Carmen García-Ruiz
María Luisa Marina

Departamento de Química Analítica, Facultad de Química, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain

Received June 8, 2005
Revised September 1, 2005
Accepted September 1, 2005

Review

Sensitive chiral analysis by capillary electrophoresis

In this review, an updated view of the different strategies used up to now to enhance the sensitivity of detection in chiral analysis by CE will be provided to the readers. With this aim, it will include a brief description of the fundamentals and most of the recent applications performed in sensitive chiral analysis by CE using offline and online sample treatment techniques (SPE, liquid–liquid extraction, microdialysis, *etc.*), on-column preconcentration techniques based on electrophoretic principles (ITP, stacking, and sweeping), and alternative detection systems (spectroscopic, spectrometric, and electrochemical) to the widely used UV-Vis absorption detection.

Keywords: Detection sensitivity / Isotachophoresis / Offline and online sample treatment techniques / Review / Sensitive chiral analysis / Stacking
DOI 10.1002/elps.200500418

1 Introduction

For some applications in the field of chiral analysis by CE it is necessary to achieve a high sensitivity of detection. As examples, it can be mentioned the determination of the enantiomeric purity of a drug, the determination of chiral compounds in biological samples, the analysis of environmental samples containing low levels of chiral pesticides or pollutants, and the analysis of food samples. Thus, the analysis of the stereochemical purity of compounds is of critical importance in chiral drug synthesis and development as well as for quality control of drug substances. In analytical chemistry the enantiomeric impurity (ei) is usually quantified as the percentage of one enantiomer (*i.e.*, the *S* form) in the mixture (*R* and *S* forms), Eq. (1).

$$\text{ei} = [S/(R + S)] \times 100 \quad (1)$$

Since nowadays, a high percentage of chiral drugs is commercialized as pure enantiomers, the determination of their purity is essential. The ICH guidelines on impurities (Topics Q3A and B) (International Conference on Harmonisation, http://www.ich.org; checked on March 2005) can be applied to study the enantiomeric impurities as impurities for achiral drugs. They define certain thresholds for the content of impurities above which they should be identified and/or quantified. These thresholds have recently been revised (February 2002 and 2003) establishing that for drug substances where the maximum daily dose (MDD) is 2 g/day or below, impurities must be reported if they are present above 0.05%, identified if above 0.10%, and qualified if above 0.15%. Obviously, to accomplish these requirements the sensitivity of detection has to be taken into consideration [1–3]. On the other hand, the determination of chiral compounds in biological samples (plasma, urine, cerebrospinal fluid, tissues, cells, *etc.*) is one of the most interesting applications of chiral CE. The analysis of these small mass/volume samples requires appropriate selectivity (usually a sample treatment is used to avoid matrix interferences) and sensitivity [4]. In addition, chiral analysis of environmental samples by CE is a clear challenge nowadays. The main problem is the high sensitivity required for the detection of herbicides, fungicides, and organic persistent pollutants in environmental samples such as water, soil, and slug [5]. In fact, the levels of pollutants in environmental samples are below ppbs levels, that is, molar concentrations lower than 3×10^{-9} M for chiral pollutants with a molecular weight of ~300 g/mol. Finally, the determination of the enantiomeric purity of food components or the analysis of chiral compounds in food samples, which among others, enables obtaining important information on adulterations and food proces-

Correspondence: Professor Dr. María Luisa Marina, Departamento de Química Analítica, Facultad de Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, E-28871 Alcalá de Henares, Madrid Spain
E-mail: mluisa.marina@uah.es
Fax: +34-91-8854-971

Abbreviations: **CM-β-CD**, carboxymethylated-β-CD; **DM-β-CD,** heptakis(2,6-di-*O*-methyl)-β-CD; **FT-IR**, Fourier transform infrared; **HP-β-CD**, 2-hydroxypropyl-β-CD; **LLE**, liquid–liquid extraction; **LPME**, liquid-phase microextraction; **NACE**, nonaqueous CE; **QA-β-CD**, 2-hydroxypropyltrimethylammonium-β-CD; **SC**, sodium cholate; **SLE**, solid–liquid extraction

sing, also require appropriate sensitivity. In all these cases, an enhancement of the sensitivity of detection achieved by UV-Vis absorption detection, which is the most widely used in CE, is needed. An improvement of the sensitivity of detection may be achieved by means of three different strategies: (i) the sample treatment which can be made offline and online, (ii) the use of on-column sample preconcentration techniques based on electrophoretic principles, and (iii) the use of alternative detection systems to the UV-Vis absorption detection.

With the aim of providing to the readers an updated view of the different strategies used up to now to enhance the sensitivity of detection in chiral analysis by CE, this review will include a brief description of the fundamentals and most of the recent applications performed in sensitive chiral analysis by CE using offline and online sample treatment techniques, on-column preconcentration techniques, and alternative detection systems to the widely used UV-Vis absorption detection. In addition, a brief discussion of the CE working modes employed for chiral analysis will previously be included in order to establish the context of the different applications shown along this manuscript.

2 Separation modes in chiral analysis by CE

Chiral separations performed by CE in aqueous media may be included in the EKC mode because the discrimination of the enantiomers of a chiral compound is due to their different interaction with a chiral selector, that is, enantiomers are distributed in a different way between the bulk solution and the chiral selector according to a chromatographic mechanism [6–8]. It is important to note that the chiral separation principle is absolutely the same in the enantiomeric separation of a charged chiral analyte with a neutral chiral selector and in that of an uncharged chiral analyte with a charged chiral selector. However, there are some authors who have included those chiral separations with neutral chiral selectors into the CZE format. On the other hand, although much less used, CEC and nonaqueous CE (NACE) are also employed for chiral analysis.

The following paragraphs deal with these three different working modes in chiral electrophoretic separations, briefly describing the chiral selectors most widely used up to now as well as their applicability range in chiral analysis.

2.1 EKC

In EKC one or several chiral selectors are added to the BGE obtaining a separation buffer at a certain pH with capability for chiral discrimination. Many chiral selectors are commercially available [8–12]. Table 1 shows the most widely used up to now and the applicability range in chiral EKC.

CDs, which have the shape of a truncated cone, are the most important chiral selectors in CE. In addition to naturally occurring CDs (also called native CDs: α-CD, β-CD, and γ-CD) there is a big number of CD derivatives available, which can have high water solubility and enable to enhance the enantioselectivity achieved by native CDs. The neutral CD derivatives 2-hydroxypropyl-β-CD (HP-β-CD), heptakis(2,6-di-*O*-methyl)-β-CD, also called dimethyl-β-CD (DM-β-CD), and heptakis(2,3,6-tri-*O*-methyl)-β-CD, also denominated trimethyl-β-CD (TM-β-CD) have been widely used for the enantioseparation of charged chiral compounds in the pharmaceutical, biomedical, and environmental fields. HP-β-CD has been the most employed derivatized CD in food analysis. The separation of the enantiomers of neutral compounds (not achievable by using CDs of neutral nature) and also of charged analytes has been achieved by employing charged CDs, although they have the drawback of increasing the current intensity of the separation buffer. The most employed anionic CD derivatives are carboxymethylated-β-CD (CM-β-CD), sulfated-β-CD, and sulfobutyl ether-β-CD (SBE-β-CD). Finally, 2-hydroxy-propyl-trimethylammonium-β-CD (QA-β-CD) and 6-monodeoxy-6-monoamino-β-CD (β-CD-NH_2) are the most used cationic CDs. From all these commercially available CD derivatives, anionic CDs have been much more used than cationic CD derivatives. From the above-mentioned CD derivatives, HP-β-CD, CM-β-CD, sulfated-β-CD, SBE-β-CD, and QA-β-CD are randomly substituted CDs, that is, they are mixtures of many isomeric forms differing in the degree of substitution and in the position of the substituents. As a consequence, considerable variability in the selectivity obtained from different commercial suppliers and even from batch to batch for the same supplier can be observed.

The macrocyclic polyether, which has shown to be effective as chiral selector in CE is the crown ether (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ($18C_6H_4$). It enables the enantiomeric discrimination of amino acids and other compounds with primary amine groups [9] and has the advantage of not interfering with UV detection [13].

Bile salts are natural chiral surfactants (see Table 1) showing an interesting enantioseparation power for compounds having a rigid structure of fused rings [9, 14] whose separation with CDs can be difficult.

Synthetic chiral surfactants, also called polymeric surfactants, can derive from natural sugars (alkyl-glucoside and steroidal glucoside type surfactants) [15, 16] or from

Table 1. Chiral selectors most widely used and applicability range of the different working modes in chiral CE

CE mode	Chiral selector	Applicability
EKC	CDs: Neutral nature: α-CD, β-CD, γ-CD, 2-hydroxypropyl-β-CD (HP-β-CD), heptakis(2,6-di-*O*-methyl-β-CD (dimethyl-β-CD, DM-β-CD), heptakis(2,3,6-tri-*O*-methyl-β-CD (trimethyl-β-CD, TM-β-CD) Anionic nature: carboxymethyl-β-CD (CM-β-CD), sulfated-β-CD, sulfobutylether-β-CD (SBE-β-CD) Cationic nature: quaternary ammonium-β-CD (QA-β-CD), 6-monodeoxy-6-monoamino-β-CD (β-CD-NH_2)	Broad applicability range for neutral and charged chiral compounds. Chiral selectors most used in CE with about 90% of the applications.
	Crown ether: (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid ($18C_6H_4$)	Restricted to chiral compounds containing primary amine groups.
	Natural chiral surfactants (bile salts): sodium cholate (SC); sodium deoxycholate (SDC); sodium taurocholate (STC); sodium taurodeoxycholate (STDC) Synthetic chiral surfactants (polymeric surfactants): polysodium *N*-undecanoyl-L-leucylvalinate (poly-L-SULV)	Bile salts are appropriate for chiral analytes with rigid structure of fused rings. Polymeric surfactants are adequate for the enantiomeric separation of cationic and neutral chiral analytes whereas anionic chiral analytes are difficult to resolve. Compatible with MS detection.
	Macrocyclic antibiotics: ristocetin A, vancomycin, teicoplanin	Problems of light absorption and adsorption to the capillary walls. Adequate for neutral and anionic chiral compounds of a wide range of structures.
	Proteins: serum albumin	Problems of light absorption and adsorption to the capillary walls. Appropriate for neutral and ionic chiral compounds.
	Polysaccharides: neutral nature: dextrin, dextran; anionic nature: chondroitin, heparin	Neutral polysaccharides are appropriate for anionic and cationic chiral compounds. Anionic polysaccharides are adequate for neutral and charged chiral analytes.
CEC	Packed capillary columns: CDs, polysaccharides, ligand-exchange-type selectors, macrocyclic antibiotics, proteins	Used for neutral and ionic chiral compounds. Compatible with MS detection
	Open-tubular capillary columns: CDs, proteins	
	Monolithic capillary columns: CDs, crown ethers, ligand-exchange type selectors, macrocyclic antibiotics	
NACE	CDs: β-CD, heptakis-(2,3-dimethyl-6-sulfato)-β-CD, heptakis-(2,3-diacetyl-6-sulfato)-β-CD, octakis-(2,3-dimethyl-6-sulfato)-γ-CD, octakis-(2,3-diacetyl-6-sulfato)-γ-CD, QA-β-CD	Mainly used for basic chiral compounds (basic drugs) and some acidic and neutral compounds
	Ion-pairing compounds: (+)- or (−)-camphorsulfonate and quinine derivatives (*tert*-butyl carbamoylated quinine, 1-adamantyl carbamoylated quinine, *trans*-1,4-cyclohexylene-bis (carbamoylated-11-dodecylthio-dihydroquinine)	

amino acids (*N*-(*n*-alkanolyl)- or *N*-(*n*-alkyloxycarbonyl)-amino acids) [15–17]. Polymerized surfactants, also denominated micelle polymers, are a type of synthetic chiral surfactants, which have shown advantages over micelles of low molecular weight surfactants such as be compatible with MS detection as is the case of poly-L-SULV (see Table 1) [15, 18]. Synthetic chiral surfactants have given rise to promising results [19, 20] but have the drawback of not being commercially available in many cases.

From the two main types of macrocyclic antibiotics used in CE, glycopeptides (ristocetin A, vancomycin, and teicoplanin are the most effective ones) and ansamycins [9, 21–25], the first ones are more effective for neutral and anionic solutes while ansamycins are more useful for cationic analytes. Strong adsorption to the inner wall of fused-silica capillaries, low chemical stability because they can be degraded at high temperatures and acid or basic pHs, and absorption in the low UV range causing low sensitivity are the main drawbacks derived from the use of these chiral selectors, which have to be employed under controlled experimental conditions in order to avoid some of these problems.

Serum albumins are plasma proteins, which have been the most employed proteins for chiral separations by CE [26]. Since enantiomeric separations are based on the differential affinity of the protein for each enantiomer, this separation mode is called affinity CE (ACE) [27]. As in the case of macrocyclic antibiotics, adequate experimental conditions have to be used in order to avoid the interaction of proteins with the wall of the capillary and to minimize the background UV absorption of protein solutions (usual concentrations are lower than 100 μM).

Finally, enantioseparation of chiral compounds by EKC can also be achieved using neutral (dextrins and dextrans [28, 29]) or charged (heparin and chondroitin sulfates [30]) polysaccharides, forming ligand-exchange complexes [31, 32], or with chiral calixarenes or ergot alkaloids [32].

2.2 CEC

CEC, which is a hybrid technique of CE and HPLC, is characterized by (i) high separation efficiency due to the plug profile of the mobile phase driven by the EOF, (ii) a wide range of HPLC chiral stationary phases, which can be transferred to CEC to provide broad enantioselectivity, (iii) low solvent consumption and low sample requirements, and (iv) easy coupling to MS. Enantioseparations are possible in three different types of columns: (i) packed capillary columns, (ii) open-tubular capillary columns, and (iii) monolithic capillary columns [33–36]. In addition, achiral packed capillaries in combination with BGEs where the chiral selector is added have also been used for enantioseparations by CEC.

Stationary phases previously employed in chiral HPLC, such as CDs and their derivatives [37, 38], macrocyclic antibiotics [39], proteins [26], polysaccharides [40–42], and ligand-exchange type selectors [43] (see Table 1) have been adapted for chiral CEC using packed capillary columns. Open-tubular capillary columns have been the less used for the enantiomeric separation of chiral compounds by CEC. CDs and proteins (see Table 1) have generally been used as chiral selectors [35].

Many of the chiral selectors employed to prepare packed capillary columns have also been used in order to prepare chiral monolithic capillary columns for CEC (see Table 1) [35, 36, 44–49].

In spite of the drawbacks of CEC compared with CE in chiral analysis (CEC is less flexible and enables to achieve lower peak efficiency than CE [50]), CEC is entering in the field of practical applications [35, 36, 51–54].

2.3 Nonaqueous CE

The elimination of the aqueous media in NACE provides additional selectivity with respect to that obtained in aqueous CE, and favors the analysis of solutes with poor water solubility [55]. The main chiral selectors used in NACE for chiral separations are CDs and their derivatives (see Table 1) [56]. Ion-pairing compounds, such as camphorsulfonate enantiomers [57] and quinine derivatives, have also been employed [58–62].

3 Sensitive chiral analysis by CE

An enhancement of the sensitivity can be obtained in chiral analysis by CE by using three different strategies: (i) offline and online sample treatment techniques, (ii) on-column sample preconcentration techniques, and (iii) alternative detection systems to on-column UV-Vis absorption detection. Our goal is to provide the readers an updated overview on the use of these three different strategies to improve sensitivity detection in chiral analysis by CE.

3.1 Offline and online sample treatment techniques for sensitive chiral analysis by CE

To achieve adequate detection sensitivity and separation selectivity, the analysis of real samples in CE usually requires efficient sample treatment to remove interfering

solutes, inorganic and organic salts, and particulate matter. Sample preparation has been mainly achieved offline, but a few online sample treatment systems coupled to CE have also been developed. As a consequence, a brief description of those offline sample treatment techniques and their applications for sensitive chiral analysis as well as a more detailed description of those few works dealing with the use of sample treatment techniques coupled online to CE for sensitive chiral analysis will be presented as follows.

3.1.1 Offline sample treatment techniques for sensitive chiral analysis by CE

The offline sample treatment techniques mainly used for the preconcentration and extraction of the enantiomers of a chiral compound in different matrices are SPE, liquid–liquid extraction (LLE), liquid-phase microextraction (LPME), solid–liquid extraction (SLE), and microdialysis [63].

Table 2 groups the main applications performed using offline sample treatment techniques for sensitive chiral analysis by CE. Briefly, offline SPE has been used for the extraction and preconcentration of drugs in biological samples and pesticides in water and food samples. Thus, the detection from 3×10^{-8} to 5×10^{-6} M of different drugs in plasma [64], serum [65], and urine [66, 67] has been performed. In addition, the detection up to 10^{-10} or 10^{-7} M of phenoxy acid herbicides in spiked water samples [68, 69] as well as the detection of the pesticides vinclozolin ($\sim 10^{-6}$ M), maleic hydrazide and imazalil (3×10^{-7} M), in beverages and vegetables [70–72] has been achieved. Offline LLE has been employed for the extraction and preconcentration of drugs in biological samples and pollutants in culture media. The detection up to 10^{-8}–10^{-6} M of drugs (such as disopyramide, praziquantel, fluoxetine, ibuprofen, ketoprofen, tramadol, and ofloxacin) in plasma [73–76], serum [75, 77], and cells [78], and up to micromolar concentrations of the persistent pollutants polychlorinated biphenyls or of thiobencarb sulfoxide in culture media [79, 80] has been performed. Although much less used, as it can be observed in Table 2, SLE, LPME, and microdialysis have also been employed for the preparation of samples for sensitive chiral CE. SLE has allowed the extraction and preconcentration up to 5×10^{-7} M of pesticides in spiked soils [80, 81] and in foods as potatoes and onions [71]. LPME has been used for the extraction and preconcentration of the drugs citalopram and desmethylcitalopram in human plasma detecting concentrations up to 7×10^{-9} M [82]. Finally, sample preparation of biological samples by microdialysis has enabled the isolation and detection up to 3×10^{-9} M of the drug isoproterenol [83, 84].

3.1.2 Online coupling sample treatment systems to CE for sensitive chiral analysis

Nowadays, the online coupling of sample treatment systems to CE have a great interest because it allows the automatization of the analytical process (from sample preparation to data treatment), which is a current trend in analytical chemistry. Nevertheless, only six original papers (reported from 1996 to 2003) dealing with the analysis of chiral compounds have been found in the literature. Due to the current interest in the development and application of online systems, a description of the different systems found in the literature to achieve chiral analysis by CE will be presented here.

Pálmarsdóttir *et al.* [85, 86] demonstrated the high selectivity and sensitivity obtained in bioanalysis using the supported liquid membrane (SLM) technique coupled online with CE through a microcolumn LC (CLC) interface. The system utilized two selective, sequential enrichment steps before the third analyte focusing and separation step with a double-stacking preconcentration. By this way, a total concentration $\sim$40 000 times ($\sim$6 times by the SLM treatment, $\sim$17 times by micro-CLC focusing, and $\sim$400 times by the double-stacking preconcentration) was achieved and 2.5×10^{-10} M of the enantiomers of bambuterol in human plasma were detected obtaining appropriate selectivity [85].

The rapid determination of aspartate enantiomers in tissue samples from rats by microdialysis coupled online with CE has been reported by Thompson *et al.* [87]. The microdialysis probe was inserted into a homogenized tissue sample, which allowed generation of a continuous sample stream that was filtered and deproteinated before the CE separation. With this system, where microdialysis was coupled online with derivatization (with *o*-phthalaldehyde in the presence of β-mercaptoethanol) and CE with LIF detection, aspartate enantiomers ($\sim 10^{-6}$ M) were resolved in less than 3 s. On the other hand, a similar setup for online microdialysis-CE was used for the analysis of serine enantiomers in tissue homogenates. In this case, D-serine was resolved from L-serine and other primary amines commonly found in biological samples in less than 22 s. In addition, D-serine was determined in larval tiger salamander retinal homogenates [88].

A system with online derivatization coupled to CE has been developed for the enantiomeric separation of carnitine enantiomers by Mardones *et al.* [89]. The enantiomers of carnitine were derivatized with 9-fluorenylmethyl chloroformate (FMOC) in a flow system working online with the capillary electrophoretic equipment. LODs of 5 μM of both isomers were obtained by this method

Table 2. Offline sample treatment techniques employed for sensitive chiral analysis by CE

Sample treatment	Analyte and sample	Separation buffer	Detection (UV), nm	LOD, M	Ref.
SPE	Adrenoreceptor antagonist in plasma	Formic acid-ammonia (pH 4) + HP-β-CD (3.5 mM)	200	–	[64]
SPE	Ondansetron in human serum	Phosphate (pH 2.5) + 15 mM DM-β-CD	254	$\sim 3 \times 10^{-8}$	[65]
SPE	Pentobarbital in serum	Phosphate (pH 9.0) + 40 mM HP-γ-CD	254	$\sim 5 \times 10^{-6}$	[65]
SPE	Methadone and its primary metabolite (2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine) in urine	Phosphate (pH 3.0) + 4.3 mM HP-γ-CD	200	–	[66]
SPE	3,4-Methyl-enedioxymethamphetamine in human urine	Phosphate (pH 2.5) + 30 mM HP-γ-CD	195	$\sim 10^{-7}$	[67]
SPE	Chlorophenoxy acid herbicides (2,4-CPAA, 2,4,5-CPAA, 2,4-CPPA, 2,4,5-CPPA, 2,4-CPBA, 2-CPMBA) in spiked lake water	Phosphate (pH 5.6) + α-CD (4 mM) + β-CD (1 mM)	200	$\sim 10^{-7}$	[68]
SPE	Aryloxypropionic (mecoprop, fenoprop); aryloxyphenoxy-propionic acidic herbicides (fluazifop and haloxyfop) in spiked ground and river waters	Boric/acetic/ phosphoric (pH 5) + vancomycin + γ-CD	230	$\sim 10^{-10}$	[69]
SPE + fractionation with HPLC	Vinclozolin in wine	Phosphate/borate (pH 8.5) + SDS + γ-CD (50 mM)	203	$\sim 10^{-6}$	[70]
SLE/SPE	Maleic hydrazide in potatoes, onions	Phosphate (pH 7) + SC (40 mM)	220	–	[71]
LLE/SPE	Imazalil in orange	Phosphate (pH 3) + SDS + HP-β-CD (4 mM)	200	$\sim 3 \times 10^{-7}$	[72]
Sample cleanup/LLE	Disopyramide and mono-*N*-dealkyl-disopyramide in human plasma	Acetate (pH 5) + sulfated β-CD (0.2%)	214	$\sim 10^{-7}$	[73]
LLE	Praziquantel and *trans*-4-hydroxy-praziquantel in human plasma	Borate (pH 10) + sulfated β-CD (2%) + SDC (20 mM)	214	$\sim 6 \times 10^{-8}$	[74]
LLE	Fluoxetine and norfluoxetine in plasma and serum	Phosphate (pH 2.5) + DM-β-CD (0.5 mg/mL) + phosphated-γ-CD (0.6 mg/mL)	195	–	[75]
LLE	Ibuprofen in human plasma	Phosphoric acid-tetra-ethylammonium (pH 2.6) + sulfated β-CD (2%)	220	$\sim 3 \times 10^{-7}$	[76]
LLE	Ketoprofen in human serum	Phosphate-triethanolamine (pH 5) + TM-β-CD (50 mM)	253	$\sim 5 \times 10^{-7}$	[77]
LLE	Tramadol and its metabolites	Borate (pH 10.2) + CM-β-CD 20 mg/mL	214	$\sim 10^{-6}$	[150]

Table 2. Continued

Sample treatment	Analyte and sample	Separation buffer	Detection (UV), nm	LOD, M	Ref.
LLE	Ofloxacin in cells	Phosphate (pH 2.8) + M-β-CD (4%)	280	$\sim 10^{-8}$	[78]
LLE	PCBs (45, 88, 91, 95, 136, 144, 149, and 176) in culture medium	MES (pH 6.5) + β-CD (10 mM) + CM-β-CD (20 mM)	230	$\sim 10^{-6}$	[79]
LPME	Citalopram and desmethyl-citalopram in human plasma	Phosphate (pH 2.5) + ACN (12%) + sulfated-β-CD (1%)	200	$\sim 7 \times 10^{-9}$	[82]
SLE/LLE	Thiobencarb sulfoxide in spiked soil or culture medium	Phosphate/borate (pH 8.5) + SDS + HP-γ-CD (60 mM)	220	$\sim 5 \times 10^{-6}$	[80]
SLE	Free acid herbicides (haloxyfop, fluazifop, fenoxaprop and flamprop free acids); phenoxy acid herbicides (diclofop, mecoprop, dichlorprop, fenoprop, PPA) in spiked soil	Boric/acetic/phosphoric (pH 5) + vancomycin (6 mM)	230	$\sim 5 \times 10^{-7}$	[81]
Microdialysis	Isoproterenol in rats	Lithium acetate (pH 4.75) + M-β-CD	Ampero-metric	$\sim 3 \times 10^{-9}$	[83]
Microdialysis	Isoproterenol in human plasma	Lithium acetate (pH 4.75) + M-β-CD	Ampero-metric	$\sim 3 \times 10^{-9}$	[84]

LLE, liquid–liquid extraction; LPME, liquid-phase microextraction; SLE, solid–liquid extraction. 2,4-CPAA, (2,4-dichlorophenoxy) acetic acid; 2,4,5-CPAA, (2,4,5-trichlorophenoxy) acetic acid; 2,4-CPPA, 2-(2,4-dichlorophenoxy)propionic acid; 2,4-CPBA, 2-(2,4-dichlorophenoxy)butyric acid; 4-CPMBA, 2-(4-chlorophenoxy)-2-methylbutyric acid; 2,4,5-CPPA, 2-(2,4,5-trichlorophenoxy)propionic acid; PPA, 2-phenoxypropionic acid; PCBs, polychlorinated biphenyls.

allowing the determination of D-carnitine in excess of L-carnitine in synthetic samples demonstrating that the maximum ratio determined was 1:100 (D:L).

On the other hand, miniaturized SPE coupled online with CE with on-column UV absorption detection has enabled to enhance the concentration sensitivity for terbutaline, used as model compound, by a factor of 7000 [90]. The extractor consisted of a short length (1–3 mm) of a capillary (200 μm ID) packed with C18 alkyl-diol silica (d_p 12 μm) connected to a 50 μm ID separation capillary and glass-fiber filters retaining the sorbent (see Fig. 1a). Preparation and performance of the enrichment capillary is shown in Fig. 1b and includes washing, wetting, conditioning, sorption, washing, filling, and desorption. The concentration LOD for terbutaline in aqueous solution with this online enrichment (10 min × 140 kPa pressure injection) was 0.6 nM compared to 4.4 μM (1 s × 3.4 kPa pressure injection) without enrichment. In addition, Fig. 1c shows that this enrichment procedure was successfully adapted to the separation of terbutaline enantiomers using DM-β-CD as chiral selector with a high efficiency.

3.2 On-column preconcentration techniques in sensitive chiral analysis by CE

On-column preconcentration techniques based on electrophoretic principles used in chiral analysis by CE are ITP, stacking, and sweeping. We will describe them briefly previous to the description of the applications performed for sensitive chiral analysis by CE.

In ITP the sample preconcentration is based on the differences in the mobilities of the ions and of the analytes in a discontinuous electrophoretic buffer (leading and terminating buffers). When the voltage is applied to the end of the capillary, a potential gradient is generated along the capillary because the leading buffer contains ions with higher mobility than the analytes, and the terminating buffer contains ions of lower mobility being the analytes focused in zones. Although ITP may be performed in the same capillary where the electrophoretic separation is achieved, which is denominated transient ITP, two different capillaries may be coupled online, one for the ITP preconcentration and the other for the electrophoretic separation [91].

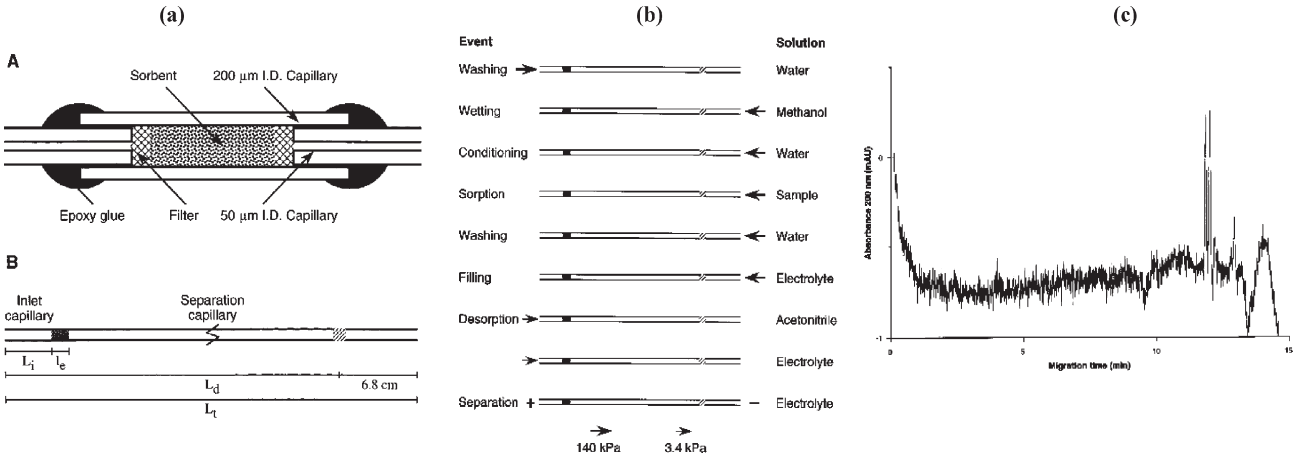

Figure 1. (a) Cross-section of (A) the extractor and (B) the enrichment capillary where L_t is the enrichment capillary total length, L_d is the length to the detector, L_i is the length of the inlet capillary, and l_e is the extractor length. (b) Sample enrichment procedure for terbutaline dissolved in water (arrows indicate flow directions). (c) Electropherogram of terbutaline enantiomers separated by online SPE-CE using an enrichment capillary: L_t 58.0 cm, L_d 51.2 cm, L_i 5.5 cm, l_e 2.5 mm, wash: water × 1.6 min × 140 kPa, wetting: methanol × 2.4 min × 140 kPa, conditioning: water × 2.4 min × 140 kPa, injection: 100 nM *rac*-terbutaline in water × 1.0 min × 140 kPa, wash/filling: 40 mM potassium phosphate (pH 6.4) × 0.1 min × 140 kPa, 15 mM DM-β-CD in 40 mM potassium phosphate (pH 6.4) × 0.7 min × 140 kPa, desorption: ACN × 40 s × 3.4 kPa followed by 15 mM DM-β-CD in 40 mM potassium phosphate (pH 6.4) × 4.0 min × 3.4 kPa, other electrophoretic conditions: separation temperature, 25°C; applied voltage, 14 kV; UV detection at 200 nm. Reprinted from [90], with permission.

On-column preconcentration by sample stacking is based on the injection of a sample zone prepared in a matrix with a higher resistance, that is, with minor conductivity, than the separation buffer. Thus, when the voltage is applied between the ends of the capillary, the sample ions acquire electrophoretic mobilities higher in the sample region than in the separation buffer region in such a way that sample reduces its mobility in the latter region and is focused in a thick zone between both regions. In this preconcentration mode, also called normal stacking mode (NSM), the sample is dissolved in water, a buffer with lower concentration than the separation buffer or solvents (*i.e.*, ACN) and is injected hydrodynamically (usually applying a pressure for a certain time) in the separation capillary. Although in hydrodynamic injection no more than 3–4% of the total length of the capillary may be filled with sample to do not loose efficiency, under sample stacking conditions, the capillary may be filled with sample up to 10–20% of the total capillary without any loss of efficiency. Nevertheless, it is also possible to fill the total capillary if after that, there is a step of elimination of the matrix applying reverse polarity previously to the application of the normal polarity for the electrophoretic separation. This preconcentration mode is called reverse electrode polarity stacking mode (REPSM). It is also possible to use micelles with reverse migration and to work with reverse polarity for the preconcentration and electrophoretic separation. In this case, the preconcentration is called stacking with reverse migrating micelles (SRMM) and may include a water plug in which case it is denominated stacking with reverse migrating micelles and a water plug (SRW). Nevertheless, when the sample is injected electrokinetically (applying a voltage for a certain time), the analytes are introduced into the capillary depending on the EOF of the sample, and of the charge and mobility of the analytes. If the preconcentration of the analytes is made during the electrokinetic injection, then the preconcentration is performed by field-amplified sample stacking (FASS), also called field-enhanced sample injection (FESI), or field-amplified sample injection (FASI) [91–93].

Sweeping is a preconcentration technique, which enables an exceptional increase in the detection sensitivity (>1000 times) for those analytes with a high solute-pseudostationary phase association constant (usually micelles or CDs) [94, 95]. In fact, the resulting length of the swept zones (l_{sweep}) can be approximated by the following equation (Eq. 2)

$$l_{sweep} = l_{inj} \cdot (1/1 + k) \quad (2)$$

where l_{inj} is the length of the injected sample zone and k is the analyte retention factor. Although the first applications were performed using a sample matrix with the same conductivity than the separation buffer [94], this sample matrix may have different conductivity [96] but it is very important that the sample matrix does not contain the

pseudostationary phase, which is the component producing the preconcentration of the analyte by sweeping the sample matrix.

Table 3 shows the main applications performed for sensitive chiral analysis by CE using on-column preconcentration techniques based on electrophoretic principles. LODs up to 10^{-8} M for methadone enantiomers [97] and for the enantiomers of tryptophan and norleucine derivatized in urine samples and complex ionic matrices [98, 99] have been achieved using ITP as preconcentration technique. Stacking preconcentration has allowed the detection of drugs in plasma samples obtaining LODs in the range from 2×10^{-9} to 4×10^{-9} M [84–86, 100], which were appropriate for the analysis of biological samples. As an interesting example, Fig. 2 illustrates the detection of 2×10^{-7} M of terbutaline enantiomers when using double-stacking preconcentration followed by the CE enantiomeric separation. On the other hand, the effect of ACN and salt in the sample matrix on the stacking and separation of naphthyl derivatives has provided an improvement of peak efficiency and thus concentration detection sensitivity when the sample injection size was relatively large. However, further investigations may be done to clarify this effect which has not been observed for other molecules [101]. Finally, sweeping preconcentration of the phenoxyacid herbicide fenoprop enabled its detection at ppb levels ($\sim 10^{-8}$ M) [94].

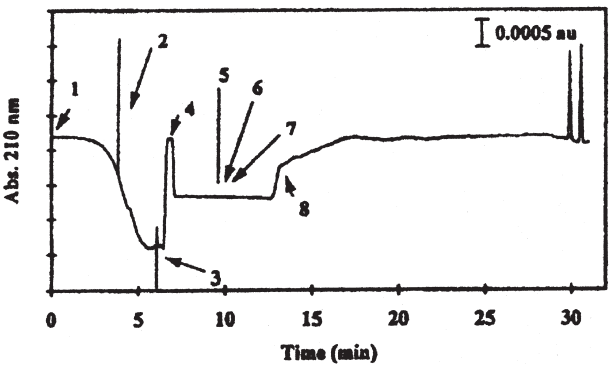

Figure 2. Electropherogram obtained for terbutaline (200 nM of each enantiomer) using double-stacking procedure followed by the CE enantiomeric separation. Arrows indicate the different events: (1) stacking step 1 begins; (2) stacking peak of positive species; (3) voltage off, backpressure off; (4) zone of 5 mM phosphate buffer pH 7.5; (5) back-pressure off; (6) voltage on, stacking step 2 at the inlet end of the capillary; (7) backpressure on; (8) backpressure off, final separation step begins. Electrophoretic conditions: separation buffer, 100 mM phosphate (pH 2.5) containing 10 mM DM-β-CD; separation temperature, 20°C; applied voltage for the electrophoretic separation, 24 kV; UV detection at 210 nm. Reprinted from [151], with permission.

3.3 Alternative detection systems to on-column UV-Vis absorption detection for sensitive chiral analysis by CE

UV-Vis absorption detection is the most available and used detection in CE. Unfortunately, the concentration sensitivity achieved by this on-column detection is limited by the optical pathlength (*b*) of the capillary, which corresponds to its inner diameter. Although for chiral compounds with chromophore groups, LODs in the micromolar range may be achieved, there are cases in which this concentration sensitivity is not achievable (compounds with low values of molar absorptivity, ϵ) or is insufficient (biological, environmental, food samples, *etc.*). In order to increase the absorbance signal observed ($A = \epsilon bc$), several strategies to increase the pathlength have been developed. One of these strategies has been the development of special designs of the detection window but they have been scarcely used (bubble cells, Z-shaped cells). In addition, in chiral analysis by CE they may decrease the enantiomeric resolution when the separated zones containing the enantiomers reach the detection window.

Other option to overcome the poor concentration sensitivity, achieved by on-column UV-Vis absorption detection in CE, is the selection of alternative detection systems. Up to now, spectroscopic detectors, mass spectrometers, and electrochemical detectors have been used.

The spectroscopic detectors alternative to UV-Vis absorption detectors used in chiral analysis by CE have been LIF, phosphorescence, Fourier transform infrared (FT-IR), and NMR detection.

LIF detection is one of the most attractive detection systems for sensitive chiral analysis by CE. In fact, it enables to obtain high sensitivity and additional selectivity with respect to UV-Vis absorption detection, which is a "quasi" universal detection system. The basic instrumentation used for LIF detection requires an excitation source (laser), a detection cell (the own capillary), an optic system for the excitation and collection of the fluorescence emitted, and a photomultiplier tube connected to an acquisition data system, usually a computer [102]. Table 4 shows most of the applications in the field of enantioseparations by CE with LIF detection. Although LIF detection has been used for the analysis of pesticides and drugs, the main application is the detection of amino acid enantiomers. In fact, very favorable LODs have been obtained (4×10^{-10}–10^{-8} M) for the detection of the enantiomers of amino acids derivatized previously to the injection in the electrophoretic system [103–108]. The detection of the natural amino acid L-tryptophan together with its enantiomer (D-form) has been performed in human

Table 3. On-column preconcentration techniques employed for sensitive chiral analysis by CE

Online preconcentration	Analyte and sample (remarks)	Detection (UV), nm	LOD, M	Reference
ITP	L-Tryptophan and norleucine labeled with 2,4-dinitrophenyl in urine and complex ionic matrices	200	$\sim 10^{-8}$	[98, 99]
ITP	Methadone	200	$\sim 10^{-8}$	[97]
Sample stacking	Isoproterenol in plasma (pharmacokinetic study)	Ampero-metric	$\sim 3 \times 10^{-9}$	[84]
FASS	Adrenoreceptor antagonist in plasma	200	$\sim 4 \times 10^{-9}$	[100]
Double stacking	Terbutaline, bambuterol, ephedrine, brompheniramine, and propranolol	210	$\sim 10^{-8}$	[151]
Double stacking	Bambuterol in plasma	210	$\sim 2 \times 10^{-9}$	[85, 86]
Stacking with ACN	Naphthyl enantiomers and dansylated amino acid	220	–	[101]
Sweeping	Fenoprop	210	$\sim 10^{-8}$	[94]

Dansylated amino acid, amino acid derivatized with dansyl chloride.

urine [106]. On the other hand, the detection up to $\sim 7 \times 10^{-7}$ M of fenprocoumon [109] in urine samples as well as naproxen in liver and kidney tissues ($\sim 3 \times 10^{-7}$ M) [110] has been achieved with good separation selectivity. Figure 3 shows the electropherograms of a urine sample from a healthy volunteer after oral administration of tramadol hydrochloride (150 mg) compared to blank urine.

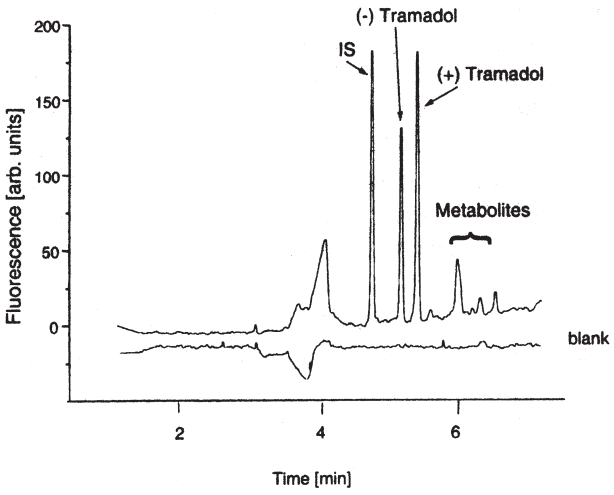

Figure 3. Electropherogram corresponding to a urine sample of a healthy volunteer collected 7–8 h after oral administration of 150 mg tramadol hydrochloride compared to that of blank urine. Electrophoretic conditions: fused-silica capillary, l_d = 55 cm, l_t = 75 cm, and 50 μm ID; separation buffer, 25 mM borax (pH 10.0) containing 40 mg/mL CM-β-CD; separation temperature, 19°C; applied voltage, 25 kV; sample injection 50 mbar for 6 s. LIF detection with λ_{em} at 257 nm. Reprinted from [111], with permission.

The absence of interferences from peaks of the metabolites is clearly demonstrated [111]. This is an interesting case where the drug tramadol and its metabolites show native fluorescence, their direct detection being possible by LIF. On the other hand, it is remarkable that the LODs in the nanomolar range were achieved for a group of phenoxy acid herbicides derivatized with the 7-amino-naphthalene-1,3-disulfonic acid (ANDSA), which are appropriate for the analysis of environmental samples [112].

The novel application of quenched phosphorescence detection to the chiral analysis field has been reported recently for the first time [113]. The basic instrumentation is the same as for LIF detection [114] but a UV-Vis lamp has usually been employed [115–118]. There are two additional settings in phosphorescence not used in fluorescence: the delay time (time of waiting after the lamp pulse to remove all fluorescence from the signal) and the gating time (time of measurement of the phosphorescence signal), which have to be considered. In addition, it is very important to consider that in phosphorescence measurements the deoxygenation of the buffer solution by a constant nitrogen flow is essential in order to avoid oxygen quenching. Under deoxygenated conditions, the dynamic quenching by camphorquinone of the strong phosphorescence emission of brominated naphthalenesulfonate present in the buffer solution (25 mM borate buffer at pH 8.5 with 10 mM CM-β-CD and 20 mM α-CD) enabled to obtain LODs ($\sim 7 \times 10^{-7}$ M) three orders of magnitude lower than the conventional UV absorption detection at 200 nm. Under these conditions the stereoselective degradation of camphorquinone by yeast was demonstrated. Thus, Fig. 4 illustrates the variation of peaks correspond-

Table 4. LODs obtained in sensitive chiral analysis by CE using alternative detection systems to on-column UV-Vis absorption detection.

Detection system	Analyte and sample (remarks)	LOD, M	Reference
LIF (λ_{exc} = 442 nm; λ_{exc} = 490 nm)	CBI-amino acids (Thr, Asp, Ile, Tyr, Phe, Ser, Leu, Val, Met, and Arg)	$\sim 4 \times 10^{-10}$	[103]
Diode LIF (λ_{exc} = 635 nm)	CBI-amino acids (Ala, Glu, Val, Phe, Tyr, and Trp)	$\sim 2 \times 10^{-8}$	[104]
LIF (λ_{exc} = 457 nm)	CBI-amino acids (Tyr, Ile, Asp, Met, Trp, and Phe)	3×10^{-8}	[105]
LIF (λ_{exc} = 457 nm)	CBI- tryptophan in biological samples	3×10^{-8}	[106]
LIF (λ_{exc} = 457.9 nm; λ_{em} = 495 nm)	CBI-aspartic acid in rat brain	$\sim 10^{-8}$	[107]
LIF (λ_{exc} = 488 nm; λ_{em} = 520 nm)	FITC-amino acids (Pro, Asp, Ser, Asn, Glu, Ala, and Arg) in orange juices and concentrates	$\sim 3 \times 10^{-9}$	[108]
LIF (λ_{exc} = 325 nm; λ_{em} = 405 nm)	Fenprocoumon in urine	$\sim 7 \times 10^{-7}$	[109]
LIF (λ_{exc} = 325 nm; λ_{em}>366 nm)	Naproxen in liver and kidney tissues	$\sim 3 \times 10^{-7}$	[110]
LIF (λ_{exc} = 257 nm)	Tramadol and *O*-demethyl tramadol glucuronide in human urine (direct detection of these fluorescent drugs)	$\sim 2 \times 10^{-7}$	[111]
LIF (λ_{exc} = 325 nm; λ_{em} = 420 nm)	ANDSA-phenoxy acids (silvex, mecoprop, diclorprop, 2,4-CPAA, 2,4,5-CPAA, PPA, 2-CPPA, 3-CPPA, and 4-CPPA)	$\sim 10^{-9}$	[112]
Quenched phosphorescence	Camphorquinone in culture medium	$\sim 7 \times 10^{-7}$	[113]
NMR	Alprenolol (online coupling of ITP-NMR)	$\sim 10^{-7}$	[119]
FT-IR	3,5-dinitrobenzoil leucine (DNB-Leu) (enantioseparation by NACE)	($\sim 10^{-3}$)	[120]
ESI-MS	Terbutaline and ephedrine in spiked urine (direct coupling CE-ESI-MS)	$\sim 10^{-7}$	[135]
ESI-MS	Tramadol and its main phase I metabolites (use of the partial-filling technique)	$< 5 \times 10^{-7}$	[145]
ESI-MS-MS	Adrenoreceptor antagonist (use of the partial-filling technique)	$\sim 2 \times 10^{-8}$	[152]
ESI-MS	Clenbuterol in plasma (use of the partial-filling technique)	$\sim 10^{-6}$	[142]
ESI-MS	Amphetamines, venlafaxine, tropane alkaloids, methadone and its metabolites in serum (use of the partial-filling technique)	$\sim 10^{-8}$	[143]
ESI-MS	Amino acids and neurotransmitters in blood cells (use of a crown ether compatible with MS as chiral selector and buffer)	$\sim 10^{-9}$	[136]
ESI-MS	Underivatized amino acids (use of a crown ether as chiral selector and a sheathless interface)	$\sim 10^{-9}$	[137]
ESI-MS	FITC-amino acids (Asp, Glu, Ser, Asn, Ala, Pro, and Arg)	$\sim 10^{-6}$	[139]
ESI-MS	Methamphetamine and its metabolites in urine	$\sim 2 \times 10^{-7}$	[140]
ESI-MS	Methamphetamine and related compounds in urine	$\sim 5 \times 10^{-8}$	[141]
ESI-MS-MS	Three basic drugs in *in vivo* samples (use of a home-made interface)	$\sim 10^{-5}$ (10^{-8})	[144]
Amperometric	Isoproterenol in plasma/rats	$\sim 3 \times 10^{-9}$	[83, 84]
Amperometric	Promethazine	5×10^{-8}	[147]
Amperometric (interdigitated electrodes)	Neurotransmitters (isoproterenol, epinephrine, and norepinephrine)	5×10^{-6}	[148]

FT-IR, Fourier transform infrared; CBI-amino acids, cyanobenzoisoindole-labeled amino acids after derivatization with naphthalene-2,3-dicarboxaldehyde; FITC-amino acids, FITC-labeled amino acids; ANDSA-phenoxy acids herbicides, 7-aminonaphthalene-1,3-disulfonic acid-labeled phenoxy acid herbicides; 2-CPPA, 2-(2-chlorophenoxy)propionic acid; 3-CPPA, 2-(3-chlorophenoxy)propionic acid; 4-CPPA, 2-(4-chlorophenoxy)propionic acid; other abbreviations as in Table 2.

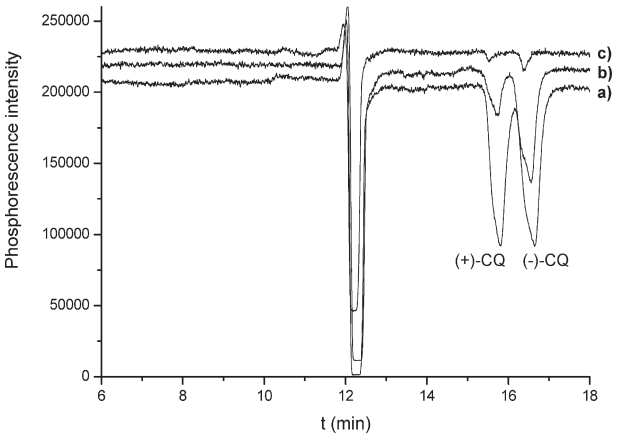

Figure 4. Electropherograms showing camphorquinone enantiomers in control (a), incubation medium with yeast (b), and extract obtained from yeast cells (c), after 24 h of incubation (direct injection of 100 times dilutions in methanol). Electrophoretic conditions: fused-silica capillary, l_d = 60 cm, l_t = 100 cm, and 50 μm ID; separation buffer, 25 mM borate (pH 8.5) containing 10 mM CM-β-CD and 20 mM α-CD with the phosphorophore 1-bromonaftalenosulfonate (1 mM); separation temperature, 25°C; applied voltage, 20 kV; sample injection 50 mbar for 6 s. Quenched phosphorescence detection in deoxygenated conditions, λ_{ex} = 294 nm, delay time: 0.05 ms and gating time: 5.00 ms. Reprinted from [113], with permission.

ing to camphorquinone enantiomers in the control, culture medium, and cells after 24 h of incubation, showing that the biodegradation of (1*S*)-(+)-camphorquinone was faster than that of the (1*R*)-(−)-enantiomer [113].

The use of NMR and FT-IR in CE for the detection of the enantiomers of a chiral compound has been reported very recently [119, 120]. Although these spectroscopic detectors provide structural information, their sensitivity is rather poor, especially for FT-IR-CE (see Table 4). However, the LOD achieved by this technique has been included in Table 4 since it is the only application of FT-IR-CE reported in chiral analysis by CE. Online ITP-NMR can be recorded with CE coupled to microcoil NMR probes, formed by directly wrapping a coated Cu wire around a capillary where the separation capillary is inserted, which provides a sensitivity enhancement inversely proportional to the coil diameter with respect to the standard-sized Helmholtz coil [121–123]. Online ITP-NMR has been used for the separation and concentration of alprenolol enantiomers. Figure 5 illustrates the online ITP-NMR spectra of alprenolol enantiomers as a function of runtime when they are separated using a leading electrolyte (acetate buffered to pD 6.0 with acetic acid in D_2O) containing α-CD and sulfated β-CD as chiral selectors. This figure shows the progress of ITP stacking for *S*-alprenolol from NMR spectra recorded from ~59.3 to ~61.8 min (see Fig. 5a) and for *R*-alprenolol from ~62.2 to ~65.7 min (see Fig. 5b). Concentrations determined for both enantiomers (25 mM for the *S*-enantiomer and 28 mM for the *R*-enantiomer) indicated a concentration by ITP ~200-fold taking into account that each enantiomer was injected at a concentration of 125 μM. Finally, alprenolol NMR resonances were observed around 1.6, 3.3, 4.2, 4.4, 5.9, 6.9, and 7.1 ppm. This system has also enabled the study of intermolecular interactions between the CDs and the analyte from the NMR spectra observing that aromatic and methyl moieties of *R*- and *S*-alprenolol are identified as two important sites that bind with these CDs (α-CD and sulfated-β-CD) [119]. The hyphenation of CE and FT-IR detection has been made using a flow cell in CE where the IR beam was focused using an external optical focusing unit built in-house and described in detail in [124]. This online coupling of CE-FT-IR has allowed the separation and online distinction of the 3,5-dinitrobenzoyl leucine (DNB-Leu) enantiomers using NACE with *O*-(*tert*-butyl carbamoyl) quinine as chiral selector. Figure 6 depicts the comparison of the IR spectra of DNB-(*R*)-Leu and DNB-(*S*)-Leu dissolved in the carrier electrolyte containing 4 mM of chiral selector. It can be observed a shift in the asymmetric stretch vibration of the carboxylate at 1606/cm as well as a slight change around 1290/cm. Also, the C=O band at 1713/cm is clearly visible in the spectrum of the (*S*)-enantiomer, but the band with the shoulder at 1736/cm is not visible in the spectrum of the (*R*)-enantiomer. Therefore, this novel and attractive detection technique for CE has provided qualitative stereochemical information on the interactions between the chiral selector and the enantiomers [120].

The coupling of CE to MS is a trend in sensitive chiral analysis by CE, which is being used with more frequency during last years. This detection system is universal, selective, and enables obtaining structural information. Table 4 shows the LODs obtained by CE-MS in sensitive chiral analysis by CE. The works included in this table are those in which the lowest LODs were obtained. MS detection has been employed in other works on chiral analysis by CE but LODs close to or above 10^{-5} M were generally obtained [125–133]. The basic instrumentation in CE-MS [134] allows, once the electrophoretic separation is performed, the introduction of the analytes in the mass spectrometer using an interface, usually a sheath liquid interface, which enables the establishment of the electrical contact and the nebulization of the solution. Then, the ionization of the analytes, normally by ESI, is performed before the MS detection (usually by quadrupole or IT). Although in some works a low concentration of chiral selector is directly introduced in the MS obtaining satisfactory results [135], in most works the introduction of the chiral selector in the MS detector is avoided or chiral selectors compatible with MS detection, such us

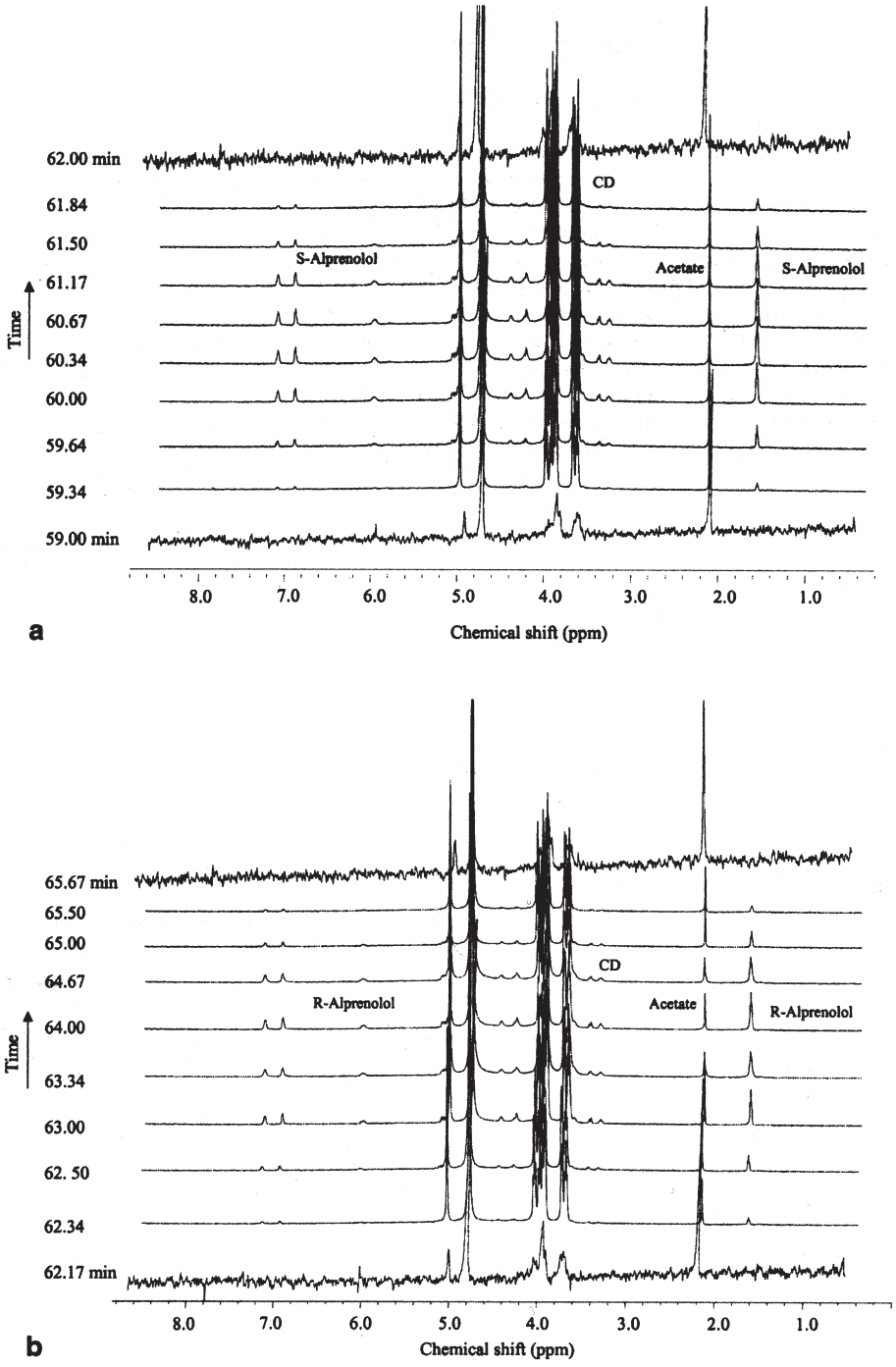

Figure 5. On-flow ITP-NMR spectra of aprenolol enantiomers as a function of runtime. (a) *S*-Alprenolol, spectra at the bottom (59.0 min) and the top (62.0 min) mark the boundaries of the sample plug. Estimated concentration at the peak maximum ~25 mM. (b) *R*-Alprenolol, spectra at the bottom (62.2 min) and the top (65.7 min) mark the boundaries of the sample band. Estimated concentration at the peak maximum ~28 mM. Electrophoretic conditions: fused-silica capillary, l_d = 58 cm and 50 μm ID; leading buffer, 150 mM acetate (buffered to pD 6.0 with acetic acid in D_2O) containing 70 mM α-CD and 0.5 mM sulfated β-CD; terminating electrolyte, 10 mM acetic acid in D_2O; applied voltage, 20 kV. ITP-NMR using a microcoil probe tuned to 500 MHz (reprinted from [119], with permission).

crown ethers [136, 137] are employed. In fact, the introduction of nonvolatile chiral selectors in MS can affect the sensitivity and stability of this detector [138]. In order to avoid the introduction of chiral selectors to the MS, partial-filling and counter-current migration techniques have been used. In the partial-filling technique, a zone of the capillary where the enantiomeric separation takes place is filled with buffer containing the chiral selector, while the zone close to the MS detector, which the separated enantiomers cross before being introduced in the detector, is filled with buffer without chiral selector. The counter-current migration technique is based on the control of the EOF to avoid the chiral selector from reaching the MS detector.

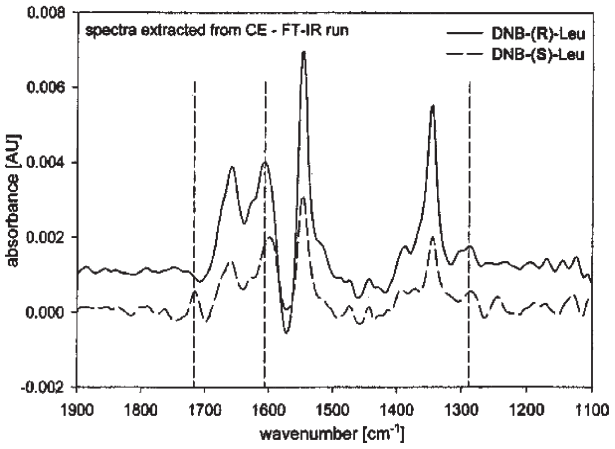

Figure 6. IR spectra of DNB-(*R*)-Leu and DNB-(*S*)-Leu (3 g/L each) dissolved in separation buffer. Electrophoretic conditions: fused-silica capillary, l_d = 34 cm, l_t = 55 cm, and 50 μm ID; separation buffer, 100 mM octanoic acid, 22 mM ammonium hydroxide containing 4 mM *O*-(*tert*-butyl carbamoyl quinine) in ethanol:methanol (60:40); separation temperature, 18°C; applied voltage, −20 kV; hydrodynamic injection by lifting the capillary at the injection side. FT-IR detection using an FT-IR transmission cell with a pathlength of 25 μm, HeNe laser modulation frequency of 150 kHz. Coaddition of 100 scans for each spectrum with a spectral resolution of 8/cm. Reprinted from [120], with permission.

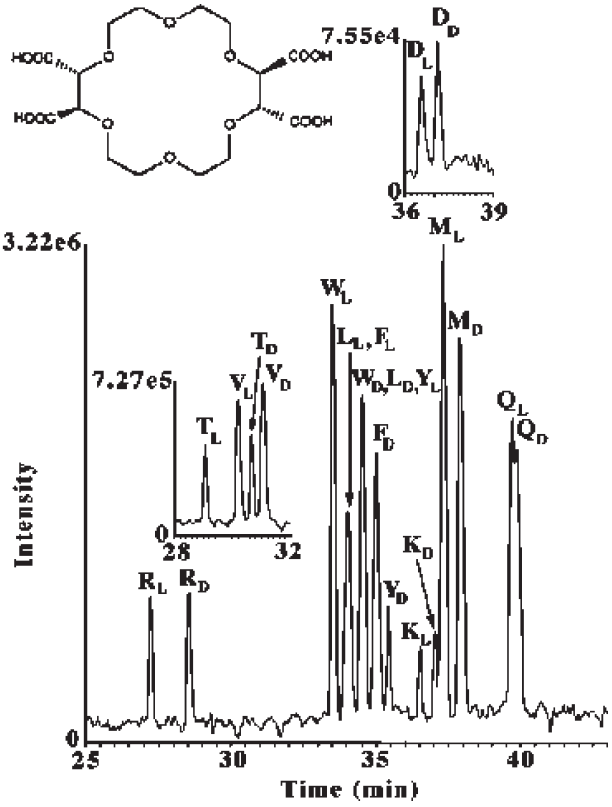

Figure 7. Separation of 11 amino acid enantiomers using a 30 mM $18C_6H_4$ solution as the buffer/chiral selector reagent (top left, shows the chemical structure of $18C_6H_4$). Electrophoretic conditions: fused-silica capillary, $l_d = l_t$ = 115 cm with 20 μm ID; separation buffer, 30 mM $18C_6H_4$; run voltage, 30 kV (augmented with 10 psi of inlet pressure); temperature, 25°C; injection of 2 nL. ESI-MS conditions: SIM positive ion mode (*m/z* range 74.5–250). Reprinted from [137], with permission.

With MS detection, LODs up to 10^{-9} M have been obtained (see Table 4). Although possible, it is not an easy task to improve the detection sensitivity achieved by MS compared with UV-Vis absorption detection for compounds with high molar absorptivity. This is mainly due to the following reasons: (i) the ionization yields achieved during ionization, (ii) the suitability of the BGE, which should be volatile and not to produce interferences, (iii) the dilution produced by the additional liquid employed in the sheath liquid interface used for ESI, which is mainly used in CE-MS, and (iv) the solute nature; thus apolar compounds require an atmospheric pressure chemical ionization (APCI) interface. A high sensitivity of detection was achieved by ESI-MS using a sheathless interface. This setup was used for the separation of 11 underivatized amino acids (2.5×10^{-8} M each one) using a crown ether as BGE and chiral selector (see Fig. 7). Since using the crown ether (30 mM $18C_6H_4$) as BGE/chiral selector, the absolute intensities obtained were only about four times lower than those obtained under formic acid (1 M), it was found to be compatible with ESI-MS detection [137]. It is important to remark that derivatization of samples may increase sensitivity in MS detection because it enables to analyze them in a mass region with lower noise. Thus, two different derivatization protocols for amino acids using dansyl chloride (DNS) and FITC were compared observing better mass sensitivity for FITC-amino acids (LODs in the μM range) [139]. CE-ESI-MS has also been used for the sensitive determination of chiral compounds in biological samples such as urine [135, 140, 141], plasma [142], serum [143], and *in vivo* samples [144], and for the identification of drugs and their metabolites [145, 140].

Electrochemical detection has not been much used in chiral analysis by CE. In electrochemical detection (where amperometric, voltamperometric, conductimetric, and potentiometric detection are included) only amperometric detection has been used for the detection of enantiomers separated by CE. In amperometric detection, carbon or metallic electrodes of different geometries (disk, cylindric, and tubular) may be used and their potential should be isolated from the applied voltage of the electrophoretic system. With this aim, the electrochemical detection is

performed at the end or out of the separation capillary [146]. Table 4 shows the LODs obtained in sensitive chiral analysis by CE using amperometric detection. LODs up to 3×10^{-9} M have been achieved. Thus, several drugs (promethazine, neurotransmitters such as isoproterenol, epinephrine, and norepinephrine) have been detected from 3×10^{-9} to 5×10^{-6} M [83, 84, 147, 148]. An interesting example is shown in Fig. 8, which shows the enantioseparation of a mixture of the racemates of isoproterenol, epinephrine, and norepinephrine in tris-phosphate buffer (pH 2.5) with DM-β-CD. In this case, the detection was performed by using end-column microfabricated interdigitated electrodes, which enabled to obtain LODs of 5 μM for the three neurotransmitters [148]. Finally, it is remarkable that electrochemical detection seems to be very promising in CE microchips due to its compatibility with microfabrication techniques [149].

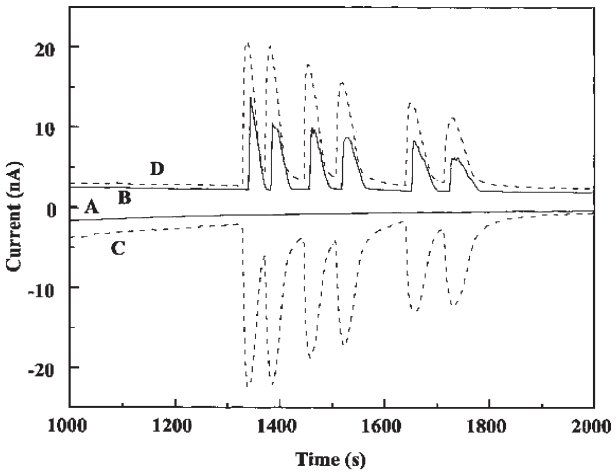

Figure 8. Enantiomeric separation of three neurotransmitters (separation order: norepinephrine, epinephrine, and isoproterenol) in Tris-phosphate buffer (pH 2.5) with DM-β-CD using end-column microfabricated interdigitated electrodes. Electrophoretic conditions: fused-silica capillary, $l_d = l_t = 80$ cm with 20 μm ID; separation buffer, 50 mM Tris-phosphate buffer (pH 2.5) with 15 mM DM-β-CD; run voltage, 25 kV; temperature, 25°C; electrokinetic injection, 25 kV for 20 s. Electrochemical detection in an end-column microfabricated chip. Reprinted from [148], with permission.

4 Conclusions

On-column UV-Vis absorption detection is the most widely used detection system in CE but its sensitivity depends on the optical pathway, which corresponds to the inner diameter of the capillary (usually, 50 or 75 μm). Although for some chiral compounds with chromophore groups LODs $\sim 10^{-6}$–10^{-5} M may be obtained, there are many cases in which this detection sensitivity is not achieved (compounds with low molar absorptivity) or is insufficient (biological samples, environmental samples, enantiomeric purity determinations, food analysis, *etc.*).

The use of offline sample treatment techniques has enabled to achieve appropriate concentration LODs with UV-Vis absorption detection. It is important to emphasize that the use of online sample treatment techniques in CE has shown to be a good strategy for the analysis of biological samples. A much more simple strategy is the use of online preconcentration techniques based on electrophoretic principles, which are usually performed in the same separation capillary.

The alternative detection systems to UV-Vis absorption detection used in sensitive chiral analysis by CE are spectroscopic detection (LIF, quenched phosphorescence, and NMR), MS detection, and amperometric detection. FT-IR detection has recently been employed in chiral analysis but sensitivity obtained has to be improved. Although the use of LIF detection is the best alternative for many applications (sensitive detection of amino acids enantioseparated by CE), MS detection is being more utilized in last years. However, the problem associated to the use of nonvolatile chiral selectors, as CDs, in CE-MS provokes that their introduction in the mass spectrometer should be avoided. It is interesting to remark that some of the last research works are focused on the development of chiral selectors compatible with MS detection, this aspect being of great interest in the coupling CE-MS.

Finally, the best LOD reported in this review is $\sim 10^{-10}$ M using an online coupling of SPE with a CE system and UV-Vis absorption detection [90]. However, lower LODs are expected to be possible by combining several of the described strategies, that is, by combining sample treatment techniques, on-column preconcentration techniques, and alternative detection systems to UV-Vis absorption, combinations that look very promising to enable the improvement of sensitivity in chiral analysis by CE.

5 Future prospects

The sensitive analysis of chiral compounds in complex matrices, *i.e.*, in biological samples, usually requires a sample treatment step before the injection in the CE system, and as a consequence, although challenging, the online coupling of sample treatment systems to CE seems to be very promising because it enables the automatization of the analytical process. However, this strat-

egy needs special setups while the use of on-column preconcentration techniques based on electrophoretic principles (mainly those based on special injection techniques such as stacking and sweeping) is more available and easier, and only needs an appropriate knowledge of the sample and the matrix. The development of new applications in the chiral field with new spectroscopic detectors such as phosphorescence, NMR, and FT-IR can be expected in the near future. In addition, new detectors such as circular dichroism will be coupled to CE for chiral analysis. The development of new applications in the chiral field by CE-MS and the study of new chiral selectors compatible with this detection system is also a future prospect in sensitive chiral analysis by CE. Finally, microchip electrophoresis uses tiny micromachined devices to perform chiral separations in seconds, the development and application of different approaches to improve the detection sensitivity being very challenging.

M. L. Marina thanks the Ministry of Science and Technology (Spain) for the research project BQU2003–03638. C. García-Ruiz also thanks the same Ministry for the Ramón y Cajal program (RYC-2003–001).

6 References

[1] Schmitt, U., Branch, S. K., Holzgrabe, U., *J. Sep. Sci.* 2002, *25*, 959–974.

[2] Blomberg, L. G., Wan, H., *Electrophoresis* 2000, *21*, 1940–1952.

[3] Riekkola, M. L., Wiedmer, S. K., *Process Control. Qual.* 1997, *10*, 169–180.

[4] Zaugg, S., Thormann, W., *J. Chromatogr. A* 2000, *875*, 27–41.

[5] Eash, D. T., Bushway, R. J., *J. Chromatogr. A* 2000, *880*, 281–294.

[6] Chankvetadze, B., *J. Chromatogr. A* 1997, *792*, 269–295.

[7] Chankvetadze, B., *Trends Anal. Chem.* 1999, *18*, 485–498.

[8] Chankvetadze, B., Blaschke, G., *J. Chromatogr. A* 2001, *906*, 309–363.

[9] Blanco, M., Valverde, I., *Trends Anal. Chem.* 2003, *22*, 428–439.

[10] Rizzi, A., *Electrophoresis* 2001, *22*, 3079–3106.

[11] Szejtli, J. J., *Chem. Rev.* 1998, *98*, 1743–1753.

[12] Fanali, S., *J. Chromatogr. A* 1997, *792*, 227–267.

[13] Rizzi, A., *Electrophoresis* 2001, *22*, 3079–3106.

[14] Terabe, S., Shibata, M., Miyashita, Y., *J. Chromatogr.* 1989, *480*, 403–411.

[15] Otsuka, K., Terabe, S., *J. Chromatogr. A* 2000, *875*, 163–178.

[16] El Rassi, Z., *J. Chromatogr. A* 2000, *875*, 207–233.

[17] Yarabe, H. H., Billiot, E., Warner, I. M., *J. Chromatogr. A* 2000, *875*, 179–206.

[18] Palmer, C. P., *Electrophoresis* 2002, *23*, 3993–4004.

[19] Billiot, E., Macossay, J., Thibodeaux, S., Shamsi, S. A., Warner, I. M., *Anal. Chem.* 1998, *70*, 1375–1381.

[20] Rugutt, J. K., Yarabe, H. H., Shamsi, S. A., Billodeaux, D. R., Fronczek, F. R., Warner, I. M., *Anal. Chem.* 2000, *72*, 3887–3895.

[21] Ward, T. J., Farris III, A. B., *J. Chromatogr. A* 2001, *906*, 73–89.

[22] Aboul-Enein, H. Y., Ali, I., *Chromatographia* 2000, *52*, 679–691.

[23] Desiderio, C., Fanali, S., *J. Chromatogr. A* 1998, *807*, 37–56.

[24] Armstrong, D. W., Nair, U. B., *Electrophoresis* 1997, *18*, 2331–2342.

[25] Fangmin, H., He, H., *Chin. J. Anal. Chem.* 2002, *30*, 621–626.

[26] Tanaka, Y., Terabe, S., *J. Biochem. Biophys. Methods* 2001, *48*, 103–116.

[27] Haginaka, J., *J. Chromatogr. A* 2000, *875*, 235–254.

[28] Nishi, H., Kuwahara, Y., *J. Biochem. Biophys. Methods* 2002, *48*, 89–102.

[29] Nishi, H., Kuwahara, Y., *J. Pharm. Biomed. Anal.* 2001, *27*, 577–585.

[30] Nishi, H., Nakamura, K., Nakai, H., Sato, T., *Anal. Chem.* 1995, *67*, 2334–2341.

[31] Schmid, M. G., Grobuschek, N., Lecnik, O., Gübitz, G., *J. Biochem. Biophys. Methods* 2001, *48*, 143–154.

[32] Gübitz, G., Schmid, M. G., *J. Chromatogr. A* 1997, *792*, 179–225.

[33] Gübitz, G., Schmid, M. G., *Biopharm. Drug Dispos.* 2001, *22*, 291–336.

[34] Gübitz, G., Schmid, M. G., *Enantiomer* 2000, *5*, 5–11.

[35] Kang, J., Wistuba, D., Schurig, V., *Electrophoresis* 2002, *23*, 4005–4021.

[36] Wistuba, D., Schurig, V., *Electrophoresis* 2000, *21*, 4136–4158.

[37] Schurig, V., Wistuba, D., *Electrophoresis* 1999, *20*, 2313–2328.

[38] Wistuba, D., Cabrera, K., Schurig, V., *Electrophoresis* 2001, *22*, 2600–2605.

[39] Carter-Finch, A. S., Smith, N. W., *J. Chromatogr. A* 1999, *848*, 375–385.

[40] Girod, M., Chankvetadze, B., Blaschke, G., *J. Chromatogr. A* 2000, *887*, 439–455.

[41] Mayer, S., Briand, X., Francotte, E., *J. Chromatogr. A* 2000, *875*, 331–339.

[42] Mangelings, D., Hardies, N., Maftouh, M., Suteu, C., Massart, D. L., Heyden, Y. V., *Electrophoresis* 2003, *24*, 2567–2576.

[43] Lämmerhofer, M., Tobler, E., Lindner, W., *J. Chromatogr. A* 2000, *887*, 421–437.

[44] Lämmerhofer, M., Peters, E. C., Yu, C., Svec, F., Fréchet, J. M. J., Lindner, W., *Anal. Chem.* 2000, *72*, 4614–4622.

[45] Wistuba, D., Schurig, V., *Electrophoresis* 2000, *21*, 3152–3159.

[46] Quaglia, M., De Lorenzi, E., Sulitzky, C., Massolini, G., Sellergren, B., *Analyst* 2001, *126*, 1495–1498.

[47] Chen, Z. L., Hobo, T., *Electrophoresis* 2001, *22*, 3339–3346.

[48] Kang, J. W., Wistuba, D., Schurig, V., *Electrophoresis* 2002, *23*, 1116–1120.

[49] Chen, Z. L., Ozawa, H., Uchiyama, K., Hobo, T., *Electrophoresis* 2003, *24*, 2550–2558.

[50] Fanali, S., Catarcini, P., Blaschke, G., Chankvetadze, B., *Electrophoresis* 2001, *22*, 3131–3151.

[51] Fujimoto, C., *Anal. Sci.* 2002, *18*, 19–25.

[52] Lämmerhofer, M., Svec, F., Fréchet, J. M. J., Lindner, W., *Trends Anal. Chem.* 2000, *19*, 676–698.

[53] Dermaux, A., Sandra, P., *Electrophoresis* 1999, *20*, 3027–3065.

[54] Zheng, J., Shamsi, S. A., *Anal. Chem.* 2003, *75*, 6295–6305.

[55] Fillet, M., Servais, A. C., Crommen, J., *Electrophoresis* 2003, *24*, 1499–1507.

[56] Wang, F., Khaledi, M. G., *J. Chromatogr. A* 1998, *817*, 121–128.

[57] Bjørnsdottir, I., Hansen, S. H., Terabe, S., *J. Chromatogr. A* 1996, *745*, 37–44.

[58] Piette, V., Fillet, M., Lindner, W., Crommen, J., *J. Chromatogr. A* 2000, *875*, 353–360.

[59] Piette, V., Lämmerhofer, M., Lindner, W., Crommen, J., *Chirality* 1999, *11*, 622–630.

[60] Piette, V., Lämmerhofer, M., Lindner, W., Crommen, J., *J. Chromatogr. A* 2003, *987*, 421–427.

[61] Piette, V., Lindner, W., Crommen, J., *J. Chromatogr. A* 2000, *894*, 63–71.

[62] Piette, V., Lindner, W., Crommen, J., *J. Chromatogr. A* 2002, *948*, 295–302.

[63] Kataoka, H., *Trends Anal. Chem.* 2003, *22*, 232–244.

[64] Grard, S., Morin, P., Ribet, J. P., *Electrophoresis* 2002, *23*, 2399–2407.

[65] Siluveru, M., Stewart, J. T., *J. Chromatogr. B* 1997, *691*, 217–222.

[66] Lanz, M., Thormann, W., *Electrophoresis* 1996, *17*, 1945–1949.

[67] Lanz, M., Brenneisen, R., Thormann, W., *Electrophoresis* 1997, *18*, 1035–1043.

[68] Hsieh, Y. Z., Huang, H. Y., *J. Chromatogr. A* 1996, *745*, 217–223.

[69] Polcaro, C. M., Marra, C., Desiderio, C., Fanali, S., *Electrophoresis* 1999, *20*, 2420–2424.

[70] Kodama, S., Yamamoto, A., Saitoh, Y., Matsunaga, A., Okamura, K., Kizu, R., Hayakawa, K., *J. Agric. Food Chem.* 2002, *50*, 1312–1317.

[71] Kubilius, D. T., Bushway, R. J., *J. Liq. Chromatogr. Rel. Technol.* 1999, *22*, 593–601.

[72] Kodama, S., Yamamoto, A., Ohura, T., Matsunaga, A., Kanbe, T., *J. Agric. Food Chem.* 2003, *51*, 6128–6131.

[73] Jabor, V. A. P., Lanchote, V. L., Bonato, P. L., *Electrophoresis* 2001, *22*, 1406–1412.

[74] Jabor, V. A. P., Bonato, P. S., *Electrophoresis* 2001, *22*, 1399–1405.

[75] Desiderio, C., Rudaz, S., Raggi, M. A., Fanali, S., *Electrophoresis* 1999, *20*, 3432–3438.

[76] Jabor, V. A. P., Lanchote, V. L., Bonato, P. S., *Electrophoresis* 2002, *23*, 3041–3047.

[77] Glowka, F. K., *J. Pharm. Biomed. Anal.* 2002, *30*, 1035–1045.

[78] Awadallah, B., Schmidt, P. C., Wahl, M. A., *J. Chromatogr. A* 2003, *988*, 135–143.

[79] García-Ruiz, C., Andrés, R., Valera, J. L., Laborda, F., Marina, M. L., *J. Sep. Sci.* 2002, *25*, 17–22.

[80] Kodama, S., Yamamato, A., Matsunaga, A., Okamura, K., Kizu, R., Hayakawa, K., *J. Sep. Sci.* 2002, *25*, 1055–1062.

[81] Desiderio, C., Polcaro, C. M., Padiglioni, P., Fanali, S., *J. Chromatogr. A* 1997, *781*, 503–513.

[82] Andersen, S., Halvorsen, T. G., Pedersen-Bjergaard, S., Rasmussen, K. E., Tanum, L., Refsum, H., *J. Pharm. Biomed. Anal.* 2003, *33*, 263–273.

[83] Hadwiger, M. E., Park, S., Torchia, S. R., Lunte, C. E., *J. Pharm. Biomed. Anal.* 1997, *681*, 621–629.

[84] Hadwiger, M. E., Torchia, S. R., Park, S., Biggin, M. E., Lunte, C. E., *J. Chromatogr. B* 1996, *681*, 241–249.

[85] Pálmarsdóttir, S., Mathiasson, L., Jonsson, J. A., Edholm, L.-E., *J. Capil. Electrophor.* 1996, *5*, 255–260.

[86] Pálmarsdóttir, S., Mathiasson, L., Jönsson, J. Å., Edholm, L.-E., *J. Chromatogr. B* 1997, *688*, 127–134.

[87] Thompson, J. E., Vickroy, T. W., Kennedy, R. T., *Anal. Chem.* 1999, *71*, 2379–2384.

[88] O'Brien, K. B., Esguerra, M., Klug, C. T., Miller, R. F., Bowser, M. T., *Electrophoresis* 2003, *24*, 1227–1235.

[89] Mardones, C., Ríos, A., Valcárcel, M., Cicciarelli, R., *J. Chromatogr. A* 1999, *849*, 609–616.

[90] Petersson, M., Wahlund, K.-G., Nilsson, S., *J. Chromatogr. A* 1999, *841*, 249–261.

[91] Urbánek, M., Křivánková, L., Boček, P., *Electrophoresis* 2003, *24*, 466–485.

[92] Kim, J. B., Terabe, S., *J. Pharm. Biomed. Anal.* 2003, *30*, 1625–1643.

[93] Chien, R.-L., *Electrophoresis* 2003, *24*, 486–497.

[94] Quirino, J. P., Terabe, S., *Science* 1998, *282*, 465–468.

[95] Quirino, J. P., Terabe, S., *Anal. Chem.* 1999, *71*, 1638–1644.

[96] Quirino, J. P., Kim, J.-B., Terabe, S., *J. Chromatogr. A* 2002, *965*, 357–373.

[97] Lanz, M., Caslavska, J., Thormann, W., *Electrophoresis* 1998, *19*, 1081–1091.

[98] Danková, M., Kaniansky, D., Fanali, S., Ivanyi, F., *J. Chromatogr. A* 1999, *838*, 31–43.

[99] Fanali, S., Desiderio, C., Olvecka, E., Kaniansky, D., Vojtek, M., Ferancova, A., *J. High Resolut. Chromatogr.* 2000, *23*, 531–538.

[100] Grard, S., Morin, P., Ribet, J. P., *Electrophoresis* 2002, *23*, 2399–2407.

[101] Choy, T. M. H., Chan, W. H., Lee, A. W. M., Huie, C. W., *Electrophoresis* 2003, *24*, 3116–3123.

[102] Kuijt, J., García-Ruiz, C., Stroomberg, G. J., Marina, M. L., Ariese, F., Brinkman, U. A. Th., Gooijer, C., *J. Chromatogr. A* 2001, *907*, 291–299.

[103] Ueda, T., Kitamura, F., Mitchell, R., Metcalf, T., Kuwana, T., Nakamoto, A., *Anal. Chem.* 1991, *63*, 2979–2981.

[104] Kaneta, T., Shiba, H., Imasaka, T., *J. Chromatogr. A* 1998, *805*, 295–300.

[105] Liu, Y. M., Zhao, S. L., *LC GC North Am.* 2001, *19*, 414–420.

[106] Zhao, S. L., Liu, Y. M., *Electrophoresis* 2001, *22*, 2769–2774.

[107] Zhao, S. L., Feng, Y. Z., LeBlanc, M. H., Liu, Y. M., *J. Chromatogr. B* 2001, *762*, 97–101.

[108] Simó, C., Barbas, C., Cifuentes, A., *J. Agric. Food Chem.* 2002, *50*, 5288–5293.

[109] Chankvetadze, B., Burjanadze, N., Blaschke, G., *Electrophoresis* 2001, *22*, 3281–3285.

[110] Albrecht, C., Thormann, W., *J. Chromatogr. A* 1998, *802*, 115–120.

[111] Soetebeer, U. B., Schierenberg, M. O., Schulz, H., Andresen, P., Blaschke, G., *J. Chromatogr. B* 2001, *765*, 3–13.

[112] Mechref, Y., El Rassi, Z., *Anal. Chem.* 1996, *68*, 1771–1777.

[113] García-Ruiz, C., Siderius, M., Ariese, F., Gooijer, C., *Anal. Chem.* 2004, *76*, 399–403.

[114] Kuijt, J., Ariese, F., Brinkman, U. A. T., Gooijer, C., *Electrophoresis* 2003, *24*, 1193–1199.

[115] Kuijt, J., Brinkman, U. A. Th., Gooijer, C., *Anal. Chem.* 1999, *71*, 1384–1390.

[116] Kuijt, J., Brinkman, U. A. Th., Gooijer, C., *Electrophoresis* 2000, *21*, 1305–1311.

[117] Kuijt, J., van Teylingen, R., Nijbacker, T., Ariese, F., Brinkman, U. A. Th., Gooijer, C., *Anal. Chem* 2001, *73*, 5026–5029.

[118] Kuijt, J., Arraez Roman, D., Ariese, F., Brinkman, U. A. Th., Gooijer, C., *Anal. Chem* 2002, *74*, 5139–5145.

[119] Jayawickrama, D. A., Sweedler, J. V., *Anal. Bioanal. Chem.* 2004, *378*, 1528–1535.

[120] Hinsmann, P., Arce, L., Svasek, P., Lämmerhofer, M., Lendl, B., *Appl. Spectrosc.* 2004, *58*, 662–666.

[121] Olson, D. L., Peck, T. L., Webb, A. G., Magin, R. L., Sweedler, J. V., *Science* 1995, *270*, 1967–1970.

[122] Kautz, R. A., Lacey, M. E., Wolters, A. M., Foret, F., Webb, A. G., Karger, B. L., Sweedler, J. V., *J. Am. Chem. Soc.* 2001, *123*, 3159–3160.

[123] Wolters, A. M., Jayawickrama, D. A., Larive, C. K., Sweedler, J. V., *Anal. Chem.* 2002, *74*, 2306–2310.

[124] Kölhed, M., Hinsmann, P., Svasek, P., Frank, J., Karlberg, B., Lendl, B., *Anal. Chem.* 2002, *74*, 3843–3848.

[125] Gaus, H. J., Gogus, Z. Z., Schmeer, K., Behnke, B., Kovar, K. A., Bayer, E., *J. Chromatogr. A* 1996, *735*, 221–226.

[126] Lu, W. Z., Cole, R. B., *J. Chromatogr. B* 1998, *714*, 69–75.

[127] Tanaka, Y., Kishimoto, Y., Terabe, S., *J. Chromatogr. A* 1998, *802*, 83–88.

[128] Javerfalk, E. M., Amini, A., Westerlund, D., Andren, P. E., *J. Mass Spectrom.* 1998, *33*, 183–186.

[129] Tanaka, Y., Otsuka, K., Terabe, S., *J. Chromatogr. A* 2000, *875*, 323–330.

[130] Cherkaoui, S., Veuthey, J. L., *J. Pharm. Biomed. Anal.* 2002, *27*, 615–626.

[131] Shamsi, S. A., *Anal. Chem.* 2001, *73*, 5103–5108.

[132] Rudaz, S., Calleri, E., Geiser, L., Cherkaoui, S., Prat, J., Veuthey, J.-L., *Electrophoresis* 2003, *24*, 2633–2641.

[133] Iwata, Y. T., Kanamori, T., Ohmae, Y., Tsujikawa, K., Inoue, H., Kishi, T., *Electrophoresis* 2003, *24*, 1770–1776.

[134] Olivares, J. A., Nguyen, N. T., Yonker, C. R., Smith, R. D., *Anal. Chem.* 1987, *59*, 1230–1232.

[135] Sheppard, R. L., Tong, X. C., Cai, J. Y., Henion, J. D., *Anal. Chem.* 1995, *67*, 2054–2058.

[136] Moini, M., Schultz, C. L., Mahmood, H., *Anal. Chem.* 2003, *75*, 6282–6287.

[137] Schultz, C. L., Moini, M., *Anal. Chem.* 2003, *75*, 1508–1513.

[138] Shamsi, A. A., *Electrophoresis* 2002, *23*, 4036–4051.

[139] Simó, C., Rizzi, A., Barbas, C., Cifuentes, A., *Electrophoresis* 2005, *26*, 1432–1441.

[140] Iio, R., Chinaka, S., Tanaka, S., Takayama, N., Hayakawa, K., *Analyst* 2003, *128*, 646–650.

[141] Iio, R., Chinaza, S., Takayama, N., Hayakawa, K., *Anal. Sci.* 2005, *21*, 15–19.

[142] Toussaint, B., Palmer, M., Chiap, P., Hubert, P., Crommen, J., *Electrophoresis* 2001, *22*, 1363–1372.

[143] Cherkaoui, S., Rudaz, S., Varesio, E., Veuthey, J. L., *Electrophoresis* 2001, *22*, 3308–3315.

[144] Kindt, E. K., Kurzyniec, S., Wang, S. C., Kilby, G., Rossi, D. T., *J. Pharm. Biomed. Anal.* 2003, *31*, 893–904.

[145] Rudaz, S., Cherkaoui, S., Dayer, P., Fanali, S., Veuthey, J. L., *J. Chromatogr. A* 2000, *868*, 295–303.

[146] Baldwin, R. P., *Electrophoresis* 2000, *21*, 4017–4028.

[147] Wang, R. Y., Lu, X. N., Wu, M. J., *J. Sep. Sci.* 2001, *24*, 658–662.

[148] Male, K. B., Luong, J. H. T., *J. Chromatogr. A* 2003, *1003*, 167–178.

[149] Wang, J., *Talanta* 2002, *56*, 223–231.

[150] Kurth, B., Blaschke, G., *Electrophoresis* 1999, *20*, 555–563.

[151] Pálmarsdóttir, S., Edholm, L.-H., *J. Chromatogr. A* 1995, *693*, 131–143.

[152] Grard, S., Morin, P., Dreux, M., Ribet, J. P., *J. Chromatogr. A* 2001, *926*, 3–10.

