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Liquid-phase microextraction combined with capillary electrophoresis, a promising tool for the determination of chiral drugs in biological matrices

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Abstract

A disposable device for liquid-phase microextraction (LPME) based on porous polypropylene hollow fibres has recently been introduced. In the present paper, LPME was combined with capillary electrophoresis (CE) and the combination was for the first time evaluated for chiral determination of drugs in biological matrices. The chiral antidepressant drug mianserin was selected as model compound. The mianserin enantiomers were extracted from 0.5 ml of plasma added internal standard and made alkaline with 0.25 ml of 2 *M* NaOH. The unionised analytes were extracted into di-*n*-hexyl ether impregnated in the pores of the hollow fibre, and into an acidic solution inside the hollow fibre. This resulted in a three-phase system where the extracts were aqueous, and hence directly compatible with the CE system. Efficient sample clean-up was seen and the extraction recovery was 80% for both enantiomers. Discrimination between the enantiomers in the extraction system was not observed. The limit of quantitation ($S/N=10$; 12.5 ng/ml for both enantiomers) and the limit of detection ($S/N=3$; 4 ng/ml for both enantiomers) were below the therapeutic range for mianserin. The method was validated and successfully applied to determine *R*- and *S*-mianserin in plasma samples from seven patients treated with mianserin, indicating that LPME–CE is a promising combination for analysis of racemic drugs present in low concentrations in biological matrices.

Keywords: Liquid-phase microextraction; Extraction methods; Enantiomer separations; Hollow fibres; Antidepressants; Mianserin

1. Introduction

Chiral drugs are in most cases administered as racemates although the two enantiomers often exhibit different behaviour in terms of pharmacological action, therapeutic efficiency and biological processes. The optimisation of fast chiral analytical methods offering high efficiency and resolution is therefore important to study the pharmacokinetic and pharmacodynamic properties of racemic drugs and to control the enantiomeric purity of pharmaceutical preparations.

Stereospecific drug monitoring is widely accomplished by the use of chromatographic methods, which require either rather expensive chiral stationary phases or the conversion of the enantiomers into diastereomers with a chiral reagent. During the past few years, chiral separations by capillary electrophoresis (CE) have been studied extensively and shown to provide low cost analysis with high

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efficiency and resolution together with selectivity and short analysis time [1–17]. However, so far the use of CE in therapeutic drug monitoring has been limited due to high concentration detection limits.

Recently a new and disposable device for sample preparation based on polypropylene hollow fibres was introduced [18–26]. The technique, liquid-phase microextraction (LPME), offers high preconcentration and efficient sample clean up. 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 (25 μl). By the use of an aqueous acceptor phase direct compatibility with CE is achieved, and 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 been successfully combined with CE for nonchiral therapeutic drug monitoring [21]. The developed method enabled determination of the model compound citalopram in the complete therapeutic range, and resulted in limits of quantitation (LOQs) comparable with previous published high-performance liquid chromatography (HPLC) methods. As a follow-up to this work, the possibilities of utilising LPME as a sample preparation technique prior to chiral determination by CE was investigated in the present work. The combination of LPME with chiral CE will take maximum advantage of both techniques. High enrichment and efficient sample clean-up by LPME will make it possible to perform efficient low cost chiral separation by CE despite low analyte concentrations and/or limited availability of sample volume. In addition the extract is directly compatible with the separation system and the total amount of organic solvents used is almost negligible.

To demonstrate the potential for LPME–CE in chiral therapeutic drug monitoring a method was developed utilising the chiral drug mianserin as a model compound. Mianserin is a tetracyclic antidepressant drug formulated as a racemic mixture. It was chosen as the model compound since the two enantiomers exhibit different degree of antidepressant activity. Several behavioural tests indicate that the *S*-enantiomer is much more active than the *R*-enantiomer [27–30]. In addition the metabolism of the two enantiomers is different [31,32] and an interindividual variation in the ratio of *S*- and *R*-mianserin is described [32–34]. Separation of *R*- and *S*-mianserin is hence of interest in therapeutic drug monitoring as individual determination of the enantiomers will reflect the antidepressant activity better than the determination of the total mianserin concentration.

This was the first time LPME was combined with CE for chiral determination. The combination was validated for the determination of *R*- and *S*-mianserin in plasma, and afterwards the method was utilised for the determination of the two enantiomers in seven samples from patients treated with mianserin. Two metabolites were identified and it was possible to detect differences in the metabolism of the two enantiomers. The results showed that LPME–CE is a promising combination for chiral determination, especially when high preconcentration is necessary due to low analyte concentrations and/or limited amounts of sample available.

2. Experimental

2.1. Chemicals

Mianserin was obtained from Sigma (St. Louis, MO, USA) and methamphetamine (>99%) (internal standard) from Norsk Medisinaldepot (Oslo, Norway). For structures see Fig. 1. Paracetamol and risperidone were purchased from Sigma. Citalopram was a gift from H. Lundbeck (Copenhagen, Denmark). Alimemazine, ergotamine, lamotrigine, levomepromazine, metoprolol, olanzapine and zopiclone were obtained by extraction with ethanol from tablets: alimemazine from Vallergan 5 mg (Aventis Pharma), ergotamine from Anervan (Recip), lamotrigine from Lamictal 25 mg (Glaxo Wellcome), levomepromazine from Nozinan 5 mg (Pharmacia & Upjohn), metoprolol from Selo-Zok 50 mg (AztraZeneca), olanzapine from Zyprexa 5 mg (Lilly) and zopiclone from Imovane 5 mg (Novartis). All tablets were obtained from Norsk Medisinal-

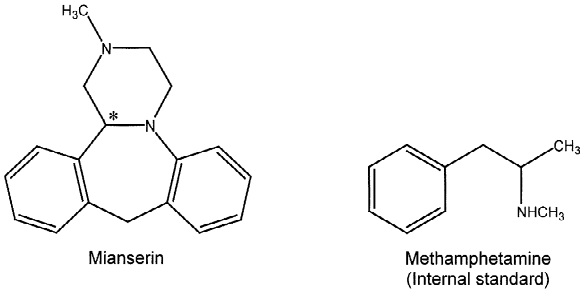

Fig. 1. Structures of mianserin and internal standard (methamphetamine). The stereocentre of mianserin is indicated with an asterisk.

depot. Di-*n*-hexyl ether (≥97%), glyceryl tributyrate (≥98%), di-*n*-butyl ether (≥99.5%), 1-chloropentane (~99%) and 2-octanone (>97%) were purchased from Fluka (Buchs, Switzerland). 1-Octanol (99%) was obtained from Aldrich (Sigma–Aldrich, Steinheim, Germany). Hydrochloric acid, orthophosphoric acid, sodium phosphate, sodium hydroxide, sulfuric acid, and toluene, all of analytical grade, and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Triethylamine was purchased from Prolabo (Fontenay-Sous-Bois, France). Hydroxypropyl-β-cyclodextrin (eCAP) was obtained from Beckman (Fullerton, CA, USA). Ethanol (96%) was purchased from Arcus (Oslo, Norway). All aqueous solutions were prepared with water purified with an EASYpure RO system (Barnstead, Dubuque, IA, USA). Plasma samples from patients treated with mianserin were supplied by Diakonhjemmet University Hospital (Oslo, Norway).

2.2. Liquid-phase microextraction

The disposable LPME device is illustrated in Fig. 2. The sample solution was transferred to a 1.5-ml sample vial with a screw top/silicone septum (Chromacol, Trumbull, CT, USA). Two 0.8 mm O.D. syringe needles (Terumo, Leuven, Belgium) were inserted through the silicone septum, one served to introduce the acceptor solution into the hollow fibre prior to extraction while the other needle was utilised for collection of the acceptor solution after extraction. An 8 cm piece of the porous polypropylene hollow fibre Q3/2 Accurel KM (Membrana, Wuppertal, Germany) connected

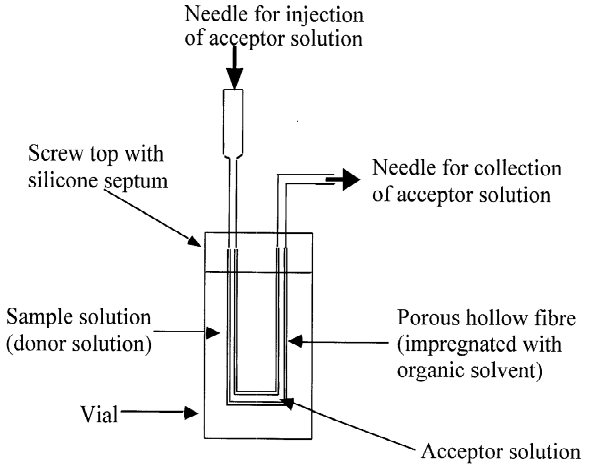

Fig. 2. Principle of LPME.

the ends of the two needles. The inner diameter of the fibre was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm.

Prior to extraction the sample vial was filled with 0.5 ml plasma and made alkaline with 0.25 ml of 2 *M* NaOH. Water (0.25 ml for the optimisation of the extraction conditions) or internal standard (0.25 ml of 140 ng/ml methamphetamine for the quantitative analysis) was added. A new length of the polypropylene hollow fibre was placed between the two needles 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 ultrasonification. After impregnation, 25 μl of acceptor solution was injected into the hollow fibre with a microlitre syringe and the fibre was placed in the sample solution. During extraction, the samples were vibrated using a Vibramax 100 (Heidolph, Kelheim, Germany) at 1500 rpm. After extraction the acceptor solution was flushed into a 200-μl vial/insert (Advanced Biotechnologies, Surrey, UK) 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

CE was performed with a MDQ instrument (Beckman) equipped with a UV detector. Separations were accomplished on a 75 μm I.D. fused-silica capillary

(BGB Analytik, Anwil, Switzerland) with an effective length of 40 cm (total length 50.2 cm). The running buffer consisted of 75 m*M* phosphoric acid adjusted to pH 3.0 with concentrated triethylamine, and contained 2 m*M* hydroxypropyl-β-cyclodextrin. The buffer was filtered through a 0.45-μm filter (Minisart RC 25, Sartorius, Göttingen, Germany). Before analysis the capillary was conditioned with 0.1 *M* NaOH for 30 min at 20 p.s.i., water for 20 min at 20 p.s.i., and then electroconditioned with the running buffer for 45 min at 25 kV and 5 p.s.i. (1 p.s.i.=6894.76 Pa). During analysis the instrument was operated at 20 kV, generating a current level of approximately 75 μA. All samples were introduced by hydrodynamic injection for 20 s at 0.5 p.s.i. Detection was accomplished at a wavelength of 200 nm utilising a 100×800 μm slit.

2.4. Identification of the enantiomers of mianserin

Pure enantiomers were not commercially available for mianserin. The order of migration was based on the migration order in the separation system described by Eap et al. [35].

2.5. Calculation of extraction recoveries

The extraction recovery (R) 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 ($n_{a,\,final}$):

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

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 extract (acceptor phase) and the initial analyte concentration within the sample, respectively.

3. Results and discussion

LPME and CE were for the first time combined for chiral determination of drugs in biological matrices. The goal was to take advantage of the strengths of both techniques, and hence develop a powerful chiral method for quantification of drugs present in low concentrations in biological matrices. A chiral method for the determination of mianserin, an antidepressant drug in plasma was developed. Mianserin was chosen as the model compound since the two enantiomers exhibit different degree of antidepressant activity, *S*-mianserin being much more active than *R*-mianserin [27–30]. In addition interindividual variation in the S/R ratio is described [32–34], hence determination of the enantiomers individually will reflect the antidepressant activity better than the determination of the total mianserin concentration.

In LPME of the mianserin enantiomers prior to CE, the analytes were extracted from an aqueous sample solution, through an organic solvent impregnated in the pores of the hollow fibre and into an aqueous acceptor solution. In order to promote extraction of the analytes of interest, pH of the sample solution was modified to deionise the analytes and reduce the solubility in the sample solution. For the enantiomers of the basic drug mianserin, this was done by alkalinisation of the sample solution. The analytes were then extracted into the organic solvent in the pores of the hollow fibres where the solubility of the unionised analytes was high. By using an acceptor phase with even higher solubility of the analytes, i.e., using an acceptor phase with a pH that ensured ionised analytes further extraction was facilitated. For the mianserin enantiomers a low-pH acceptor phase was used. In this three-phase system the analytes were simultaneously extracted and back-extracted, and hence the samples were efficiently cleaned up. The selectivity of the three-phase LPME system was high; only substances that were deionised in the sample solution and soluble in the organic solvent were extracted into the organic solvent. Further extraction was dependent on reionisation in the acceptor solution. In LPME high enrichment is possible based on a large volume difference between the volume of the sample solution and acceptor phase. In these experiments the size of the sample solution was 0.5 ml and the size of the acceptor phase was 25 μl resulting in a maximum enrichment factor of 20.

3.1. Separation of mianserin enantiomers

The mianserin enantiomers were separated in a slightly changed version of a previous published method [35]. A shorter capillary was used (40 cm effective length compared to 56 cm), having a larger internal diameter (75 μm vs. 50 μm). Unlike the capillary used in Ref. [35] the 75-μm I.D. capillary did not have extended light path in the detection window. The composition of the running buffer was not changed, but due to the use of a shorter capillary, the voltage was reduced from 30 to 20 kV resulting in a current of approximately 75 μA. Efficient separation between *R*- and *S*-mianserin was maintained (Fig. 3). By thorough conditioning of the capillary prior to each analysis sequence, it was not necessary to change buffer between every analysis. UV detection at 200 nm was performed instead of at 211 nm. The samples were injected for 20 s at 0.5 p.s.i. to achieve satisfactory quantification and detection limits.

The stability of the separation system was investigated by repeatedly injection ($n=18$) of a standard solution containing 10 μg/ml *R*- and *S*-mianserin and internal standard. The reproducibility of the relative migration time of the two enantiomers compared with internal standard was at the 1.0% level.

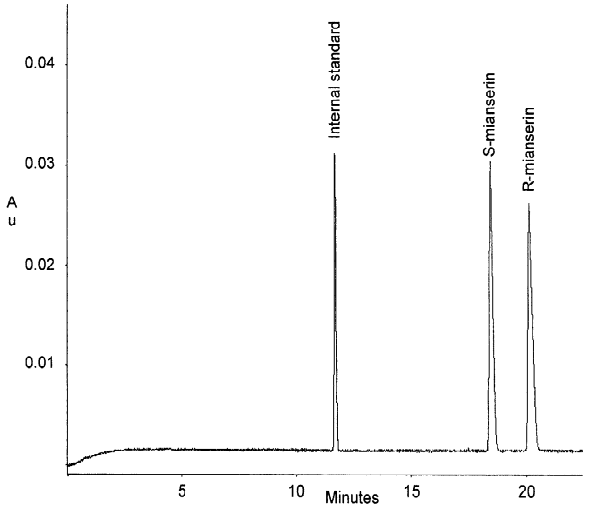

Fig. 3. Electropherogram showing the separation of internal standard, and *R*- and *S*-mianserin.

3.2. Extraction of mianserin enantiomers

R- and *S*-mianserin were extracted from 0.5 ml samples. First the extraction conditions were optimised for water and then the conditions were applied to plasma samples. During the optimisation of the extraction recoveries it was important to ensure that the recovery of the two enantiomers was equal. The water samples were spiked with racemic mianserin (*R*-mianserin:*S*-mianserin, 1:1) and, different extraction recoveries would indicate that the enantiomers behaved different in the extraction set-up due to different stereochemistry.

The optimisation of the extraction parameters started with optimisation of the organic phase immobilised in the pores of the hollow fibre. 100 ng/ml racemic mianserin was extracted from 0.5 ml plasma. The solution was made alkaline with 0.25 ml of 2 *M* NaOH and 0.25 ml of water was added for dilution (total sample volume: 1 ml). The acceptor phase was 25 μl of 0.1 *M* HCl. Extractions were performed for 45 min. Three of the solvents (di-*n*-hexyl ether, 1-octanol and 2-octanone) were chosen based on earlier results [18,21] and in addition five other solvents (toluene, glyceryl tributyrate, di-*n*-butyl ether and 1-chloropentane) were tested. The solvents were immiscible with water, of low volatility, and easily impregnated on the polypropylene hollow fibre. LPME of *R*- and *S*-mianserin was accomplished with all solvents (Table 1). However, both enantiomers were most efficiently extracted using di-*n*-hexyl ether in the pores of the hollow fibre,

Table 1
Extraction recoveries of mianserin from water utilising different organic solvents immobilised in the pores of the hollow fibre[a]

Organic solvent	Extraction recovery (%, $n=2$)[b]
Di-*n*-hexyl ether	74
Toluene	60
Glyceryl tributyrate	58
Di-*n*-butyl ether	42
1-Chloropentane	39
1-Octanol	20
2-Octanone	16

[a] All extractions were performed with 0.1 *M* HCl as the acceptor phase and a total concentration of 0.5 *M* NaOH in the sample solution. Extractions were performed for 45 min.

[b] The recoveries of *R*- and *S*-mianserin were identical.

providing 74% extraction recovery for both enantiomers. No differences between the extraction recoveries for the *R*- and *S*-enantiomers were seen, indicating that the enantiomers did not behave different in the extraction set-up.

After optimisation of the organic solvent the acceptor phase was optimised by investigation of five different acceptor solutions (Table 2). The sample solution consisting of 100 ng/ml racemic mianserin was made alkaline with 0.25 ml of 2 *M* NaOH and 0.25 ml water was added. Di-*n*-hexyl ether was used as the organic solvent. Extractions were performed for 45 min. Three acceptor phases consisted of dilute HCl (0.01, 0.1 and 1 *M*), one consisted of 10 m*M* phosphate buffer, pH 3.3, and one of 10 m*M* sulfuric acid. Both HCl (0.1 and 0.01 *M*) and phosphate buffer (at a lower pH, 2.75) had previously shown to efficiently extract basic drugs from biological matrices [18,21,23,24]. The HCl solutions and the sulfuric acid provided an acceptor phase of very low pH, while the phosphate buffer was chosen due to its capability to provide a relatively low-pH medium of high buffer capacity. Highest recoveries (85% for both enantiomers) were seen utilising 0.01 *M* HCl as the acceptor phase. Both 0.1 *M* HCl and 10 m*M* sulfuric acid resulted in relatively high extraction recoveries (>70%), whereas the extraction recoveries using 10 m*M* phosphate buffer, pH 3.3, as the acceptor phase were lower; 48% for both enantiomers. It was not possible to determine extraction recoveries utilising 1 *M* HCl as the acceptor phase. The high ionic strength of this solution resulted in poor electrophoretic behaviour of the extract in the CE system.

In addition to the organic solvent and the acceptor phase, the amount of NaOH in the sample solution was optimised (Table 3). A 100 ng/ml solution of racemic mianserin (0.5 ml) was used, and before extraction the solution was diluted to a total of 1 ml with NaOH and water. The organic solvent was di-*n*-hexyl ether and the acceptor phase consisted of 0.01 *M* HCl. Extractions were performed for 45 min. Three different amounts of NaOH were used, resulting in a total concentration of 0.125, 0.5 and 1 *M* NaOH in the diluted sample solution (total 1 ml). Highest extraction recoveries (85% for both enantiomers) were seen utilising 0.5 *M* NaOH as the total concentration of NaOH, which corresponded to addition of 0.25 ml of 2 *M* NaOH. This was in accordance with similar experiences with other drugs [21,24–26].

Table 3
Extraction recoveries of mianserin in water utilising different concentrations of NaOH in the sample solution[a]

Concentration of NaOH (*M*)	Extraction recovery (%, $n=2$)[b]
0.125	73
0.5	85
1	77

[a] All extractions were performed with di-*n*-hexyl ether immobilised in the pores of the hollow fibre and 0.01 *M* HCl as the acceptor solution. Extractions were performed for 45 min.
[b] The recoveries of *R*- and *S*-mianserin were identical.

The optimisation experiments resulted in the following extraction conditions for *R*- and *S*-mianserin in water samples: 0.5 ml of sample solution was added 0.25 ml of 2 *M* NaOH and diluted with water to 1 ml. Di-*n*-hexyl ether was utilised as the organic solvent and the acceptor phase consisted of 25 μl of 0.01 *M* HCl. After 45 min of extraction the extraction recovery for both enantiomers was 85%.

After optimisation of the extraction recoveries from water, the conditions were applied to plasma

Table 2
Extraction recoveries of mianserin in water utilising different acceptor phases inside the hollow fibre[a]

Acceptor solution	Extraction recovery (%, $n=2$)[b]
0.01 *M* HCl	85
0.1 *M* HCl	74
1 *M* HCl	n.d.
10 m*M* Phosphate buffer, pH 3.3	48
10 m*M* H_2SO_4	72

[a] All extractions were performed with di-*n*-hexyl ether immobilised in the pores of the hollow fibre. The concentration of NaOH in the sample solution was 0.5 *M*. Extractions were performed for 45 min.
[b] The recoveries of *R*- and *S*-mianserin were identical.

samples. The degree of plasma protein binding of mianserin is approximately 90% [36]. In order to ensure correct determination of each enantiomer of mianserin the drug–protein interactions have to be eliminated. Insufficient reduction of drug–protein interactions is indicated by a lower extraction recovery from plasma compared with water, and/or by differences in the recoveries of the two enantiomers in plasma. The two enantiomers may have different affinity for the plasma proteins due to different stereochemistry and hence the recoveries may differ between the enantiomers. However, the extraction recovery for both enantiomers in plasma was 80%, which were considered equal to the recovery from water (85%), and as a proof of complete elimination of drug–protein interactions. Dilution of the sample with water followed by alkalisation with NaOH provided efficient breakage of the interactions of mianserin with plasma proteins, and the conditions could hence be applied directly to plasma samples without further modifications.

3.3. Validation

Prior to analysis of patient samples the method was validated. Repeatability, linearity, limit of detection and limit of quantification were determined for *R*- and *S*-mianserin. To all samples 0.25 ml 140 ng/ml internal standard (methamphetamine) was added. Repetitive extractions (25, 80 and 135 ng/ml racemic mianserin) varied between 8.9 and 11.0% for *R*-mianserin and between 8.8 and 12.3% for *S*-mianserin (Table 4). The standard curves for *R*- and *S*-mianserin were linear from 2.5 to 85 ng/ml (corresponding to 5–170 ng/ml total mianserin) ($r=$ 0.9994 for both enantiomers) and covered an enlarged range of the therapeutic window (25–115 ng/ml total mianserin concentration) [33,37–39]. The LOQ ($S/N=10$) was estimated to 12.5 ng/ml for both enantiomers, while the limit of detection ($S/N=3$) was 4 ng/ml for both enantiomers utilising UV detection at 200 nm. The validation emphasises that LPME–CE is a powerful combination for chiral analysis of drugs in biological matrices. The excellent chiral separation (shown in Fig. 3) may hence be utilised in therapeutic drug monitoring of mianserin enantiomers since LPME provides sufficient enrichment for determination of the enantiomers in the lower region of the therapeutic window.

Table 4
Within-day repeatability of *R*- and *S*-mianserin from human plasma samples[a]

Racemic mianserin concentration (ng/ml)	RSD[b] (%)	
	R-Mianserin	*S*-Mianserin
25	8.9	12.3
80	10.0	8.8
135	11.0	10.9

[a] Internal standard added.
[b] Based on six repetitive extractions.

3.4. Additional investigations prior to analysis of patient samples

The newly developed method was used to determine the concentrations of *R*- and *S*-mianserin in seven patients treated with 30–90 mg racemic mianserin daily. All patients were administered other drugs in addition to mianserin, like patients treated with psychotropic drugs frequently are. It was therefore important to investigate two additional parameters prior to analysis of the patients' plasma samples.

First, the influence of other drugs on the extraction recoveries of *R*- and *S*-mianserin was investigated. Plasma samples spiked with 100 ng/ml racemic mianserin (and added internal standard) were added citalopram, lamotrigine, metoprolol and paracetamol in either low or high therapeutic concentrations and the recoveries were compared with the recoveries of plasma samples with no additions except for mianserin and internal standard. A slight increase in the recoveries of *R*- and *S*-mianserin was seen with other drugs added (Table 5). The results were compared by two-sample *t*-test ($\alpha=0.05$) and the differences were not significant. The recoveries of the internal standard were constant. The presence of other drugs in the patient samples would hence not affect the extraction recoveries of the analytes.

Second, the influence of other drugs on the separation system was investigated. Alimemazine, citalopram, ergotamine, lamotrigine, levomepromazine, metoprolol, olanzapine, risperidone, sertraline and zopiclone were drugs taken by the patients that were likely to be extracted in the LPME system. The influence of these drugs (except for citalopram and sertraline which had been investigated previously

Table 5
Comparison of extraction recoveries of *R*-mianserin, *S*-mianserin and internal standard from plasma after addition of citalopram, lamotrigine, metoprolol and paracetamol in low or high therapeutic concentrations prior to extraction

Drugs added	Extraction recovery; %, $n=5$ (RSD, %)		
	R-Mianserin	*S*-Mianserin	Internal standard
35 ng/ml Citalopram 2 μg/ml Lamotrigin 50 ng/ml Metoprolol 5 μg/ml Paracetamol	75 (7.9)	76 (8.6)	97 (2.6)
250 ng/ml Citalopram 15 μg/ml Lamotrigin 500 ng/ml Metoprolol 30 μg/ml Paracetamol	76 (7.6)	77 (6.4)	95 (3.8)
No drugs added	69 (11.7)	69 (10.8)	97 (1.2)

[35]) on the separation system was tested. Standards containing 10 μg/ml racemic mianserin, internal standard and each of the other drugs at a turn were injected directly into the separation system. None of the compounds interfered with the determination of either *R*- and *S*-mianserin or the internal standard.

3.5. Analysis of patient samples

After investigation of these parameters the patient samples were analysed. All patients showed a higher concentration of *S*-mianserin than *R*-mianserin, and in addition a variation (1.8–4.7) in the ratio of *S*- and *R*-mianserin was seen (Table 6). Both results corresponded with previous reports, which have shown that the *S*/*R* ratio of mianserin in plasma from patients treated with racemic mianserin usually is larger than one and that interindividual variation in the ratio is seen [32–34]. This interindividual variation in the *S*/*R* ratio of mianserin combined with the fact that *S*-mianserin is the most active enantiomer with respect to antidepressant effect [27–30] indicates that determination of *S*- and *R*-mianserin enantiomers reflects the antidepressant activity more correctly than determination of the total mianserin concentration.

The metabolism of the two enantiomers differs [31,32]. The only metabolites seen in the present separation system, which was a slightly changed version of a previously described system [35], were the *N*-desmethylmianserin enantiomers. The method was not developed for determination of these metabolites, but by comparison with the migration order in the previously described system [35] it was possible to identify the *N*-desmethyl metabolites. For all patients the amount (area) of *R*-*N*-desmethylmianserin was larger than for *S*-*N*-desmethylmianserin (Fig. 4). This was in agreement with

Table 6
Concentration of *R*- and *S*-mianserin in patient samples

Patient	Mianserin dose daily (mg)	*R*-Mianserin (ng/ml)	*S*-Mianserin (ng/ml)	Enantiomeric ratio (*S*/*R*)
1	30	n.q.	22	>1.8
2	30	n.q.	22	>1.8
3	60	18	22	1.2
4	60	19	59	3.1
5	60	13	42	3.2
6	60	n.q.	47	>3.8
7	90	n.q.	57	>4.7

n.q.: Detectable, but not quantifiable.

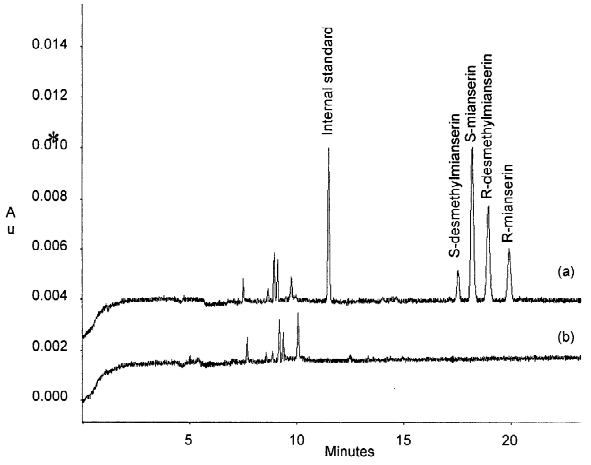

Fig. 4. Electropherograms of LPME extracts from (a) a plasma sample from patient 4 (*R*- and *S*-*N*-desmethylmianserin was identified by comparison with the separation system in Ref. [35]), (b) a blank plasma sample.

previous reports, which have shown that the rate of N-demethylation is higher for *R*-mianserin than for *S*-mianserin. This has been shown in rats and mice, and in human liver microsomes and by the use of eight recombinant human cytochrome P450 isoforms [31,40–42].

4. Conclusions

In the present work LPME was combined with CE for the chiral determination of drugs in biological matrices for the first time. LPME resulted in high preconcentration and efficient sample clean-up, while efficient chiral resolution was obtained in the CE system. Determination of the model compounds (*R*- and *S*-mianserin) in the low-ng/ml level was possible without further preconcentration/sample preparation. LPME did not discriminate between the two enantiomers during extraction, and the extraction recoveries from plasma were comparable with the recoveries from water samples. The method was validated and used for determination of the enantiomers in plasma samples from patients treated with the racemic mianserin. The results showed that LPME–CE is a promising combination for the chiral separation of drugs in biological matrices.

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