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Liquid-phase microextraction and capillary electrophoresis of citalopram, an antidepressant drug

Trine Grønhaug Halvorsen*, Stig Pedersen-Bjergaard, Knut E. Rasmussen

School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway

Abstract

A newly developed disposable device for liquid-phase microextraction (LPME) was evaluated for the capillary electrophoresis (CE) of the antidepressant drug citalopram (CIT) and its main metabolite *N*-desmethylcitalopram (DCIT) in human plasma. CIT and DCIT were extracted from 1 ml plasma samples through hexyl ether immobilised in the pores of a porous polypropylene hollow fibre and into 25 μl of 20 m*M* phosphate buffer (pH 2.75) present inside the hollow fibre (acceptor phase). 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 owing to high partition coefficients, CIT and DCIT were enriched by a factor of 25 to 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. Since the extracts were aqueous, they were injected directly into the CE instrument. Limits of quantification ($S/N=10$) for CIT and DCIT in plasma were 16.5 ng/ml and 18 ng/ml respectively, while the limits of detection ($S/N=3$) were 5 ng/ml and 5.5 ng/ml respectively. This enabled CIT (and DCIT) to be analysed within the therapeutic range by LPME–CE and detection limits were comparable with previously reported HPLC methods.

Keywords: Liquid-phase microextraction; Citalopram; *N*-Desmethylcitalopram

1. Introduction

Capillary electrophoresis (CE) is a highly attractive separation technique, which can be applied in several modes of separation. The technique is fast and offers high separation efficiencies with minimal sample and solvent consumption. In spite of this, the applicability of CE may be reduced due to inherently high concentration detection limits. This is frequently a problem for the analysis of drugs in human plasma, and in these cases, analyte enrichment is crucial.

For the enrichment of drugs from plasma, either solid-phase extraction (SPE) or liquid–liquid extraction (LLE) is traditionally applied [1]. SPE provides extensive sample clean-up and the final extracts eluted from SPE columns are normally compatible with CE. However, small sample volumes only are available of human plasma (1–2 ml) and the final extracts are typically transferred from the SPE columns in 0.25–0.5 ml of eluent. Thus, because of the low volume ratios between the samples and the final extracts, relatively low enrichment factors are obtained without partial evaporation of the sample extracts. As for SPE, also LLE provides extensive sample clean-up for human plasma samples and the technique is relatively simple and inexpensive. However, high analyte enrichments

*Corresponding author. Fax: +47-22-85-4402.

E-mail address: t.g.halvorsen@farmasi.uio.no (T.G. Halvorsen).

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may be difficult because of the limitations in sample material and the compatibility with CE is relatively poor; the organic solvents used for extraction are immiscible with water and are not directly injectable in CE. Thus, prior to the CE step, the extracts have to be evaporated followed by reconstitution in an aqueous phase.

Recently, we have developed a simple and disposable device for liquid-phase microextraction (LPME) reducing the analyte enrichment problems of LLE from small biological samples and eliminating the compatibility problems of LLE with CE [2,3]. LPME was accomplished in conventional 4-ml vials utilising a porous polypropylene hollow fibre as compartment for the final extract; each sample was transferred to a vial, pH was adjusted to deionise the analyte within the sample, and the analytes were extracted through an organic solvent in the pores of the hollow fibre and into a new aqueous phase (acceptor phase) inside the hollow fibre. With a high volume ratio between the samples (1–2 ml) and the acceptor phase (10–25 μl), high enrichment factors were reported both for the basic drug methamphetamine [2] and for several acidic non-steroid anti-inflammatory drugs (NSAIDs) [3].

Although LPME was found to be effective for the basic drug methamphetamine, no information is currently available on LPME of highly hydrophobic drugs. Therefore, in the present work attention was focused on LPME of the antidepressant drug citalopram and its major metabolite as model compounds of relatively high hydrophobicity. The fundamental extractability by LPME, LPME from human plasma samples and optimisation studies for citalopram were the major objectives of the present work.

2. Experimental

2.1. Chemicals

Citalopram hydrobromide (CIT), *N*-desmethylcitalopram hydrochloride (DCIT) and the internal standard (I.S.) (structures see Fig. 1) were obtained from H. Lundbeck (Copenhagen, Denmark). FC-135, which consisted of fluoroalkyl quartenary ammonium iodides in isopropyl alcohol (produced by 3M, Zwijndrecht, Belgium), was obtained from Kemi-

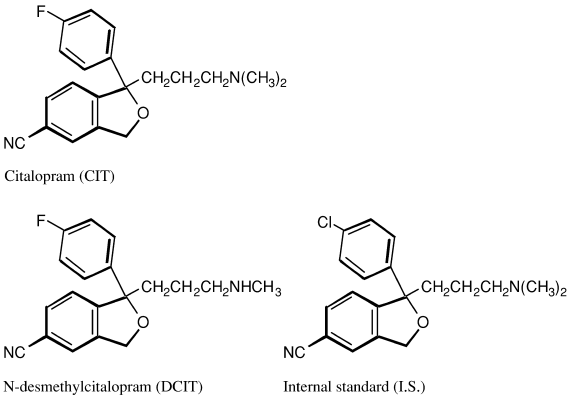

Fig. 1. Structures of Citalopram, *N*-desmethylcitalopram and internal standard.

Intressen AB (Solna, Sweden). Sodium phosphate, sodium hydroxide, sodium acetate, ortho-phosphoric acid, hydrochloric acid and TRIS (Tris(hydroxymethyl)aminomethan), all of analytical grade and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). Tween 20, 1-octanol (>99.5%) and 2-octanone (>97%) were obtained from Fluka (Buchs, Switzerland). Sodium dodecyl sulphate (approx. 99%) and hexyl ether (purity not reported) were purchased from Sigma (St. Louis, MO, USA). Acetic acid (≥99.8%) of analytical grade was obtained from Prolabo (Rohne-Poulenc LTD, Manchester, UK) and sodium borate (>99.5%) of analytical grade was obtained from Mallinckrodt (Paris, KY, USA). All aqueous solutions were prepared with water purified with an EASYpure RO system (Barnstead, Dubuque, IA, USA).

2.2. Liquid-phase microextraction

The disposable LPME device is illustrated in Fig. 2. The sample solution was transferred to a 4-ml amber vial with a screw top/silicone septum (Supelco, Bellefonte, PA, USA). Two 0.8-mm O.D. syringe needles (Terumo, Leuven, Belgium) were inserted through the silicon septum; one served to introduce the acceptor solution into the hollow fibre prior to extraction while the second needle was utilised for collection of the acceptor solution after extraction. The ends of the two needles were connected to an 8-cm piece of Q3/2 Accurel KM polypropylene hollow fibre (Akzo Nobel, Wuppertal,

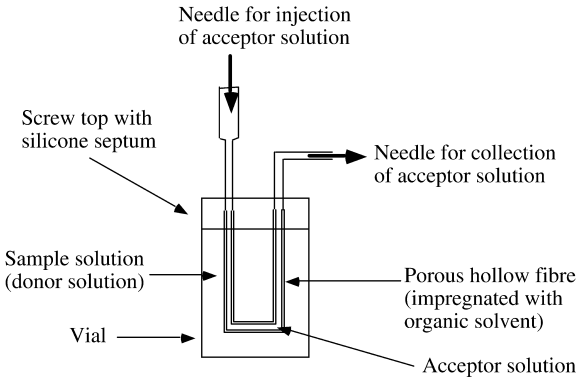

Fig. 2. Basic principle of LPME.

Germany). The inner diameter of the hollow fibre was 600 μm, the thickness of the wall was 200 μm and the pore size was 0.2 μm.

Extractions were performed according to the following scheme: a 1-ml sample solution was transferred to the vial. The solution was diluted with 2.73 ml water and 20 μl of 10 μg/ml I.S. and 0.25 ml of 2 *M* NaOH were added. A new 8-cm length of hollow fibre was placed between the two needle ends and dipped for 5 s into the organic solvent for impregnation. Subsequently, 5 s of ultra sonification in a water bath removed the excess of solvent. After impregnation, 25 μl of acceptor solution was injected into the hollow fibre with a microlitre syringe and the fibre was subsequently placed in the sample solution. During extraction, the solution was vibrated using a Vibramax 100 (Heidolph, Kelheim, Germany). After extraction, the acceptor solution was flushed into a 200-μl vial/insert (Advanced Biotechnologies Ltd., Surrey, UK) for the capillary electrophoresis instrument by applying a small pressure with a 5-ml syringe on the inlet needle of the hollow fibre. Each piece of hollow fibre was used only for a single extraction.

2.3. Capillary electrophoresis

Capillary electrophoresis 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). For the analysis of CIT, separations were performed with an effective length of 40 cm (total length of 50.2 cm) utilising 50 m*M* acetate adjusted to pH 4.6 as the separation buffer. For the simultaneous analysis of CIT, DCIT and the I.S., a 30-cm effective length capillary was utilised (40.2 cm total length) in combination with 75 m*M* TRIS–acetic acid pH 4.6, 3% (w/v) Tween 20 and 75 mg/l FC-135 as the separation buffer. Before analysis the separation buffer was filtrated through a 0.45-μm filter (Minisart RC 25, Sartorius AG, Göttingen, Germany). For CIT analysis, the instrument was operated at 30 kV, generating a current level of approximately 45 μA. For separation of CIT, DCIT and I.S. the instrument was operated at 20 kV, generating a current level of approximately 85 μA. All samples were introduced by hydrodynamic injection at 0.5 p.s.i. for 5 s. Detection was accomplished at 200 nm utilising a 100×800-μm slit.

3. Results and discussion

3.1. Basic principle

The principle of the disposable LPME device was thoroughly discussed in Ref. [2] and is briefly illustrated in Fig. 2. Analytes were extracted from the aqueous sample solution through a porous hollow fibre into an aqueous acceptor phase. The hollow fibre was impregnated with an organic solvent immiscible with water and functioned as a barrier between the sample and the acceptor phase. Since the analytes of interest were basic substances, the donor solution was made alkaline to deionise the analytes and consequently reduce the solubility within the sample solution. The deionised analytes were extracted from the sample solution into the organic solvent impregnated in the pores of the hollow fibre and further into the inside of the hollow fibre containing a small volume of an aqueous acidic solution providing high solubility for the analytes (acceptor phase). Due to the acidic nature of the acceptor phase, the analytes became ionised and were prevented from re-entering the organic solvent; hence, the three-phase system provided simultaneous extraction and back-extraction.

Analyte enrichment occured due to high partition coefficients of the analytes and high volume ratio

Table 1
Extraction recoveries of citalopram from water with different organic solvents immobilised within the pores of the hollow fibre[a]

Solvent	Extraction recovery (%)	RSD (%, $n=4$)
Hexyl ether	81	6.3
1-Octanol	55	35.2
2-Octanone	78	9.9

[a] All extractions were performed with 20 m*M* phosphate buffer pH 2.75 as the acceptor phase. Extraction: 45 min at 600 rpm of vibration.

between the sample and the acceptor solution. Theoretically, with a 100% extraction recovery, an enrichment factor of 40 was achievable (sample: 1 ml, acceptor phase: 25 μl). The LPME set-up also served as a method for sample clean-up and provided very clean extracts. Salts and proteins were prevented from entering the organic phase immobilised in the pores of the hollow fibre and remained within the sample. In addition, due to the three-phase nature of the LPME system, the majority of other endogenic compounds also remained in the sample.

3.2. Preliminary experiments

The preliminary experiments were conducted with CIT in pure water samples to study the fundamental extractability by LPME. During all the preliminary experiments, the donor solution consisted of 1 ml of 100 ng/ml CIT in water, which was diluted with 2.75 ml water and made alkaline with 250 μl of 2 *M* NaOH to ensure analyte deprotonation. In a first experiment, attention was focused on optimisation of the organic phase immobilised within the pores of the hollow fibre. Based on earlier results [2], hexyl ether, 1-octanol and 2-octanone were tested. The solvents had three important characteristics that made them suitable candidates for impregnation; they were easily immobilised on the polypropylene hollow fibre, they were immiscible with water and they were of very low volatility. As illustrated in Table 1, LPME of CIT was accomplished with all the three solvents. However, superior results were obtained with hexyl ether both with respect to extraction recovery and repeatability. Consequently, the hollow fibres were treated with hexyl ether during the rest of this study.

In addition to the organic solvent, the acceptor phase was optimised for the LPME of CIT. Six different acceptor solutions were tested, three consisting of dilute HCl (1, 10 and 100 m*M* respectively) and three consisting of phosphate buffers at pH 2.75 (5, 10 and 20 m*M* respectively). The HCl solutions were selected to provide an acceptor phase with a very low pH, while the idea of the phosphate buffers was to provide a low-pH medium of high buffer capacity. As illustrated in Table 2, LPME of CIT was accomplished with all the acceptor solutions tested. The highest extraction recovery was obtained with 20 m*M* phosphate buffer and consequently, this solution was utilised as acceptor phase during the rest of the study. The 20 m*M* phosphate solution provided an 81% extraction recovery for CIT and was found to be an acceptable medium for sample introduction; with higher concentrations of phosphate, anti-stacking and analyte defocusing may occur during the initial step of the capillary electrophoresis resulting in low separation efficiencies.

Table 2
Extraction recoveries of citalopram from water with different acceptor phases inside the hollow fibre[a]

Acceptor solution (m*M*)	Extraction recovery (%)	RSD (%, $n=4$)
Phosphate pH 2.75		
20	81	7.1
10	56	11.5
5	43	4.7
HCl		
100	65	14.6
10	65	10.6
1	54	9.4

[a] All extractions were performed with hexyl ether immobilised in the pores of the hollow fibre. Extraction: 45 min at 600 rpm of vibration.

Using the donor phase described above, hexyl ether immobilised on the hollow fibre and 20 m*M* phosphate buffer pH 2.75 as the acceptor phase, the extraction time was optimised in a third experiment (Fig. 3). The amount of CIT extracted by LPME increased with increasing exposure time from 5 to 30 min. After 45 min, the extraction recovery stabilised at about 81%, providing an analyte enrichment factor of 32. Based on this, the extraction time was selected to be 45 min. Although the extraction time was relatively long, a large number of different samples was extracted simultaneously resulting in a high sample capacity.

3.3. Separation of CIT, DCIT and I.S.

In plasma samples from patients treated with CIT, the main metabolite, DCIT, is normally found in addition to CIT. Therefore, to proceed with LPME from plasma samples a CE system that separated CIT and DCIT was necessary. In addition, for quantitative purposes, CIT and DCIT had to be separated from the selected I.S. (structures in Fig. 1). Standard alkaline separation buffers for capillary zone electrophoresis and micellar electrokinetic chromatography failed owing to peak tailing or co-migration between the compounds of interest. However, successful separation was achieved within 15 min utilising a separation buffer with 75 m*M* TRIS–acetic acid pH 4.6, 3% (w/v) Tween 20 and 75 mg/l FC-135. The non-ionic surfactant Tween 20 served to effectively resolve CIT, DCIT and I.S., while the fluorinated surfactant FC-135 served to suppress peak tailing for the basic drugs.

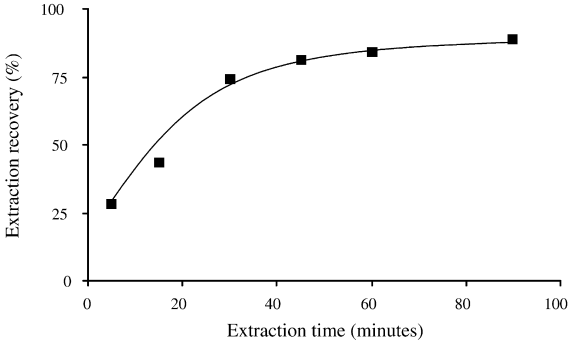

Fig. 3. Extraction recoveries of CIT from pure water versus extraction time.

3.4. Simultaneous liquid-phase microextraction of CIT, DCIT and I.S.

Based on the experiments described above with CIT, the LPME experiments were continued with simultaneous extraction of CIT, DCIT and I.S. Using the optimal conditions for CIT, extraction recoveries for CIT, DCIT and I.S. from pure water were 74, 25 and 70%, respectively. While the results were acceptable for CIT and I.S., further efforts were conducted to enhance the extraction recovery of DCIT. In a first experiment, the extraction time was increased from 45 min to 2 h; this provided a 47% extraction recovery of DCIT. In a second experiment, the rate of sample vibration during extraction was increased from 600 rpm to 1200 rpm, which provided a 50% extraction recovery of DCIT. Combining both efforts, the extraction recovery increased to 55% which was the maximum value obtainable from pure water samples. At these conditions, the extraction recoveries for CIT and I.S. remained almost unaffected at the 70% level.

In a subsequent experiment, CIT, DCIT and I.S. were extracted from human plasma for 45 min at both 600 rpm and 1200 rpm. In the former case, the extraction recoveries for CIT, DCIT and I.S. were 44, 25 and 28% respectively, while the results were improved to 60% for all of the analytes for a sample-vibration rate of 1200 rpm. This demonstrated the high importance of extensive vibration in order to counteract the effect of drug–protein binding, which is approximately 50% for CIT in human plasma [4]. As illustrated in Fig. 4, the extraction recovery increased with increasing extraction time up to 60 min for plasma samples, while no further improvements were observed utilising longer extraction times. Thus, optimal conditions for LPME of CIT, DCIT and I.S. from plasma samples included a 60-min extraction time and vibration at 1200 rpm. Due to parallel extraction of 20–30 samples, high extraction throughput was ensured despite the relatively long extraction time. Using the optimal conditions for CIT, DCIT and I.S. in human plasma, extraction recoveries were 76, 62 and 61% respectively, which is high considered LPME is a mi-

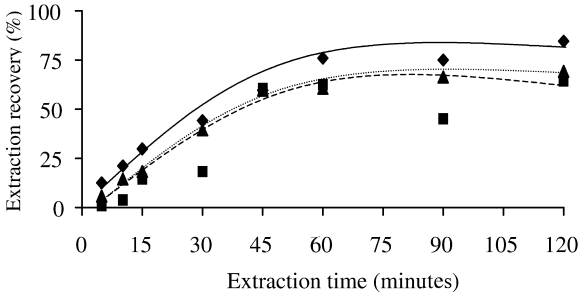

Fig. 4. Extraction recoveries of CIT, DCIT and I.S. from human plasma versus extraction time. All extractions performed at 1200 rpm. CIT – ♦ – (———), DCIT – ■ – (·····) and I.S. – ▲ – (- - - -).

croextraction technique. The extraction recoveries corresponded to enrichment factors in the range of 25–30.

3.5. Validation

In order to proceed with the current evaluation of LPME, repeatability, linearity, limit of detection and limit of quantification were determined for CIT and DCIT in human plasma. To all samples, 20 μl of 10 μg/ml I.S. was added as internal standard. Repetitive extractions (20, 100 and 300 ng/ml CIT and DCIT) varied between 3.6 and 11.0% (CIT) and between 12.8 and 19.0% (DCIT) (Table 3). The standard curves for CIT and DCIT were linear from 20 to 1000 ng/ml (r=0.998 and 0.994 respectively). The excellent linearity was obtained in an extended range as compared to the therapeutic window (20–110 ng/ml; [5]) and indicated no capacity problems due to acceptor phase saturation during LPME. The limit of quantification (S/N=10) was estimated to be 16.5 ng/ml for CIT and 18 ng/ml for DCIT, while

Table 3
Within-day repeatability for LPME–CE of citalopram and *N*-desmethylcitalopram from human plasma samples[a]

Analyte concentration (ng/ml)	RSD (%)	
	Citalopram	*N*-desmethylcitalopram
20	11.0	19.0
100	3.6	12.8
300	5.8	13.3

[a] Internal standard added. Results based on six repetitive injections.

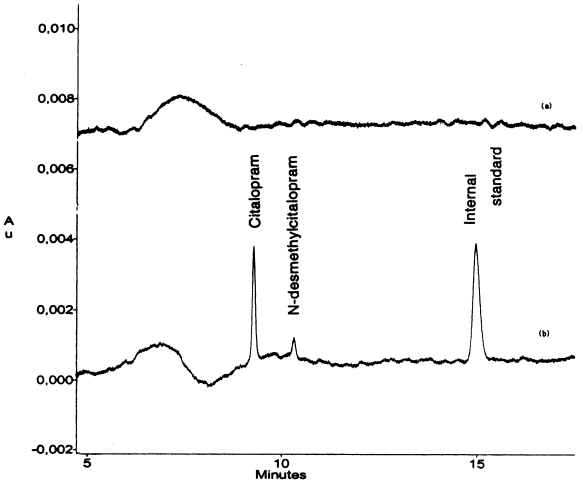

Fig. 5. LPME–CE from (a) blank plasma sample (b) a plasma sample from a patient treated with 40 mg citalopram daily. All extractions performed at 1200 rpm for 60 min.

the limit of detection (S/N=3) was 5 ng/ml for CIT and 5.5 ng/ml for DCIT with UV detection at 200 nm. CE in combination with bioanalysis often suffers from high limits of detection and quantification. The limits estimated in this work (low ng/ml level) were satisfactory as determination and quantification of CIT in the therapeutic range was enabled and the LPME–CE results were comparable with the data obtained by HPLC [6–10].

3.6. Patient sample

A real plasma sample from a patient treated with 40 mg citalopram daily was analysed in addition to a drug free sample (Fig. 5). As illustrated for the drug-free sample, the extracts were clean and no peaks were observed for matrix components. The high selectivity of LPME was further supported by the analysis of the patient sample; although this patient was treated with trimeprazine and chlorpromazine in addition to CIT, only peaks for CIT, DCIT and I.S. were detected in the electropherogram. The concentration of CIT was determined to 135 ng/ml by LPME–CE.

4. Conclusions

The present work has demonstrated the possibility

of LPME of hydrophobic drugs prior to CE-analysis. Extensive vibration (and prolonged extraction time) were shown to be important factors for obtaining high extraction recoveries from human plasma. High preconcentration enabled quantification in the therapeutic range and sample clean-up was highly efficient with no interfering peaks from matrix components.

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