Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
33 (2003) 263–273
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Stereospecific determination of citalopram and desmethylcitalopram by capillary electrophoresis and liquid-phase microextraction

Solveig Andersen [a,b,*], Trine Grønhaug Halvorsen [a], Stig Pedersen-Bjergaard [a], Knut E. Rasmussen [a], Lars Tanum [b], Helge Refsum [b]

[a] School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway
[b] Department of Psychopharmacology, Diakonhjemmet University Hospital, P.O. Box 85 Vinderen, 0319 Oslo, Norway

Received 21 January 2003; received in revised form 30 March 2003; accepted 6 April 2003

Abstract

A chiral capillary electrophoresis (CE) system allowing simultaneous enantiomer determination of citalopram (CIT) and its pharmacologically active metabolite desmethylcitalopram (DCIT) was developed. Excellent chiral separation was obtained using 1% sulfated-β-cyclodextrin (S-β-CD) as chiral selector in combination with 12% ACN in 25 mM phosphate pH 2.5. Samples were prepared by liquid-phase microextraction (LPME) based on a rodlike porous polypropylene hollow fibre. CIT and DCIT were extracted from 1 ml plasma made alkaline with NaOH, into dodecyl acetate impregnated in the pores of a hollow fibre, and into 20 mM phosphate pH 2.75, inside the hollow fibre. The acceptor solution was directly compatible with the CE system. Efficient sample clean-up was seen, and the recoveries were 46 and 29% for the enantiomers of CIT and DCIT, respectively, corresponding to 31 and 19 times enrichment. The limit of quantification (S/N = 10) was < 11.2 ng/ml, intra-day precision was $< 12.8\%$ RSD, and inter-day precision was $< 14.5\%$ RSD, for all enantiomers. The validated method was successfully applied to simultaneous determination of enantiomer concentrations of CIT and DCIT in plasma samples from nine patients treated with racemic citalopram. The results confirm LPME-CE as a suitable and promising tool for enantiomeric determination of chiral drugs and metabolites in biological matrices.

Keywords: Liquid-phase microextraction; Capillary electrophoresis; Citalopram and desmethylcitalopram; Chiral separations; Sulfated-β-CD; Organic modifier

1. Introduction

The antidepressive drug citalopram (CIT) is a selective and potent serotonin reuptake inhibitor. Potentiation of serotonergic neurotransmission gives a broad spectrum of therapeutic activity in depression, anxiety, obsessional and impulse con-

* Corresponding author. Fax: +47-22-029993.
E-mail address: solveig.andersen@diakonsyk.no (S. Andersen).

0731-7085/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00264-4

trol disorders [1,2]. CIT is a chiral compound. The pharmacological effect of CIT is related mainly to S-CIT and to some extent to S-desmethylcitalopram (S-DCIT) [3,4]. CIT is currently marked as both a racemic mixture and as a single enantiomer. Failure to account for stereoselectivity in pharmacodynamic and pharmacokinetic parameters and neglect of the possible clinical relevance of DCIT may lead to inaccurate therapeutic drug monitoring and nonoptimal treatment.

Currently used methods for stereospecific drug monitoring of CIT and DCIT, are mainly based on chromatographic techniques, which require either rather expensive chiral stationary phases or conversion of the enantiomers into diastereomers with a chiral reagent [5–11]. During the past few years, capillary electrophoresis (CE) has established itself in the area of chiral separations. The separation of enantiomers by CE has been studied extensively and shown to provide low cost analysis with high efficiency and resolution combined with selectivity and short analysis times [12–17]. No method for enantiomer determination of CIT (and DCIT) based on CE has, to our knowledge, been published.

Enantioseparations by CE are generally achieved by adding a chiral selector to the running buffer [12–17]. Cyclodextrins (CDs) are among the most prevalent selectors used in chiral CE. Selection of the appropriate selector is of paramount importance in order to achieve successful separation of enantiomers. In some cases, however, enantioseparation cannot be achieved by merely adding a chiral selector. When necessary, numerous additives like surfactants, organic modifiers or a second chiral selector can be used to optimise the experimental conditions for chiral and achiral resolution. The additives can influence several parameters such as the electroosmotic flow (EOF), the viscosity, the stability of the selector–analyte complex, the solubility of either analytes and/or selectors, the analysis time and the conductivity of the buffer [12–17].

Therapeutic monitoring of CIT requires a highly sensitive analytical method because of the low plasma levels (typically 25–160 ng/ml for the racemate) resulting from therapeutic doses [5–11]. Because CE is performed with UV detection directly on the narrow fused-capillary used for separation and because only nanoliter volumes of sample are injected in traditional CE, most concentration detection limits are relatively high. In order to achieve concentration detection limits enabling quantification over the entire therapeutic range, preparation of patient samples is crucial before analysis by CE. Liquid-phase microextraction (LPME) is a new sample preparation technique offering high analyte preconcentration and efficient sample clean-up. A new and disposable device for LPME based on polypropylene hollow fibres, was recently introduced [18,19]. In LPME analytes are extracted from small volumes of biological samples through an organic solvent in the pores of a porous hollow fibre and into a μl volume of acceptor phase inside the hollow fibre. High enrichment (20–160 times) is a result of the volume difference between the sample solution (0.5–4 ml) and the acceptor solution (15–25 μl). By the use of an aqueous acceptor phase, direct compatibility with CE is achieved. In addition, the analytes are extracted in a three-phase system where simultaneous extraction and back extraction is performed. This results in clean extracts, which requires no further pre-treatment prior to injection into the CE system. LPME has already demonstrated high potential for sample preparation of biological fluids prior to CE [18–24]. Recently, LPME and CE were for the first time successfully combined for chiral determination of drugs in biological matrices with focus on mianserin [25].

In the present project, LPME-CE was further developed and investigated as a combination for enantiomeric determination of drugs and metabolites in biological matrices. A modified version of the LPME device previously described was introduced and its eligibility studied in combination with a chiral CE system.

2. Experimental

2.1. Chemicals

Escitalopram oxalate (S-citalopram oxalate) (S-CIT), R-citalopram oxalate (R-CIT), S-desmethyl-

citalopram fumarate (S-DCIT), R-desmethylcitalopram fumarate (R-DCIT), racemic citalopram hydrobromide (CIT), racemic N-desmethylcitalopram hydrochloride (DCIT), and a racemic mixture of LU-10-202 (internal standard (I.S.)) (structures see Fig. 1) were a gift from H. Lundbeck (Copenhagen, Denmark). Sodium phosphate, sodium hydroxide, ortho-phosphoric acid, 1,4-dioxan, all of analytical grade, acetonitrile and methanol of HPLC grade, tetrahydrofuran (THF) ($\geq$99.7%), and *N*,*N*-dimethylformamid ($\geq$ 99.5%) were obtained from Merck (Darmstadt, Germany). Dodecyl acetate, poly(vinyl alcohol) (PVA) (98–99% hydrolysed, average Mw 31 000–50 000), and sulfated-β-cyclodextrin (S-β-CD) (typical substitution 7–11 moles/mole β-CD) were purchased from Aldrich (Sigma–Aldrich GmbH, Steinheim, Germany). 2-Propanol was obtained from A/S Vinmonopolet (Norway). Dimethylsulphoxide (DMSO) ($\geq$98%), N-methylformamid ($\geq$99%), Tween 20 (analytical grade), and 3-*N*,*N*-dimethylmyristylammoniumpropanesulphate (MAPS) were purchased from Fluka (Buchs, Switzerland). Formamide was obtained from Koch-Light Laboratories Ltd (England). α-Cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), and hydroxypropyl-β-cyclodextrin (HP-β-CD) (all eCAP™) were purchased from Beckman (Fullerton, CA, USA). Carboxymethylated-β-cyclodextrin (CM-β-CD) (>97%) was obtained from Cyclolab (Budapest, Hungary). All aqueous solutions were prepared with water purified with an EASYpure RO system (Barnstead, Dubuque, IA, USA).

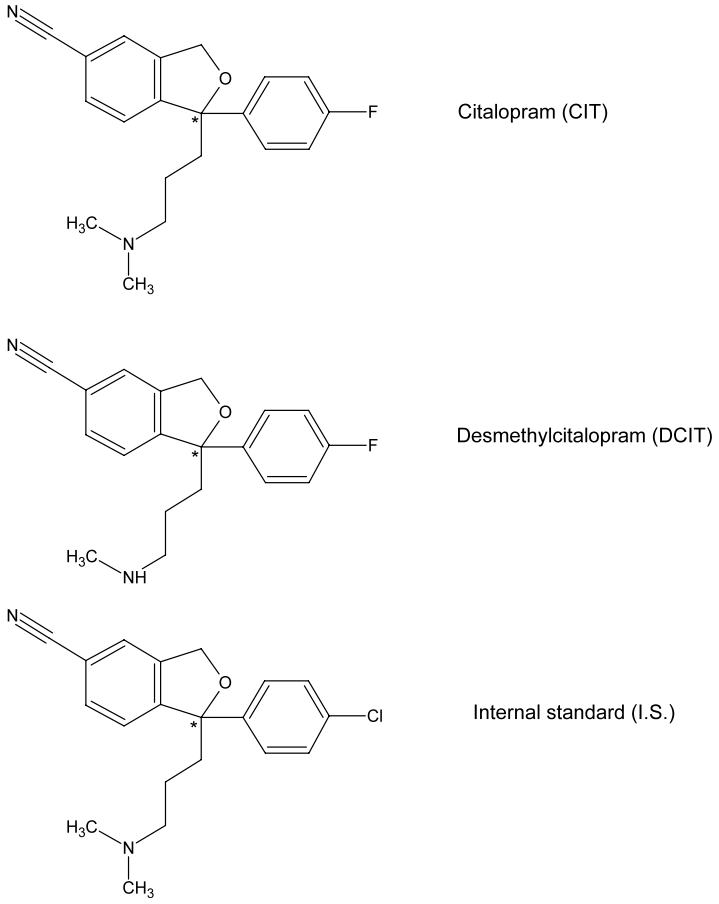

Fig. 1. Structures of citalopram, *N*-desmethylcitalopram and internal standard (stereocentres indicated with asterisks).

2.2. Liquid-phase microextraction

The disposable LPME device is illustrated in Fig. 2. LPME was carried out in conventional 1.5-ml sample vials with screw tops/silicone septums (Chromacol Ltd., Trumbull, CT, USA). A polypropylene precision moulded tip specially designed for Finnpipettes (Labsystems, Helsingfors, Finland) was inserted through the silicone septum. The tip served to support the hollow fibre, to introduce the acceptor solution into the hollow fibre prior to extraction and to collect the acceptor solution after extraction. A manually cut 18 mm piece of a polypropylene hollow fibre (Varian, Harbor City, CA, USA) was placed at the end of the tip. The inner diameter of the fibre was 1.2 mm, the pore size was 0.2 μm and the thickness of the wall was 200 μm. The end of each fibre was closed by mechanical pressure supported by gluing.

Prior to extraction the sample vial was filled with 1 ml plasma made alkaline with 100 μl ml of 2 M NaOH. The solution was diluted with 150 μl water and 250 μl of 600 ng/ml internal standard. A new 18 mm length of the polypropylene hollow fibre with a sealed end was placed at the end of the tip and dipped for 5 s in organic solvent to immobilise the solvent in the pores of the hollow fibre. Excess solvent was removed by 15 s of ultra sonification. After impregnation, 15 μl of acceptor solution was injected into the hollow fibre using a micro litre syringe (Hamilton, Bonaduz, Switzerland). The needle of the syringe was lead via the supporting tip, to the bottom fibre before introducing the acceptor solution. The syringe was withdrawn and the rodlike tip-fibre-assembly was placed in the sample solution. Extractions were performed for 45 min. During extraction, the samples were vibrated using a Vibramax 100 (Heidolph, Kelheim, Germany) at 1500 rpm. After extraction the acceptor solution was withdrawn from the fibre by the micro litre syringe and transferred into a 200 μl vial/insert (Advanced Biotechnologies Ltd., Surrey, UK). Each piece of hollow fibre, tip and sample vial were used only for a single extraction.

2.3. Capillary electrophoresis

CE was performed with a MDQ instrument (Beckman, Fullerton, CA, USA) equipped with an UV detector. Separations were accomplished in a 75 μm i.d. fused-silica capillary (BGB Analytik AG, Anwil, Switzerland) with an effective length of 40 (total length 50.2 cm) or 50 cm (total length 60.2 cm). The running buffer consisted of 25 mM sodium phosphate adjusted to pH 2.5 with concentrated ortho-phosphoric acid and added 1% S-

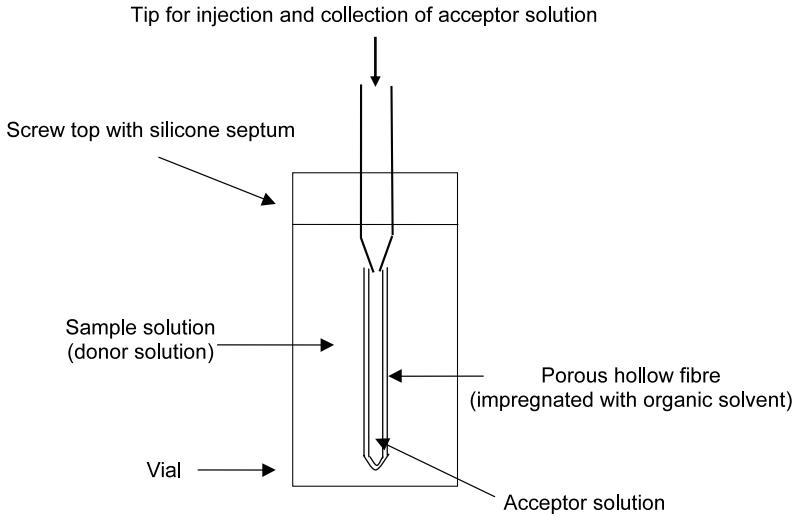

Fig. 2. Principle of LPME.

β-CD, 12% ACN and 0.1% PVA. The buffer was filtered through a 0.45 μm filter (Minisart RC 25, Sartorius AG, Göttingen, Germany). Before analysis the capillary was conditioned with 0.1 M NaOH for 2 min at 20 p.s.i, water for 15 min at 50 p.s.i and then electroconditioned with 25 mM phosphate pH 2.5 containing 0.1% PVA for 30 min at 25 kV and 5 p.s.i. Between each run the capillary was rinsed with 25 mM phosphate pH 2.5 containing 0.1% PVA for 5 min at 50 p.s.i and with the chiral running buffer for 2 min at 20 p.s.i. During analysis the instrument was operated at −20 kV (reversed polarity), generating a current level of approximately −60 μA. All samples were introduced by hydrodynamic injection for 20 s at 0.7 p.s.i. Detection was accomplished at 200 nm utilising a 100 × 800 μm slit.

2.4. Calculation of extraction recoveries

The extraction recovery (ER) was defined as the percentage of the total analyte amount $n_{s,initial}$ (originally present in the sample), which was transferred to the extract (acceptor phase) at the end of the extraction ($n_{a,final}$):

$$ER = (n_{a,\ final}/n_{s,\ initial})\cdot 100\%$$

$$= (V_a/V_s)\cdot(C_{a,\ final}/C_{s,\ initial})\cdot 100\% \quad (1)$$

where V_a and V_s are the volumes of acceptor solution and sample solution (donor solution), respectively, and $C_{a,\ final}$ and $C_{s,\ initial}$ are the final analyte concentration in the sample extract (acceptor phase) and the initial analyte concentration within the sample, respectively.

2.5. Solid-phase extraction and high-performance liquid chromatography

A modified version of a published method combining automatic solid phase extraction (SPE) and high-performance liquid chromatography (HPLC) was used for the achiral analysis of the patient samples [26]. The plasma volume used was 1.5 ml and CIT and DCIT were detected at an excitation wavelength of 266 nm and an emission wavelength of 302 nm. The remainder of the method was performed as described in the article.

2.6. Biological samples

Spiked plasma samples in different concentrations were prepared by adding appropriate aliquots of 0.1 mg/ml stock solutions of CIT and DCIT in methanol to drug-free human plasma. For the validation studies and the analysis of patient samples, internal standard was added from a 0.1 mg/ml stock solution in methanol.

Nine plasma samples from depressed patients treated with racemic CIT were collected between 12 and 24 h after drug administration. The patients were 18–54 years old, and the administered doses varied between 20 and 80 mg/day. The samples were obtained under routine therapeutic drug monitoring conditions.

The stock solutions and biological samples were stored at +5 °C and protected from light.

3. Results and discussion

3.1. Separation of citalopram and desmethylcitalopram enantiomers

No method for enantiomer determination of CIT (and DCIT) based on CE has, to our knowledge, been published. A stereospecific method for simultaneous enantiomer determination of CIT and DCIT by CE was therefore developed. Method development was performed using a fused-silica capillary with 40 cm effective length and 75 μm internal diameter. The voltage applied was ±20 kV. Several cyclodextrins were tested at different concentrations and in different buffers in an attempt to find a suitable chiral selector. The native CDs and the CD derivatives DM-β-CD, HP-β-CD and CM-β-CD gave neither achiral nor chiral separation. S-β-CD has shown superiority over native CDs and neutral CD derivatives in chiral separation of several analytes [27–29], and was therefore tested. As shown in Fig. 3a, good chiral separation was obtained with 1% (w/v) S-β-CD in 25 mM phosphate pH 2.5. However, R-CIT and R-DCIT, were not separated. Further development was therefore performed using S-β-CD in 25 mM phosphate pH 2.5 as chiral selector.

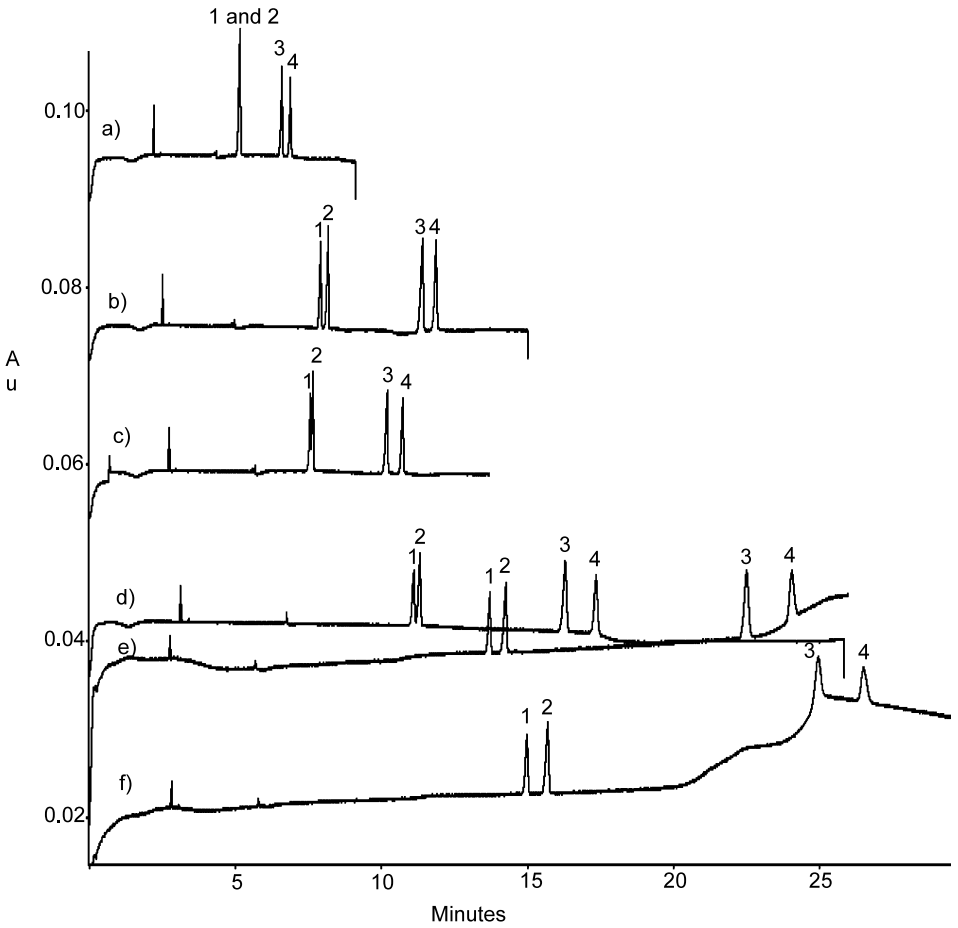

Fig. 3. Electropherograms showing the separation of CIT and DCIT using different separation buffers. All analyses were performed with reversed polarity. Injection: 5 s at 0.5 psi. Capillary: 40 cm (effective length), 75 μm i.d. 1 = R-DCIT, 2 = R-CIT, 3 = S-CIT, and 4 = S-DCIT. (a) 25 mM phosphate pH 2.5+1% S-β-CD; (b) 25 mM phosphate pH 2.5+1% S-β-CD+10% ACN; (c) 25 mM phosphate pH 2.5+1% S-β-CD+10% methanol; (d) 25 mM phosphate pH 2.5+1% S-β-CD+10% isopropanol; (e) 25 mM phosphate pH 2.5+1% S-β-CD+10% dioxan; (f) 25 mM phosphate pH 2.5+1% S-β-CD+10% THF.

Analysis using S-β-CD in phosphate pH 2.5, were performed with the anode at the detector end of the capillary (reversed polarity). At pH 2.5, the electroosmotic flow (EOF) is suppressed. S-β-CD is a negatively charged chiral selector at pH ≥ 2, migrating towards the detector. The basic analytes CIT and DCIT are positively charged, migrating towards the injector end of the capillary, away from the detector. With the electrophoretic mobility of the analytes directed towards the injector end, the only possibility for analyte migration towards the detector is through interaction with the negatively charged CD. The interactions between the polynegatively charged CD and the cationic racemates are probably due to hydrophobic driven inclusion complexation as well as electrostatic interactions that may stabilise the complexes [29,30]. The movement of the chiral selector in the opposite direction to that of the analytes, represents the ideal condition in order to achieve good chiral resolution because it causes an increase of the mobility difference between free and complexed analyte [14,31]. Experiments were done to optimise the concentration of S-β-CD. The chiral selector concentrations studied were 0.02, 0.2, 1 and 2%. The lower two concentrations

of S-β-CD suffered from long analysis times and broad peaks. Increasing the amount of S-β-CD to 2%, increased the current generated to an unacceptable level (>100 μA) due to a dramatic increased ionic strength of the buffer, without improving the resolution significantly. Based on these experiments, 1% S-β-CD was selected as concentration of the chiral selector.

Surfactants, organic modifiers and neutral CDs were tested in combination with 1% S-β-CD in phosphate pH 2.5 in an attempt to obtain achiral separation of R-CIT and R-DCIT. The most promising results were obtained with organic modifiers added to the chiral buffer. Different organic modifiers were added at 10% concentration for comparison. Addition of methanol, acetonitrile, tetrahydrofuran, isopropanol, and dioxan all increased achiral and chiral separation. The degree of influence, however, differed. The resulting separations are shown in Fig. 3b–f. The addition of organic modifiers probably improved the chiral resolution by decreasing the magnitude of the CD-analyte binding constant. The most common explanation of this is that the organic modifier reduces the affinity of the analyte for the CD cavity and increases it for the bulk buffer. Alternatively, the modifier is thought to compete with the solute for the CD cavity [30]. The reduction of the binding constants also explains the increase in migration time observed. By reducing the CD-CIT binding constant in a different degree than the CD-DCIT binding constant, the achiral separation was improved as well as the chiral separation. The buffer systems containing organic modifiers, all gave the same migration pattern. The chiral separation of desmethylcitalopram enantiomers was better than the chiral separation of citalopram enantiomers, and the CIT enantiomers migrated between the enantiomers of DCIT. ACN was chosen as the organic modifier for further use, because of efficient resolution at reasonable analysis time. The optimum organic content was found by varying the concentration of ACN while keeping the S-β-CD concentration constant (1%). As shown in Fig. 4, separation and migration time increased with increasing organic content. Buffers containing more than 12% ACN resulted in partial comigration of S-CIT and S-DCIT. Consequently, 12% ACN was chosen as organic modifier concentration.

During method development, problems with unstable and irreproducible current, which in turn affected the separation, was observed. This was thought to arise from adsorption of S-β-CD on the capillary wall. Adsorption of charged additives such as sulfated cyclodextrins onto the capillary's inner surface has been described [30,32]. To reduce the possible interactions between the cyclodextrin and the capillary wall, the capillary was dynamically coated with PVA. PVA at 0.1% (w/v) was added to the running buffer containing 1% S-β-CD and 12% ACN in 25 mM phosphate pH 2.5. Between each run, the capillary was rinsed with 0.1% PVA in 25 mM phosphate pH 2.5 for 5 min at 50 p.s.i. before a 2 min rinse with the running buffer at 20 p.s.i. This procedure stabilised the CE system.

As an internal standard, a chiral derivative of citalopram was used (Fig. 1). Due to partially comigration of one of the two enantiomers of this compound with R-DCIT in the capillary used during method development, the effective length of the capillary was increased from 40 to 50 cm. This resulted in baseline separation of the enantiomers of the internal standard, CIT and DCIT and excellent chiral resolution. Increasing the length of the capillary also increased the migration times. However, analysis time less than 20 min was considered satisfactory.

3.2. Extraction of citalopram and desmethylcitalopram enantiomers

Since the introduction of the hollow fibre based LPME device [18,19], work has been in continuous progress to further evaluate the concept of LPME and to develop a fully automate version of the device. A modified version of the LPME device was introduced in this work (Fig. 2). The modified LPME device utilises a 1.8 cm fibre with a rodlike configuration instead of the previously used 8 cm fibre with a hairpin bend. A rodlike configuration offers several advantages over hairpin configured fibres. Easier liquid handling is achieved because the acceptor solution is introduced and withdrawn

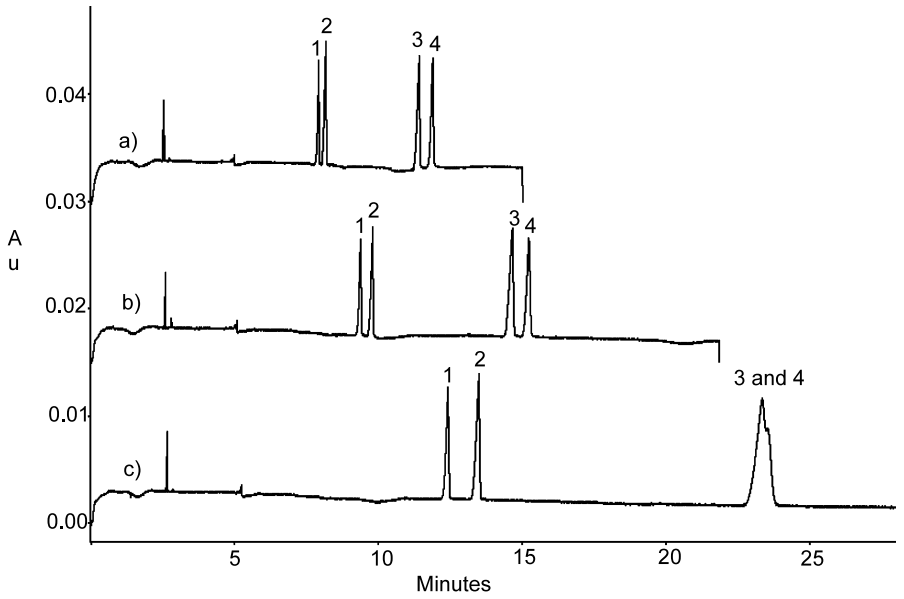

Fig. 4. Electropherograms showing the separation of CIT and DCIT with 25 mM phosphate pH 2.5+1% S-β-CD containing different amounts of ACN. Injection: 5 s at 0.5 psi. Capillary: 40 cm (effective length 50.2 cm), 75 μm i.d. (reversed polarity). 1 = R-DCIT, 2 = R-CIT, 3 = S-CIT, and 4 = S-DCIT. (a) 10% ACN; (b) 12% ACN; (c) 15% ACN.

with a micro litre syringe. In addition, the short rodlike fibre is compatible with smaller sample volumes, it makes LPME easier to automate and it improves the mechanical stability of the device. LPME of CIT and DCIT using older versions of the LPME device has already been described [20,21]. The published LPME methods were modified and adapted to the use of shorter fibres and smaller sample vials. Comparison of extraction results using hexyl ether (used in the previous publication) and dodecyl acetate as the organic phase, proved dodecyl acetate to be superior to hexyl ether as far as both extraction recovery and precision are concerned. Dodecyl acetate was therefore used as the organic phase in these experiments.

Enantiomers of CIT and DCIT were extracted from 1 ml plasma samples added 250 μl of 600 ng/ml internal standard, diluted with 150 μl water and made alkaline with 100 μl of 2 M NaOH. The acceptor phase was 20 mM phosphate pH 2.75. Due to the low solubility of the analytes within the alkaline donor phase and the correspondingly high solubility in the acidic acceptor solution, the analytes were extracted from the donor solution through the organic solvent in the pores of the hollow fibre and into the acceptor solution. The aqueous extracts were directly compatible with the CE system. The three-phase system resulted in efficient sample clean-up, illustrated by the absence of peaks in the electropherogram of the LPME extract of the blank plasma sample (Fig. 5b). The recoveries were 46 and 29% for the enantiomers of CIT and DCIT, respectively, corresponding to 31 and 19 times enrichment. The recovery was constant at the three concentration levels tested (25, 80 and 160 ng/ml racemic CIT and DCIT). The difference in recovery for CIT and DCIT is probably due to the difference in lipid solubility between the two compounds. The desmethylated metabolite is less lipophillic than the parent drug. The lipid solubility of the analytes is an important parameter affecting the equilibrium processes, and thereby the recovery, in the extraction process. The difference in recovery obtained in this project, corresponds with a previous publication on LPME of CIT and DCIT [20]. No differences in extraction recoveries for the enantiomers of neither CIT nor DCIT were seen, indicating absence of stereoselectivity in the

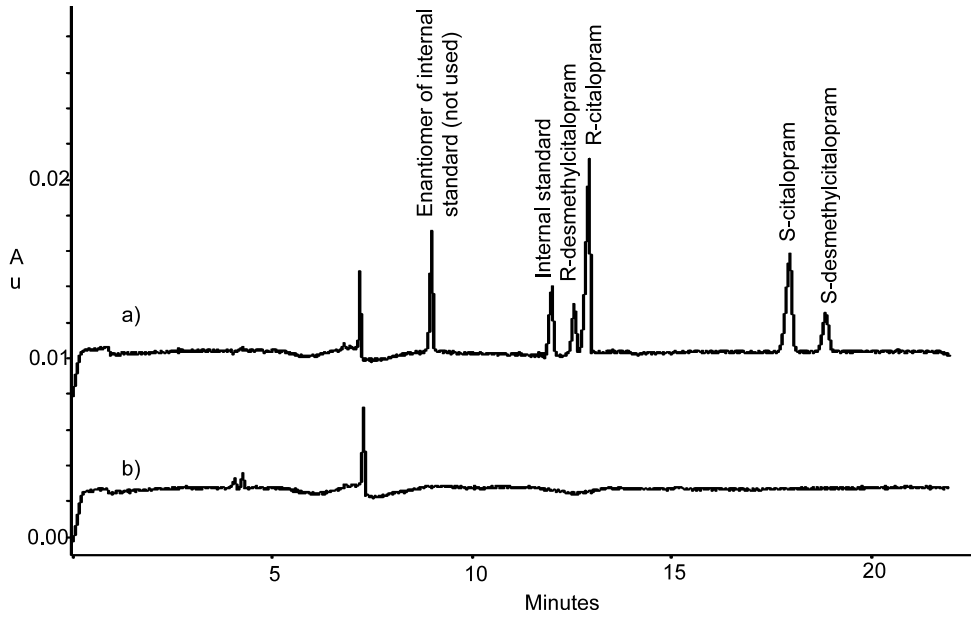

Fig. 5. Electropherograms of LPME extracts from patient sample and blank plasma sample. Injection: 20 s at 0.7 psi. Capillary: 50 cm (effective length 60.2 cm), 75 μm i.d. (reversed polarity). (a) Patient plasma sample (patient 8); (b) blank plasma sample.

extraction set-up. Methanol (25%) and NaCl (30%) were added to the donor solution, in subsequent and separate extractions, in an experiment to increase the extraction recoveries. However, no further enrichment was obtained.

3.3. Validation

The method was validated prior to analysis of patient samples. Intra- and inter-day precision, intra-day accuracy, linearity, limit of detection (LOD) and limit of quantification (LOQ) were determined for both enantiomers of CIT and DCIT. To all samples 0.25 ml of 600 ng/ml internal standard was added. The precision and accuracy validation data are summarised in Tables 1 and 2, respectively. The intra-day precision was $< 12.8\%$ RSD and the inter-day precision was $< 14.5\%$ RSD for all enantiomers at the three concentrations tested. The intra-day accuracy varied between 99 and 119% for the enantiomers at the two concentrations tested, indicating acceptable method accuracy. The calibration curves were linear ($r > 0.9997$ for CIT enantiomers and $r >$

Table 1
Intraday and interday precision of CIT and DCIT enantiomers from human plasma

Racemic concentrations (ng/ml)	Intraday, RSD (%) ($n = 6$)				Interday, RSD (%) ($n = 6$)			
	S-CIT	R-CIT	S-DCIT	R-DCIT	S-CIT	R-CIT	S-DCIT	R-DCIT
25	7.0	7.9	9.9	12.8	11.8	8.4	14.5	12.3
80	4.7	5.1	9.3	8.8	6.7	8.0	8.6	5.4
160	8.4	8.2	10.3	12.1	7.4	5.9	10.3	8.0

Table 2
Intraday accuracy of CIT and DCIT enantiomers from human plasma ($n = 3$)

Racemic concentrations (ng/ml)	S-CIT (%)	R-CIT (%)	S-DCIT (%)	R-DCIT (%)
95	113	119	103	114
175	102	102	102	99

Table 3
Results from analysis of patient plasma samples

Patient	Dose racemic CIT (mg)	S-CIT (ng/ml)	R-CIT (ng/ml)	S-DCIT (ng/ml)	R-DCIT (ng/ml)	Ratio CIT (S/R)	Total CIT (ng/ml)	Total CIT by HPLC (ng/ml)	Bias (%)
1	20	20	31	20	29	0.6	51	53	−3.8
2	20	16	20	16	18	0.8	36	31	16.1
3	30	18	32	14	26	0.6	50	41	22.0
4	40	17	27	26	40	0.6	44	42	4.8
5	40	24	42	28	33	0.6	66	60	10.0
6	40	73	70	21	21	1.0	142	150	−5.3
7	45	26	44	21	23	0.6	70	86	−18.6
8	60	47	81	32	36	0.6	128	126	1.6
9	80	30	49	38	30	0.6	78	74	5.4

0.997 for DCIT enantiomers) over the entire concentration range studied (25, 80, 120, 160, 240 and 320 ng/ml racemic CIT and DCIT). The LOQs (S/N = 10) were 5.2, 4.4, 11.2 and 8.9 ng/ml and the LODs (S/N = 3) were 1.6, 1.4, 3.4 and 2.7 ng/ml for S-CIT, R-CIT, S-DCIT and R-DCIT, respectively. The validation data are expected to improve significantly when the future commercialised devices replace the homemade devices used in this project.

3.4. Analysis of patient samples

The validated method was applied to the analysis of plasma samples from nine patients treated with racemic CIT. The measured steady-state concentrations are listed in Table 3. The concentrations obtained by the chiral LPME-CE method were compared with the concentrations obtained by a routine achiral HPLC method. Plasma levels assayed by the two procedures were within ±22.0% for CIT. This further indicates acceptable method accuracy. In all patient samples the concentration of S-CIT, the active enantiomer, was lower or equal to those of R-CIT. The S/R ratio of both CIT and DCIT ranged from 0.6 to 1.0 with a mean of 0.7 and 0.8, respectively. This approximates to other publications [5–11]. The results indicate that stereospecific determination of S-CIT (and S-DCIT) may lead to better correlation between plasma concentrations and therapeutic response.

4. Conclusion

The present work has demonstrated high preconcentration and efficient sample clean-up of LPME allowing simultaneous enantiomer determination of CIT and DCIT by CE. Efficient chiral and achiral resolution was obtained using S-β-CD in combination with ACN in the CE buffer. The results also indicate that the modified and rodlike LPME device is a favourable configuration resulting in a more useful LPME device. Analysis of patient samples showed that the concentration of the active S-CIT in patients treated with racemic CIT approximates 40% of the total CIT concentration measured by achiral methods. The results confirm LPME-CE as a suitable tool for enantio-

meric determination of chiral drugs and metabolites in biological matrices.

Acknowledgements

The authors acknowledge H. Lundbeck for the kind gift of their substances.

References

[1] P. Baumann, Int. Clin. Psychopharmacol. 11 (1996) 5–11.
[2] P.J. Goodnick, B.J. Goldstein, J. Psychopharmacol. 12 (1998) S5–S20.
[3] J. Hyttel, K.P. Bogeso, J. Perregaard, C. Sanchez, J. Neural. Transm. Gen. Sect. 88 (1992) 157–160.
[4] R.M. Lane, G.B. Baker, Cell. Mol. Neurobiol. 19 (1999) 355–372.
[5] B. Rochat, M. Amey, H. Van Gelderen, B. Testa, P. Baumann, Chirality 7 (1995) 389–395.
[6] J.P. Foglia, B.G. Pollock, M.A. Kirshner, J. Rose, R. Sweet, B. Mulsant, Psychopharmacol. Bull. 33 (1997) 109–112.
[7] D. Haupt, J. Chromatogr. B 685 (1996) 299–305.
[8] O.V. Olesen, K. Linnet, J. Chromatogr. B 675 (1996) 83–88.
[9] B. Rochat, M. Amey, P. Baumann, Ther. Drug. Monit. 17 (1995) 273–279.
[10] J. Sidhu, M. Priskorn, M. Poulsen, A. Segonzac, G. Grollier, F. Larsen, Chirality 9 (1997) 686–692.
[11] Z. Zheng, M. Jamour, U. Klotz, Ther. Drug. Monit. 22 (2000) 219–224.
[12] A. Amini, Electrophoresis 22 (2001) 3107–3130.
[13] B. Chankvetadze, G. Blaschke, J. Chromatogr. A 906 (2001) 309–363.
[14] S. Fanali, J. Chromatogr. A 875 (2000) 89–122.
[15] M. Fillet, P. Hubert, J. Crommen, J. Chromatogr. A 875 (2000) 123–134.
[16] N. Matthijs, C. Perrin, M. Maftouh, D.L. Massart, Y.V. Heyden, J. Pharm. Biomed. Anal. 27 (2002) 515–529.
[17] S. Zaugg, W. Thormann, J. Chromatogr. A 875 (2000) 27–41.
[18] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650–2656.
[19] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Grønhaug, J. Chromatogr. A 873 (2000) 3–11.
[20] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87–93.
[21] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. B 760 (2001) 219–226.
[22] T.S. Ho, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 963 (2002) 3–17.
[23] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579–585.
[24] S. Pedersen-Bjergaard, T.S. Ho, K.E. Rasmussen, J. Sep. Sci. 25 (2002) 141–146.
[25] S. Andersen, T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 963 (2002) 303–312.
[26] L. Kristoffersen, A. Bugge, E. Lundanes, L. Slørdal, J. Chromatogr. B 734 (1999) 229–246.
[27] P.K. Owens, A.F. Fell, M.W. Coleman, J.C. Berridge, J. Chromatogr. A 797 (1998) 187–195.
[28] E. Schneiderman, A.M. Stalcup, J. Chromatogr. B 745 (2000) 83–102.
[29] A.M. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1360–1368.
[30] S.R. Gratz, A.M. Stalcup, Anal. Chem. 70 (1998) 5166–5171.
[31] A.B. Bergholdt, K.W. Jørgensen, L. Wendel, S.V. Lehmann, J. Chromatogr. A 875 (2000) 403–410.
[32] Y. Daali, S. Cherkaoui, P. Christen, J.-L. Veuthey, Electrophoresis 20 (1999) 3424–3431.