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Review

Liquid separation techniques coupled with mass spectrometry for chiral analysis of pharmaceuticals compounds and their metabolites in biological fluids

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Abstract

Determination of the chiral composition of drugs is nowadays a key step in order to determine purity, activity, bioavailability, biodegradation, etc., of pharmaceuticals. In this article, works published for the last 5 years on the analysis of chiral drugs by liquid separation techniques coupled with mass spectrometry are reviewed. Namely, chiral analysis of pharmaceuticals including, e.g., antiinflammatories, antihypertensives, relaxants, etc., by liquid chromatography–mass spectrometry and capillary electrophoresis–mass spectrometry are included. The importance and interest of the analysis of the enantiomers of the active compound and its metabolites in different biological fluids (plasma, urine, cerebrospinal fluid, etc.) are also discussed.

Keywords: LC–MS; CE–MS; Enantiomers; Drugs; Capillary electrophoresis; Biological fluids; Hyphenated techniques; Liquid chromatography; Metabolites; Sample preparation; CEC–MS

Contents

1. Introduction

In the past 30 years, there has been a growing interest in the pharmaceutical industry and analytical laboratories for the analysis and quantification of the enantiomers of chiral drugs, being this chiral analysis nowadays required by pharmaceutical regulatory authorities. This is basically due to the different therapeutic effects that two enantiomers can have. Whereas one can have beneficial applications, the other can have none or even adverse effects.

Numerous analytical techniques have been developed to respond to this requirement and among them, liquid separation techniques such as liquid chromatography (LC) and capillary electrophoresis (CE) have been demonstrated to play an important role [1].

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Recently a new step has been taken with the detection of chiral compounds in biological fluids [2], a topic of paramount importance for the pharmaceutical industry. The key application is the possibility, e.g., to determine in vivo the pharmacokinetic properties of a drug, including absorption, distribution, metabolism and excretion (ADME) [3]. This allows a better understanding of the action of chiral drugs and their metabolites, to determine which enantiomer is the most efficient in a racemic mixture, and so, propose a better and/or safer treatment based on a single enantiomer. Other applications include studies of chiral inversion in vivo and detection of chiral biomarkers [4]. In comparison to the analysis of chiral drugs directly dissolved in a solvent, their analysis in biological samples can be extremely problematic. Biological fluids (i.e. urine, plasma, etc.) are extremely complex matrices and contain low-molecular mass compounds, salts and proteins. Some of those can be at high concentration. Different problems can be expected; first of all the chromatograms (or electropherograms) can become extremely complex, and co-elution (or co-migration) become frequent and difficult to detect. Secondly, analytes in those matrices can form complexes especially with metal ions, and thus their analysis time can be shifted, which may lead to false negatives without a proper analytical tool. Moreover, the pharmaceutical of interest and its metabolites will frequently have to be accurately measure 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.

The sample preparation is an important step, as it will allow simplification of the matrix. This step includes, e.g., proteins removing, desalting, liquid–liquid extraction (LLE) or solid phase extraction (SPE) [5,6]. Sample pre-treatments are generally time consuming and as a result it is often assumed that the sample preparation is the longest step in the whole analytical method. As mentioned above, among the liquid separation techniques developed to achieve the chiral separation of drugs, liquid chromatography (LC) and capillary electrophoresis (CE) have been demonstrated to play an important role [1] themselves or combined to mass spectrometry (MS) techniques. One of the major breakthroughs for the determination of chiral analytes in biological matrix has been the development of new MS interfaces, especially the development of atmospheric pressure ionization (API) sources such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) that simplified the compatibility between the separation step working in the liquid phase and the mass spectrometer detector working in the gas phase.

MS allows an unambiguous assignment of the different chromatographic or electrophoretic peaks. For instance, comparisons of the mass spectra of the main peaks with their corresponding standard spectra allows knowing without ambiguity their identity and if there is, e.g., co-elution. The full mass spectra and MS/MS spectra can also give information about the structure of the metabolites. MS/MS allows selection of one specific ion/fragment, this permits obtaining a very clean chromatogram where, with a careful selection, the only peaks will be the ones of the analytes of interest.

It was believed that the development of MS, MS/MS and MS^n 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 detector. Moreover, presence of co-migrating species can reduce the MS signal and give errors on the measure (matrix effect) [7]. Also, non-volatile components will accumulate at the ion-source reducing the signal too. Therefore, it is clear that although MS detection allows simplification of the pre-treatment and separations steps, they are still very important steps of any method development.

2. Scope

This review will cover the papers published from 2000 to April 2005 on liquid separation techniques coupled with mass spectrometry for chiral analysis of pharmaceuticals compounds and their metabolites in biological fluids. The liquid separation techniques reviewed will include liquid chromatography (LC), capillary electrophoresis (CE) and capillary electrochromatography (CEC). This review will be separated in two main parts. The first one will be focus on the pre-treatment step, and the second part on the separation and detection step. The papers based on liquid chromatography separation techniques are summarized in Table 1, including other useful data as pharmaceutical analyzed, type of column and mobile phase used, sample preparation applied, API interface, matrix and limit of quantification (LOQ) achieved. The one related to capillary electrophoresis and electrochromatography are summarized in Table 2, providing in this case information on the pharmaceutical analyzed, type of capillary, background electrolyte (BGE), sample preparation applied, API interface used, the matrix analyzed and LOQ achieved.

3. Sample preparation

3.1. Plasma and serum sample

For the analysis of plasma and serum samples, direct injection into the separation instrument is usually avoided, as their high proteins content will clog the column or capillary. Although protein precipitation is usually the first step of the sample preparation procedure, in some applications where sensitivity and robustness are not so important it can be the only step. For instance, Rashed et al. [46] used only protein precipitation as sample clean-up to analyze L-pipecolic acid, an important biochemical marker for the diagnosis of peroxisomal disorders, in human plasma. Although relatively high limits of quantification (LOQ) were found using this procedure [46], the LOQ was still five times higher than the average concentration found in human plasma from healthy volunteers. Similarly, carvidol [20] and gemifloxacin [24] enantiomers have been successfully analyzed after a simple protein precipitation. In the case of gemifloxacin [24], a new antibiotic, Ramji et al. monitor both enantiomers and their metabolites after oral and intravenous injection in rat and

Table 1
Chiral separation in biological matrix by LC–MS

Pharmaceutical	Column	Mobile phase	Sample preparation	API interface	Matrix	References	LOQ
12-HETE	Chiral CD-ph	Methanol:water:acetic acid (65:35:0.02)	SPE	ESI	Urine	[11]	NR
2-Hydroxyglutaric acid	Chirobiotic R	Triethylammonium acetate (pH 7):methanol (9:1)	Filtration	ESI	Urine	[12]	NR
Albuterol	Chirobiotic T	Methanol, 0.02% formic acid, 0.1% ammonium formate	SPE	ESI	Plasma	[13]	NR
Albuterol	Chitobiotic T	Methanol:acetic acid:ammonium (1000:5:1)	SPE	APCI	Plasma	[14]	0.25 ng/ml
Amlodipine	Chiral AGP	Ammonium acetate (10 mM, pH 4.5):1-propanol (99:1)	SPE	APCI	Plasma	[15]	0.1 ng/ml
Anticholinergic analogs	Cyclobond I 2000 β-CD	Acetonitrile:methanol:acetic acid:TEA (95:5:0.5:0.3)	SPE	ESI	Serum	[16]	1 ng/ml
Azelastine	Chiralpak AD	Ammonium acetate:ACN:methanol (80:12:8)	LLE	ESI	Plasma	[17]	40 ng/ml
Baclofen	Crownpak CR	Ammonium acetate:methanol (9:1)	Ultra-centrifugation	APCI	Plasma, CSF	[18]	0.15 ng/ml
Buvicaine	ChromTeck AGP	3% *n*-propanol in water + 5 mM ammonium acetate	LLE	ESI	Urine	[19]	NR
Carvedilol	C18 (GITC as chiral reagent)	Water:CAN (50:50)	Protein precipitation	ESI	Plasma	[20]	0.2 ng/ml
CGS 26214	Chiral AGP	3% *n*-propanol, 0.03% ammonium acetate in water	SPE	ESI	Plasma	[21]	0.4 ng/ml
Chiral sulfoxide drug candidate	Chiralpak-AD	2-Propanol:*iso*-hexane (80:20)	SPE	APCI	Plasma	[22]	5 ng/ml
Felodipine	Chiralcel OJ-R	2-Propanol:*iso*-hexane (11:89)	LLE	ESI	Plasma	[23]	0.1 ng/ml
Gemifloxacin	Crownpak CR	NR	Protein precipitation	ESI	Plasma	[24]	10 ng/ml
Glyceric acid	Chirobiotic	0.1% Triethylammonium acetate (pH 4.1):methanol (9:1)	NR	ESI	Urine	[25]	NR
Ibuprofen	Chiralpak-AD-RH	Methanol:water + 0.1% phosphoric acid (8:2 v/v)	LLE	ESI	Plasma	[26]	0.12 μg/ml
Ketamine	Chiral AGP	Propanol:ammonium acetate (10 mM, pH 7.6) (6:94)	SPE	ESI	Plasma	[27]	1 ng/ml
Ketoprofen	Chirex 3005	Ammonium acetate (30 mM, pH 3.5):methanol (5:96)	SPE	ESI	Plasma	[28]	0.05 ng/ml
Lercanidipine	Chiralpak AD	Hexane:ethanol:diethylamine (95:5:01)	LLE	ESI	Plasma	[29,30]	25 ng ml
Methadone	Chiral AGP	Ammonium formate:methanol	SPE	ESI	Plasma	[31]	0.1 ng/ml
Methadone	Chiral AGP	ACN:ammonium acetate buffer (10 mM, pH 7.0)	Centrifugation	ESI	Saliva	[32]	5 ng/ml
Methadone	Chiral AGP	Isopropyl:ammonium acetate (10 mM, pH unadjusted) (12:88)	LLE	ESI	Plasma	[33]	5 ng/ml
Methylphenidate	Chirobiotic V	Methanol + 0.05% ammonium TFA	LLE	APCI	Fetal tissues	[34]	0.218 ng/g
Methylphenidate	Chirobiotic V	Methanol + 0.05% ammonium TFA	LLE	APCI	Plasma	[35–39]	1 ng/ml
Metrifonate	ChiralPak AS	Ethanol with 1% water:*n*-heptane (25:75)	LLE	ESI	Blood, brain tissues	[40]	5 ng/ml, 7.5 ng/g
MK-0767	Kromasil CHI-DMB	Hexane:isopropanol (+0.1% formic acid) (81:19)	LLE	APCI	Plasma	[41]	1 ng/ml
Nefopam	Chirobiotic V	Methanol + 0.1% ammonium TFA	LLE	ESI	Plasma, urine	[42]	0.9 ng/ml
Omeprazole	Chiralpak AD-RH	ACN:ammonium acetate (50 mM, pH 4.65) (36:65)	SPE	ESI	Plasma	[43]	N/A
PGE-9509924	C18 (FLEC as derivatizing agent)	Methanol:ACN:water:formic acid (40:40:20:0.1)	SPE	APCI	Plasma	[44]	25 ng/ml
Pindolol	Chiral drug (β-CD)	Ammonium acetate (10 mM):ACN (50:50)	Cation exchange	ESI	Serum, urine	[45]	0.13 ng/ml
Pipecolic acid	Chirobiotic T	Methanol:water (60:40)	Protein precipitation	ESI	Plasma	[46]	30 ng/ml
Propanolol	Chirobiotic T	Methanol, 0.035% ammonium TFA	SPE	APCI	Plasma	[47]	2 ng/ml
TaClo	Chiralcel OD-R	Water:ACN (60:40), 0.1% TFA	SPE	ESI	Blood clots	[48]	50 pg/g
Terbutaline	Chirobiotic T	Methanol, 0.1% ammonium TFA	SPE	APCI	Plasma	[49]	1 ng/ml
Tramadol	Chiralpak AD	*Iso*-hexane:ethanol:diethylamine (97:3:0.1)	SPE	APCI	Plasma	[50]	0.5 ng/ml
Verpomil	Chiral AGP	ACN:ammonium acetate (20 mM, pH 7.4) (15:85)	LLE	ESI	Plasma	[51]	0.1 ng/ml

NR: the result or condition was not reported.

Table 2
Chiral separation in biological matrix by CE–MS and CEC–MS

Pharmaceutical	Capillary	BGE	Sample preparation	API interface	Matrix	Reference	LOQ
Clenbuterol	CE, no coating	Ammonium acetate buffer (10 mM, pH 2.5):methanol (80:20) + 40 mM DM-β-CD	SPE	ESI	Plasma	[52]	490 ng/ml
PD0217015 and PD0217016	CE, no coating	ACN:ammonium acetate buffer (5 mM):acetic acid:HS-β-cyclodextrin (25:75:1:0.3)	LLE	ESI	Plasma	[53]	10 ng/ml
Methadone	CE, polyvinyl alcohol coated	Ammonium acetate (40 mM, pH 4) + 1 mg/ml CM-β-CD	LLE	ESI	Serum	[54]	NR
Tramadol	CE, polyvinyl alcohol coated	Ammonium acetate buffer (40 mM, pH 4) + 2.5 mg/ml SBE-β-CD	LLE	ESI	Plasma	[55]	NR
Warfarin	CEC, packed with (3*R*,4*S*)-Whelk-O1	Ammonium acetate buffer (5 mM, pH 4.0):ACN (30:71)	SPE	ESI	Plasma	[56]	25 ng/ml

NR: the result or condition was not reported.

dogs. But whereas a chiral LC–MS method, with a simple protein precipitation was used for the analysis of the enantiomers, the analysis of metabolites required a solid phase extraction (SPE) treatment of the samples. SPE is the most widely used sample clean up techniques. This is probably due to its relative simplicity, its large range of application and the possibility to use semi-automated apparatus (96-well plate and robotized arm, for example) or to develop on-line coupling approaches [8]. The method consists of four steps: conditioning, sample load, washing step and elution. More information about sample clean-up in electromigration, chromatographic and mass spectrometric separation methods can be found in the review by Gilar and co-workers [9].

Eichold and co-workers [28] used LC–MS following an automated SPE procedure with a 96-well plate to measure the ketoprofen enantiomeric concentration in human plasma. Ketoprofen is an anti-inflammatory marketed as a racemic mixture. However, it is accepted that the (*S*) form is the one active, and it is therefore desirable to perform enantioselective concentration measurements in plasma. The combination of chiral LC–MS and semi-automated SPE allows them to obtain a very low LOQ with a relatively high throughput. Thus, they were able to monitor the concentration of both enantiomers in plasma following oral and topical administration. Another popular set-up is on-line-SPE either in a single column or column-switching (CS) configurations (see Fig. 1). In the first step the sample is injected into the SPE column, where analytes of interest are retained and matrix components are washed out. The chiral column is usually conditioned in the same time with the mobile phase. Then the SPE column is connected to the chiral column, and analytes are eluted. Wu et al. [13] used a similar set-up to analyze albuterol enantiomers, a drug used to help asthma patients, in dog plasma. They design a high throughput method with a sample turnout of 8 min. Xia and co-workers [47,49] used an on-line dual column-switching approach in conjunction with chiral LC–MS. This allows them to use a SPE column coupled on-line with the chiral column for the extraction/separation while the other column was equilibrated. In this way a significant increasing of sample throughput was achieved without sacrificing sensitivity or precision. They validate their approach by quantifying propranolol enantiomers in rat plasma [47] and terbutaline enantiomers in human plasma [49]. Other examples of on-line-SPE-LC–MS can be found in Table 1.

Liquid–liquid extraction (LLE) is an alternative to SPE when more robust and sensitive LC–MS methods are needed. LLE is considered more time and resources consuming than SPE but can potentially (depending on the choice of solvent) provides cleaner extracts. Use of liquid-handling workstation permits to work in semi-automated way. This approach has been used by Bakhtiar et al. [34–39] to develop a chiral LC–MS method for the quantification of methylphenidate enantiomers, a drug prescribed for the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy, in plasma. They applied this method to numerous toxycokinetic studies.

Although SPE and LLE are the main techniques used for sample clean-up other approaches exist. Goda et al. [18] use an ultrafiltration membrane and ultra-centrifugation to clean-up plasma and cerebrospinal fluid (CSP) samples. Their results showed that no matrix effects and no co-eluting peaks interfere with the enantiomeric quantification of baclofen, a muscle

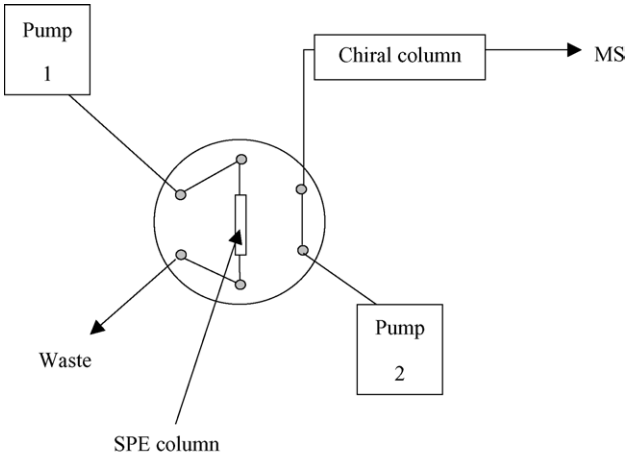

Fig. 1. On-line SPE with column-switching configuration.

relaxant. Motoyama and colleagues [45] used a simple centrifugation and filtration step before injecting the serum sample onto a pre-column with a strong cation exchange stationary phase. The purified eluent was then injected to the chiral column using a column-switching system. They applied this approach to the enantiomeric determination of pindolol in human serum, and obtain a low LOQ and a good intra- and inter-day reproducibility.

3.2. Urine and other biological matrices

Urine is a matrix usually devoid of proteins and, although SPE and LLE are still the two preferred sample clean-up method for sensitive applications, when a high throughput is preferable a simple filtration might be sufficient. This has already been demonstrated by Rashed et al. [12,25] with the analysis of the enantiomers of 2-hydroxyglutaric acid [12] and glyceric acid [25]. Each of these enantiomers is a specific biomarker of an inherited metabolic disease (D-2-hydroxyglutaric aciduria, L-2-hydroxyglutaric aciduria, D-glyceric aciduria and L-glyceric aciduria, respectively). To obtain a simple chromatogram, they develop a MS–MS method using multiple reactions monitoring mode (MRM). They were able to diagnosis the disease with this simple sample clean-up (i.e., filtration).

4. Separation and detection

4.1. Liquid chromatography

Liquid chromatography (LC) is one of the main separation techniques in liquid phase. Chiral separation can be achieved in two ways, either using a chiral reagent, or using a chiral stationary phase (CSP). Due to the extra time required for the chiral derivatization and the wide choice of CSP column available in the market, the uses of chiral reagents tend to disappear. Yang et al. [20] used 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) as a chiral reagent to separate and quantify enantiomers of carvedilol in human plasma. The separation was performed on an Ace 3 C18 column and the eluent was transferred to the MS using an atmospheric pressure chemical ionization (APCI) source. Carvedilol is an anti-hypertensive agent that has been used in treatment of congestive heart failure. In order to determine the stereospecificity exhibited in the metabolism and disposition of carvedilol, an analytical method for the quantitative analysis of each enantiomer was required. On the other hand, Zoutendam et al. [44] used (−)-1-(9-fluorenyl)ethyl chloroformate (FLEC) as a chiral reagent to develop an analytical technique that will be used for the enantiomeric pharmacokinetics studies of the enantiomers in plasma. The column used was in this case a C18, and the MS was interfaced using an electrospray ionization (ESI) source.

Table 1 shows that various CSPs have been successfully used for pharmacokinetic studies. The choice of the mobile phase is determined among other factors by the MS interface. In general the atmospheric pressure chemical ionization (APCI) source is not suitable with flammable solvent and, therefore, it is difficult to apply together with normal phase chiral chromatography. For instance, tramadol, MK-0767 and a chiral sulfoxide drug candidate were separated by normal phase with an APCI source. Miller-Stein and Fernandez-Metzler [22] mixed the eluent at the output of the column with an aqueous solution before the APCI source to allow the enantiomeric pharmacokinetic and metabolic characterizations of a sulfoxide drug candidate using normal phase chiral LC–MS. A similar approach has been used by Yan et al. [41]. Ceccato et al. [50] analyzed both enantiomers of tramadol and its metabolites by setting the probe at a low temperature. Similarly, when using an electrospray ionization (ESI) source with flammable solvents some risk can exist. To overcome this risk, Jabor et al. [29,30] used a make-up flow that was mixed with the eluent. The mixed flow was then split before being transferred to the MS source. Using this approach they develop a new method to quantify enantiomers of lercanidipine, a drug used for the treatment of hypertension, in human plasma [29]. They applied this method to the enantioselective pharmacokinetics of lercanidipine in healthy volunteers, and show that the pharmacokinetics was enantioselective following a single dose of the racemic drug.

4.2. Capillary electrophoresis

Capillary electrophoresis is a growing analytical technique with numerous advantages. It exhibits much higher efficiency and faster migration time than LC and need lower volume of samples (few nanoliters). Moreover, since the chiral selectors are mixed with the background electrolyte (BGE), it is easy to try numerous chiral selectors at different concentrations. The volume in the column is also very low what makes affordable to try expensive chiral selectors. Blocking is less likely to occur in CE–MS as the column is open, thus can allow a simplified sample clean-up procedure. The main disadvantage is the relatively poor limit of detection. CE and LC are complementary techniques. Whether for application needing a very low LOQ, LC can be the method of choice, for fast and efficient applications CE can provide a very interesting alternative. Heinemann [17] compared the separation of azelastine and three of its metabolites by CE and HPLC. As it can be seen in Fig. 2, the advantage in term of speed, efficiency and chiral separation using CE is striking. However, in that work [17] only the LC method was interfaced to MS and, therefore, limits of quantification could not be compared between the two methods (HPLC–MS versus CE–MS).

ESI is the interface most frequently applied for CE–MS coupling and, as a consequence, it is important to insure that only volatile components enter the ESI-MS interface. Different approaches have been used to avoid that the chiral selector enter the source as for instance the partial filling technique, the use of charged chiral selector which will migrate away from the MS or the employ of counter-current modes [10]. Toussaint et al. [52] used neutral dimethyl-β-cyclodextrin in acidic conditions with a partial filling technique to separate clenbuterol enantiomers, a drug used in the treatment in the treatment of pulmonary diseases, in human plasma. Clenbuterol was used as a test chiral analyte to show that CE–MS can be applied to the determination of chiral drugs in complex mixtures. The influence of the BGE

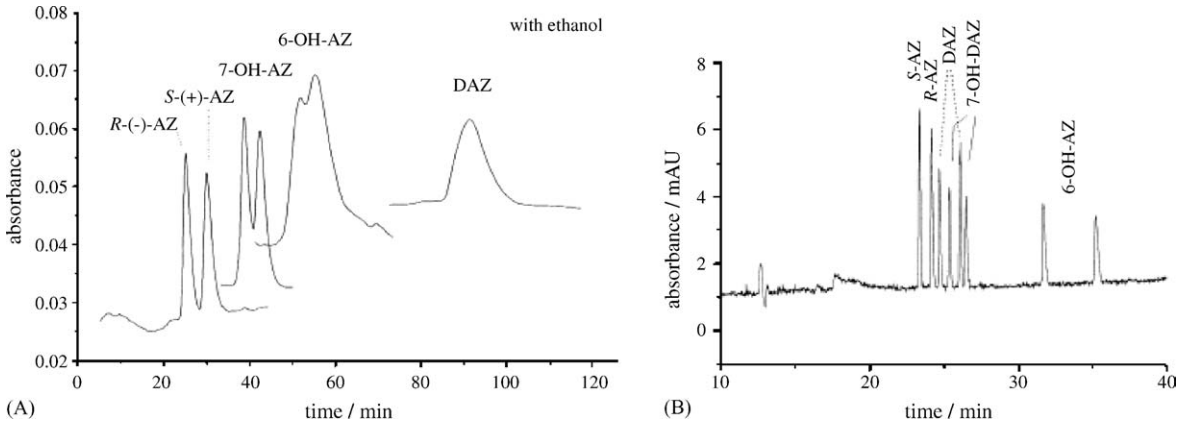

Fig. 2. (A) HPLC separation of the enantiomers of azelastine (AZ) and its metabolites on a Chiralpak AD column (25034.6 mm, 10 mm material) with a flow-rate of 1.2 ml/min and UV detection at 235 nm with *n*-hexane–ethanol–diethylamine (97:3:0.7 v/v). (B) Chiral CE separation of the standard mixture in an uncoated fused-silica capillary using UV detection at 214 nm. Buffer: H_3PO_4 100 mM, pH 5.25 with triethanolamine, 3% methanol, 14 mg/ml CM-β-CD and 16 mg/ml β-CD. Field strength: 1426 V/cm. Redrawn from Heinemann et al. [17].

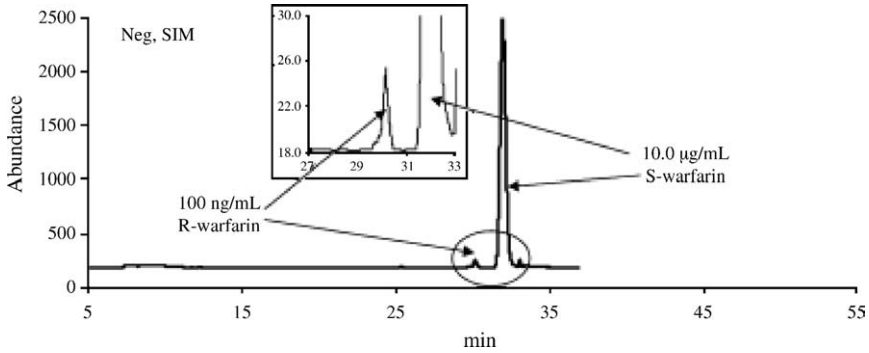

Fig. 3. Analysis of human plasma spiked with 0.1 μg/ml (*R*)-warfarin and 10.0 μg/ml (*S*)-warfarin. The inset shows the expanded version of enantiomeric peaks. Conditions: mobile phase, ACN/H_2O (70:30 v/v) containing 5 mM NH_4OAc, pH 4.0; injection, 12 bar for 3 min; sheath liquid, 70% (v/v) CH_3OH containing 5 mM NH_4OAc (pH 8.5), flow rate 7.5 μl/min. MS spray chamber parameters: drying gas flow rate 5 l/min; drying gas temperature, 350 °C; nebulizer pressure, 0.276 bar (4 psi); capillary voltage, −2500 V; fragmentor voltage, 91 V. Redrawn from Zheng and Shamsi [56].

composition on the sensitivity of MS detection was also investigated. Rudaz and co-workers [55] used charged cyclodextrin and a polyviniyl coated capillary for the stereoselective analysis of tramadol, and analgesic formulated as a racemic mixture and its main metabolites in plasma sample. They succeed in separating and detecting both enantiomers and metabolites in a plasma sample collected from a healthy volunteer 2 h after oral administration of 100 mg of tramadol hydrochloride. Kindt et al. [53] developed a partial filling CE–MS approach to quantify the enantiomers of a novel drug in plasma, and to determine its potential for chiral interconversion. Using a similar approach, Cherkaoui and colleagues [54] separated enantiomers of methadone in human serum.

4.3. Capillary electrochromatography (CEC)

Capillary electrochromatography can be defined as an analytical procedure in which LC and CE are put together. In CEC the separation is achieved in a packed column, but the transport is due to the electroosmotic flow induced by the electric field. This allows a similar separation process as in LC with a significant increase in efficiency. Zheng and Shamsi [56] designed a CEC–ESI-MS method to quantify the enantiomers of warfarin, an oral anticoagulant, in human plasma. Both enantiomers exhibit marked differences in their pharmacokinetics and pharmacodynamics properties. In addition, the importance of monitoring the concentration ratio of (±)-warfarin in patients with thromboembolic disease was demonstrated. CEC capillaries packed with (3*R*,4*S*)-Whelk-O1 chiral stationary phase were used for simultaneous enantioseparation of warfarin and its internal standard coumachlor. They investigated the influence of the column fabrication, as well as the influence of the composition of the mobile phase. The method was applied to the analysis of human plasma spiked with warfarin enantiomers (see Fig. 3).

5. Conclusions and perspectives

It is clear that nowadays the use of LC–MS for chiral separation of drugs and metabolites is more widely used than CE–MS techniques (compare Tables 1 and 2). However, the important advantages that CE and CEC techniques can provide (e.g., faster, more efficient and cheaper analysis) make foreseeable that these techniques coupled with MS become more and more widely applied. Moreover, the high throughput shown by using microchips together with different detectors for chiral separations [57–63], could be corroborated in the non-distant future

by using microchip-MS couplings [64,65] for chiral analysis of drugs and their metabolites in complex matrices.

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