CHIRAL CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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Abbreviations:

BNP, Binaphthyl-2,2'diylhydrogen phosphate; **BOH**, 1,1'-binaphthol; **CDMPC**, cellulose tris(3,5-dimethylphenylcarbamate); **CIS**, coordination ion spray; **CMBCD**, carboxymethyl-β-cyclodextrin; **CS**, chiral selector; **18C6TCA**, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid; **DASBCD**, heptakis(2,6-diacetyl-6-sulfato)-β-cyclodextrin; **DIKGA**, (2)-2,3:4,6-di-O-isopropylidene-2-keto-L-gulonic acid; **DMBCD**, heptakis(2,6-di-O-methyl)-β-cyclodextrin; **DMSBCD**, heptakis(2,3-di-O-methyl-6-O-sulfo)-β-cyclodextrin; **HPBCD**, hydroxypropyl-β-CD; **HSBCD**, highly sulfonated β-cyclodextrin; **HSGCD**, highly sulfated γ-cyclodextrin; **MBCD**, methyl-β-cyclodextrin; **PABCD**, 6-monodeoxy-6-mono(3-hydroxy)propylamine-β-cyclodextrin; **PEAs**, phenylethylamines; **poly-L,L-SUCLV**, polysodium N-undecenoxycarbonyl-L,L-leucyl-valinate; **poly-L-SUCAAS**, polysodium N-undecenoxycarbonyl-L-amino acid sulfates; **poly-L-SUCL**, polysodium N-

undecenoxycarbonyl-L-leucinate; **poly-L-SUCLS**, polysodium N-undecenoxycarbonyl-L-leucine sulfate; **poly-L-SUCV**, polysodium N-undecenoxycarbonyl-L-valinate; **poly-L-SUL**, polysodium N-undecanoyl-L-leucinate; **poly-L,L-SULV**, polysodium N-undecenoyl-L,L-leucyl-valinate; **poly-L-SUV**, polysodium N-undecanoyl-L-valinate; **QABCD**, quaternary ammonium β-cyclodextrin; **SBCD**, sulfated β-cyclodextrin; **SBEBCD**, sulfobutyl ether β-cyclodextrin; **SuccGCD**, succinyl-γ-cyclodextrin; **TMABCD**, 2-hydroxypropyltrimethylammonium-β-cyclodextrin; **TMBCD**, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin.

Keywords: Enantiomers, chiral analysis, CEC-MS, MEKC-MS, CZE-MS

ABSTRACT

This review article addresses the developments and applications of capillary electromigration methods coupled on-line with mass spectrometry for chiral analysis. The multiple enantiomeric applications of this hyphenated technology are covered including chiral analysis of drugs, food compounds, pesticides, natural metabolites, etc., in different matrices such as plasma, urine, medicines, foods, etc. This work intends to provide an updated overview (including works published till September 2009) on the principal chiral applications carried out by CZE-MS, CEC-MS and MEKC-MS, discussing their main advantages and drawbacks in all their different areas of application as well as their foreseeable development in the non-distant future.

CONTENTS

1. INTRODUCTION

In the past thirty years, there has been a growing interest in the separation and quantification of enantiomers from many different fields including pharmaceutical, clinical, environmental and food analysis. Thus, chiral analysis is nowadays required by pharmaceutical regulatory authorities due to the different therapeutic effects that enantiomers can have. Whereas one can have the desired biological activity, the other can have none or even adverse effects. Also, in food analysis, enantioselective separations can be used e.g., for identifying adulterated foods and beverages or monitoring microbiological contamination [1].

Numerous analytical techniques have been developed so far to respond to these requirements [2-4]. In this regard, the use of capillary electrophoresis (CE) has shown impressive possibilities for the enantioselective separation of chiral compounds as can be deduced from the large number of reviews published on this topic in the last 10 years (see Table 1) [5-29]. CE exhibits high efficiency, fast migration times and needs low volume of samples (few nanoliters). Moreover, since the chiral selectors are mixed with the background electrolyte (BGE) in chiral CZE and MEKC or forming the stationary phase in chiral-CEC, it is easy to try numerous chiral selectors at different concentrations. The volume in the column is very low and makes affordable to try expensive chiral selectors. The main disadvantage is the relatively poor limit of detection of CE.

On the other hand, analysis of chiral compounds in real samples can be extremely problematic, electropherograms can be very complex, and co-migration becomes frequent and difficult to detect giving rise to unwanted matrix effects. Besides, the analytes of interest will frequently need to be accurately measured at very low concentrations. Therefore, to obtain the

required robustness and sensitivity in a reasonable time, all steps of the analytical method should be optimized. Those steps include the sample preparation, the chiral separation and the detection [3].

One of the major breakthroughs of CE for the determination of chiral analytes in real matrices has been its on-line coupling to MS using electrospray ionization interfaces (ESI). MS allows an unambiguous assignment of the different electrophoretic peaks while MS/MS spectra can also give information about the structure of the analytes. Therefore, the combination of chiral-capillary electromigration methods with mass spectrometry brings about a very powerful hyphenated technique able to provide high sensitivity and selectivity, while it allows solving the identification problems associated with unknown chiral compounds in real samples. Interestingly, it was believed that the development of modern MS, MS/MS and MS^n instruments able to provide everyday better accuracy and mass resolution would permit a direct analysis without the need of a separation and/or sample pre-treatment. However, it has been demonstrated to be unrealistic and more in the case of chiral analysis, where both enantiomers will have the same mass spectra and, therefore, baseline separation will have to be obtained before the MS detection. Moreover, presence of co-migrating species can reduce the MS signal and give errors on the measure (matrix effect) [30]. These limitations can be overcome by using hyphenated techniques as CE-MS.

In spite of all these advantages, it has been repeatedly indicated that one of the main problems during any chiral analysis by CE-MS is the contamination of the ionization source induced by the chiral selector used in the BGE (typically nonvolatile cyclodextrin (CD) derivatives) [31]. As a result, different procedures have been developed in order to overcome this limitation, as reviewed by several authors (see Table 1). Thus, as will be discussed below, different

solutions have been proposed including the use of chiral-CEC-MS in which the fixed chiral stationary phase does not move within the capillary avoiding any interference with the ionization source. However, the peak efficiency obtained by using CEC-MS is usually low, while its robustness still needs to be improved. On the other hand, in CZE-MS and MEKC-MS is common the use of charged CDs, macrocyclic antibiotics, and/or the most frequent partial-filling technique (PFT), being their main goal to prevent the entrance of the chiral selector into the ionization source. In general, these approaches are able to overcome the MS contamination problem although they result in a different selectivity, lower resolution, and lower peak capacity than in normal chiral CZE or MEKC.

2. SCOPE

This review covers the papers published till September 2009 on capillary electromigration techniques coupled with mass spectrometry for enantiomeric analysis of chiral compounds in different matrices. The separation techniques reviewed include capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) on-line coupled to mass spectrometry (MS). This review is divided in three main parts, the first one focuses on the use of chiral CZE-MS, by far the approach most frequently used when combining chiral capillary electromigration analysis and MS. The second and third parts are devoted to the chiral applications of MEKC-MS and CEC-MS, respectively. Useful data on analytes, sample matrices, BGEs (or types of column used in CEC), instruments and interfaces are provided in tables to make easier to the reader the search for a specific application. The review ends with some concluding remarks and future outlooks on the expected developments and applications of these hyphenated methodologies in chiral analysis.

3. CHIRAL CAPILLARY ZONE ELECTROPHORESIS-MASS SPECTROMETRY (CHIRAL-CZE-MS).

The most often used approach for chiral separations by CE-MS is direct enantioseparation by capillary zone electrophoresis-mass spectrometry (CZE-MS) adding to the BGE chiral selectors that involve transient non-covalent complexes formed in dynamic equilibrium. This direct chiral CE mode is also called chiral capillary electrokinetic chromatography (cEKC) and can also include the use of chiral micelles that will be revised in section 4. The main advantage of chiral analysis by CE is the flexibility on the use of a great variety of chiral selectors at low concentrations, reducing in this way the cost of the method. However, the main difficulty of the direct chiral approach by CZE-MS is the contamination of the ion source, since chiral selectors are usually non-volatile compounds. Among chiral selectors, cyclodextrins (CDs) have been demonstrated to be useful compounds for achieving good chiral resolution of a large variety of chiral molecules, mostly because their good solubility in water and the variety of cyclodextrins commercially available [26-28]. CDs are largely the most used compounds among chiral selectors in CE applications due to their particular physico-chemical properties and their great enantiorecognition capability. Moreover, CDs can be obtained with many different properties by modifying the hydroxyl groups of the CD structure. However, other chiral selectors are used for chiral separations in CE, such as chiral micelles, crown ethers, macrocyclic antibiotics, peptides, etc. In Table 2, an overview of the main chiral CZE-MS applications published till September 2009 is presented [32-63]. The majority of the CZE-MS works published until now describe applications in drug development and drug quality control, pharmacokinetic and pharmacodynamic studies. For instance, Lu and Cole [34] studied the suppression of MS analyte signal caused by the

introduction of CDs into the ion source and the dependence of analyte signal intensity and CD concentration. They obtained good chiral separations with acceptable sensitivities of terbutaline and ketamine using 5 and 15 mM heptakis(2,6-di-O-methyl)-β-cyclodextrin (DMBCD), respectively, and of propanolol with 20 mM of hydroxypropyl-β-CD (HPBCD), in acidic volatile buffers and using no special approach to avoid the entrance of CDs in the ion source. The massive entrance of cyclodextrins into the ion source can be reduced using acidic electrolytes. Thus, Lio *et al.* [35] used 1 M formic acid at pH 2.2 to minimize electroosmotic flow inside the capillary. A combination of two different CDs, namely, 3 mM of β-CD and 10 mM DMBCD was used in that work to improve the enantiomeric resolution of amphetamine, methamphetamine, dimethylamphetamine and *p*-hydroxymethamphetamine. The chiral CZE-MS method was applied to the analysis of these compounds in urine from addicts preceded by a solid-phase extraction (SPE) step to remove ammonia [35]. NACE-MS coupling using keptakis(2,6-diacetyl-6-sulfato)-β-cyclodextrin (DASBCD) as chiral selector in an acidic methanolic BGE, has been demonstrated to be useful for the quantitative determination of low levels of the enantiomers of the basic drug salbutamol in human urine previously submitted to a SPE procedure. The obtained LOQ were 20 ng/mL for both R and S salbutamol enantiomers [36].

The analysis of chiral compounds in foods and beverages is a very useful tool to asses their quality, corroborate their authenticity or detect microbiological contaminations. Moreover, determination of specific enantiomers or enantiomer ratios can provide valuable information about adulterations, control of fermentation processes and products, study of the effect of storage time, monitoring of age, etc. Among the food constituents, amino acids represent a very important group of chiral compounds. The potential of enantioseparation by CZE-MS of seven chiral amino acids and one achiral amino acid to detect adulterations in orange juices

was demonstrated [37]. A polymer coating of the inner capillary wall was used in this case to reduce the EOF and minimize in this way the entrance of the neutral β-CD into the ion source [37]. In a recent paper, the non-protein chiral amino acid ornithine was determined by CZE-MS in beers submitted to different fermentation processes. By operating the instrument in tandem mode (MS/MS), a significant increase in the signal was achieved, obtaining LOD of $2.5 \cdot 10^{-9}$ M, two orders of magnitude lower than the LOD obtained with UV detection [38]. The use of modified cyclodextrins was investigated for the separation of five chiral amino acids in vinegars and soybeans by CZE-MS (Figure 1). The new synthesised 3-monodeoxy-3-monoamino-β-cyclodextrin could bring additional ionic interactions due to its positive charge, increasing in this way the selector-analyte complex formation, and allowing in this way the use of low concentration of chiral selectors for their use in the CZE-MS coupling [39]. Analysis of chiral amino acids is also important in clinical studies and neuroclinical applications. Moini *et al.* [40] demonstrated that the chiral crown ether (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18C6TCA) can act as an excellent background electrolyte acting also as a complexation chiral agent for the separation of 11 D/L enantiomers of amino acids and 2 small neurotransmitters by CZE-MS. Amino compounds were monitored with high sensitivity by MS as AA/18C6TCA complexes, reaching nM concentration detection limits.

In order to minimize or avoid the contamination of the ESI-MS with the chiral selector, and improve therefore the sensitivity and the stability of the MS signal, different approaches and strategies have been employed in CZE-MS.

3.1 Chiral selector counter migration.

To avoid in a simple way the entrance of the non-volatile chiral selectors into the ion source counter migration of charged chiral selectors can be used. Charged chiral selectors are of special importance since additional electrostatic interactions with oppositely charged enantiomers can contribute in the chiral recognition. Moreover, they can act as "carrier" for analytes, as it was first noticed by Terabe [64], making possible the chiral separation of neutral enantiomers. Apart of the higher solubility and improved chiral recognition of many charged CDs compared with neutral ones, one of the most important advantages of charged cyclodextrins derivatives is that they can be used in the counter-current mobility in CZE-MS. In these cases, the effective mobility of the charged chiral selector is towards the capillary inlet in order to avoid the contamination of the ion source of the MS with the chiral selector. However, the use of these charged chiral selectors brings about an increasing of the electrical conductivity of the BGE while they can adsorb onto the capillary wall reducing separation efficiency and resolution. Readers interested on this topic can take resort to the very interesting review recently published by Chankvetadze on the use of charged chiral selectors in CE [29].

The positively charged 2-hydroxypropyltrimethylammonium-β-CD (TMABCD) [41] and 6-monodeoxy-6-mono(3-hydroxy)propylamino-β-CD (PABCD) [45], have been used in the counter-current mobility approach for the separation of tropic acid and five acidic drugs (ibuprofen, fenoprofen, flurbiprofen, ketoprofen and indoprofen), respectively. The use of coated capillaries was necessary in both cases in order to avoid the adsorption of the CDs into the inner capillary wall and the subsequent generation of anodic electroosmotic flow.

Also negatively charged cyclodextrins derivatives have been used in CZE-MS trying to reduce the ion source contamination problem. Schulte *et al.* [41] reported the chiral separation

of mianserine, dimimethidene and chlorpheniramine chiral drugs using 0.2 mg/mL of carboxymethyl-β-CD (CMBCD) in an ammonium acetate buffer at pH 3.5. Although higher concentration of CDs (3 mg/mL CMBCD) was needed for the separation of etilefrine enantiomers, migration of the CDs towards the anode avoided the negative effects of the presence of chiral selector in the ion source [41]. The same counter migration principle using highly sulfonated-β-CD (HSBCD) was used to determine the potential chiral interconversion of a novel chiral drug in plasma samples [42]. The anionic heptakis(2,3-di-O-methyl-6-O-sulfo)-β-CD (DMSBCD) was used in a non-aqueous buffer for the chiral separation of mebeverine and five related compounds of pharmaceutical importance (Figure 2). Some ionic suppression was observed in this case from the sodium counter ion of the anionic CD that migrated towards the MS. Nevertheless, the LODs were in the sub-µg/mL range [44].

Since macrocyclic antibiotics, contain ionizable functional groups, the counter-current mobility principle can also be applied to prevent their entrance into the ion source. Using this approach, vancomycin was used for the enantioseparation of racemic non-steroidal anti-inflammatory drugs, as well as two metabolites of the non-steroidal anti-inflammatory drug etodolac in human urine [46].

3.2 Partial filling technique.

The partial filling technique (PFT) proposed by Hjerten and co-workers [65] to keep the UV-absorbing chiral selectors away from the detection window has been proved to be very useful to prevent entering non-volatile chiral selectors into the MS. Several works have been published regarding the use of neutral chiral selectors and applying the PFT with percentages of the capillary filled from 50 to 90% [47-55]. Most of the works reported comprise

pharmaceutical and clinical research including enantioselective metabolism, bioavailability, and elimination of chiral drugs. One of the first CZE-MS methods using PFT employed methyl-β-cyclodextrins (MBCD) for chiral separation of drugs [47]. Since then, several research groups used this approach to avoid the entrance of the neutral cyclodextrins in the ion source [47-55]. HPBCD was used in CZE-MS for the chiral separation of a chiral adrenoreceptor antagonist, filling 80% of an uncoated capillary [50]. When decreasing the percentage of filled capillary lower resolution between enantiomers was obtained but higher peak efficiencies and signal/noise ratios were observed. Using MS detection sensitivity was enhanced by a factor of 30 compared to CZE-UV, reaching LOD of each adrenoreceptor antagonist enantiomer of 5 ng/mL [50]. PFT using DMBCD as chiral selector was also very useful for the determination by CZE-MS of enantiomers of clenbuterol and salbutamol in plasma, reaching LOD values of 0.22 µg/mL [52]. A NACE-MS method filling 55% of the capillary with the chiral selector (2)-2,3:4,6-di-O-isopropylidene-2-keto-L-gulonic acid (DIKGA) was proposed to separate pronethalol enantiomers [54]. In a recent work [55], the separation of 5 chiral dipeptides was accomplished by filling 53% of the capillary with the chiral crown ether 18C6TCA in a low pH buffer. Despite of the low percentage of the capillary filled a good separation of the chiral peptides was obtained.

Many chiral CZE-MS works published so far combine CS counter migration and PFT. Thus, positively charged chiral selectors were combined with polyacrylamide-coated capillaries to suppress EOF and avoid adsorption of the chiral selectors onto the inner capillary wall. The separation of tropic acid was studied by Tanaka *et al.* [48] using 70% of the capillary filled with quaternary ammonium β-CD (QABCD) as chiral selector. In the same work, several methods were also described using the chiral selector avidin, which migrated towards the cathode (injection end) at pH 6. Using a 70% filled capillary with the chiral selector good

stereoselective resolution of acidic pharmaceuticals was obtained, including arylpropionic acids ibuprofen, ketoprofen, and the anticoagulant warfarin.

The negatively charged sulfobutyl ether β-CD (SBEBCD) was used to obtain baseline resolution of tramadol and 5 of its phase I metabolites. A 90% filled polyvinylalcohol-coated capillary with the CS was used for the determination of these compounds in plasma collected 2 h after the administration of 100 mg of tramadol hydrochloride to a healthy volunteer [56]. The chiral separation of the antidepressant drug venlafaxine and 3 of its phase I metabolites was studied by filling 90% of a PVA-coated capillary wit 40 mM ammonium acetate buffer at pH 4 containing 2 mg/mL of CMBCD. All compounds excepting one of the three metabolites were well resolved [53]. Sulfated β-CD (SBCD) showed to be more effective for the counter current PFT than the negatively charged SBEBCD and CMBCD for the enantioseparation of the three anaesthetic drugs ketamine, prilocaine and mepivacaine [57].

When no capillary coating is used, BGE pHs lower than 2.5 were used in order to avoid the entrance of the CDs into the ion source by the effect of the EOF. Moreover, there is a trend on the use of lower percentage of filled capillary with the CS when no capillary coating is used [58-63]. For example, Schappler *et al.* [60] developed a CE-MS method to analyze plasma samples containing five amphetamine derivatives and the two drugs tramadol and methadone. 50% of the capillary was filled with 0.15% highly sulfated γ-CD (HSGCD) in a 20 mM ammonium formate buffer at pH 2.5, obtaining a LOD of 0.5 ppb for each enantiomer (Figure 3). In a recent paper, the determination of D and L carnitine in different infant formulas is described [63]. Succinyl-γ-cyclodextrin (SuccGCD) was used filling 50% of the capillary with this CS in a 0.5 M ammonium formate BGE at pH 2.5. In order to improve sensitivity and selectivity of the CZE-MS method, MS/MS experiments with an ion trap analyzer were

carried out. Thus, a 100-fold sensitivity enhancement with respect to UV detection was obtained, achieving LOD of 100 ng/g for D-carnitine.

3.3 Other approaches.

Although in a much less extent, indirect chiral separation has also been used in CZE-MS. Indirect chiral separation implies the formation, using pure chiral reagents, of covalent diastereomers that inherently show different electrophoretic mobilities in achiral BGEs. The main drawbacks of this approach are that a high enantiomeric purity of the derivatization reagent is needed while the derivatization procedure is usually a time-consuming step. Thus, Day *et al.* [66] described the chiral derivatization of D/L-selenomethionine with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent). The obtained diastereomers were then analyzed by CZE-ICP MS using a 30 mM ammonium phosphate buffer at pH 3.3 and working in reverse CE polarity. The method allowed the analysis of the derivatized selenomethionine diastereoisomers in spiked selenized yeasts samples, together with small peptides containing selenium present in this kind of samples.

Other strategies have also been developed to avoid the entrance of the chiral selector in the ion source. As stated above, anionic CS are used in normal polarity CE mode to avoid the contamination of the MS with these non-volatile compounds, however, the simultaneous analysis of nine amphetamines, reported by Iwata *et al.* [67] was only allowed when the anionic HSGCD was used in reversed polarity CE. In these conditions the amphetamine (positively charged) and HSGCD (negatively charged) complexes migrated towards the ion source. It was found that the amphetamines were dissociated from the HSGCD in the ion source. Working in the positive ESI mode the positively charged amphetamines entered into

the MS analyzer while dissociated or HSGCD excess were not introduced into the MS reducing its deleterious influence. On the other hand, due to the counter migration of the analytes and the CS, long analysis times were required (about 50 min).

Non-continuous flow CZE-MS was applied for the separation of omeprazole enantiomers using 5 mM heptakis-(6-sulfo)-β-CD in a 10 mM ammonium acetate buffer at pH 5.8 [68]. To minimize the contamination of the MS with the CDs the CE separation was performed with the ESI and sheath flow switched off during the CE separation. After 30 min, the ESI voltage and sheath flow were applied allowing the separated omeprazole enantiomers to enter into the MS analyzer.

4. CHIRAL MICELLAR ELECTROKINETIC CHROMATOGRAPHY-MASS SPECTROMETRY (CHIRAL-MEKC-MS).

Although novel MEKC-based methodologies have recently been proposed for chiral analysis, the combination of MEKC methods with MS detection has still some important problems. Thus, one of the main limitations of this coupling is the lack of compatibility of the most widely used surfactants in MEKC separation with mass spectrometers. Generally, surfactant monomers suppress the ionization of the analyte in the spray chamber, which in turn, provides an increased chemical noise in the electrospray reducing sensitivity of the whole MEKC-MS analysis. In last years, the use of high-molecular-mass micelle polymers (also referred to as micelle polymers or molecular micelles) as pseudostationary phase has demonstrated to be an interesting alternative to more conventional micelles for the analysis of enantiomers with MEKC-MS (the main works published so far on chiral-MEKC-MS are summarized in Table 3 [69-76]). These micellar systems are synthesized from surfactants having a polymerizable

group and the main advantages of these structures in MEKC-MS include: (i) a simplified and enhanced binding between the analyte and the micelle; (ii) they can be used at any polymeric surfactant concentration due to zero critical micelle concentration, and therefore, better S/N ratio are usually obtained in MEKC-ESI-MS analyses; (iii) molecular micelles are stable in the presence of organic solvents due to the covalent bond formed between monomers; (iv) the better stability of micelles prevents dissociation of monomers during electrospray process and also, micelles produce less background interference due to their high molecular weight; (v) due to their lower surface activity, molecular micelles provide less ion suppression and more stable electrospray.

Chiral separations with MEKC-MS were first reported by Shamsi [69] who showed the feasibility of the molecular micelles with polysodium N-undecanoyl-L-valinate (poly-L-SUV) as pseudostationary phase for the separation of binaphthol (BOH) enantiomers. Electrospray parameters (voltage, nebulizing gas pressure, drying gas temperature, drying gas flow rate, sheath liquid flow rate) affecting the sensitivity were first examined and after that, other separation conditions (pH, micelle polymer concentration, and volatile background electrolyte concentration) were evaluated to obtain the best possible resolution of enantiomers without sacrificing sensitivity. The micelle polymer concentration was found to have a significant impact on chiral resolution and S/N ratio of BOH, providing good results at concentrations around 0.2% (w/v) poly-L-SUV.

Later, the versatility of new alkenoxy amino acid molecular micelle, i.e., polysodium N-undecenoxycarbonyl-L-leucinate (poly-L-SUCL) as chiral selector in MEKC-MS was investigated for the simultaneous enantioseparation of eight structurally similar β-blockers [70]. Under optimum sheath liquid and spray chamber conditions, the effect of MEKC

separation parameters was studied to find a good compromise between enantioseparation and sensitivity. Separation parameters as pH, volatile BGE concentration, polymeric surfactant concentration and, nebulizing gas pressure demonstrated to play an important role in the chiral resolution, S/N ratio of the analytes and analysis time. In general, a decreased chiral resolution was observed at basic pH values, lower BGE concentrations and nebulizing gas pressures, whereas the variation on chiral resolution when poly-L-SUCL concentration demonstrated to be analyte dependent. More precisely, chiral resolution values of hydrophilic β-blockers were increased while those of hydrophobic β-blockers were decreased at higher surfactant concentrations. Also, a comparison of monomeric and polymeric L-SUCL under optimum experimental conditions demonstrated that, generally, polymer micelles showed better performance than unpolymerized micelles at the same monomer concentration. Authors explained these results as a consequence of the tendency of unpolymerized micelles to break down once they are into the ESI chamber, thus resulting in unstable electrospray, which in turn, increases background noise of the mass spectrometer.

Next, Shamsi and co-workers extended their experiments to study the applicability of six amino acid-based polymeric surfactants to the simultaneous enantioseparation of three chiral compounds, i.e., two benzodiazepines and one benzoxazocine with MEKC-MS [71]. The amino acid-based polymeric surfactants included three carbamate-type polymers [poly-L-SUV, polysodium N-undecanoyl-L-leucinate (poly-L-SUL), and polysodium N- undecenoyl-L,L-leucyl-valinate (poly-L,L-SULV)], and three amide-type polymers [poly-L-SUCL, polysodium N-undecenoxycarbonyl-L-valinate (poly-L-SUCV), and polysodium N-undecenoxycarbonyl-L,L-leucyl-valinate (poly-L,L-SUCLV)]. MEKC-MS separations with the six different polymer micelles of a mixture containing three pairs of stereoisomers of chiral drugs provided different selectivity. The carbamate-type polymers showed slightly

longer retention, but good chiral resolution for the two benzodiazepines, whereas no chiral separation for the cationic and the most retained benzoxazocine drug. On the other side, dipeptide polymeric surfactants (poly-L,L-SUCLV and poly-L,L-SULV) showed higher S/N ratios than single-amino acid polymeric surfactants in most cases. In this study, the addition of methanol and 2-propanol as organic modifiers to the running buffer increased the migration times of all three solutes in a linear way, whereas the addition of ACN showed a maximum for the migration time of the solutes at 10% v/v ACN, and then a further increase of ACN content resulted in shorter migration times. Authors suggested that the differential effect of ACN in this separation system might be due to a decreased association of solutes to polymeric surfactant at higher ACN concentrations. Under optimum conditions, the developed MEKC-MS method provided LODs ~1.8 µg/mL for the analytes that were nearly three to four-fold better than those obtained with chiral MEKC-UV.

In a separate study, the same group reported on the application of the poly-L,L-SULV polymeric surfactant for the separation and determination of warfarin enantiomers in human plasma with MEKC-MS [72]. Using poly-L,L-SULV, a decreased chiral resolution was observed when the pH of the running buffer was increased from 5.5 to 8.0. Authors explained this loss of resolution by the enhancement of electrostatic repulsive interactions between the polymeric micelle due to the higher effective charge of the analyte and surfactant at less acidic pH values. On the other side, as buffer pH increases, the S/N ratio increases possibly due to a decrease of migration times and a better ionization of analytes under negative ion mode at the interface. After systematic optimization, the developed MEKC-MS method was applied to the analysis of warfarin enantiomers in human plasma samples spiked with different amounts of enantiomers. The sensitivity obtained with the chiral MEKC-MS method (LOD 0.1 µg warfarin enantiomers/mL) was comparatively better to the one obtained with

chiral MEKC-UV (LOD 5 µg/mL) demonstrating the good possibilities of MEKC-MS for the monitoring of this anticoagulant drug in plasma samples.

Further studies by the same group with polysodium N-undecenoxycarbonyl-L-amino acid sulfates (poly-L-SUCAAS) demonstrated the good performance and versatility of these polymeric micelles for chiral MEKC-UV analysis of ten structurally different phenylethylamines (PEAs) under acidic conditions (pH 2.0-3.0) [73]. To investigate the applicability of poly-L-SUCAAS as a pseudostationary phase to MEKC-MS under acidic conditions, Rizvi *et al.* used polysodium N-undecenoxycarbonyl-L-leucine sulfate (poly-L-SUCLS) for the enantioseparation of pseudoepinephrine in human urine samples. Severe arching problems were encountered possibly due to the formation of very strong ion pairs with negatively charged micelles and the positively charged analytes at low pH. The addition of 1% valeric acid (v/v) to the sheath liquid improved the sensitivity as this ion-pairing reagent competes for the ion pair formation with the charged compounds. After optimization of sheath liquid and MS spray chamber parameters, the reported LODs were almost 16 times lower (LOD 325 ng/mL) for the analyses performed at pH 2 than at pH 8.0 (LOD 5.2 µg/ml).

Last developments in this field have been focused on the application of multivariate approaches for the study and optimization of the most relevant parameters affecting the chiral separation in MEKC-MS with polymeric micelles. Thus, Hou *et al.* [74] developed a MEKC separation method with MS detection and the use of poly-L-SUCL surfactant for the simultaneous separation of four pairs of ephedrine stereoisomers. In this study, the parameters affecting MEKC separation of the analytes were first investigated and then, a central composite design was carried out in order to evaluate independently the effects and interactions between the spray chamber parameters and between sheath liquid conditions. In

the first method development stage, MEKC experiments with different organic modifiers in the BGE revealed similar results to those previously reported for the chiral MEKC separation of benzodiazepines and benzoxazocine [71]. Using the optimized MEKC conditions, the response surface 3-D contour plots obtained from the central composite design experiments indicated the optimum conditions for the best sensitivity. Next, the developed method was applied to the analysis of ten enantiomers of ephedrine and related compounds in standard reference materials with different enantiomeric composition (Figure 4) [75]. In this study, the comparison between MEKC-MS and MEKC-UV indicated that MS detection provides better sensitivity, but a slightly lower dynamic range for quantitation. Authors reported %RSDs values lower than 0.77% and 1.80% for migration time within the same day and different days, respectively, and the overall limits of detection ranged from 0.00037 to 12.49 mg/g.

More recently, central composite design has also been applied to evaluate the importance of selected MEKC-MS parameters for the simultaneous chiral separation of BOH and 1,1'-binaphthyl-2,2'diylhydrogen phosphate (BNP) enantiomers [76]. In this case, a first experimental design for MEKC optimization, based on six factors and three-levels, was proposed. The analysis of data obtained from 86 MEKC-MS runs suggested that nebulizer pressure had the most significant influence on the chiral resolution. Voltage and polymeric surfactant concentration had more pronounced effect on the chiral resolution of BOH and (±) BNP, respectively, whereas ammonium acetate concentration and temperature did not affect the chiral separation of the analytes significantly. Same methodology was used to evaluate the effect of the sheath liquid and spray chamber parameters on S/N ratio. Finally, the optimum conditions, a combination of the optimal from the three parts of the study (MEKC, sheath liquid and spray chamber optimization), were used to run 20 replicate runs. The obtained

enantiomeric resolutions for BOH and BNP differed 4% and 26%, respectively, from the predicted values, whereas the migration times were 3% higher than the predicted value.

5. CHIRAL CAPILLARY ELECTROCHROMATOGRAPHY-MASS SPECTROMETRY (CHIRAL-CEC-MS).

The application of CEC-MS to the analysis of chiral compounds has been explored in different ways by several research groups as can be seen in Table 4 [77-83]. Thus, Schurig and Mayer [77] developed a CEC-MS method based on an open-tubular capillary internally coated with polysiloxane-bonded permethyl-β-cyclodextrin as chiral stationary phase for the enantioseparation of the sedative-hypnotic drug hexobarbital in human urine. To avoid co-elution of urine components with the same mass, a liquid-liquid extraction step of the sample was included prior to its injection into the capillary column. In this case, the detection system consisted of an electrospray interface coupled to a quadrupole mass spectrometer. Thus, hexobarbital enantiomers were detected within 2 minutes at concentrations below the therapeutic levels (20-50 ng/mL).

Von Brocke *et al.* [78] reported on the on-line coupling of pressure supported CEC using packed capillaries with ESI-MS and coordination ion spray (CIS)-MS for the chiral analysis of barbiturates and chlorinated alkyl phenoxypropanoate enantiomers. To achieve their separation, in-lab prepared capillaries (100 µm ID), packed with permethylated β-cyclodextrin-modified silica (5 µm, 300 Å) were used. The use of a coordination ion solution (sheath flow) is one of the main features of the CEC-CIS-MS coupling because this ensures stable spray conditions and acts as complexing reagent to provide ionization of analytes. Hence, many coordination compounds are available for introducing a charge to the analytes

providing different selectivity. In their study, authors investigated the separation and detection of hexobarbital and mephobarbital with CEC-CIS-MS using silver(I), cobalt(II) and copper(II) salts, whereas the results demonstrated that the use of lithium(I) salt was impracticable. On the other hand, chlorinated alkyl prenoxypropanoates showed high affinity to lithium salts, providing better sensitivity to the detection of these enantiomers.

Several works have been published describing the use of capillary packed with different chiral stationary phases for the analysis of enantiomers. Zheng and Shamsi [79], for example, investigated the effect of different column fabrication on the CEC-MS analysis of warfarin and coumachlor enantiomers. Using a 0.5 μm (3R,4S)-Whelk-O1 chiral stationary phase, the results suggested that externally tapered capillary columns provided better reproducibility (RSDs ~ 5%) than untapered columns (RSDs ~ 31%) for the migration time. This observation is possibly due to the formation of bubbles in the open segment of the untapered columns, which has been associated with the retaining frit between the packed and open segments of the capillary. In contrast, this problem was avoided in tapered columns because the end acts as a back-pressure resistor and suppressed the bubble formation. Also, the influence of acetonitrile content, buffer pH, and ionic strength on CEC separation in tapered columns was explored. Then, other parameters associated with MS detection were investigated to maximize the sensitivity of the method. Authors proved the applicability of the optimized CEC-MS method for the detection or warfarin enantiomers by analyzing spiked human plasma samples, previously treated with C18 solid-phase extraction columns. In subsequent studies, the same group improved the fabrication of packed capillaries by developing a novel type of more robust columns, suitable for ESI-MS coupling [80]. Using a simplified fabrication procedure, it was possible to obtain internally tapered columns with an opening of ~7-10 μm i.d., which were subsequently packed with vancomycin chiral stationary

phase. The performance of the new columns was tested under both reversed-phase and polar organic phase modes of CEC-MS. A comparison between externally and internally tapered columns demonstrated that the latter columns provided better electrospray stability and reproducibility. The longer lifetime of the internally tapered column, especially under polar organic conditions, was also beneficial for the analysis of eight β-blockers enantiomers, suggesting much better ruggedness, as compared to externally tapered columns. In addition, authors demonstrated a batch-to-batch column reproducibility lower than 4.1% (RSD obtained with four columns and 20 injections of eight β-blockers on each column). In a further study, the effect of different parameters related with the mobile phase, stationary phase, sheath liquid, and ESI spray chamber were investigated in order to obtain high resolution and sensitivity of eight β-blockers with CEC-MS and internally tapered columns [81]. The use of long packed columns (90 cm) enabled larger volume injections without overloading, at the expense of longer analysis times. In general, an increase in mobile phase ionic strength enabled much robust current and EOF, and sample stacking, which resulted in enhanced sensitivity. Thus, a mobile phase based on methanol-ACN (70:30, v/v) containing 1.6% (v/v) acetic acid and 0.2% (v/v) triethylamine provided a good compromise between resolution and analysis time. The quantitative capabilities of the method were also demonstrated over a wide concentration range (3-600 μM), and more interestingly, the developed CEC-MS method allowed the detection of traces (0.1% enantiomeric impurity) in non-racemic mixtures. In a separate report, preliminary sequential optimization of CEC-MS parameters, including mobile phase composition, column temperature and electric field strength was carried out to obtain the separation of eight β-blockers, i.e., four stereoisomers of labetalol and four steroisomers of nadolol [82]. Then, a second optimization procedure was established in order to reduce the analysis time and improve the sensitivity. To achieve this, a first multivariate design was used, considering the three most important factors (organic

composition, percent of acetic acid and percent of triethylamine) on the analysis time. To increase the S/N ratio, two more multivariate studies were subsequently run, one for sheath liquid parameters optimization (addition of ammonium acetate and acetic acid) and the second one for the spray chamber parameters optimization (nebulizer pressure, drying gas flow rate, and temperature). The multivariate optimization led for a reduction of 15 min in the overall analysis time (from 75 to 60 min) of the eight enantiomers and also, average S/N ratios greater than 1000 were obtained.

Recently, Zheng *et al.* [83] have investigated the capabilities of sulfated and sulfonated polysaccharide as chiral stationary phases for CEC-MS analysis of a broad range of compounds. In their work, authors presented new alternative cellulose tris(3,5-dimethylphenylcarbamate)-based stationary phases (6-SO_4-CDMPC and CDMPC-SO_3) with good potential for faster CEC enantiomeric separations under normal phase conditions. The results demonstrated a ~50% reduction in the analysis time of several enantiomers when using 6-SO_4-CDMPC and CDMPC-SO_3, which suggested a good correlation between the magnitude of EOF and the content of ionizable groups on the chiral stationary phases. However, a loss of resolution was observed in the analyses performed with 6-SO_4-CDMPC possibly due to the different content of ionizable groups and cellulose structure compared to that in CDMPC-SO_3. Experiments with the latter negatively charged polysaccharide and mobile phases with different ionic strength (2-8 mM ammonium acetate) suggested the existence of cationic-exchange mechanisms on the separation of pindolol enantiomers with CDMPC-SO_3. Authors also explored the effect on the enantioseparation of silica particle pore size chiral stationary phase loading, demonstrating good resolving power for a wide range of acidic, neutral and basic compounds with 20% stationary phase loadings (5 μm particles, 1000 Å). The organic composition of sheath liquid demonstrated to have a strong impact in

the S/N ratio of normal phase CEC-MS analyses. Under normal phase conditions, the presence of IPA in the sheath liquid enhanced the detection sensitivity, probably due to a better miscibility of hexane with 2-propanol than with methanol. Nevertheless, authors indicated that normal phase mode generally provided the lowest sensitivity of warfarin and Troger's base enantiomers because hexane produces ESI-MS signal suppression when it is used in the mobile phase (Figure 5).

6. CONCLUDING REMARKS AND FUTURE OUTLOOKS.

Compared with the chiral applications of GC-MS or HPLC-MS, CE-MS can be considered as a novice also for chiral analysis. In this regard, the high resolving power, rapid method development, easy sample preparation and low operation expense (allowing the use of sophisticated and/or very expensive chiral selectors) of CE have made of this technique a very interesting alternative for chiral analysis. However, the combination of CE and MS still needs to demonstrate its huge potential for chiral analysis trying to overcome its main limitations linked to the robustness of the CE-MS interface and the low compatibility between the chiral selector and the ionization source. These limitations are still key issues to be solved and they clearly need to be worked out before both CE-MS and its application for chiral analysis can be considered routine approaches.

Some other new and interesting developments and uses within the chiral-CE and CE-MS domains will foreseeably be important application areas for chiral-CE-MS in the non-distant future. These developments include chip-based enantioselective separations [84], synthesis of new chiral phases (based e.g., on molecularly imprinted polymeric monoliths [85] or organogels

[86]), high-throughput chiral analysis by capillary array electrophoresis [87], chiral-CE-MS applications in metabolomics [88,89] or applications of chiral-CE-MS in the new Foodomics field [90]. The development of these new approaches will make broader the applications area of this technique while allowing to study from a different perspective new fields of research.

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Authors declare no conflict of interest.

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Table 1: Reviews published in the last 10 years (1999-2009) on chiral capillary electromigration methods including information on chiral-capillay electromigration-MS analysis.

Subject	Publication Year	Reference
Enantioseparations using capillary electromigration techniques	1999	[5]
Polymeric surfactants in electrokinetic chromatography	1999	[6]
Enantioresolution of pharmaceutical compounds by capillary electrophoresis	1999	[7]
Enantioselective determination by capillary electrophoresis with cyclodextrins	2000	[8]
Enantioseparations using CE techniques in nonaqueous buffers	2000	[9]
Enantiomer separation of drugs by capillary electromigration techniques	2000	[10]
Enantioseparations by capillary electrochromatography	2001	[11]
Enantioseparation of chiral drugs by electromigration techniques	2001	[12]
Enantioseparations in capillary electromigration techniques	2001	[13]
Chiral CE-MS: modes and applications	2002	[14]
Enantiomer migration order in chiral capillary electrophoresis	2002	[15]
Chiral electromigration methods in food analysis	2003	[1]
Enantioresolutions by capillary electrophoresis using glycopeptide antibiotics	2004	[16]
Capillary electrochromatography coupled to mass spectrometry	2004	[17]
CE-MS for small achiral and chiral solutes	2004	[18]
Enantioseparation in CEC using polysaccharide-type chiral stationary phases.	2004	[19]
Extraction-separation techniques coupled to mass spectrometry	2005	[20]
Chiral molecular recognition for the MS detection and analysis of enantiomers	2005	[21]
Chiral analysis of pollutants and their metabolites by CE methods	2005	[22]
Capillary electrophoresis with mass spectrometric detection	2006	[23]
Chiral separation using capillary electromigration techniques	2007	[24]
The story of 20 and a few more years of enantioseparations by CE	2007	[25]
Cyclodextrins in capillary electrophoresis enantioseparations	2008	[26]
The role of cyclodextrins in chiral capillary electrophoresis	2008	[27]
Chiral separations by CE employing cyclodextrins.	2009	[28]
Separation of enantiomers with charged chiral selectors in CE	2009	[29]

Table 2. Chiral-CZE-MS applications

Chiral Analyte	Sample	Capillary	BGE and CE polarity	PFT	MS	Ref.
I. No CS counter migration, no partial filling technique						
Terbutaline and ephedrine	Urine	Bare	5 mM sodium phospahate pH 2.5, 5 mM DMBCD. +30 kV	-	ESI-QqQ (+)	[32]
Dichlorprop, fenoprop and mecoprop herbicides	-	Bare	50 mM ammonium acetate buffer pH 4.6, 20 mM TMBCD. - 27.5 kV	-	ESI-IT (-)	[33]
Terbutaline and Ketamine	-	Bare	5 mM ammonium acetate, 0.8 M acetic acid, 80% (v/v) methanol, 5-15 mM DMBCD. +25 kV	-	ESI-Q (+)	[34]
Propanolol	-	Bare	5 mM ammonium acetate, 0.8 M acetic acid, 80% (v/v) methanol, 20 mM HPBCD. +25 kV	-	ESI-Q (+)	[34]
Methanfetamine and metabolites	Urine	Bare	1 M formic acid pH 2.2, 3 mM β-CD, 10 mM DMBCD. + 30 kV	-	ESI-IT (+)	[35]
Salbutamol	Urine	Bare	10 mM ammonium formate, 15 mM DASBCD, in methanol. + 25 kV (+16 mbar)	-	ESI-IT (+)	[36]
6 Amino acids	Orange juice	Polymer-coated	100mM ammonium acetate pH 6, 5mM β-CD. - 15 kV	-	ESI-IT (+)	[37]
Ornithine	Beer	Bare	50 mM ammonium carbonate pH 10, 0.75 mM γ-CD. + 25 kV	-	ESI-IT (+)	[38]
5 Amino acids	Vinegar, soybeans	Bare	50 mM ammonium hydrogen carbonate pH 8, 0.5 mM charged-CD. + 30 kV	-	ESI-TOF (+)	[39]
11 amino acids and 2 neutrotransmitters	Red blood cells	Bare	30 mM 18C6TCA. + 30 kV	-	ESI-IT (+)	[40]
II. CS counter migration						
Tropic acid	-	PAA-coated	10 mM acetic acid-ammonium acetate pH 5, 8 mg/mL TMABCD. -16 kV	-	ESI-IT (-)	[41]
Etilefrine, mianserine, dimimethidene and chlorpheniramine	-	Bare	10 mM acetic acid-ammonium acetate pH 3.5, 0.2-3 mg/mL CMBCD. + 20 kV	-	ESI-IT (+)	[41]
1 Chiral drug	Plasma	Bare	5 mM ammonium acetate pH 6, 1% acetic acid, 25% methanol, 0.3% HSBCD. + 25 kV (+1 psi)	-	ESI-Q (+)	[42]
Methanfetamine and metabolites	Urine	Bare	1 M ammonium formate pH 2, 1.5 mM DASBCD. + 30 kV	-	ESI-IT (+)	[43]

Mebeverine and 5 related chiral compounds	-	Bare	0.75 M formic acid, 30 mM potassium camphorsulfonate, 30 mM HDMSBCD, in methanol. + 30 kV	-	ESI-IT (+)	[44]
Ibuprofen, fenoprofen, flurbiprofen, ketoprofen and indoprofen	-	PVA-coated	20 mM ammonium acetate, 20 mM PABCD, in methanol. -30 kV (+15 mbar)	-	ESI-IT (-)	[45]
12 Arylpropionic acids	Urine	PAA-coated	50 mM ammonium acetate pH 4.8, 5 mM vancomycin. - 20 kV	-	ESI-IT (-)	[46]
III. No CS counter migration + Partial filling technique						
Bupivacaine and ropivacaine	-	PAA-coated	30 mM acetic acid, 100 mg/mL MBCD. + 30 kV	80%	ESI-QqQ (+)	[47]
Camphorsulfonic acid	-	PAA-coated	40 mM ammonium formate pH 4, 50 mM DMBCD. - 30 kV	70%	ESI-QqQ (-)	[48]
3 racemic compounds with a primary amino group	-	PAA-coated	40 mM ammonium formate pH4, 2-5 mM 18C6TCA. + 25 kV	70%	ESI-QqQ (+)	[49]
Adrenoreceptor antagonist	-	Bare	50 mM ammonium formate pH 4, 10 mM HPBCD. + 25 kV	80%	ESI-QqQ (+)	[50]
R/S-Methadone	-	PVA-coated	20 mM ammonium acetate pH 4, 18 mg/mL HPBCD. + 20 kV	90%	ESI-Q (+)	[51]
Clenbuterol and salbutamol	Plasma	Bare	10 mM ammonium acetate pH 2, 20% (v/v) methanol, 40 mM DMBCD. + 30 kV (+10 mbar)	66%	ESI-QqQ (+)	[52]
6 Amphetamines and derivatives	-	PVA-coated	40 mM ammonium acetate pH 3, 24 mg/mL HPBCD. + 30 kV	90%	ESI-Q (+)	[53]
Phonethalol	-	Bare	20 mM ammonium acetate in methanol-2-propanol (75:25, v/v), 80 mM DIKGA. + 25 kV	55%	ESI-Q-TOF (+)	[54]
5 chiral dipeptides	-	Bare	3 M acetic acid pH 2, 5 mM 18C6TCA. + 20 kV	53%	ESI-Q (+)	[55]
IV. CS counter migration + Partial filling technique						
Tropic acid	-	PAA-coated	40 mM ammonium formate pH 5, 5 mM QABCD. - 30 kV	70%	ESI-QqQ (-)	[48]
Ibuprofen and ketopfrofen	-	PAA-coated	40 mM ammonium formate pH 6, 10% (v/v) 2-propanol, 0.1 mM avidin. - 30 kV	70%	ESI-QqQ (-)	[48]
Warfarin	-	PAA-coated	40 mM ammonium formate pH 6, 10% (v/v) ethanol, 0.1 mM avidin. - 30 kV	70%	ESI-QqQ (-)	[48]
Tramadol and metabolites	Plasma	PVA-coated	40 mM ammonium acetate pH 4, 2.5 mg/mL SBEBCD. + 25 kV	90%	ESI-Q (+)	[56]

Methadone	-	PVA-coated	20 mM ammonium acetate pH 4, 0.25 mg/mL CMBCD. + 20 kV	70%	ESI-Q (+)	[51]
Methadone	-	PVA-coated	20 mM ammonium acetate pH 4, 0.4 mg/mL SBEBCD. + 20 kV	90%	ESI-Q (+)	[51]
Methadone and related metabolites	Serum	PVA-coated	40 mM ammonium acetate pH 4, 1 mg/mL CMBCD. + 30 kV	90%	ESI-Q (+)	[53]
Venlafaxine and related metabolites	-	PVA-coated	40 mM ammonium acetate pH 4, 2 mg/mL CMBCD. + 30 kV	90%	ESI-Q (+)	[53]
Atropine and Homatropine	-	PVA-coated	30 mM ammonium acetate pH 7, 2 mg/mL SBCD. + 30 kV	75%	ESI-Q (+)	[53]
Ketamine, prilocaine and mepivacaine	-	PVA-coated	20 mM ammonium formate pH 3, 2.5-10 mg/mL SBCD. + 30 kV	90%	ESI-Q (+)	[57]
Methadone, Tramadol, Venlafaxine, Fluoxetine	-	Bare	30 mM ammonium formate pH 2.5, 0.1-0.85% HSGCD. + 25 kV	83%	ESI-Q (+)	[58]
7 Amphetamines and related compounds	Plasma	Bare	20 mM ammonium formate pH 2.5, 0.15% HSGCD. + 30 kV	60%	ESI-Q (+)	[59]
Five amphetamine derivatives, tramadol and methadone	Plasma	Bare	20 mM ammonium formate pH 2.5, 0.15% HSGCD. +25 kV	50%	ESI-Q (+)	[60]
Baclofen	Formulation	Bare	0.25 M formic acid, 1.75 mM SBEBCD. + 25 kV	88%	ESI-IT (+)	[61]
Ecstasy and methadone	Plasma	Bare	15 mM ammonium formate pH 2.5, 0.08% HSGCD. + 30 kV	70%	ESI-Q (+)	[62]
Carnitine	Infant formula	Bare	0.5 M ammonium formate pH 2.5, 10 mM SuccGCD. + 25 kV	50%	ESI-IT (+)	[63]

Abbreviations:

CMBCD: carboxymethyl-β-CD
DASBCD: heptakis(2,6-diacetyl-6-sulfato)-β-CD
DMBCD: heptakis(2,6-di-O-methyl)-β-CD
DIKGA: (2)-2,3:4,6-di-O-isopropylidene-2-keto-L-gulonic acid
DMSBCD: heptakis(2,3-di-O-methyl-6-O-sulfo)-β-CD
HPBCD: hydroxypropyl-β-CD
HSBCD: highly sulfonated β-CD
HSGCD : highly sulfated γ-CD
MBCD, methyl-β-CD
PABCD: 6-monodeoxy-6-mono(3-hydroxy)propylamino-β-CD
QABCD: quaternary ammonium β-CD
SBEBCD: sulfobutyl ether β-CD
SBCD : sulfated β-CD
SuccGCD: succinyl-γ-CD
TMABCD: 2-hydroxypropyltrimethylammonium-β-CD
TMBCD: heptakis(2,3,6-tri-O-methyl)-β-CD
18C6TCA: (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid

Table 3. Chiral-MEKC-MS applications.

Chiral analyte	Polymer[1]	Sample	BGE composition	MS	Ref.
1,1'-binaphtol	poly-L-SUV	Test mix	20 mM NH_4OAc, 0.20% (w/v) poly-L-SUV, pH 9.2	ESI-Q (-)	[69]
Atenolol Metoprolol Carteolol Pindolol Oxprenolol Talinolol Alprenolol Propanolol	poly-L-SUCL	Test mix	25 mM each NH_4OAC and TEA, 15 mM poly-L-SUCL, pH 8.0,	ESI-Q (+)	[70]
Oxazepam Lorazepam Nefopam	poly-L-SUL poly-L-SUV poly-L-SUCL poly-L-SUCV poly-L,L-SULV poly-L,L-SUCLV	Test mix	25 mM NH_4OAC, 15 mM poly-L,L-SUCLV, pH 8.5 containing 10% v/v ACN	ESI-Q (+)	[71]
Warfarin Coumachlor	poly-L,L-SULV	Human plasma	25 mM NH_4OAC, 25 mM poly-L,L-SULV, pH 6	ESI-Q (-)	[72]
Pseudoepinephdrine	poly-L-SUCLS	Human urine	15 mM NH_4OAC, 15 mM TEA, pH 2.0	ESI-Q (+)	[73]
Ephedrine Pseudoephedrine N-methylephedrine norephedrine	poly-L-SUCL	Test mix	35 mM poly-L-SUCL, 15 mM NH_4OAc, pH 6.0 containing 30% v/v ACN	ESI-Q (+)	[74]
Ephedrine Pseudoephedrine N-methylephedrine N-methylpseudoephedrine Norephedrine Norpseudoephedrine	poly-L-SUCL	Test mix	35 mM poly-L-SUCL, 15 mM NH_4OAc, pH 6.0 containing 30% v/v ACN	ESI-Q (+)	[75]
1,1'-binaphtol 1,1'-binaphthyl-2,2'diyl hydrogen phosphate	poly-L-SUCL poly-L,L-SULV	Test mix	35 mM NH_4OAc, 27 mM surfactant, pH 10.8	ESI-Q (-)	[76]

[1] The polymer is used as micellar-system and chiral selector simultaneously. Abbreviations are indicated next:

poly-L,L-SUCLV, polysodium N-undecenoxycarbonyl-L,L-leucyl-valinate
poly-L,L-SULV, polysodium N- undecenoyl-L,L-leucyl-valinate
poly-L-SUCL, polysodium N-undecenoxycarbonyl-L-leucinate
poly-L-SUCLS, polysodium N-undecenoxycarbonyl-L-leucine sulfate
poly-L-SUCV, polysodium N-undecenoxycarbonyl-L-valinate
poly-L-SUL, polysodium N-undecanoyl-L-leucinate
poly-L-SUV, poly(sodium N-undecanoyl-L-valinate)

Table 4. Chiral-CEC-MS applications.

Chiral analyte	Chiral selector[1] (stationary phase)	Sample	BGE composition (mobile phase)	MS	Ref.
Hexobarbital	Permethylated β-CD	Human urine	10 mM NH_4OAc, pH 7	ESI-Q (+)	[77]
Hexobarbital, Mephobarbital, Fenoxaprop ethyl Ciclofopmethyl Mecoprop	Permethylated β-CD	Test mixture	0.5 mM NH_4OAc in water-metanol (40:60, v/v), pH 6.6	CIS-Q (+)	[78]
Warfarin Coumachlor	(3R,4S)-Whelk-O1 CSP	Human plasma	0.5 mM NH_4OAc in ACN-water (70:30, v/v), pH 4.0	ESI-Q (-)	[79]
Atenolol Metoprolol Tartrate Pindolol Oxprenolol Talinolol Alprenolol Propranolol Warfarin Coumachlor	Vancomycin CSP	Test mixture	MeOH-ACN (70:30, v/v) containing 1.6% HOAc and 0.2% TEA	ESI-Q (-)	[80]
Atenolol Metoprolol Pindolol Oxprenolol Talinolol Propranolol Alprenolol Carteolol	Vancomycin CSP Teicoplanin CSP Multimodal CSP	Test mixture	MeOH-ACN (70:30, v/v) containing 1.6% HOAc and 0.2% TEA	ESI-Q (+)	[81]
Nadolol Labetalol	Vancomycin	Test mixture	MeOH-ACN (40:60, v/v) containing 1.6% HOAc and 0.4% TEA	ESI-Q (+)	[82]
Basic, neutral and acidic analytes	6-SO_4- CDMPC CDMPC-SO_3	Test mixture	various conditions	ESI-Q (+) and (-)	[83]

FIGURE LEGENDS

Figure 1. CE-ESI-TOF MS extracted ion electropherograms from a vinegar. Zooms of D-Ala and D-Leu naturally found in vinegar. (A) Vinegar sample. (B) Vinegar spiked with L-Ala and D-Ala. CE-MS conditions: Bare fused-silica capillary with 50 μm ID and 85 cm of total length. Running buffer: 50 mM ammonium hydrogen carbonate at pH 8.0, 0.5 mM 3-monodeoxy-3-monoamino-β-cyclodextrin. Running voltage: 30 kV and 25º C. Sheath liquid: water-2-propanol (50:50 v/v) delivered at 0.24 mL/h. ESI polarity in the positive mode with a 0.3 bar nebulizer and 4 L/min dry gas at 180º C [39].

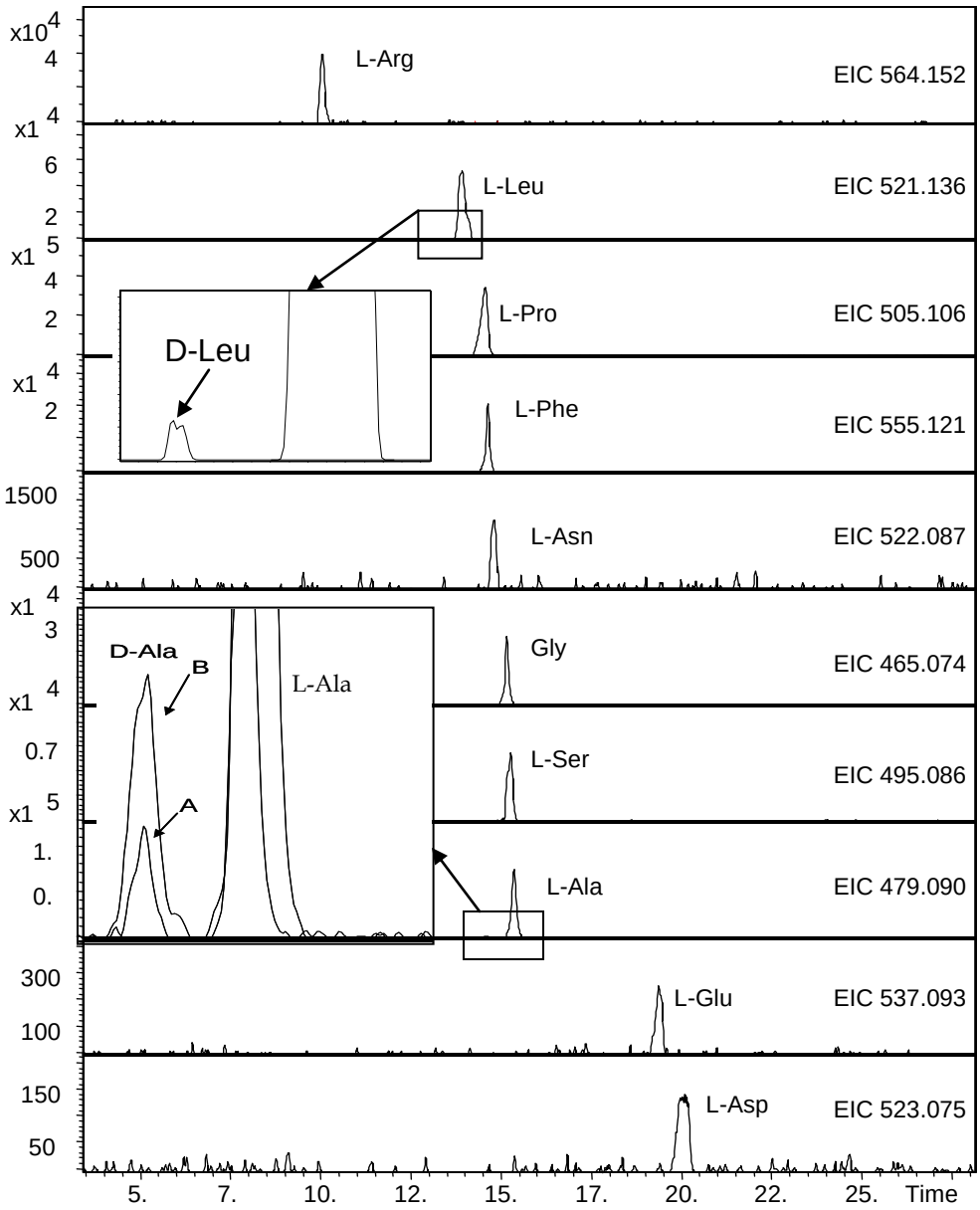
Figure 2. NAEKC-MS extracted ion electropherograms of a mixture of mebeverine and five related compounds (20 μg/ml each). BGE: 0.75 M formic acid, 30 mM HDMS-β-CD and 30 mM potassium camphorsulfonate [44].

Figure 3. Chiral analysis of amphetamine (A), methamphetamine (MA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), tramadol (TMD), and methadone (MTD) enantiomers at 2.5 ppm in plasma. CE-MS conditions: Bare fused-silica capillary of 75 cm x 50 μm ID. Running voltage: +25 kV. Injection at 50 mbar x 10 s. 50% filled with HSGCD in BGE: 20 mM ammonium formate at pH 2.5 [60].

Figure 4. MEKC-MS separation of steroisomers of ephedrine and related compounds along with internal standard. MEKC-MS conditions: 120 cm long fused silica capillary and 50 μm ID, 2 s injection at 2 mbar , 30 kV running voltage, BGE: 15 mM ammonium acetate/35 mM poly-L-SUCL containing 30% ACN at pH 6.0. Sheath liquid: methanol/water 80:20 (v/v) containing 5

mM ammonium acetate delivered at 5 μL/min, dry gas at 250º C at 8 L/min, nebulizer gas pressure at 4 psi. Redrawn from [75].

Figure 5. CEC-ESI-MS electropherograms of warfarin and Troger's base enantiomers with different modes of mobile phase using CDMPC-SO_3 as stationary phase. (a and b) normal phase; (c and d) polar organic phase; (e and f) reversed-phase; (g) S/N plot of both compounds. Redrawn from [83].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D-Leu
L-Pro
EIC 505.106
L-Phe
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EIC 522.087
D-Ala
B
L-Ala
A
Gly
EIC 465.074
L-Ser
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L-Ala
EIC 479.090
L-Glu
EIC 537.093
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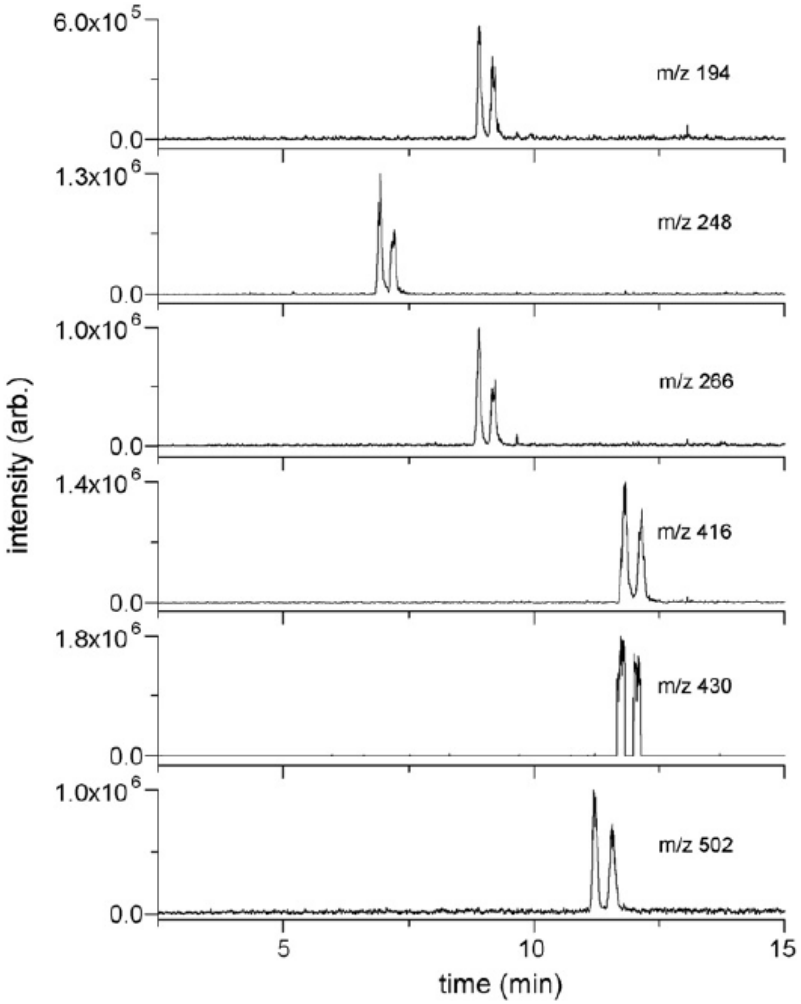
5.
7.
10.
12.
15.
17.
20.
22.
25.
Time

Figure 1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1.3x10^6
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m/z 266
1.4x10^6
0.0
m/z 416
1.8x10^6
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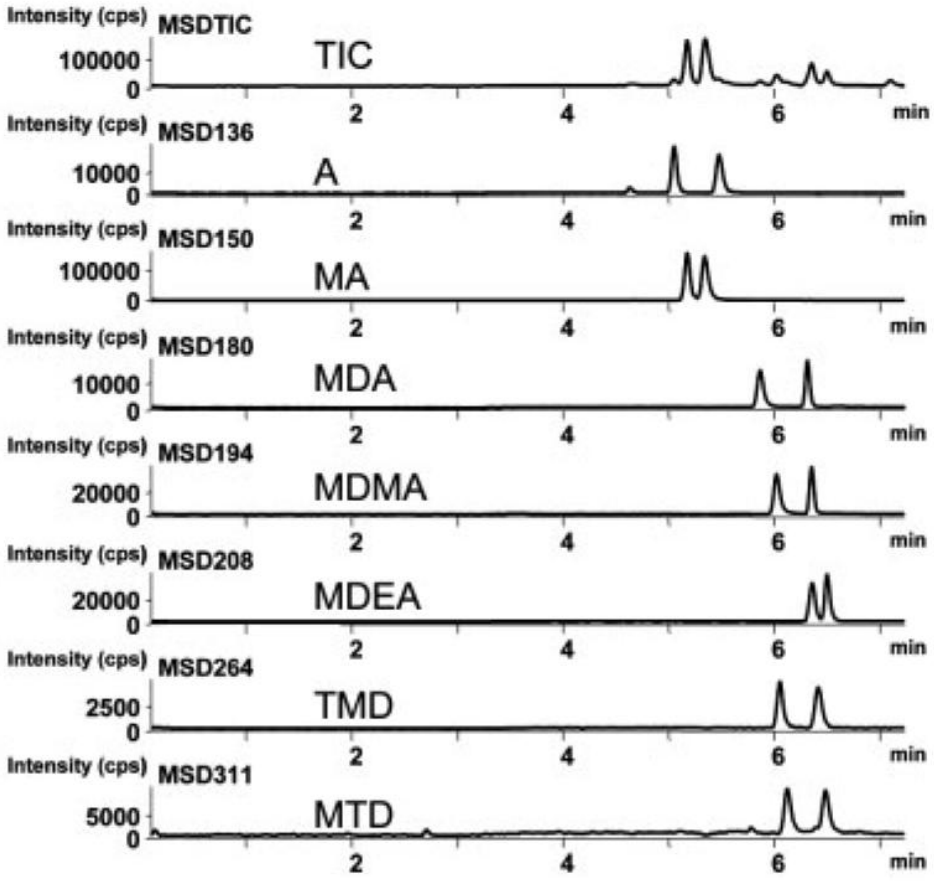
1.0x10^6
0.0
m/z 502
intensity (arb.)
5
10
15
time (min)

Figure 2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TIC
2
4
6
min
Intensity (cps)
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A
2
4
6
min
Intensity (cps)
MSD150
100000
0
MA
2
4
6
min
Intensity (cps)
MSD180
10000
0
MDA
2
4
6
min
Intensity (cps)
MSD194
20000
0
MDMA
2
4
6
min
Intensity (cps)
MSD208
20000
0
MDEA
2
4
6
min
Intensity (cps)
MSD264
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6
min
Intensity (cps)
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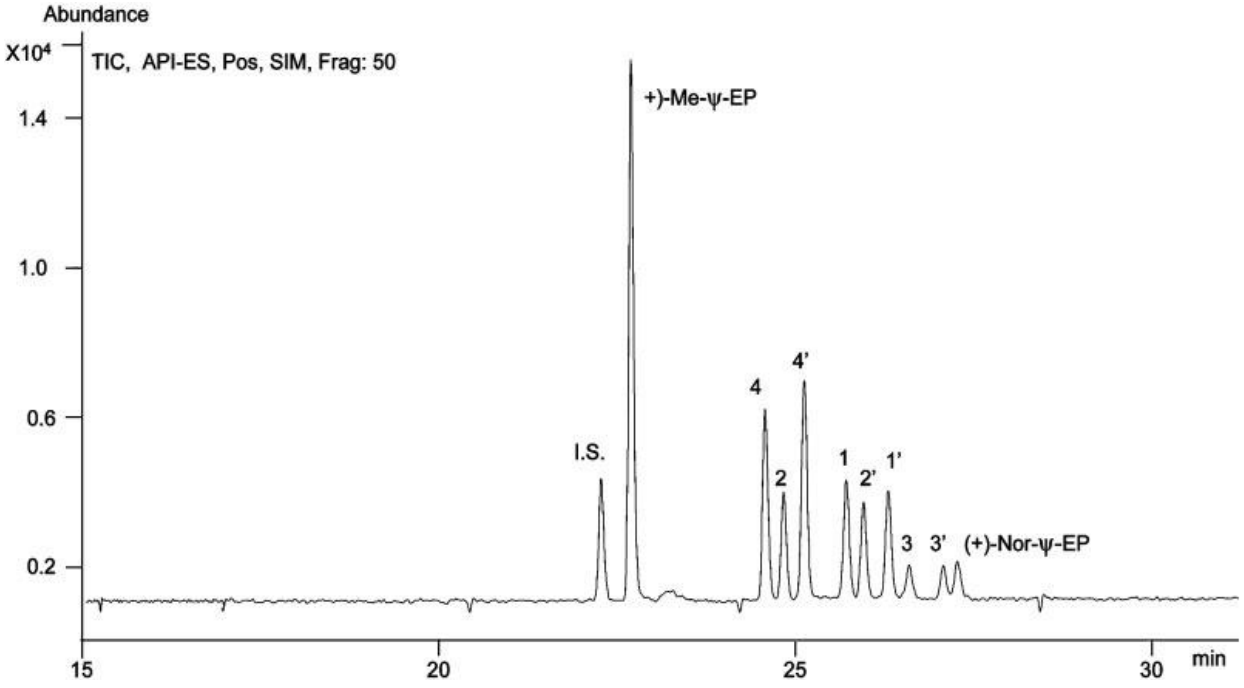
0
MTD
2
4
6
min

Figure 3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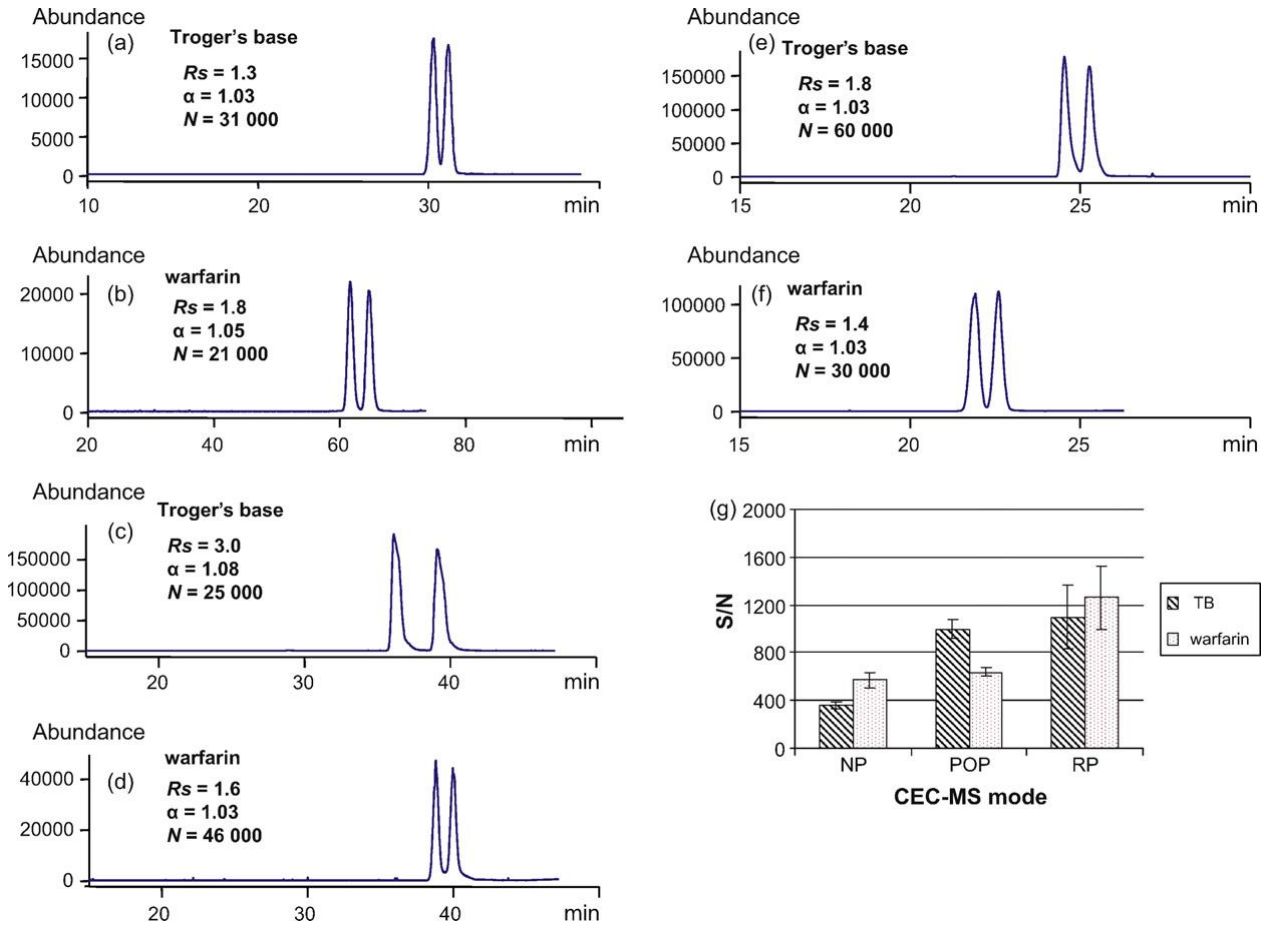
1.0
0.6
0.2
+)-Me-ψ-EP
I.S.
4
4'
2
1
2'
1'
3
3'
(+)-Nor-ψ-EP
15
20
25
30
min

Figure 4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α = 1.03
N = 31 000
min
(b) warfarin
Rs = 1.8
α = 1.05
N = 21 000
(c) Troger's base
Rs = 3.0
α = 1.08
N = 25 000
(d) warfarin
Rs = 1.6
α = 1.03
N = 46 000
(e) Troger's base
Rs = 1.8
α = 1.03
N = 60 000
(f) warfarin
Rs = 1.4
α = 1.03
N = 30 000
(g)
S/N
TB
warfarin
NP
POP
RP
CEC-MS mode

Figure 5