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Review

# Liquid–liquid extraction procedures for sample enrichment in capillary zone electrophoresis

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**Abstract**

This review article presents an overview of applications of liquid–liquid extraction (LLE) for analyte enrichment and clean-up of samples prior to capillary zone electrophoresis (CZE). The basic principles of LLE are discussed with special emphasis on analyte enrichment. In addition, attention is focused on the requirements for the final extract to be compatible with CZE. The paper discusses selected examples from the literature with special emphasis on detection limits in drug analysis and in environmental chemistry. Finally, the paper focus on alternative liquid-phase extraction concepts based on electroextraction, supported liquid membranes, and liquid-phase microextraction. 

*Keywords:* Liquid–liquid extraction; Capillary electrophoresis; Extraction methods; Reviews

## Contents



## 1. Introduction

In capillary zone electrophoresis (CZE), cationic and anionic analytes may be separated based on differences in their charge-to-size ratio, and subsequently measured on-column by UV detection. Compared with the traditional chromatographic techniques, CZE provides an alternative separation principle characterized by high separational efficiency, rapid separations, and by a low consumption of reagents as well as solvents. Based on these advantages, CZE has been implemented in a broad

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range of application areas including pharmaceuticals, proteins, peptides, agrochemicals, raw materials, water, DNA, surfactants and fine chemicals [1].

Unfortunately, CZE suffers from relatively high concentration detection limits because the sample volumes injectable under standard conditions are limited to the low-nl level, and because UV detection is accomplished directly on the capillary with a short optical path length. Thus, for trace analysis applications, the amount of analyte injected into the capillary or the detector sensitivity has to be increased. The latter aspect may be accomplished by utilizing extended light paths in connection with UV detection, or alternatively by utilizing laser-induced fluorescence (LIF) detection [2]. Both bubble cells and z-shaped cells have been utilized as extended light paths for UV detection, which typically provides an enhancement of the signal-to-noise by a factor of 3–6 [2]. Although the detector sensitivity theoretically may be further improved with the z-configuration, loss of electrophoretic resolution limits the light path in practical work. Thus, UV detection in capillary electrophoresis (CE) with extended light paths provides no substantial improvements in sensitivity. With LIF detection, extremely high mass sensitivity has been reported [2], but currently direct LIF detection is only applicable for some analytes as the number of wavelengths available with the commercial LIF detectors is limited.

The second approach, to increase the amount of analyte injected into the capillary, may be accomplished either by analyte enrichment during a sample preparation step or by extended volume injections followed by analyte focusing during the CZE analysis. The latter concept involves sample stacking, and sample stacking may take place when the sample plug is sandwiched between leading and terminating electrolytes (isotachophoretic sample stacking) [3] or when the sample is of lower conductivity than the running buffer (field-amplified sample stacking) [4,5]. Although detection limits may be improved dramatically, extensive sample stacking may be difficult in routine analysis due to several practical limitations. Isotachophoretic sample stacking suffers from difficulties in selecting proper electrolytes meeting the requirements for stacking and separation, while field-amplified sample stacking suffers from a mass loading dependency on the conductivity of the sample [5].

Although improvements of detection limits may be accomplished both by detector optimization and by sample stacking as discussed above, analyte enrichment during a sample preparation step normally is the most practical concept to overcome the sensitivity problems of CZE. Several sample preparation concepts have been utilized in combination with CZE, including solid-phase extraction (SPE), solid-phase microextraction (SPME), microdialysis, on-line preconcentration with small beds of packing material inside the separation capillary, and liquid–liquid extraction (LLE) [6]. In the present review, attention will be focused on the latter type of sample preparation for CZE. In addition to general theory and some practical points of high importance, a literature review of LLE combined with CZE has been included in the paper. Focus has been limited to analyte enrichment from aqueous samples; thus, liquid extraction of solid samples has not been included in the present work.

## 2. Analyte enrichment and sample clean-up by liquid–liquid extraction

In LLE, hydrophobic sample constituents are extracted from aqueous samples with a water-immiscible organic phase. Various volatile organic solvents are used, including pentane, hexane, diethyl ether, ethyl acetate, chloroform and methylene chloride. For an analyte *i*, the extraction process may be illustrated with the equation:

$$i_{\text{sample}} \leftrightarrow i_{\text{organic phase}} \quad (1)$$

where the subscript «sample» represents the sample solution and the subscript «organic phase» represents the organic solvent utilized for extraction. At equilibrium, the partition coefficient for the analyte *i* ($K_i$) in the two-phase system is:

$$K_i = C_{i,\text{organic phase}} / C_{i,\text{sample}} \quad (2)$$

where $C_{i,\text{organic phase}}$ is the equilibrium concentration of *i* in the extraction solvent and $C_{i,\text{sample}}$ is the equilibrium concentration of *i* in the sample phase. For successful LLE, the analyte should be extracted quantitatively from the sample and into the organic

solvent; the extraction efficiency or the recovery [$E_E$ = extracted amount of $i$/original amount of $i$ in the sample)·100%] should be close to 100%. The extraction efficiency is closely related to both the partition coefficient ($K_i$) and to the volume of organic solvent used for the extraction ($V_{organic\ phase}$):

$$E_E = 1/\left[(V_{sample}/K_i V_{organic\ phase}) + 1\right] \quad (3)$$

where $V_{sample}$ is the volume of the sample. As illustrated in Table 1, high extraction efficiencies may be obtained in general utilizing large volumes of organic solvent relative to the volume of sample (low $V_{sample}/V_{organic\ phase}$ ratio). Although compounds with high partition coefficients may be extracted effectively with high $V_{sample}/V_{organic\ phase}$ ratios, the volume of organic solvent is normally comparable or even exceeds the volume of the sample for handability reasons and in order to speed up the LLE process. For compounds actual for CZE, which are either acidic or basic compounds, pH adjustment is normally of high importance in order to ensure high partition coefficients. Thus, prior to LLE of basic compounds, pH of the sample has to be adjusted into the alkaline range, while LLE of acidic compounds has to accomplished at low pH. In the deionized forms, hydrophobic acids or bases are easily extracted into organic solvents immiscible with water. For more hydrophilic compounds in contrast, low partition coefficients are observed even for their deionized forms. In these cases, ion-pair extraction may be required to ensure a high extraction efficiency [7], or alternatively, LLE may be accomplished with very high $V_{organic\ phase}/V_{sample}$ ratios.

In addition to high extraction efficiencies ($E_E$) as discussed above, successful LLE relies on a preconcentration step to obtain high analyte concentrations in the final solutions for CZE (high analyte enrichment). Normally, the extract (organic phase) is evaporated to dryness and the residue is reconstituted in a small volume to ensure a high analyte enrichment. For practical reasons, the volume of reconstitution is normally in the range 50 to 500 μl. Thus, when the amount of sample is limited to, e.g., 1 ml, the maximum theoretical analyte enrichment factor is in the range 2–20 (with 100% extraction efficiency). This limitation is frequently the case with biological samples (especially for plasma), whereas substantially higher analyte enrichments (>20) may be obtained in cases where large sample volumes are available.

In addition to analyte enrichment, LLE provides sample clean-up. Inorganic salts are normally insoluble in the organic solvents used for LLE, and consequently they principally remain in the aqueous sample phase. For plasma samples, proteins may be of concern because they easily adsorb to the surface of the CZE separation capillary; however, also proteins are almost insoluble in the organic solvents used for LLE. Although LLE provides excellent sample clean-up for salts and biological macromolecules, a broad range of other compounds may be co-extracted during LLE. As illustrated in Table 1, even matrix components with relatively low partition coefficients may be extracted during normal LLE conditions with $V_{organic\ phase} \geq V_{sample}$.

## 3. Injection solution considerations

The organic solvents used for LLE, which are immiscible with water, are not injectable in CZE.

Table 1
Extraction efficiencies ($E_E$) as a function of the sample/extraction solvent volume ratio ($V_{sample}/V_{organic\ phase}$) and the partition coefficient ($K_i$)

| Volume ratio ($V_{sample}/V_{organic\ phase}$) | Extraction efficiency ($E_E$) (%) | | | | |
|---|---|---|---|---|---|
| | $K_i = 1$ | $K_i = 10$ | $K_i = 100$ | $K_i = 1000$ | $K_i = 10\,000$ |
| 100 | 0.99 | 9.1 | 50 | 91 | 99 |
| 10 | 9.1 | 50 | 91 | 99 | 99.9 |
| 1 | 50 | 91 | 99 | 99.9 | 99.99 |
| 0.1 | 91 | 99 | 99.9 | 99.99 | 99.999 |
| 0.01 | 99 | 99.9 | 99.99 | 99.999 | 99.9999 |

Thus, LLE for CZE has to include evaporation of the organic solvent. Reconstitution should be accomplished in a liquid phase miscible with the aqueous CZE separation buffer and providing stable current conditions during CZE. In order to obtain high analyte enrichment, reconstitution should preferably be accomplished in small volumes in the range of 50 to 500 μl. Ideally, reconstitution should be performed in pure water. This solution is easily mixed with aqueous separation buffers during CZE and provides an excellent medium for field-amplified sample stacking. However, many analytes actual for LLE and CZE exhibits low solubility in pure water, and reconstitution is normally carried out either in aqueous buffers or in one-phase mixtures of water and an organic solvent. In cases utilizing the former type of reconstitution, attention should be focused on the ionic strength of the buffer since high conductivity in the injection media may cause serious peak deterioration. For reconstitution in mixtures of water and polar organic solvents, acetonitrile is the most popular owing to attractive solubility and electrical conductance characteristics. Typically, 1:1 mixtures of water and acetonitrile are used, but even higher contents of acetonitrile may be used for reconstitution of hydrophobic analytes. Unfortunately, injection of pure solvents in aqueous CZE may be difficult owing to fluctuations of the separation current during the CZE. A solution to this problem may be to use non-aqueous CZE (NACE), where the samples may be reconstituted in the same organic solvents as used for preparation of the separation medium. This may also be an interesting option from a separational point of view since NACE has been shown to provide major selectivity differences as compared with standard aqueous CZE [8].

From a practical point of view, the evaporation step of LLE is cumbersome, and loss of analyte may occur following partial evaporation or adsorption to the equipment. This naturally limits the attractiveness of LLE for CZE. However, on the other side, LLE provides excellent clean-up for several types of matrix components as discussed above. Desalting of the sample automatically occurs during LLE because most salts are insoluble in the organic solvents used. Thus, LLE extracts normally provides an excellent medium for field-amplified sample stacking.

## 4. Applications of liquid–liquid extraction–capillary zone electrophoresis

### *4.1. Drugs from biological fluids*

The characteristics of CZE makes it a useful technique for the determination of drugs in biological samples; most drugs are ionic and well suited for CZE, separation efficiencies are high, and separation conditions can easily be adapted to optimize the resolution. The main problem is that analyte detectability expressed in concentration units is rather poor due to the low volume loadability of the CE capillary. High-performance liquid chromatography (HPLC), which tolerates sample volumes up to several hundreds of microliters, is therefore the workhorse in the bioanalysis of drugs. Because of the poor concentration sensitivity, the number of applications by CZE are still low, although the number of applications are increasing.

Plasma, serum and urine are the most common biological matrices. The high concentration of inorganic salts in urine (50–500 m*M* sodium chloride) and serum (ca. 150 m*M* sodium chloride) provides an elevated electrical conductance; basically, both sample types provides non-optimal conditions for direct field-enhanced sample stacking. In addition, the high protein content present in serum and plasma (ca. 7.5 g/l) may cause severe binding to the free silanols on the capillary wall resulting in changed migration times or the presence of interfering peaks. As the concentration of target drugs in these matrices often may be in the ng/ml range, the major challenges for a sample preparation method are removal of matrix constituents which may interfere in the CZE separation and enrichment of the analyte to a concentration which can be detected by the employed CE detector. The advantages of LLE as sample preparation method are the easy removal of inorganic salts as these are not soluble in organic solvents. In addition, LLE is an efficient sample preparation technique for the removal of plasma proteins. Enrichment is achieved after solvent evaporation and reconstitution into a smaller volume of solvent, which is suitable for injection into the CE capillary (discussed above). Due to the small sample volumes available in bioanalysis (normally 0.5–1 ml of

plasma or serum), the enrichments achieved are normally not higher than a factor of 10.

Several reviews including sample preparation techniques have been devoted to the CE analysis of drugs in biological fluids [6,9,10]. Reviews with particular focus on sample handling techniques have also been published [11,12] in addition to reviews covering selected application fields such as clinical chemistry [13,14], pharmacokinetics [15], forensic science [16] and metabolic studies [17].

An overview of published methods based on LLE as sample preparation method prior to CZE is shown in Table . Most of the applications are based on extraction of the target drugs into an organic solvent followed by solvent evaporation, reconstitution of the residue, and injection into the CZE system. The solvents typically used for LLE include *n*-hexane, cyclohexane, *n*-heptane, ethyl acetate, chlorobutane, dichloromethane and chloroform, which all are immiscible with water and of relatively low volatility. Reconstitution typically has been achieved in either pure water, dilute HCl, dilute phosphate or borate buffers, acetonitrile–water, and methanol. A selection of these applications will be discussed in detail below.

The thyreostatic drugs methylthiouracil (MTU), propylthiouracil (PTU) and thiouracil (TU) have been determined in urine by LLE and CZE [18]. The drugs were extracted from 2 ml of urine into 6 ml of ethyl acetate, the ethyl acetate phase was evaporated, and the residue was reconstituted in 150 μl of pure water. Owing to the non-conducting nature of the final extract, sample volumes of 177 nl were injected into the CZE system with highly efficient analyte stacking. The method was used for the analysis of several thousands of samples as part of a veterinary control procedure. With a 13-times enrichment, the detection limits were at the 0.3–0.5 ppm level utilizing UV detection at 276 nm. A similar method has been published for the determination of the antiviral drug (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU) and its metabolite in human plasma and urine [19] (Fig. 1). The compounds were extracted from 60-μl volumes of plasma and urine (pH adjusted to 6) into 1 ml of ethyl acetate. The ethyl acetate extracts were evaporated and reconstituted in 10 μl of pure water. The plasma method was used for measurements of both the total concentration and the concentration of the unbound drug and metabolite. The limit of quantification was 40 ng/ml for total and 10 ng/ml for free BVdU in plasma, and 170 ng/ml for BVdU in urine.

Dichloromethane extraction was used for the therapeutic drug monitoring of albendazole (ABZ) and its metabolites albendazole sulfoxide and albendazole sulfone [20]. The pH of the plasma samples (0.5 ml) was adjusted to 10.3 with carbonate buffer, and extractions were performed with 5 ml of dichloromethane. The organic phase was evaporated to dryness and reconstituted in 200 μl of *N*-methylformamide (NMF). The recovery was between 63 and 98%. NMF was an excellent solvent for reconstitution in this case as the analytes were separated by NACE using 0.036 *M* borate buffer (pH 9.9) in a mixture of methanol–NMF (1:3). Detection was UV at 280 nm. Using 0.5 ml of plasma and extract reconstitution in 200 μl of NMF, drug levels between 1.0 and 10 μ*M* were found to provide linear calibration graphs with a $8 \cdot 10^{-7}$ *M* detection limit for the principal drug.

LLE combined with back extraction into an acidic (for alkaline drugs) or basic (for acidic drugs) aqueous solution is a common sample preparation technique prior to HPLC. With back-extraction, the cumbersome procedures of solvent evaporation and reconstitution are eliminated. When using this approach prior to CZE, attention must be focused on the conductivity of the final aqueous extract as a high conductivity may reduce the efficiency of CZE through anti-stacking effects. Dextromethorphane and its metabolites were successfully extracted from plasma (1.5 ml) with 6 ml of heptane–ethyl acetate (50:50, v/v), and subsequently back-extracted to a 100 μl aqueous phase of 25 m*M* phosphate, pH 2.6 [21]. CZE was accomplished with UV detection at 195 nm using a 1.2 mm detection cell path length. The limit of detection was 0.5–1 ng/ml for both the main drug and for the metabolites. The method was used for the determination of plasma levels of dextromethorphan and its metabolites after transdermal and oral administration of dextromethorphan (Fig. 2).

One major advantage of CZE as separation method is the high chiral selectivity following addition of

Table 2
LLE–CZE applications of drugs from biological fluids

| Analytes | Sample matrix | Extraction solvent | Reconstitution solvent | Enrichment | Detection mode | Limit of quantification | Ref. |
|---|---|---|---|---|---|---|---|
| More than 400 basic drugs | Whole blood (1 ml) | 1-Chlorobutane, alkaline pH (5 ml) | 10 m*M* Phosphate buffer, pH 2.5 (30 μl) | 33 | UV | Not reported | [44] |
| 7-Hydroxycoumarin | Serum, urine (1 ml) | Diethyl ether (3.5 ml; 1.8 ml evaporated to dryness) | 25 m*M* Phosphate buffer, pH 7.5 (100 μl) | 5 | UV | 1 μg/ml | [45] |
| (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVdU)<br>(*E*)-5-(2-Bromovinyl)-uracil | Plasma, urine (60 μl) | Ethyl acetate, pH 6 (1 ml) | Water (10 μl) | 6 | UV | 40–170 ng/ml | [19] |
| Albendazole<br>Albendazole sulfoxide<br>Albendazole sulfone | Plasma (500 μl) | Dichloromethane, pH 10.3 (5 ml) | *N*-Methylformamide (200 μl) | 2.5 | UV | LOD: $8 \cdot 10^{-7}$ *M* | [20] |
| Amiodarone<br>Desethylamiodarone | Serum (20 μl) | Hexane, pH 6 (50 μl; 10 μl evaporated to dryness) | 1-Propanol–water (80:20, v/v), and 100 μmol/l $H_3PO_4$ (200 μl) | 0.02 | UV | LOD: 80 nmol/l[a] | [46] |
| Amphetamine<br>Methamphetamine<br>Ecstasy and derivates (MDA, MDMA, MDEA, MBDB) | Urine (1 ml) | 1-Chlorobutane, pH 9.2 (2 ml) | Water (50 μl) | 20 | ESI-MS | Not reported | [29] |
| D-(+)-Amphetamine<br>Methamphetamine<br>Ecstasy and derivates (MDMA, MDA)<br>Ephedrines | Urine (2 ml) | $CH_2Cl_2$ and $C_2H_4Cl_2$, pH 9 (Toxi-tubes A system) | Water (100 μl) | 20 | UV | 80–200 ng/ml | [47] |
| Amphetamine,<br>Methamphetamine<br>1-Phenylethylamine<br>2-Phenylethylamine<br>4-Hydroxyamphetamine<br>4-Hydroxymethamphetamine | Urine (5 ml) | Chloroform–2-propanol (3:1, v/v), pH 10.5 (2×2 ml) | Water (200 μl) | 25 | UV | Not reported | [48] |
| Amphetamine[b]<br>Methamphetamine[b]<br>Ecstasy and derivates[b] (MDMA, MDA, MDE)<br>Methadone[b]<br>2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolodine[b] (EDDP) | Urine (2 ml) | $CH_2Cl_2$ and $C_2H_4Cl_2$, pH 9 (Toxi-tubes A system) | Water (100 μl) | 20 | LIF | 100–200 ng/ml | [22] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Bipuvacaine | Drain fluid[c] (1 ml) | Hexane, alkaline pH (6 ml) | Mixture of separation buffer and 0.1 *M* HCl (1:1, v/v) (100 μl) | 10 | UV | Not reported | [49] |
| Carnitine<br>Acylcarnitines | Plasma, urine (500 μl) | Plasma: ethyl acetate (500 μl)<br>Urine: ethyl acetate–acetonitrile (9:1, v/v) (500 μl) | Plasma: 3% formic acid in methanol (100 μl)<br>Urine: methanol–formic acid (98.5:1.5, v/v) (200 μl) | Plasma: 5<br>Urine: 2.5 | MS | 40–400 ng/ml | [30] |
| Danorubicin<br>Doxorubicin<br>Epirubicin | Plasma (1 ml) | Chloroform (2 ml; 1.6 ml back-extracted) | 5 m*M* Phosphoric acid, pH 2.3 (100 μl) (back-extraction) | 8 | LIF | 175–250 pg/ml | [50] |
| Dextromethorphan<br>Dextrorphan<br>3-Hydroxymorphinan<br>3-Methoxymorphinan | Plasma (1.5 ml) | Heptane–ethyl acetate (50:50, v/v) (6 ml) | 25 m*M* Sodium phosphate, pH 2.6 (100 μl) (back-extraction) | 15 | UV | 1.2 ng/ml | [21] |
| Dihydrocodeine<br>Nordihydrocodeine | Plasma, urine (100 μl) | Ethyl acetate, pH 10.3 (150 μl) | Ethylene glycol (55%, v/v) containing 100 μ*M* $H_3PO_4$ (100 μl) | 1 | UV | LOD: 4–5 ng/ml[a] | [26] |
| Dihydrocodeine<br>Nordihydrocodeine | Plasma (20 μl) | Ethyl acetate, pH 11.8 (50 μl; 20 μl evaporated to dryness) | Ethylene glycol (55%, v/v) containing 100 μ*M* $H_3PO_4$ (100 μl) | 0.08 | UV | LOD: 0.3 ng/ml[a] | [51] |
| Diltiazem<br>Desacetyldiltiazem | Plasma (1 ml) | *tert.*-Butyl methyl ether (5 ml; 4 ml back-extracted) | 17 m*M* Phosphoric acid (40 μl) (back-extraction) | 20 | UV | LOD: 2 ng/ml | [52] |
| Dimethindene[b]<br>*N*-Demethyldimethindene[b] | Urine (10 ml) | *n*-Hexane (pH 9–10) (2×4 ml) | 5 m*M* Phosphate buffer, pH 3.3 (50 μl) | 200 | UV | LOD: 1–2 ng/ml | [53] |
| Doxorubicin<br>Doxorubicinol | Plasma (100 μl) | Chloroform, pH 7.4 (1 ml; 800 μl evaporated to dryness) | Acetonitrile–water (90:10, v/v) (50 μl) | 1.6 | LIF | 2 μg/ml | [28] |
| Ecstasy and derivates[b] (MDMA, MDA, MDE)<br>Ephedrine[b]<br>Amphetamine[b]<br>Methamphetamine[b] | Urine (2 ml) | Toxi-tube A system | 0.01 m*M* Phosphoric acid (100 μl) | 20 | UV | LOD: 0.1–0.2 ng/ml | [54] |
| Flurazepam<br>*N*-1-Hydroxyethylflurazepam | Urine (5 ml) | 1-Chlorobutane, pH 9.5 (3 ml) | Separation buffer (100 μl) | 50 | UV-API-MS | Not reported | [55] |
| Haloperidol<br>Reduced haloperidol[b] | Plasma (5 ml) | Heptane–isoamyl alcohol (98:2, v/v), alkaline pH (volume not reported) | 0.1 *M* HCl (100 μl) | 50 | UV | LOD: 15–30 ng/ml | [24] |
| Homatropine<br>Scopolamine<br>Neostigmine | Plasma, urine (250 μl) | Dichloromethane, alkaline pH (600 μl; 500 μl evaporated to dryness) | Terminating buffer for ITP–CZE (500 μl) | 0.4 | ITP–CZE | LOD: 100 ng/ml | [56] |
| Idarubicin | Plasma | Chloroform, pH 7.4 (1 ml; | Acetonitrile–water (95:5, v/v) (50 μl) | 1.6 | LIF | 0.5 ng/ml | [27] |

Table 2. Continued

| Analytes | Sample matrix | Extraction solvent | Reconstitution solvent | Enrichment | Detection mode | Limit of quantification | Ref. |
|---|---|---|---|---|---|---|---|
| Idarubicinol | (100 μl) | 0.8 ml evaporated to dryness) | | | | | |
| Indinavir<br>Nefinavir<br>Saquinavir<br>Ritonavir | Serum<br>(200 μl) | Ethyl acetate–hexane (9:1, v/v), pH 9.2 (1.2 ml; 1.1 ml evaporated to dryness) | Acetonitrile–water (60:40, v/v) (50 μl) | 3.7 | UV | 62.5 ng/ml | [57] |
| LSD<br>*N*-Demethyl-LSD<br>Iso-*N*-demethyl-LSD | Whole blood<br>(2 ml) | Dichloromethane, pH 9–10<br>(4 ml) | Methanol (50 μl) | 40 | LIF | 0.4–0.5 ng/ml | [58] |
| LSD | Whole blood<br>(2 ml) | Dichloromethane, pH 9–10<br>(4 ml) | Methanol (50 μl) | 40 | LIF | LOD: 0.1–0.2 ng/ml | [59] |
| Metformin | Plasma (100 μl) | Chloroform[d], pH 7.8<br>(2×1 ml) | 200 μ*M* $H_3PO_4$<br>(100 μl) | 1 | UV | 0.25 μg/ml[a] | [7] |
| Methadone[b]<br>2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolodine[b] (EDDP) | Serum, urine<br>(1 ml) | *n*-Hexane, pH 9–10 (3 ml) | Methanol<br>(serum: 200 μl;<br>urine: 1 ml) | Serum: 5<br>Urine: 1 | UV | LOD:<br>serum: 2 ng/ml<br>urine: 10 ng/ml | [60] |
| Methylphenidate | Urine<br>(4 ml) | Cyclohexane, pH 9.3 (4 ml) | Water (200 μl) | 20 | MS | 1.5 ng/ml | [61] |
| Methylthiouracil<br>Propylthiouracil<br>Thiouracil | Urine<br>(2 ml) | Ethyl acetate (6 ml) | Water (150 μl) | 13 | UV | LOD: 0.3–0.5 ppm | [18] |
| Mianserin[b]<br>Desmethylmianserin[b]<br>8-Hydroxymianserin[b] | Plasma<br>(1 ml) | (1) *n*-Heptane–ethyl acetate (80:20, v/v), pH 9.4 (6 ml)<br>(2) 0.1 *M* HCl (1.2 ml)<br>(3) Toluene–isoamyl alcohol (85:15, v/v), pH 9.4 (150 μl)[f] | Back-extraction into 0.0001% diethylamine, evaporation until approx. 50 μl left | 20 | UV | 5–15 ng/ml[f] | [25][e] |
| Naproxen | Serum (UV: 1 ml LIF: 0.25 ml) | Hexane–diethyl ether, pH 4 (UV: 3 ml, LIF: 1 ml; 2 ml and 0.6 ml evaporated to dryness, respectively) | UV: water–ethanol (1:1, v/v) (200 μl)<br>LIF: methanol (20 μl) | UV: 3.3<br>LIF: 7.5 | UV<br>LIF | UV: 0.5 μg/ml<br>LIF: 10 ng/ml | [62] |
| Oxprenolol[b] and four metabolites[b] | Urine<br>(5 ml) | Ethyl acetate (pH 10–11)<br>(8 ml) | 5 m*M* Phosphate buffer (200 μl) | 25 | UV | LOD: 0.2 μg/ml | [63] |
| Tamoxifen<br>*N*-Desmethyltamoxifen<br>4-Hydroxytamoxifen | Serum<br>(1 ml) | Hexane–isoamyl alcohol (98:2, v/v) (3×5 ml) | Methanol–acetonitrile (1:1, v/v) containing 10 m*M* ammonium acetate and 1% acetic acid (25–50 μl) | 20–40 | UV | 10 pg total injected | [64] |
| Thiopental[b]<br>Pentobarbital[b] | Plasma<br>(300 μl) | Dichloromethane, acidic pH (1 ml) | 10-Fold diluted separation buffer without chiral selector (50 μl) | 6 | UV | Not reported | [65] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Tramadol[b]<br>*N*-Demethyltramadol[b]<br>*O*-Demethyltramadol[b]<br>*N,O*-Dimethyltramadol[b]<br>*N,N,O*-Tridemethyltramadol[b] | Urine<br>(2 ml) | *n*-Hexane–ethyl acetate (80:20, v/v), alkaline pH<br>(2×4 ml) | 25 m*M* Borate buffer,<br>pH 10.1 (50 μl) | 40 | UV | 0.2 μg/ml | [23] |
| Tramadol[b]<br>*O*-Demethyltramadol[b]<br>*N*-Demethyltramadol[b]<br>*O*-Demethyl-*N*-demethyltramadol[b]<br>*N*-Bisdemethyltramadol[b]<br>*O*-Demethyl-*N*-bisdemethyltramadol[b] | Urine<br>(200 μl) | (1) Ethyl acetate–hexane (20:80, v/v), alkaline pH<br>(2) *tert*.-Butyl methyl ether, alkaline pH (1 ml)[g] | 0.01 *M* HCl (50 μl) | 4 | UV | 0.3 μg/ml | [66] |
| Tramodol[b]<br>*O*-Demethyltramadol[b]<br>*N*-Demethyltramadol[b]<br>*N*-Bis-demethyltramadol[b]<br>*O*-Demethyl-*N*-demethyltramadol[b]<br>*O*-Demethyl-*N*-bis-demethyltramadol[b] | Plasma<br>(1 ml) | *n*-Hexane–ethyl acetate (80:20, v/v) (5 ml) | 0.01 *M* HCl (100 μl) | 10 | ESI-MS | Not reported | [31] |
| Verapamil[b]<br>Norverapamil[b] | Plasma<br>(1 ml) | Hexane–isopropanol (90:10, v/v), alkaline pH<br>(5 ml) | Methanol–water (25:75, v/v) (100 μl) | 10 | UV | 2.5 ng/ml | [67] |
| Warfarin[b] | Plasma<br>(1 ml) | Dichloromethane, acidic pH<br>(5 ml) | Separation buffer<br>(50 μl) | 20 | UV | LOD: 0.2 mg/ml | [68] |
| Zopiclone[b]<br>Zopiclone *N*-oxide[b]<br>*N*-Desmethylzopiclone[b] | Urine,<br>saliva<br>(1 ml) | Chloroform–isopropanol<br>(9:1, v/v), pH 8 (5 ml) | Acetonitrile–water (1:1, v/v) (50 μl) | 20 | UV–LIF | LOD: 6 ng/ml (urine) | [69] |

[a] Sensitivity enhanced with field-amplified stacking.
[b] Enantiomeric separation.
[c] Diluted 10-fold.
[d] Ion-pair extraction with bromothymol blue after protein removal.
[e] Back-extraction of the first organic phase into the acidic aqueous phase. Re-extraction of the acidic phase with the second organic phase.
[f] Sensitivity enhanced with on-column preconcentration.
[g] Urine extracted twice. Organic layers combined and evaporated to dryness.

LOD: Limit of detection; MDA = 3,4-methylenedioxyamphetamine; MDMA = 3,4-methylenedioxymethamphetamine; MDEA = MDE = 3,4-methylenedioxyethylamphetamine; MBDB = *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine; LSD = lysergic acid diethylamide.

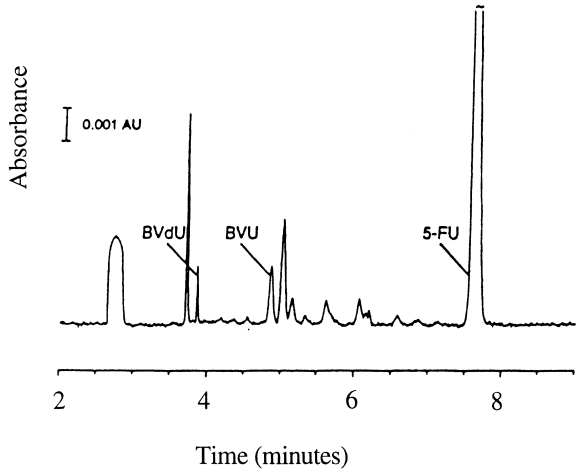


Fig. 1. LLE and CZE of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU) and the metabolite (*E*)-5-(2-bromovinyl)-uracil (BVU) in human plasma. The concentration of BVdU was 40 ng/ml and the concentration of BVU was 80 ng/ml. A 4-μg/ml amount of 5-fluorouracil (5-FU) was added as internal standard. From Ref. [19] with permission.

a chiral selector to the background electrolyte. LLE was used as sample preparation procedure for the stereoselective screening and confirmation of amphetamines, designer drugs, methadone and selected metabolites [22]. Urine samples (2 ml) were extracted at pH 9 using Toxi-Tubes A. This commercial LLE system comprises an organic solvent mixture composed of $CH_2Cl_2$ and $C_2H_4Cl_2$. After shaking and centrifugation, the organic phase was transferred to a clean tube, added two drops of 2 *M* acetic acid in ethyl acetate in order to create less volatile salts before the organic solvent was evaporated. The residue was reconstituted in 100 μl of water and analyzed by CZE with (2-hydroxypropyl)-β-cyclodextrin as the chiral selector. The chiral determination of tramadol and its metabolites have been carried out in urine by LLE and CZE using carboxymethylated β-cyclodextrin (CMB) as the chiral selector [23]. Urine samples (2 ml) were extracted with two 4-ml portions of ethyl acetate–*n*-hexane under alkaline conditions, and the residues were reconstituted in 50 μl of 25 m*M* borate buffer. With the validated assay, quantification was accomplished down to the 0.2 μg/ml level. Trace analysis of haloperidol and its chiral metabolite in plasma was performed by LLE and CZE utilizing dimethyl-

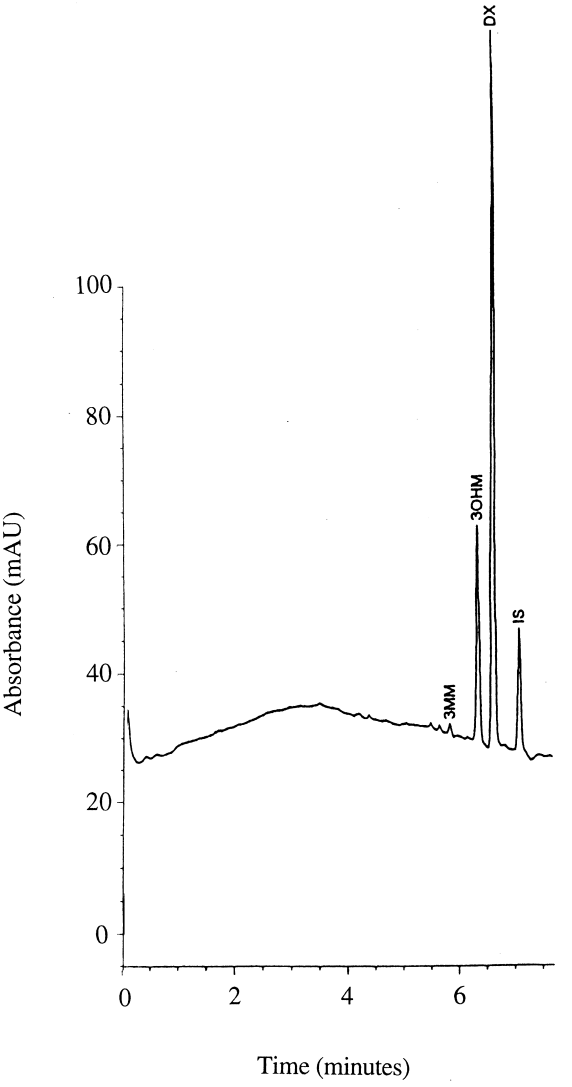


Fig. 2. LLE and CZE of dextromethorphan metabolites in human plasma from a patient 10 h after administration of the first tablet. 3MM: 3-Methoxymorphinan, 3OHM: 3-hydroxymorphinan, DX: dextrorphan, and I.S.: internal standard (levallorphan). From Ref. [21] with permission.

β-cyclodextrin as the chiral selector [24]. Plasma samples (5 ml) were extracted with heptane–isoamyl alcohol (98:2, v/v), and the dried residues were dissolved in 100 μl of 0.1 *M* HCl. The detection limits were 15 ng/ml for haloperidol and 30 ng/ml for both enantiomers. The enantiomers of mianserin and its desmethyl metabolites in plasma were determined after LLE and CZE [25]. Plasma samples

(1 ml) were extracted at pH 9.4 with 6 ml of *n*-heptane–ethyl acetate (80:20, v/v). This organic phase was subsequently back-extracted with 1.2 ml of 0.1 *M* HCl, and in a third step, the HCl phase was extracted with 150 μl of toluene–isoamyl alcohol (85:15, v/v) at pH 9.4. The organic phase was mixed with 100 μl water containing diethylamine and the organic phase was evaporated together with approximately 50 μl of the aqueous phase, while the remaining aqueous extract was utilized for injection into the CE instrument. The compounds were quantified down to the 5 ng/ml level with a validated method providing excellent selectivity (Fig. 3).

The application of stacking techniques after LLE of target drugs from biological fluids is an elegant approach to increase the loadability on the CZE capillary. Opioides were determined in μl amounts of body fluids by using this technique [26]. Using ethyl acetate extraction at pH 10.3, dihydrocodeine and nordihydrocodeine were reproducibly recovered from 20 to 100 μl of plasma, serum and urine. Application of mixed-mode polymer solid-phase resins for sample preparation was shown to provide extracts that were either too salty or contained substantial endogenous substances interfering with the opioides. The LLE sample extracts were reconstituted in 55% (v/v) ethylene glycol containing 100 μ*M* $H_3PO_4$. In the described approach, efficient concentration of cationic opiates from low conductivity extracts of body fluids was affected across a water plug, with separation taking place in a binary buffer comprising 60% (v/v) ethylene glycol, 75 m*M* $Na_2HPO_4$, and 25 m*M* $NaH_2PO_4$ (pH 7.9). UV absorption detection at 210 nm lead to the determination of ppb concentrations of these compounds with an approach that only required μl amounts of sample and organic solvents. Field-amplified sample stacking was also employed after LLE of metformin from plasma [7]. Metformin, which has low UV absorbance, was extracted from 100 μl plasma samples as an ion-pair with bromothymol blue into two 1-ml portions of chloroform. After evaporation, the extracts were reconstituted in 100 μl of 200 μ*M* $H_3PO_4$. The running buffer was 0.1 *M* phosphate buffer (pH 2.5), and the limit of quantification was 0.25 μg/ml utilizing field-amplified stacking and UV detection at 195 nm.

LIF detection is the most sensitive detection method in CE, and LLE combined with CZE and LIF detection has been successfully applied for the determination of drugs in biological fluids. Idarubicin and idarubicinol were determined in plasma after LLE with chloroform [27]. A 100-μl volume of plasma was extracted with 1 ml chloroform at pH 7.4, and subsequently the extracts were reconstituted in 50 μl of acetonitrile–water (95:5, v/v). The sample volume of 100 μl was considered a particular advantage for studies in pediatric oncology. LIF detection was carried out with an Ar laser operated at 488 nm, which provided a sensitive and selective detection method without interferences from biological fluids. The limit of quantification for idarubicin was 0.5 ng/ml. LLE–CZE–LIF was also employed for therapeutic drug monitoring of

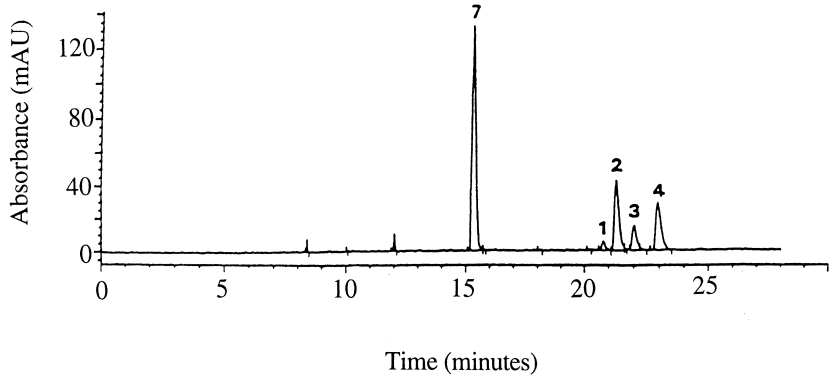


Fig. 3. LLE and CZE of mianserin and metabolites in human plasma from a patient treated with 30 mg of racemic mianserin for 50 days. Peaks: 1=(*S*)-desmethylmianserin, 2=(*S*)-mianserin, 3=(*R*)-desmethylmianserin, 4=(*R*)-mianserin. From Ref. [25] with permission.

doxoribicin in pediatric oncology [28]. Chloroform (1 ml) was used to extract doxorubicine and its main metabolite from 100 μl of plasma. After evaporation of the organic phase, the sample was reconstituted in acetonitrile–water (90:10, v/v) and injected into the CZE system by electrokinetic injection. The limit of quantification was 2 ng/ml with an Ar laser operated at 488 nm.

Electrospray ionization mass spectrometry (ESI-MS) is another specific detection system for CZE. This system has been used to determine ecstasy and other related amphetamines in urine after LLE [29]. A 1-ml volume of urine was spiked with 10 μl of internal standard, mixed with 1 ml of borate buffer (pH 9.2), and extracted with 2 ml of 1-chlorobutane. After centrifugation, the extraction tube was frozen and the organic phase was transferred to another tube, added 100 μl of methanolic HCl and evaporated to dryness. The dry residue was dissolved in 50 μl of water. No interferences from endogenous compounds were observed during analysis of the amphetamines. CZE–MS was also used to determine carnitine and acylcarnitines in biological samples after LLE [30] as well as tramadol and its main phase I metabolites [31]. In the former paper, 500 μl of plasma and urine samples were extracted with 500 μl of ethyl acetate–acetonitrile (9:1, v/v), and after evaporation the residue was reconstituted in 100 μl of 3% formic acid in methanol (for plasma) or 200 μl methanol–formic acid (98.5:1.5, v/v) (for urine). Carnitine and acetylcarnitine were determined in the concentration range of 2.7 to 108 nmol/ml. In the tramadol paper, 1-ml plasma samples were extracted with 5 ml of *n*-hexane–ethyl acetate (80:20, v/v) followed by evaporation and reconstitution of the residue in 100 μl of 0.01 *M* HCl.

### *4.2. Other applications*

CE has a unique capability for the separation of analytes of environmental concern as many of the target compounds are ionic. Several reviews have been devoted to the separation of environmental pollutants [32–35]. Mostly, the determination of analytes of environmental concern is difficult without a preconcentration and/or sample clean-up step. This is because pollutants are most often extremely diluted in the environmental media containing matrix components as well. Although LLE is effective in extracting many pollutants from real samples, the current trend is to used solid-phase extraction. As HPLC is the preferred separation method in drug analysis capillary GC is the preferred separation method in the analysis of environmental samples.

Among the few applications of LLE and CZE found, the determination of phenols, inorganic anions, and carboxylic acids in Kraft black liquors has been reported [36]. Black liquor samples (10 ml) were acidified with HCl to pH 1 and extracted with 15 ml chloroform. After centrifugation, the injection of the chloroform extract was possible without further purification. CZE separation was accomplished with a phosphate–borate electrolyte system containing 25% 2-butanol, 5% ethylene glycol, and 10% acetonitrile, and detection was UV at 214 nm. Haloacetic acids have been determined by CZE and LLE in tap water [37]. Sample volumes of 30 ml were extracted at pH 0.5 by 3 ml of methyl *tert*.-butyl ether, the organic phase was evaporated, and the residue was reconstituted in 100 μl of pure water. Subsequently, the extracts were analyzed by CZE, which enabled the haloacetic acids to be detected down to the low μg/l level.

## 5. New directions for liquid–liquid extraction and capillary zone electrophoresis

Several other approaches closely related to conventional LLE have been reported for sample enrichment prior to CZE. Among these, we have decided to focus on electroextraction combined with isotachophoresis, on extraction with supported liquid membranes, and finally on liquid-phase microextraction (LPME).

Combined electroextraction (EE) and isotachophoresis (ITP) as a fast on-line focusing step in CZE prior to ESI-MS has been described with clenbuterol, salbutamol, terbutaline and fenoterol as model compounds [38]. In EE–ITP–CZE, electroextraction was accomplished directly from ethyl acetate extracts utilizing extended electrokinetic injection (10 min) and with a hydrodynamic counterflow to avoid the introduction of ethyl acetate. After focusing the analytes by ITP, separation was accomplished by CZE. With this concept, clenbuterol, salbutamol, and

terbutaline were detected down to the $2 \cdot 10^{-9}$ mol/l level, whereas the detection limit for fenoterol was $5 \cdot 10^{-9}$ mol/l.

A second interesting approach closely related to LLE is the use of supported liquid membranes (SLMs) for sample clean-up and analyte enrichment prior to CZE [39,40]. The SLM technique involves extraction of analytes from a stream of aqueous sample (donor) into an organic solvent immobilized in a porous membrane, and subsequently back-extraction into a stagnant aqueous phase on the other side of the membrane (acceptor). Bambuterol has been extracted from human plasma samples with the SLM technique and analyzed by CZE. The electropherograms obtained after SLM enrichment were as clean as when aqueous samples containing bambuterol were processed in the same way. The low ionic strength of the SLM treated plasma samples permitted subsequent sample stacking in the CZE step, providing detection of bambuterol down to the 50 nmol/l level.

Recently, LPME has been developed as another alternative to LLE for CZE utilizing disposable and low-cost extraction devices [41–43]. LPME was performed from conventional sample vials (4 ml) containing a small piece of a porous hollow fiber. The analytes were extracted from the sample into an organic solvent (immiscible with water) immobilized in the pores of the hollow fiber, and further into an aqueous acceptor solution inside the hollow fiber. The concept was similar to the SLM technique utilizing the back-extraction principle, but with LPME, the extractions were performed with very simple and disposable equipment. The latter aspect was of high importance since this eliminated the possibility of sample cross contaminations. For CZE, the acceptor phase volume typically was 25 μl and extractions were performed from 1- to 4-ml samples. The high ratio between the sample volume and the acceptor phase volume provided an excellent basis for high analyte enrichments. This ratio in combination with the back-extraction concept ensured very efficient sample clean-up even from complex biological samples. Several basic and acidic drugs were effectively analyzed down to the low ng/ml level in both plasma and urine by utilizing LPME and CZE (Fig. 4).

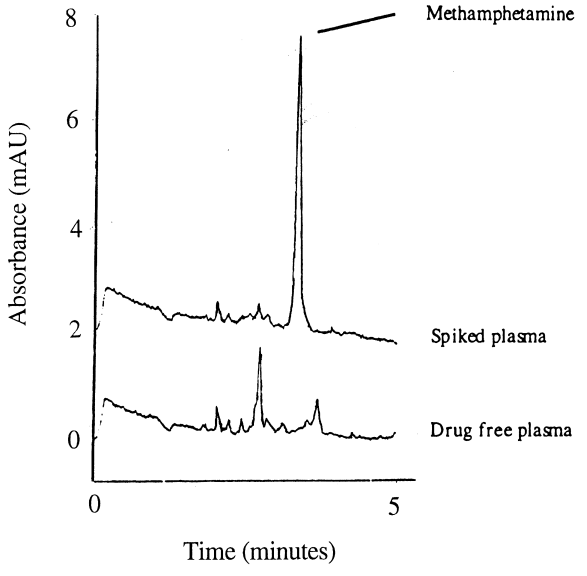


Fig. 4. LPME and CZE of methamphetamine in human plasma. The concentration of methamphetamine was 100 ng/ml. From Ref. [41] with permission.

## 6. Conclusions

The present review has focused on applications of LLE for sample enrichment in CZE. The majority of applications reported so far are related to the determination of drugs i biological samples like plasma and urine. Adjustment of pH is normally of high importance prior to extraction with the organic solvent. In order to maximize extraction recovery and time, relatively large volumes of organic solvent as compared to the sample volume are used. Following the LLE process, the organic solvent normally has to be evaporated for compatibility reasons and in order to ensure maximum analyte enrichment. Subsequently, the residue has to be reconstituted in either pure water, a weak buffer, or in mixtures of water miscible organic solvents and water. The ionic content of the final extract is of high importance in order to promote field-amplified stacking during the initial part of the CZE analysis; the latter aspect is of high importance to reach low detection limits.

In cases where the amount of sample is limited, high analyte enrichments may be difficult to obtain because reconstitution normally has be accomplished

in volumes exceeding 40 to 50 μl. For drug analysis applications, this practical point limits the analyte detectability to the low or medium ng/ml level. For environmental applications however, where higher sample volumes normally are easily available, detection limits may be reduced below the μg/l level by LLE combined with CZE.

In addition to analyte enrichment, LLE provides important sample clean-up. Both inorganic salts and biological macromolecules are effectively removed by LLE. However, organic compounds in a broad polarity range may be co-extracted owing to the relatively non-selective nature of organic solvents, and consequently may interfere in the CZE analysis. In these cases, back-extraction either in the conventional format or by LPME may be an advantage. In addition to LPME, also the SLM and the EE–ITP techniques are interesting alternatives to conventional LLE, which may increase the rate of CZE implementation in the near future.

## References

[1] K.D. Altria, S.M. Bryant, LC–GC 15 (1997) 448.
[2] G. Hempel, Electrophoresis 21 (2000) 691.
[3] P. Gebauer, W. Thormann, P. Bacek, J. Chromatogr. 608 (1992) 17.
[4] R.-L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 489A.
[5] S.H. Chen, Y.H. Chen, Electrophoresis 20 (1999) 3259.
[6] C.M. Boone, J.C.M. Waterval, H. Lingeman, K. Ensing, W.J.M. Underberg, J. Pharm. Biomed. Anal. 20 (1999) 831.
[7] J.Z. Song, H.F. Chen, S.J. Tian, Z.P. Sun, J. Chromatogr. B 708 (1998) 277.
[8] S.H. Hansen, J. Tjornelund, I. Bjornsdottir, Trends Anal. Chem. 15 (1996) 175.
[9] D.K. Lloyd, J. Chromatogr. A 735 (1996) 29.
[10] Z. Deyl, F. Tagliaro, I. Miksik, J. Chromatogr. B 656 (1994) 3.
[11] R.B. Taylor, S. Toasaksiri, R.G. Reid, Electrophoresis 19 (1998) 2791.
[12] J.R. Veraart, H. Lingeman, U.A.T. Brinkman, J. Chromatogr. A 856 (1999) 483.
[13] R. Lehmann, H.M. Liebich, W. Voelter, J. Cap. Electrophoresis 3 (1996) 89.
[14] R. Lehmann, W. Voelter, H.M. Liebich, J. Chromatogr. B 697 (1997) 3.
[15] D. Leveque, C. GallionRenault, H. Monteil, F. Jehl, J. Chromatogr. B 697 (1997) 67.
[16] L.A. Holland, N.P. Chetwyn, M.D. Perkins, S.M. Lunte, Pharm. Res. 14 (1997) 372.
[17] S. Naylor, L.M. Benson, A.J. Tomlinson, J. Chromatogr. A 735 (1996) 415.
[18] G. Vargas, J. Havel, K. Frgalova, J. Cap. Electrophoresis 5 (1998) 9.
[19] J. Olgemoller, G. Hempel, J. Boos, G. Blaschke, J. Chromatogr. B 726 (1999) 261.
[20] A. Prochazkova, M. Chouki, R. Theurillat, W. Thormann, Electrophoresis 21 (2000) 729.
[21] H.T. Kristensen, J. Pharm. Biomed. Anal. 18 (1998) 827.
[22] A. Ramseier, J. Caslavska, W. Thormann, Electrophoresis 20 (1999) 2726.
[23] B. Kurth, G. Blaschke, Electrophoresis 20 (1999) 555.
[24] S.M. Wu, W.K. Ko, H.L. Wu, S.H. Chen, J. Chromatogr. A 846 (1999) 239.
[25] C.B. Eap, K. Powell, P. Baumann, J. Chromatogr. Sci. 35 (1997) 315.
[26] A.B. Wey, C.X. Zhang, W. Thormann, J. Chromatogr. A 853 (1999) 95.
[27] G. Hempel, S. Haberland, P. Schulze-Westhoff, N. Mohling, G. Blaschke, J. Boos, J. Chromatogr. B 698 (1997) 287.
[28] G. Hempel, P. Schulze-Westhoff, S. Flege, N. Laubrock, J. Boos, Electrophoresis 19 (1998) 2939.
[29] E. Varesio, S. Cherkaoui, J.L. Veuthey, J. High Resolut. Chromatogr. 21 (1998) 653.
[30] K. Heinig, J. Henion, J. Chromatogr. B 735 (1999) 171.
[31] S. Rudaz, S. Cherkaoui, P. Dayer, S. Fanali, J.L. Veuthey, J. Chromatogr. A 868 (2000) 295.
[32] G.W. Sovocool, W.C. Brumley, J.R. Donnelly, Electrophoresis 20 (1999) 3297.
[33] E. Dabek-Zlotorzynska, Electrophoresis 18 (1997) 2453.
[34] Z. El-Rassi, Electrophoresis 18 (1997) 2465.
[35] A. Karcher, Z. El-Rassi, Electrophoresis 20 (1999) 3280.
[36] D. Volgger, A. Zemann, G. Bonn, J. High Resolut. Chromatogr. 21 (1998) 3.
[37] D. Martinez, J. Farre, F. Borrull, M. Calull, J. Ruana, A. Colom, J. Chromatogr. A 808 (1998) 229.
[38] E. Vandervlis, M. Mazereeuw, U.R. Tjaden, H. Irth, J. van der Greef, J. Chromatogr. A 712 (1995) 227.
[39] S. Palmarsdottir, B. Lindegard, P. Deininger, L.E. Edholm, L. Mathiasson, J.A. Jonsson, J. Cap. Electrophoresis 2 (1995) 185.
[40] S. Palmarsdottir, L. Mathiasson, J.A. Jonsson, L.E. Edholm, J. Chromatogr. B 688 (1997) 127.
[41] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[42] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579.
[43] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Grønhaug, J. Chromatogr. A 873 (2000) 3.
[44] J.C. Hudson, M. Golin, M. Malcolm, Can. Soc. Forensic Sci. J. 28 (1995) 137.
[45] D.P. Bogan, B. Deasy, R. O'Kennedy, M.R. Smyth, U. Fuhr, J. Chromatogr. B 663 (1995) 371.
[46] C.X. Zhang, Y. Aebi, W. Thormann, Clin. Chem. 42 (1996) 1805.
[47] A. Ramseier, J. Caslavska, W. Thormann, Electrophoresis 19 (1998) 2956.

[48] N. Kuroda, R. Nomura, O. Al-Dirbashi, S. Akiyama, K. Nakashima, J. Chromatogr. A 798 (1998) 325.
[49] H. Wolfisberg, A. Schmutz, R. Stotzer, W. Thormann, J. Chromatogr. A 652 (1993) 407.
[50] N.J. Reinhoud, U.R. Tjaden, H. Irth, J. van der Greef, J. Chromatogr. 574 (1992) 327.
[51] C.X. Zhang, W. Thormann, Anal. Chem. 70 (1998) 540.
[52] C. Coors, H.G. Schulz, F. Stache, J. Chromatogr. A 717 (1995) 235.
[53] M. Heuermann, G. Blaschke, J. Pharm. Biomed. Anal. 12 (1994) 753.
[54] F. Tagliaro, G. Manetto, S. Bellini, D. Scarcella, F.P. Smith, M. Marigo, Electrophoresis 19 (1998) 42.
[55] I.M. Johansson, R. Pavelka, J.D. Henion, J. Chromatogr. 559 (1991) 515.
[56] N.J. Reinhoud, U.R. Tjaden, J. van der Greef, J. Chromatogr. 653 (1993) 303.
[57] N. Chelyapov, S.A. Jacobs, T.J. Magee, J. Chromatogr. A 853 (1999) 431.
[58] M. Frost, H. Kohler, G. Blaschke, J. Chromatogr. B 693 (1997) 313.
[59] M. Frost, H. Kohler, Forensic Sci. Int. 92 (1998) 213.
[60] M. Frost, H. Kohler, G. Blaschke, Electrophoresis 18 (1997) 1026.
[61] G.A. Bach, J. Henion, J. Chromatogr. B 707 (1998) 275.
[62] H. Soini, M.V. Novotny, M.-J. Riekkola, J. Microcol. Sep. 4 (1992) 313.
[63] F. Li, S.F. Cooper, S.R. Mikkelsen, J. Chromatogr. B 674 (1995) 277.
[64] J.M. Sanders, L.T. Burka, M.D. Shelby, R.R. Newbold, M.L. Cunningham, J. Chromatogr. B 695 (1997) 181.
[65] S. Zaugg, J. Caslavska, R. Theurillat, W. Thormann, J. Chromatogr. A 838 (1999) 237.
[66] S. Rudaz, J.L. Veuthey, C. Desiderio, S. Fanali, J. Chromatogr. A 846 (1999) 227.
[67] J.M. Dethy, S. De Broux, M. Lesne, J. Longstreth, P. Gilbert, J. Chromatogr. B 654 (1994) 121.
[68] P. Gareil, J.P. Gramond, F. Guyon, J. Chromatogr. 615 (1993) 317.
[69] G. Hempel, G. Blaschke, J. Chromatogr. B 675 (1996) 139.