 46% for CIT enantiomers and 29% for DCIT enantiomers corresponding to 31 and 19 times enrichment.

SPE using different materials has been widely employed for sample pre-treatment in CIT and metabolites determination. Reversed-phase C_8 (Molander *et al.*, 2002) and C_{18} (Akerman *et al.*, 1998; Meng and Gauthier, 2005) applications have been most often described. The major advantage of the polymeric reversed-phase materials is the ease of use, since there is no need to keep the phases moisturized to maintain their interaction capacities. Additionally, they have both hydrophilic and lipophilic properties and are capable of capturing polar analytes. This is advantageous in the search for metabolites, which are usually more polar.

Higher recoveries, cleaner extracts, better sensitivity, better precision and reduced solvent consumption and disposal were achieved for the screening of CIT and other antidepressants with the use of the mixed SPE Bond Elut Certify compared with Chem Elut columns (Martinez *et al.*, 2004). Clean extract were also obtained in the determination of CIT and its main metabolites DCIT and DDCIT employing end-capped C2 column (100 mg of sorbent and 10 mL reservoir size) utilizing secondary silanol interactions (Carlsson and Norlander, 1997). This method presented good sensitivity and selectivity since it was noted that several anxiolytics, neuroleptics and other antidepressants did not interfere with the analytes. It was applied for the analysis of about 250 patient plasma samples. SPE based on weak cation exchange (Bond Elut CBA SPE columns) has been used for determination of CIT and 17 opium alkaloids and opioids in blood and urine samples (Dams *et al.*, 2002). Mixed mode bonded silica, C_8 + SCX, containing both reversed-phase and cationic exchange compounds, is one of the most versatile SPE products on the market due to generic extraction methods and some successful applications for cleaner extraction and recovery of basic and zwitterionic compounds from biological fluids (Liu *et al.*, 2009). The only described SPE method not based on a reversed-phase mechanism uses Extrelut-3 cartridges (Rop *et al.*, 1990). Extrelut cartridges contain kieselguhr as stationary phase and extraction is based on a normal-phase mechanism.

Automated SPE systems that allow the systematic processing of a large number of samples have been recently introduced (Vendelin-Olesen *et al.*, 2000). Fully automated online solid-phase extraction coupled to liquid chromatography tandem mass spectrometry (SPE-LC-MS/MS) was developed and validated for the direct analysis of 14 antidepressants and their metabolites in plasma. Integration of the sample extraction and HPLC separation into a single system permitted direct injection of the plasma without prior sample pre-treatment (de Castro *et al.*, 2007).

An alternative to SPE is the solid-phase microextraction (SPME). Amongst the observed benefits of SPME are its minimal sample volume requirement and that it is easily automated, which allows the preconcentration of the analytes. Since the advent of the solid-phase microextraction (SPME), initially developed by Pawliszyn (Arthur and Pawliszyn, 1990), associated with thermal desorption in GC, many authors have developed modifications of this extractive approach and associated devices to improve the availability and application of this sample preparation technique on-line coupling with a chromatograph. SPME-GC has been used for determination of six SSRI in urine samples (Salgado-Petinal *et al.*, 2005). Fibers of 65 µm polydimethylsiloxane–divinylbenzene (PDMS-DVB) were used in this method, which enabled simultaneous determination of the target SSRIs after simple *in-situ* derivatization by acetylation of fluvoxamine (FLV), fluoxetine (FLX) and sertraline (SER). A 1.5 g aliquot of sodium chloride was then added to the samples. The vial was sealed with an aluminum cap and a Teflonfaced septum. It was immersed in a water bath at 100°C and left to equilibrate for 5 min. To perform the extraction the SPME fiber was exposed to the magnetically stirred sample for 30 min. The fiber was then immediately inserted into the GC injection port and analysis was performed. Desorption time was set at 3 min. The time required for the SPME step and for GC analysis (30 min each) enabled high throughput.

For SPME-HPLC coupling, the extraction procedure is similar to that used for GC analysis. The main difference between SPME-HPLC and SPME-GC is the second step, the desorption procedure. In HPLC analysis, thermal desorption at high temperature creates practical problems such as degradation of the polymer, and furthermore, many non-volatile compounds cannot be completely desorbed from a fiber. Solvent desorption is thus proposed as an alternative method for SPME–HPLC coupling (Fig. 2). An organic solvent (static desorption) or the mobile phase (dynamic desorption) is used to desorb the analytes from the SPME fiber. In order to apply this desorption an SPME-HPLC system with a desorption chamber and a six-port valve called an SPME-HPLC interface is used. CIT has been determined by a conventional SPME-HPLC method (Silva *et al.*, 2007; Unceta *et al.*, 2008) and using a modified fiber obtained by electrochemical deposition (cyclic voltammetric) of a polypyrrole (PPY) film on a stainless-steel wire (Chaves *et al.*, 2009). The best SPME experimental conditions among those investigated for antidepressant assays were as follows: to 250 µL of plasma 4 mL phosphate buffer (pH 7) was added and the extraction was carried out at 25°C for 40 min. The

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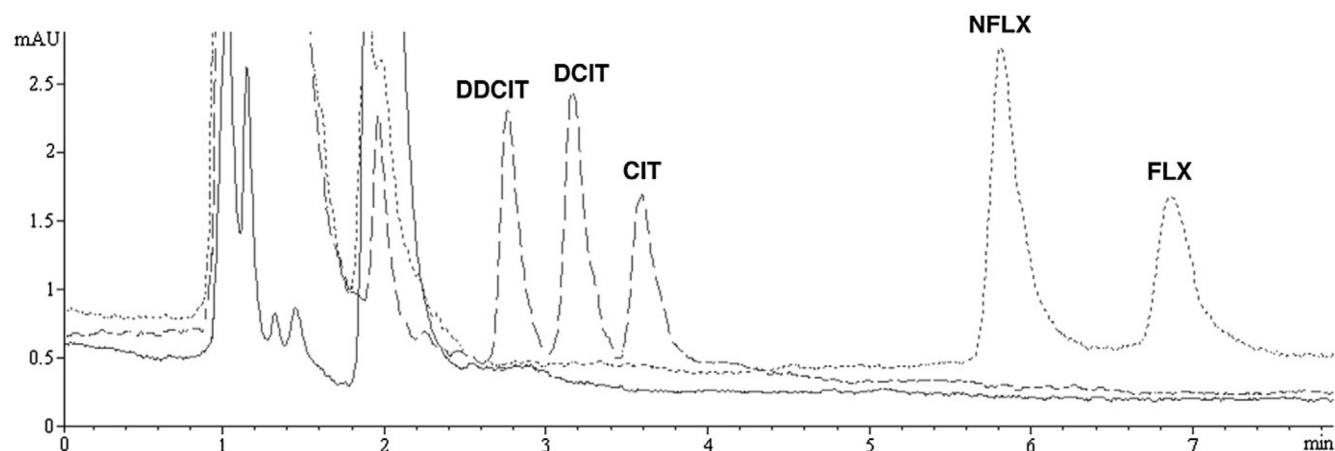

Figure 2. SPME-HPLC-DAD chromatograms obtained from blank urine sample (normal line), urine from volunteer 1 (DDCIT, 0.66 mg/L; DCIT, 0.84 mg/L; CIT, 0.48 mg/L; dashed line) and urine from volunteer 2 (NFLX, 1.07/L; FLX, 0.50 mg/L; dotted line). Reprinted with permission from Unceta *et al*. (2008).

drug liquid desorption was made on mobile phase at 25°C for 15 min. This SPME-PPY/HPLC method presented high sensitivity, precision and accuracy and enabled the quantification of antidepressants in human plasma following oral administration.

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. Conceptually, in-tube SPME should preserve the advantages of SPME and could offer improved enrichment efficiency, quantification and automation through the on-line coupling with a chromatograph. By using a piece of bonded-phase capillary GC column for sorption, a larger amount of stationary phase and a more robust film are obtained, relative to outside-coated films of conventional SPME fibers. These differences result in higher enrichment factors and longer extractor life. Since many capillary GC stationary phases are commercially available, in-tube SPME enables easy changing of the extraction-phase polarity, which extends the application range of the method (Wang and Lemmer, 1989). The technique has been successfully used employed for quantification of CIT and other nontricyclic antidepressants in human plasma using LC (Silva *et al*., 2008).

Other techniques such as sorpive stir bar extraction (SBSE) have also been used for the determination of CIT in plasma. In most cases SBSE has been used with GC in conjunction with thermal desorption systems (Crifasi *et al*., 2006). In this way, in the extraction stage, the compounds are adsorbed on the surface of the stir-bar and then thermally desorbed in an injector-type ATD (authomated thermal desorption) for GC. The process requires a later cryofocus stage with liquid nitrogen or by Peltier effect, prior to chromatographic injection (Sanchez-Ortega *et al*., 2009). The use of SBSE favors improvements in the limits of quantification, as the surface of extraction is higher than microextraction fibers. Although SBSE was initially designed for GC, it has also been used with HPLC, using chemical desorption rather than thermal. So CIT has been quantified in plasma (Chaves *et al*., 2007) and urine and brain tissue samples (Unceta *et al*., 2010b). In most cases the stir bar is coated with polydimethylsiloxane (PDMS), but recently a dual-phase of PDMS and PPY has been used to improve the efficiency of the extraction step (Melo *et al*., 2009).

On the other hand, it must be borne in mind that CIT and/or their phase I metabolites are excreted in urine as highly hydrophilic glucuronic acid or sulfuric acid conjugates which are not amenable to extraction and GC and therefore they must be cleaved prior to further workup. This can be achieved by either acidic or enzymatic hydrolysis (Peters *et al*., 2009). In the case of acid hydrolysis, urine is refluxed with concentrated hydrochloric acid, leading to cleavage of acetalyc and acylalic glucuronides and sulfuric esters. This aggressive cleavage procedure is rapid, simple and cheap, but it is often associated with the formation of artifacts or the degradation of analytes. Enzymatic cleavage is gentle compared with acid hydrolysis, but is comparatively expensive and time-consuming. It can be performed using β-glucuronidase (GRD) and/or arylsulfatase (ARS) from various species.

Therefore, in most cases, the procedures for sample pretreatment and extraction are performed together, trying to minimize the number of stages and the time of analysis. Thus, (Paterson *et al*., 2000) developed a method for the determination of drugs of abuse, which allows simultaneous extraction, derivatization and analysis of acidic, neutral and basic drugs from urine. Urine samples were subjected to enzymatic hydrolysis followed by SPE using Bakerbond narc-2 columns. The eluant was selectively derivatized with *N*-methyl-bis-trifluoroacetamide (MBTFA) and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide plus 1% trimethylchlorosilane.

Other, previously unmentioned, extraction techniques have also been used, such as solvent microextraction with back extraction (SME/BE). The extraction process was performed in a homemade pure glass vial without using a Teflon ring, usually employed in SME/BE. CIT was first extracted from 0.5 mL of plasma, modified with sodium hydroxide, into hexane. A back-extraction step was then performed into 5.2 μL of 45 mM ammonium formate solution (pH 4) using a GC microsyringe. The extraction was subsequently transferred into a liner-like vial and then injected into the HPLC system. An enrichment factor of 150 along with a good sample clean-up was obtained (Bagheri *et al*., 2008).

There are other matrices different from those of a biological nature in which it is possible to analyze CIT and their select degradates. This is the case of environmental samples and in particular aquatic matrices such as wastewater effluents (Schultz and Furlong, 2008) or seawater and sewage influents and effluents (Vasskog *et al*., 2008). The ultra trace levels of pharmaceuticals in

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these matrices require an analyte preconcentration procedure in order to obtain the required sensitivity. This procedure has usually been carried out by solid-phase extraction (SPE), which allows the preconcentration of the analytes increasing the sensitivity of the method. Schultz (Schultz and Furlong, 2008; Schultz *et al.*, 2010) employed Waters Oasis HLB solid-phase extraction cartridges (Milford, MA, USA) that were conditioned by first wetting the sorbent with 5 mL of water and 5 mL of methanol. The 1 L of acidified sample was then added to the cartridge at a flow rate of 15 mL/min. The cartridge was washed with 5 mL of 70% methanol in 2% ammonium acetate. The analytes of interest were eluted from the cartridge with 10 mL of 70% methanol in 2% acetic acid. The 10 mL extract was dried under a stream of nitrogen to a volume of 0.1 mL. This way, the authors obtained LOQ in the range of the ng/L.

A method developed by Vasskog *et al.* (2006) allowed better LOQ limits to be obtained, but it was more laborious and time-consuming since a SPE procedure followed by a LLE was required. This problem was solved by these authors with the development of a method based on a three-phase hollow-fiber supported liquid phase microextraction of 1.1 L samples, followed by HPLC-ESI-MS and which was applied for the quantification of CIT, DCIT, DDCIT and several antidepressants more (Vasskog *et al.*, 2008). A 28 cm long piece of plasmaphan polypropylene hollow fiber from Membrana (Wuppertal, Germany) was utilized for extraction. The inner diameter of the hollow fiber was 330 μm, the thickness of the wall was 140 μm and the pore size was 0.4 μm. The sensitivity was improved obtaining LOQ between 57 and 781 pg/L.

Although in these cases the technique usually chosen is SPE, there are references on the use of SPME. This is the case of the determination of 16 pharmaceutical compounds, including CIT, in influent and effluent wastewater treatment plant samples, using dual-SPME (Unceta *et al.*, 2010a), whereby the water samples are divided into two aliquots of 2 mL each and extracted by two CW-TPR fibers at different pH values (pH 3 and 11) and with an NaCl concentration of 300 g/L at 75°C for 30 min. The analytes in both fibers are desorbed one after the other in the desorption chamber in static mode with mobile phase for 10 min. The extracts are injected into an HPLC system coupled to an ion trap mass spectrometer.

Liquid Chromatography

Achiral Methods

Several analytical methods have been reported for achiral assay of CIT and/or its main metabolites using liquid chromatography (Table 1). The most common type of chromatography used with HPLC is reversed-phase chromatography containing non-polar C_{18} groups. The choice of the mobile phase depends on the compounds and the detection used and isocratic elution is preferred to gradient elution in almost all methods. Several detectors have been used in conjunction with HPLC for the analysis of these compounds.

Ultraviolet detection (UV) has been very commonly used in CIT detection, despite the limits of quantitation obtained with this technique being in general not as low as the ones obtained with FLD or MS. In the 1980s, Rop *et al.* (1985) described an isocratic reversed-phase HPLC method using a μBondapack C_{18} column for the determination of CIT, AMIT, CLM and their desmethylmetabolites The analytes were extracted from plasma samples after LLE with diethylether. Good sensitivity and selectivity were obtained since LOD were lower than 5 ng/mL for each compound and the interference of several drugs was studied. Some years later, the same authors also developed a method for the quantitation of CIT, DCIT and DDCIT after a SPE from plasma samples (Rop *et al.*, 1990). The proposed procedure was less time-consuming than the previous one and it was sensitive enough (LOQ < 0.8 ng/mL) for its application in pharmacokinetic studies and therapeutic monitoring.

Several HPLC-UV methods have been also developed for the determination of CIT and DCIT in biological matrices. While the methods developed by Olesen and Linnet (1996) and Rampono *et al.* (2000) were based on a previous LLE from serum and plasma and milk samples respectively, Akerman *et al.* (1998) employed an SPE procedure with a Bond-Elut C_{18} column for the extraction of the analytes from serum samples. The sensitivity obtained with these methods were quite similar, varying from 1 ng/mL in the method developed by Rampono *et al.* to 5 ng/mL in the method of Akerman *et al.*

Owing to the wide applicability of the UV detector, it has been deemed suitable for the development of multiresidue liquid chromatography methods. As well as several SRSIs, these procedures were developed for the screening and/or quantitation of several tricyclic and non-tricyclic antidepressants and antipsychotics (Berzas *et al.*, 2002; Catai *et al.*, 2009; Chaves *et al.*, 2007, 2009; Dallet *et al.*, 2002; Duverneuil *et al.*, 2003; Frahnert *et al.*, 2003; Lai *et al.*, 1997; Malfara *et al.*, 2007; Melo *et al.*, 2009; Silva *et al.*, 2008; Su and Hsieh, 2008; Titier *et al.*, 2003; Tournel *et al.*, 2001; Wille *et al.*, 2005). Most of these methods were applied to the determination in plasma or serum samples; only the method developed by Lai *et al.* (1997) was validated for urine samples. The limits of quantitation of CIT obtained with these methods varied between 0.35 and 50 ng/mL in plasma samples.

Fluorescence detection (FLD) presents some advantages to ultraviolet detection. In addition to higher sensitivity, FLD is more specific and selective reducing some interfering peaks. It was the first detection method used for the analysis of CIT and its desmethyl metabolite DCIT by Overo (1978). This method required the LLE extraction of the analytes with hexane from alkaline plasma and their derivatization with 9,10-dimethoxyanthracene-2-sulfonate.

Oeyehaug *et al.* (1982) also coupled liquid chromatography to FLD for the analysis of CIT and its main desmethyl metabolites DCIT and DDCIT. These compounds were extracted from alkaline plasma with diethylether and analyzed in a Spherisorb ODS column with reversed-phase liquid chromatography. The detection was performed at 240 nm for excitation and 296 nm as emission wavelength. This method enabled limits of detection to be obtained of 1 ng/mL for CIT and 0.5 ng/mL for its metabolites and it was also applied for the quantification of CIT, DCIT, DDCIT and its *N*-oxide and propionate-derivatives in urine samples.

Matsui *et al.* (1995) were able to perform a new method for the analysis of these compounds in plasma by HPLC-FLD with a successive column-switching technique. Plasma samples were injected directly onto a guard column where the analytes were retained and later eluted employing a six-port valve by the back-flush method. The compounds were analyzed at an excitation wavelength of 249 and an emission wavelength of 302 nm.

Kristoffersen *et al.* (1999) proposed a method for the simultaneous determination of the three selective serotonin reuptake inhibitors CIT, FLX, paroxetine (PRX) and their metabolites in whole blood and plasma. Sample clean-up and separation were

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achieved using a SPE method with C_8 non-endcapped columns followed by reversed-phase HPLC with FLD for CIT, PRX and their metabolites and UV detection for FLX and NFLX. The limits of quantitation obtained for CIT and its metabolites were of 0.025–0.05 μmol/L, suitable for the therapeutic drug monitoring of these compounds but not as low as the limits obtained with other fluorescence based methods.

Several FLD based methods for the quantification of CIT; DCIT and DDCIT were also performed. Ohman *et al*. (2001) made a comparison between a new on-line extraction method using an RP-C4-ADS extraction column and an off-line SPE method, showing that the two methodologies were equal in analytical precision but that the on-line method was faster and therefore superior in sample capacity per day. Waschgler *et al*. (2002) presented an analytical procedure for the simultaneous quantification of CIT, clozapine (CLZ), fluoxetine (FLX), norfluoxetine (NFLX), maprotiline (MPT), desmethylmaprotiline (DMPT) and trazodone (TRZ) in human serum using HPLC. These drugs and metabolites were extracted with two liquid–liquid extractions in the sample preparation phase and separated on a C_{18} column. Native fluorescence were detected at 260 nm and 227/300 nm for excitation and emission respectively.

Electrochemical detectors are rarely used coupled to HPLC to determine CIT. This is due to the extreme potential of oxidation or reduction of the citalopram molecule. Al-Ghazawi *et al*. (2007) developed a method for the quantification of CIT in plasma samples after LLE extraction employing amperometric detection with a LOQ of 1.493 ng/mL. This method was applied to assess the bio-equivalence of two citalopram 40 mg tablet formulations.

Since 2004, mass spectrometry (MS) has been the most commonly used detector in the determination of antidepressants, the high specificity and the lower limits of detection obtained being the main advantages with this detection technique. There are different types of mass analyzers: quadrupole mass spectrometers, suitable for quantitative analysis; time of flight (TOF) mass spectrometers, which have a high resolution and exact mass measurement capabilities; and ion trap mass spectrometers, that allow the carrying out of MS^n-analysis, performing structure elucidation studies. With a mass spectrometer, it is possible to monitor only the mass of the analyte (or the transition from one mass to another after the fragmentation of the compound). Usually, when a triple quadrupole analyzer is used, mass spectra data showed protonated molecular ion peaks at *m/z* 325, *m/z* 311 and *m/z* 297 for CIT, DCIT and DDCIT, respectively, that were used as precursor ions. Multiple rection monitoring (MRM) was used to quantify the most abundant product ions: *m/z* 325 → *m/z* 109, *m/z* 311 → *m/z* 109, and *m/z* 297 → *m/z* 109 obtained with collision energy of 20–23 eV, for CIT, DCIT and DDCIT respectively (Vasskog *et al*., 2008). This characteristic avoids the presence of interfering peaks and reduces noise, resulting in lower LOQs. In addition, an MS can identify a peak in a chromatogram using a full-scan mass spectrum. Muller *et al*. (2000) developed an LC/MS method for the identification of CIT, maprotiline (MPT), their demethylated metabolites DCIT and DMPT and pipamperone in hair samples. Electrospray ionization (ESI) with in-source collision-induced dissociaton (ESI/CID) and tandem-mass spectrometry (MS/MS) were used for drug and metabolites identification. Extracted ion chromatograms were used for the detection of the metabolites, which were also identified by their fragment-ion spectra, The lowest LOD obtained with this method was 0.1 ng/mg. This method was applied to hair samples of patients of psychiatry and suicide cases after the ultrasonication in methanol of powdered hair and the subsequent SPE.

Some years later, Kollroser and Schober (2003) developed an HPLC atmospheric pressure chemical ionization tandem mass spectrometry (APCI-MS/MS) method for the identification and quantitation of CIT, FLV and PRX in human plasma. Plasma samples were diluted in 0.1% formic acid and directly injected into the HPLC system. Proteins and other large biomolecules were removed during an on-line sample clean-up step. The LOQ obtained with this method, below 20 ng/mL, were not as good as the ones obtained with other methods. However, it permitted the direct analysis of plasma samples avoiding a time-consuming sample preparation step.

The limits of quantitation were improved in the narrow-bore LC-ESI/MS method validated by Pistos *et al*. (2004) for the quantitation of CIT in human plasma. They employed a Hypersil BDS C_8 micro-bore column (250 × 2.1 mm i.d.; 3.5 μm particle size) and a mobile phase with 10 mM ammonium formate–formic acid (pH 4.5) and acetonitrile (30:70, v/v) at a flow rate of 0.15 mL/min. The analyte and the internal standard imipramine were extracted by LLE with a mixture of hexane–heptane–isopropanol (88:10:2). The analytes were detected after selected ion-monitoring mode, at *m/z* 325 for CIT. The LOQ was 0.5 ng/mL and the accuracy and precision were acceptable.

Since the development of the method of Pistos *et al*., several LC-MS based methods have been performed for the quantitation of CIT and DDCIT (Jiang *et al*., 2010) or CIT and another pharmaceuticals (de Castro *et al*., 2007, 2008; El-Rjoob *et al*., 2010; He *et al*., 2005; Kirchherr and Kuhn-Velten, 2006; Mendes *et al*., 2005; Sauvage *et al*., 2006a, b) in plasma and serum samples.

Kirchherr and Kuhn-Velten (2006) developed a method for the simultaneous analysis of 48 antipsychotics and pharmacologically active metabolites in serum, where CIT was included, which required only protein precipitation and stepwise dilution for sample preparation. Drugs were assigned to subgroups covering low, medium and high concentrations by further dilution of the supernatant obtained after the first protein precipitation. After electrospray ionization positive ion fragments were detected in the multiple reaction monitoring (MRM) mode with a tandem mass spectrometer. The method allowed a general view on the individual intake of psychoactive drugs and its accurate quantification as well.

The procedure proposed by de Castro *et al*. (2007) permitted the analysis of CIT and 13 further antidepressants and their metabolites in plasma samples. An automated on-line SPE-LC-MS/MS method permitted direct injection of 50 μL of plasma without prior sample pre-treatment ensuring the analysis of the samples in 20 min. Several deuterated drugs were employed as internal standards. For the detection, a tandem mass spectrometer with a triple quadrupole was employed operating in electrospray in the positive ionization mode. Selectivity of the method was achieved by a combination of the retention time and two precursor-product ion transitions for the non-deuterated compounds. The LOQs were estimated to be 10 ng/mL for all compounds. These LOQs were improved by the authors in another paper in which on-line SPE-LC-MS/MS was also applied for the quantitation of several antidepressants, including CIT, in plasma and oral fluids (de Castro *et al*. 2008).

LC-MS/MS has also been applied for the determination of CIT and its process impurities in bulk drugs and pharmaceutical formulations. After the monitorizaton in an UV detector, the degra-

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dation products and unknown impurities were isolated and characterized by MS/MS (Rao *et al.*, 2008; Sun *et al.*, 2007).

Mass analyzers such as Q-TOF and IT have also been used for for screening and for the structural elucidation of the CIT and other antidepressants. Sequential product ion fragmentation experiments (MSn) have been performed in order to elucidate the degradation pathways for the $[M + H]^{(+)}$ ions and their predominant product ions in hair samples (Smyth *et al.*, 2006). Citalopram gives a protonated molecule at *m/z* 325.2 in ESI ion trap mass spectrometry (ITMS) with negligible in-source fragmentation. MS^2 results in a most abundant product ion at *m/z* 262.1 and less abundant ions at *m/z* 307.1 and 279.9. MS^3 of the former ion gives *m/z* 234.3 which in turn gives an MS^4 ion at *m/z* 215.6. QTOF-MS/MS gives ions at *m/z* 325.1716, 307.1601, 280.1179, 262.1037, 234.0711 and 215.0905.

The market entry of the techniques of ultra-high-pressure liquid chromatography (UHPLC or UPLC) has managed to reduce the time analysis and the effectiveness of the separations. Thus, methods have been developed for the determination of CIT and other compounds by UPLC-MS-MS with a total run time for all analyses of 1.2 min using BEH 1.7 micron particle C_{18} columns (Smalley *et al.*, 2009). Similarly, using Q-TOF mass analyzers, Nielsen *et al.* (2010) have developed and validated an UPLC–TOF-MS method for simultaneous screening and quantification of 52 drugs in hair, with a total chromatographic run time of 17 min and LOQ for CIT of 0.05 ng/mg.

In recent years, the interest in the analysis of pharmaceutical products in environmental waters has grown. It is thought that the continuous exposure to these compounds, even at low levels, might affect the health of wildlife and humans (Daughton and Ternes, 1999). The presence of antidepressants in environmental water results from human excretion in urine and feces in high percentages and their subsequent discharge into domestic wastewaters. These compounds are present at low levels and in a matrix where many different molecules are present. All this has led to the increased use of LC-MS and several multiresidue methods have been developed for the analysis of CIT and other pharmaceutical products in recent years (Demeestere *et al.*, 2010; Fick *et al.*, 2009; Kwon and Armbrust, 2005; Lajeunesse *et al.*, 2008; Metcalfe *et al.*, 2010; Schultz *et al.*, 2010; Unceta *et al.*, 2010a; Vasskog *et al.*, 2006, 2008).

Chiral Methods

With regard to the monitoring of (*R*) and (*S*) enantiomers of CIT and of their metabolites, different HPLC stereoselective methods have been developed as shown in Table 1. In 1995, Rochat *et al.* (1995a) developed a HPLC-FLD method for the assay of the enantiomers of CIT, DCIT, DDCIT and CIT-PROP in plasma after their separation on a Chiralcel OD column. The LOQs in plasma samples were 15, 4, 5 and 2 ng/mL for CIT, DCIT, DDCIT and CIT-PROP respectively. Except CIT, all the metabolites were derivatized with achiral reagents, DCIT and DDCIT with heptafluorobutyric acid and CIT-PROP with iodomethane. However, in a later publication, the authors presented a procedure where the underivatized enantiomers of CIT, DCIT and DDCIT were analyzed using the reversed-phase mode HPLC with an acetylated β-cyclobond column (Rochat *et al.*, 1995b). They also obtained a slightly better LOQ of 3 ng/mL for plasma samples.

From this time on, different chiral stationary phases (CSPs) were used in the enantioseparation of (*R*) and (*S*)-CIT and/or its metabolites. A Macrocyclic antibiotic CSP Chirobiotic V column has been employed coupled to FLD (Kosel *et al.*, 1998), UV detector (Zheng *et al.*, 2000) and MS (MacLeod *et al.*, 2007) and different cellulose based-Chiralcel columns have been applied also with FLD (Rochat *et al.*, 1995a, b) and MS (Rocha *et al.*, 2007). As an alternative to the high cost of the chiral columns, El-Gindy *et al.* (2006) developed a HPLC-UV method based on the addition of beta-cyclodextrin as a chiral additive to the mobile phase. However, this method was validated only for the enantioselective separation of (*R*) and (*S*)-CIT.

Another option for the enantioselective determination is the derivatization of the analytes with a chiral reagent to form diastereoisomeric derivatives and their chromatographic separation in an achiral column. Owing to the studies that reported novel methods for treating depression and other CNS disorders using enantiomerically enriched demethyl- and didemethylmetabolites of CIT (Bush *et al.*, 2003), Millan *et al.* (2008) described a procedure for the separation of the enantiomers of DCIT and DDCIT. This assay involved a previous LLE of the analytes from plasma and brain tissue samples followed by a pre-column chiral derivatization with (–)-(*R*)-1-(1-naphthyl)ethyl isocyanate and the separation on a normal-phase silica column. However, in this case the derivatization of CIT with this chiral reagent was not possible because of its tertiary amine structure.

Some works combine methods of screening and confirmatory tests for the discrimination of enantiomers. Thus Carlsson *et al.* (2009) investigated (*S*)-CIT present in forensic autopsy cases positive for the presence of CIT in routine screening using a non-enantioselective bioanalytical method. Fifty out of the 270 samples found positive by gas chromatography–nitrogen-phosphorus detection were further analyzed using enantioselective high-performance liquid chromatography. The 50 cases were genotyped for CYP2D6 and CYP2C19, as these isoenzymes are implicated in the metabolism of CIT and (*S*)-CIT. In samples positive for racemic citalopram using the screening method for forensic autopsy cases, up to 20% would have been misinterpreted in the absence of an enantioselective method. An enantioselective method is thus necessary for correct interpretation of autopsy cases, after the enantiomer has been introduced to the market.

Gas Chromatography

Gas chromatography has not been as widely used as liquid chromatography for the determination of CIT and/or its metabolites (Table 2). GC-MS in the full-scan electron impact (EI) ionization mode is considered the reference technique for drug screening procedures since it plays an important role in the analysis of drugs in forensic toxicology and doping control. The main advantage of this technique is that it provides comparable GC-MS spectra on all commercially available instruments, since the standardized ionization EI mode produces unique and uniform mass fragmentation patterns. As a consequence, large mass spectral libraries have been constructed which enable the identification of unknown compounds. Although this technique is limited to volatile or semi-volatile compounds, advances in derivatization methods for converting non-volatile and/or thermally labile analytes to a suitable form for GC analyses have extended its use to a wider variety of drugs and metabolites (Song *et al.*, 2004).

The first method based on GC-MS proposed for quantitative determination of CIT, DCIT, DDCIT and CIT-PROP in plasma samples was developed by Reymond *et al.* (1993). After the addition of a separate internal standard for each drug, LLE was used to

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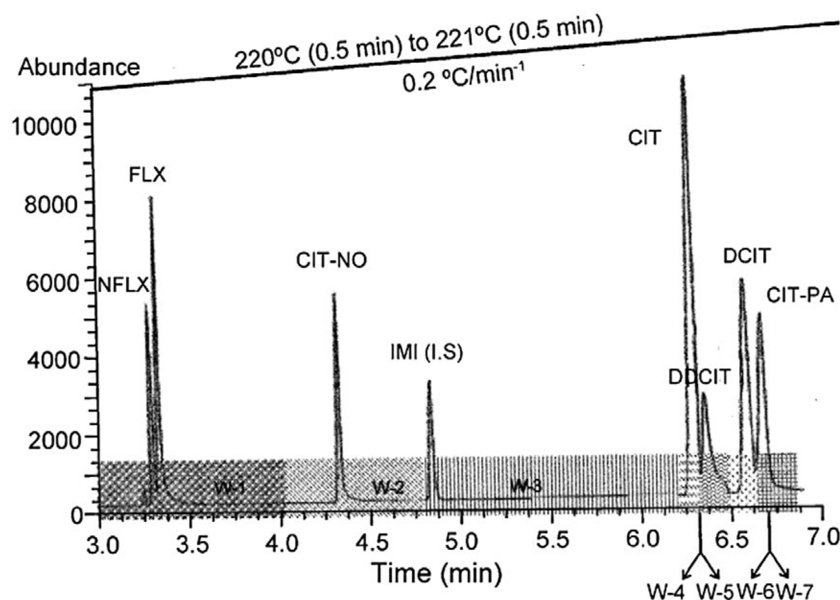

Figure 3. MS-SIM chromatogram of a NFLX (2 mg/L), FLX (0.5 mg/L), CITNO (2 mg/L), CIT (0.05 mg/L), DDCIT (4 mg/L), DCIT (0.5 mg/L), CIT-PA (2 mg/L) and IMI (0.5 mg/L) spiked standard solution, obtained under the selected GC–MS-SIM conditions. W1–W7 = working windows for monitoring the selected ions in SIM mode. Reprinted with permission from Nevado *et al*. (2006a).

separate the basic compounds from the acid ones. The demethylated amines are derivatized with trifluoroacetic anhydride, and the acid metabolite with methyl iodide. GC-MS is performed in the electron impact mode, as mass spectrometry by the chemical ionization mode (methane and ammonia) was unsuitable. The limits of quantification were 1 ng/mL for CIT and DCIT and 2 ng/mL for the other metabolites in plasma samples.

From this time on, several methods were published for the quantification of CIT and its metabolites in plasma (Eap *et al*., 1998; Wille *et al*., 2007) and urine samples (Nevado *et al*., 2006a, b). The method developed by Nevado *et al*. was based on a capillary gas chromatography with mass spectrometry detection in selected ion monitoring in electron (SIM) mode for the analysis of CIT, FLX and all of their metabolites in urine samples. An optimized SPE procedure was applied which provided a preconcentration factor of 10 and no derivatization step was required. This way, limits of detection (LODs) between 0.7 ng/L (CIT) and 33.6 μg/L (CIT-PROP) were reached (Fig. 3).

A GC-MS method for the simultaneous determination of the 13 antidepressants and nine of their active metabolites, including CIT, DCIT and DDCIT, in plasma using different ionization modes was developed and validated (Wille *et al*., 2007). Sample preparation consisted of a strong cation exchange mechanism and derivatization with heptafluorobutyrylimidazole. Identification and quantification were based on SIM using EI and chemical ionization (CI) modes. Calibration by linear and quadratic regression for electron and chemical ionization, respectively, utilized deuterated internal standards. Limits of quantitation were established between 5 and 12.5 ng/mL in EI and positive ionization CI (PICI), and 1 and 6.25 ng/mL in negative ionization CI (NICI). During validation stability, sensitivity, precision, accuracy, recovery and selectivity were evaluated for each ionization mode and were demonstrated to be acceptable for most compounds. This paper draws attention to the advantages and disadvantages of different ionization modes in the GC-MS analysis of these antidepressants in plasma.

Several multiresidue methods that include CIT based on GC-MS have also been developed (Gunnar *et al*., 2004; Nevado *et al*., 2006a, b; Paterson *et al*., 2000, 2004; Salgado-Petinal *et al*., 2005). The study presented by Paterson *et al*. (2004) showed that GC-MS with ion trap detection can be used for screening post mortem blood. The method described was used to simultaneously screen for unknowns, identify basic drugs present and semi-quantitate 14 drugs commonly encountered in coroner's toxicology, including CIT. Post mortem blood samples were extracted by LLE procedure using diethylether followed by back extraction into 0.1 M HCl. The procedure is routinely used for coroner's toxicology; semi-quantitation is used to speed-up the throughput of cases where drugs are an incidental finding and for cases where the amount of sample submitted for analysis is too small to allow for screening, identification and quantitation on separate sample volumes.

In addition to mass spectrometry, other detection techniques have also been employed coupled to GC for the determination of CIT. Nitrogen–phosphorous detection (NPD) was applied by Lacassie *et al*. (2000) and Martinez *et al*. (2004) for the determination of CIT and DCIT in serum and CIT and other antidepressants in blood respectively. However, the sensitivity obtained with these detection techniques was not as good as the ones obtained with mass spectrometry.

Electrodriven Methods

Achiral Methods

Despite capillary electrophoresis (CE) not having been as widely used as HPLC for the analysis of CIT, some interesting methods have been developed (Table 3). Although the resolution

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obtained with these methods is better than HPLC or GC, they present a major drawback that higher LOQs are obtained with respect to the above techniques.

Halvorsen *et al*. (2001) developed a disposable device for liquid-phase microextraction (LPME) for the CE-UV detection-based analysis of CIT and DCIT in human plasma. Separations were accomplished in a 75 µm i.d. fused-silica capillary. For the simultaneous analysis of CIT, DCIT and the internal standard, a 30 cm effective length capillary was utilized (40.2 cm total length) using 75 mM TRIS–acetic acid pH 4.6, Tween 20 (3% w/v) and 75 mg/L FC 135 as the separation buffer. The instrument was operated at 20 kV, generating a current level of approximately 85 mA. CIT and DCIT were extracted from 1 mL plasma samples through hexyl ether immobilized in the pores of a porous polypropylene hollow fiber and into 25 mL of 20 mM phosphate buffer (pH 2.75) present inside the hollow fiber (acceptor phase).

Micellar electrokinetic chromatography (MEKC) (Labat *et al*., 2002; Pucci *et al*., 2002; Su and Hsieh, 2008) and capillary zone electrophoresis (CZE) (Flores *et al*., 2004) with diode array detection have been among other tools used for the separation of several antidepressants, including CIT. The method developed by Labat *et al*. (2002) consisted if the use of an uncoated fused-silica capillary (600 mm, 75 mm i.d.) and a migration buffer of 20 mM sodium borate, pH 8.55, with 20 mM SDS and 15% isopropanol, at an operating voltage of 25 kV. The column temperature was maintained at 40°C. Injection in the capillary was performed in the hydrodynamic mode (0.5 psi, 15 s). In these conditions, the migration time of the antidepressants was less than 11 min. Samples (1 mL) were extracted with diethyl ether (5 mL) at pH 9.6 and reconstituted in diluted migration buffer. The LOQs ranged between 20 and 30 ng/mL for all the molecules. This method allowed the determination of some of these compounds in biological fluids (blood, urine) in post-mortem cases.

Su and Hsieh (2008) employed an efficient on-line preconcentration method, cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography (CSEI–sweeping-MEKC), for the analysis of SSRIs. Under the optimized separation parameters, the enrichment factors for these five compounds when using CSEI–sweeping-MEKC fell within the range from 5.7×10^4 to 1.2×10^5 relative to the use of MEKC; relative to the use of sweeping-MEKC, the gains were from 1.1×10^3 to 2.3×10^3. In this way, the LODs for the five SSRIs in human plasma ranged from 0.35 to 1.5 ng/mL.

Chiral Methods

Electrodriven methods are efficient and reliable analytical methods for the determination of enantiomers. Enantioseparations by CE are generally achieved by adding a chiral selector to the running buffer, which reduces the costs of analysis with respect to techniques such as HPLC chiral column. Cyclodextrins (CDs) are among the most prevalent selectors used in chiral CE. Mandrioli *et al*. (2003) developed a stereoselective analytical method for the chiral and achiral separation of CIT, DCIT and DDCIT, based on CZE coupled to a DAD and using a dual CD system as the chiral selector. Baseline enantioseparation of the racemic compounds was achieved in less than 6 min using a fused-silica capillary, filled with a background electrolyte consisting of a 35 mM phosphate buffer at pH 2.5 supplemented with 1% w/v β-CD sulfate and 0.05% w/v β-CD at 25°C and applying a voltage of −20 kV. A fast separation method for CIT was also optimized where *S*- and *R*-CIT were resolved in less than 1.5 min using short-end injection (8.5 cm, effective length) running the experiments in a background electrolyte composed of a 25 mM citrate buffer at pH 5.5 and 0.04% w/v β-CD sulfate at a temperature of 10°C. LOQs were above 0.5 µg/mL. The method showed good repeatability of peak heights and migration times and is able to separate CIT enantiomers even when they are present in a 1:100 ratio, thus allowing for the quality control of pharmaceutical formulations containing resolved enantiomers.

In some cases enantioseparation cannot be achieved by merely adding a chiral selector and it is necessary to add additives like surfactants, organic modifiers or a second chiral selector. The method developed by Sungthong *et al*. (2008) required the addition of two chiral selectors for the enantiomeric purity of (*S*)-CIT in bulk drugs and tablets. Separations were carried out with 20 mM phosphate buffer, pH 2.5, containing 0.5 mg/mL β-CD and 22 mg/mL sulfated-β-CD as background electrolyte in a 50 µm, 47/40 cm fused-silica capillary, and an applied voltage of −20 kV and a temperature of 28°C were used. The assay was validated for the (*R*)-enantiomer of CIT and the enantiomers of the impurity citadiol in the range of 2.5–150 µg/mL and 2.5–50 µg/mL, respectively.

β-CD sulfate in combination with acetonitrile in 25 mM phosphate pH 2.5 was also employed as a chiral selector by Andersen *et al*. (2003) for the enantioselective separation of CIT and DCIT. This method was applied for the simultaneous determination (*R*)- and (*S*)-CIT and DCIT in plasma samples from patients treated with racemic citalopram pre-treated with an LPME-based method.

Instead of sulfated-β-CD, Nevado *et al*. (2005) used carboxymethyl-γ-cyclodextrin as a single chiral selector for the separation of S and R-CIT. In preliminary studies, the authors assayed neutral CDs such as β-CD, methyl-β-CD, dimethyl-β-CD, hydroxypropyl-β-CD and γ-CD, resulting in achiral separations in all the cases, even when these CDs were combined with anionic surfactants such as SDS. Different mixtures between neutral CDs, organic modifiers and other chiral selectors such as bile salts were assayed in order to reach the enantiomeric separation. On the other hand, assays using carboxymethyl-γ-CD (CM-γ-CD) (anionic cyclodextrin) produced very good results in the enantiomeric separation. In an early study, the authors used this CD for the enantiomeric determination of *R*- and *S*-CIT in pharmaceutical formulations. In 2006, they published a study of five stereoselective methods based on different background electrolytes composed of CM-γ-CD as a chiral selector and other selectivity additives, such as methylcellulose, hydroxylpropylmethyl cellulose, a crown ether, and amylose, to achieve the enantiomeric screening of CIT and its DCIT, DDCIT and CIT-NO metabolites (Berzas-Nevado *et al*., 2006). The best conditions for the enantioselective separation of these compounds were short-end anodic hydrodynamic injection (6 s, 0.7 psi); as background electrolyte pH 5, 20 mM phosphate buffer, 0.2% w/v CM-γ-CD, 0.05% w/v hydroxylpropylmethyl cellulose; voltage of 28 kV with a ramp applied (0.4 s); cartridge temperature of 20°C. The UV detection was performed at 205 nm. The analytes were extracted from urine samples after an SPE procedure which enabled the preconcentration of the analytes obtaining LOQs as low as 80–340 µg/L.

Conclusions and Outlook

The initially developed analytical methods were based on HPLC and CE coupled to ultraviolet or fluorescence detectors and on GC coupled to MS. Most of these methods allowed the determination

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of CIT and its main metabolites in biological fluids and tissues. The sensitivity and selectivity presented by these methods seemed to be suitable for their application in pharmacokinetic studies. In recent years, ultraviolet and fluorescence detectors in liquid chromatography have given way to mass spectrometry since it has been necessary to develop screening methods for forensic and environmental applications. This technique has allowed the development of suitable analytical methods for the identification of the analytes present in biological and environmental samples in low concentrations. Sample pre-treatment techiniques as SPME, LPME and SBSE offer higher sensitivity for quantification of CIT and its metabolites. However, these methods are in general quite time-consuming or labor- intensive and introduce variability in recovery. To increase the efficiency of the sample preparation step, an alternative is to use automated on-line extraction methods, which increase the reliability of the results. This approach could be a suitable option for the determination of CIT and its metabolites in multiresidue methods applied to forensic samples. Therefore, future analytical methods could be focused on the screening and quantitation of these drugs in biological samples in a simple, easy and reliable way.

The launch of (*S*)-CIT or escitalopram promoted the publication of several enantioselective methods for the determination of the enantiomers of CIT and/or of its metabolites. Most of the liquid chromatography methods developed with this aim were based on the use of chiral columns. As an alternative to the high cost of these columns, the derivatization of the analytes with a chiral reagent to form diastereoisomeric derivatives followed by chromatographic separation in an achiral column was proposed. Nevertheless, this procedure has been successfully applied only to DCIT and DDCIT since the derivatization of CIT with the chiral reagent was not possible because of its tertiary amine structure.

Enantioseparation by capillary electrophoresis was achieved by adding a cyclodextrin to the running buffer and was suitable for the separation of the CIT as well as of its metabolites. The use of these chiral selectors in liquid chromatography was limited to the enantioselective determination of (*S*)-CIT and (*R*)-CIT. However, these methods are limited to UV detectors, which do not guarantee the identification of the analytes. Therefore, new approaches using chiral selectors or derivatization chiral agents suitable for MS should be determined for the enantioseparation not only of CIT enantiomers but also of DCIT and DDCIT enantiomers as an alternative to the chiral columns.

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