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Review

Enantiomer separation of drugs by capillary electromigration techniques

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Abstract

The review summarizes the most recent developments in the field of enantioseparation of chiral drugs using capillary electromigration techniques. The basic principles of enantioseparations in CE are discussed. Recent developments in sample introduction, separation and detection in capillary electrophoresis and capillary electrochromatography are summarized. The applications are arbitrarily divided into the following three groups: (a) racemates and artificial mixtures of enantiomers, (b) drug forms and (c) chiral drugs and their metabolites in biological fluids. Among the various techniques involved the relatively new developments such as CEC in aqueous and nonaqueous buffers, on-line CE–MS coupling, etc. are emphasized

Keywords: Reviews; Enantiomer separation; Drugs

Contents

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1. Introduction

Capillary electrophoresis (CE) is increasingly used for enantioseparations of chiral compounds and it seems that the prediction ''The high theoretical plate counts (100 000–200 000 plates in a typical capillary) make CE particularly amenable to separating chiral molecules'' [1] becomes more realistic than the rather pessimistic evaluations such as ''problems with capillary electrophoresis have inhibited its progress, and have stopped it developing into a major analytical technique'' [2]. After almost 15 years following the very first chiral separation using CE [3] no stagnation appears for chiral CE applications. The contribution of CE in the overall number of analytical chiral separation is continuously increasing and it is obviously established as one of the major techniques in the field.

What are the challenges of CE in the very competitive field of chiral separations? The most important is a complemenarity of this technique to other separation techniques. CE is more suitable for charged and polar compounds where chromatographic techniques are not very strong.

Is there any unique feature that is offered only by CE and not by other analytical enantioseparation technique? Apparently, there is. At first, it is the extremely high peak efficiency. From the viewpoint of a separation this means that in a given chiral selector/selectand pair one can detect in CE the enantioseparation (chiral recognition) that is impossible to detect using any other instrumental technique. This extremely high resolution power can be amplified further by a large excess of a chiral selector that can be used in CE. This is difficult to manage in separation techniques with immobilized chiral selectors. In addition, a combination of chiral selectors is much easier in CE compared to chromatographic techniques. Another important advantage of CE considering the presently tight laboratory budgets and strict environmental limitations is its miniaturized size and consequently, lower costs of use and less environmental pollutions.

Despite the aforementioned advantages, CE is obviously not a technique that is absolutely free from disadvantages. There are still many problems and challenges. However, the evaluation of the present state of the art in the way: ''Standard deviations of CZE analytical results are typically one full order of magnitude greater than those obtained with HPLC'' [4] seems to be a strong underestimation not only of the present state but also of the future potential of this definitely promising technique.

Recently the requirements for a quantitative analytical method were described in the following concise and elegant way: An analytical technique should ''(1) be easy to use and inexpensive to operate; (2) provide accurate and precise quantitative results; (3) be extremely good at solving at least one important analytical problem'' [4]. Is CE a technique that may qualify as such for analytical scale enantioseparations? The authors are tend to give a positive answer. Below some arguments for this judgement are discussed together with problems that are still evident in chiral CE.

2. Basic concepts of enantioseparation in capillary electrophoresis

The enantioseparation mechanism in capillary electrophoresis (CE) is chromatographic [5,6] while the migration mechanism is electrophoretic. To clearly recognize this concept is very important from both, fundamental and practical points of view. At first, it makes clear that the well accepted idea about the nonselective role of the electroosmotic flow (EOF) (which is undoubtedly true for the separations based on the electrophoretic mechanism) does not apply to chiral CE separations. For enantiomers both, the EOF and the electrophoretic mobility of the analyte are equally nonselective (non-stereoselective). What may distinguish enantiomers in chiral CE is their interaction with a chiral selector. However, this is a chromatographic separation mechanism and the fact that in CE one has not a really stationary but a pseudostationary phase does not make any principal difference from the mechanistic point of view. Weather or not the selector–selectand interaction will be stereoselective is absolutely independent from the fact, does an analyte migrate by its own electrophoretic mobility (μ_{ef}), the EOF (μ_{EOF}) or their combination ($\mu_{ef}+\mu_{EOF}$) [5,6].

The practical consequence of the aforementioned is that the criticism, ''the efficiency of CZE is high but, the flexibility of chromatography in adjusting

separation factors is lost'' [4] does not apply to chiral separations because they rely on a chromatographic separation principle. Moreover, the flexibility of chiral CE from this point of view is even higher because the selector–selectand interactions can be intensified by using a large excess as well as a combination of chiral selectors.

3. Potential of capillary electrophoresis in enantioseparation of chiral drugs

The high peak efficiency which inherently characterizes CE is exactly what is required for chiral separations [1]. Identity of enantiomers in achiral medium and in the most cases rather small differences in their behavior in chiral medium results a small thermodynamic enantioselectivity in their noncovalent interaction with chiral selectors. The low thermodynamic selectivity of recognition combined with high separation efficiency may turn the separations that are impossible to observe by chromatographic techniques well observable in CE. This is a major advantage of CE compared to other instrumental techniques. Comparative characteristics of CE as a tool for analytical enantioseparations are given below. Criteria such as suitability to the samples of interest, success rate (separation power, sensitivity), reliability of results (accuracy, reproducibility), simplicity to learn and use it, speed (to learn a technique and method development) environmental pollution (hazardous materials used and their scale), costs (of equipments, required consumables such as chiral selectors, buffers, accessories, etc.) and acceptance in academic and industrial environment will be discussed.

Chiral CE may be used in an early stage of drug development (synthesis of a chiral drug candidate), formulation studies, in preclinical and clinical phase 1 to phase 3 studies, drug storage and use.

In the drug development stage the enantiomers are determined as pure chiral compounds or as a mixture together with other synthetic intermediates and side products. For this kind of samples CE offers advantages compared to gas chromatography (GC) (except few anesthetic gases) and is at least, not less suitable than HPLC. The requirements such as high separation efficiency, high selectivity, etc. may be even better met by CE than by HPLC. The current generation of CE instruments is automated, as are HPLC and GC equipments.

As the latest developments demonstrate, chiral CE may be also useful for micropreparative collection of drug enantiomers in amounts that may be sufficient for initial pharmacological screening [7–9].

Drug forms commonly contain a matrix (cellulose derivatives, starch, etc.) which may create certain problems in CE because high-molecular-mass compounds tend to be adsorpted on the capillary inner wall and to affect the EOF. Indeed, high-molecular-mass compounds may create problems also in HPLC (contamination of the column, filters, irreversible adsorption, etc). Possible solutions for these problems in CE may be a precoating of the capillary inner wall in a way which inhibits the interaction of matrix ingredients of drug forms with the capillary wall. Alternatively, the matrix ingredients can be removed by sample pretreatment (membrane filtration, liquid–liquid extraction, etc.).

In a preclinical and clinical phase 1 to phase 3 studies a chiral drug candidate and its possible metabolites (phase I and Phase II) have to be determined in biological media such as plasma, serum, urine, saliva, cerebrospinal fluid and tissue homogenates. Most of these sample matrixes (especially urine) are better compatible with CE rather than chromatographic techniques. High-molecular-mass ingredients of plasma may cause problems similar to the aforementioned polysaccharides due to adsorption on the capillary inner wall. Several solutions of this problem are discussed below.

The parent drug may be polar or may undergo metabolic transformations to polar compounds. This relates especially to phase II metabolites such as glucoronides, sulfates, mercapturates, etc. CE is the technique of choice for the analysis of charged compounds.

It is certainly unimaginable in GC and extremely difficult in chiral HPLC to achieve the enantioseparation of a chiral drug and its phase I and phase II metabolites in a single run. However, this is possible in chiral CE. In Fig. 1 the simultaneous enantioseparation of phase I and phase II metabolites of chiral antihistaminic drug dimethindene (DM) is shown as an example [10].

Thus, CE is at least as well suitable for bioanalyti-

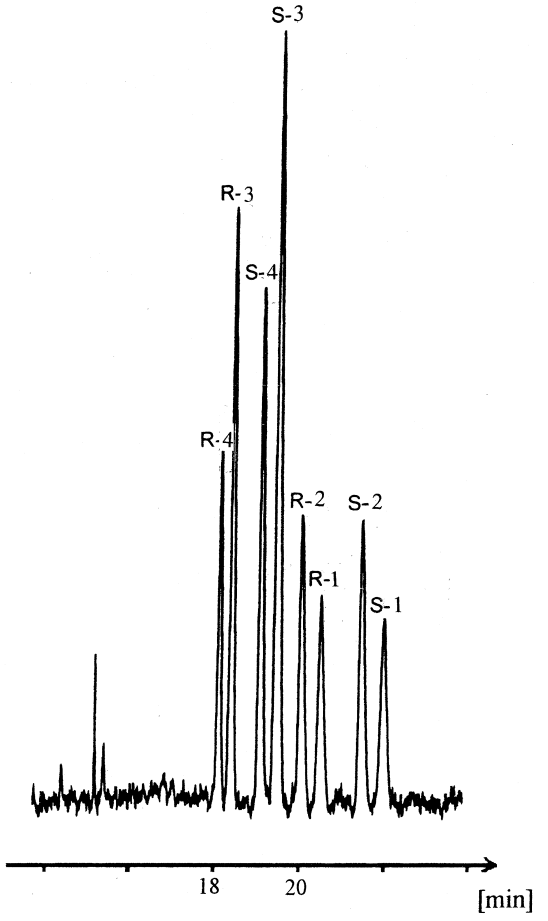

Fig. 1. Simultaneous separation and enantioseparation of phase 1 and phase 2 metabolites of antihistaminic drug dimethindene. (1) 6-hydroxydimethindene; (2) 6-hydroxy-*N*-demethyldimethindene; (3) 6-hydroxydimethindene-glucoronide; (4) 6-hydroxy-*N*-demethyldimethindene-glucoronide. The separation was performed in a fused-silica capillary of 47 cm (effective length 40 cm)×50 μm I.D. Anode and cathode buffers: 50 m*M* potassium phosphate buffer pH 3.3; Run buffer: 50 m*M* potassium phosphate pH 6.0 containing 15 m*M* CM-β-CD. Applied field strength 400 V/cm. (From Ref. [10] with permission).

cal samples as HPLC is. However, CE may cover better whole range of related compounds (native drug, its phase I and phase II metabolites) possessing different polarities.

One of the important criteria for the selection of an analytical technique is the success rate. In other words, what is the probability to solve a chiral separation problem using a given technique. The number of commercially available chiral stationary phases (CSP) for HPLC approaches two hundreds at present. Very effective CSPs exist for both normal- and reversed-phase HPLC. The number of commercially available chiral selectors (CSs) in CE is lower, and in general, CE does not offer a higher success rate compared to HPLC for a separation of a mixture of two enantiomers. However, the superiority of chiral CE compared to HPLC becomes evident as soon as one needs to separate and simultaneously enantioseparate multicomponent mixtures containing the parent drug, its phase 1 and phase 2 metabolites or synthetic intermediates. One example is described in Fig. 2 [11]. The enantioseparation of thalidomide (TD), (the drug that seems to be the most cited example of enantioselective drug toxicity but racemizes rapidly in vivo), is not a problem either in HPLC [12–14] or in CE [15]. However, rather more difficult is to simultaneously separate and enantioseparate TD and its biologically relevant metabolites that were recently detected in incubation mixtures of racemic TD with fraction S9 from human liver and plasma samples from male volunteers who had received racemic TD orally [16]. On the other hand, the teratogenity mechanisms of TD needs to be clarified due to the distinct anti-inflammatory activity of this drug for the treatment of leprosy [17], and recently discovered inhibition of the HIV-1-virus [18], the suppression of the release of tumor necrosis factor-α (TNF-α) [19], etc. Recently, TD has been approved by the United States Food and Drug Administration (FDA) for the treatment of erythema nodosum leprosum [20]. All experiments to separate and enantioseparate TD and its three hydroxylated metabolites in a single run using either HPLC with chiral columns or CE with single chiral selector in normal polarity mode were unsuccessful. However, all of them were resolved in a single run using carrier mode CE with a combination of two chiral selectors (Fig. 2). A combination of two chiral selectors based on column-switching is also possible in chromatographic techniques. However, this is associated with several technical problems (high back pressure, increased dead volumes, additional peak dispersion, etc.). In addition, chiral selectors can be mixed in CE in any desired ratio (only limited by the solubility). This is difficult and at least very time consuming in HPLC and GC.

If one compares the chiral selectors exhibiting the

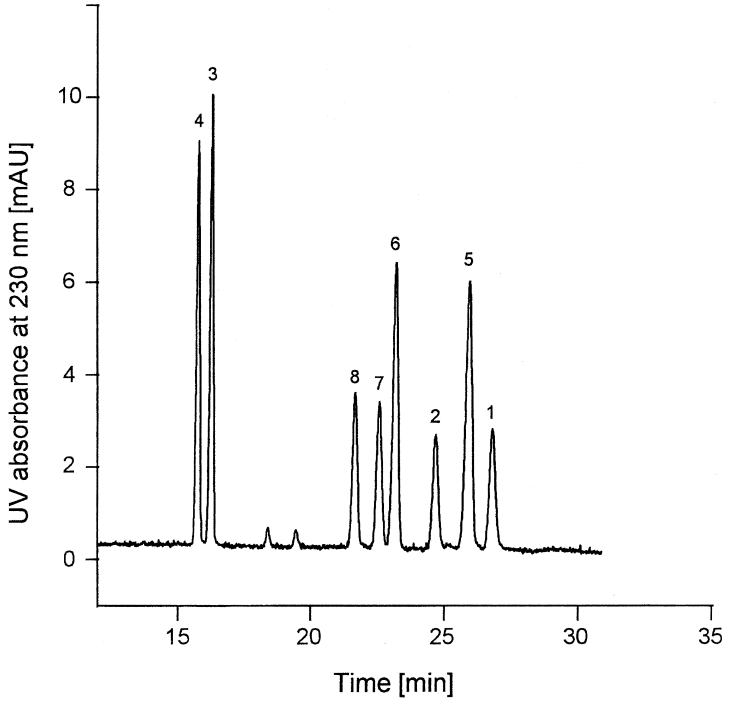

Fig. 2. Simultaneous separation and enantioseparation of thalidomide [1,2] and its metabolites 5-hydroxythalidomide [3,4] and diastereomeric 5′-hydroxythalidomides [5–8]. Separation was performed in polyacrylamide-coated capillary of 30 cm (effective length 22 cm)×50 μm I.D. Buffer; 50 m*M* potassium phosphate pH 6.0 containing 20 mg/ml SBE-β-CD and 10 mg/ml β-CD. Applied voltage 25 kV. Detection on the anodic side of the capillary. (From Ref. [11] with permission).

same thermodynamic enantioselectivity of recognition (α) in an ideal experiment, then it is clear that the rate of success of an enantioseparation is much higher in CE than in HPLC. This advantage steams from the fact that peak efficiency is much higher in CE and the resolution factor increases proportional to $N^{1/2}$. In addition, selector–selectand interactions may be enormously intensified in CE by increasing the concentration of chiral selector. This may be certainly limited by the solubility of a chiral selector, high viscosity of BGE or high current in case of charged chiral selectors. However, available frames are quite wide in order to optimize a separation. Thus, the separation power of CE and the tools and tricks available in order to adjust a chiral separation is definitely larger than those of HPLC or in any other chromatographic techniques.

Reproducibility of migration times and the separation as a whole has been a matter of uncertainty in CE for a long time. However, as most recent developments indicate this is not a critical issue any more. On one hand, 75–80% of all CE separations currently are performed using capillaries from the same manufacturer (Polymicro Technologies, Phoenix, AZ, USA). On the other hand, several techniques became available for an elimination of the contribution of the capillary wall to the mobility of an analyte either by adjusting the mobility and direction of the EOF independently of the original chemistry and morphology of capillary inner surface or by its suppression. Further, an increasing number of well characterized, single component chiral selectors entered the market recently [21]. This also facilitates the development of well-reproducible chiral CE methods.

Above only one dimension of an electropherogram has been discussed. However, it is actually a two-dimensional graph. The separation (migration time) axis illustrates the strength of CE. In contrast, the orthogonal axis where a signal intensity is plotted

has been and still remains somewhat its weakness. This is a challenging field for a coming years. There are several innovative developments described in the recent literature and certainly there is no reason to make a conclusion like this ''It is unlikely that chiral CE will be used in drug bioanalysis because enantiomers must be determined at very low levels, often ng/ml or lower'' [22]. A drug concentration at the nanogram level in one milliliter is also not an easy task for a determination using HPLC which is a well accepted technique in the field. On the other hand, a concentration ng/ml or even lower is not the limit of detection (LOD) in CE. Furthermore, there are several possibilities of sample preconcentration such as on capillary solid-phase (micro)extraction, membrane filtration/preconcentration and sample stacking. In addition, one has to consider also a wide variety of a detection cells with an extended optical path length on the market. In principle, one may have even the same path length in CE as in HPLC providing that a separation factor allows this. As mentioned above, it is not difficult to adjust a separation in chiral CE. Further, comparing the LOD and the limit of quantitation (LOQ) in CE to HPLC, one has to consider that a sample zone elutes as a much sharper peak in a significantly shorter period of time and is less diluted in CE, than in HPLC. Therefore, as our experience shows even without any sophisticated cell design, sample stacking, etc. one may observe a LOQ in CE comparable to HPLC. However, the most important argument for a bright future of chiral CE in drug bioanalysis is that this technique is compatible with such a sensitive, specific and universal detection method as mass spectrometry (MS) [23,24].

Another aspect of sensitivity that can not remain outside the scope of this discussion is following: In the majority of cases it is rather important to detect an impurity of the minor enantiomer in the presence of the major one. Therefore, it may happen that the detection limit of one enantiomer in the presence of the other one will be a more critical issue than the overall LOD or LOQ of the method. In this case a separation factor becomes as important for the final success as the overall sensitivity of the system. This happens because the separation factor must allow sufficient difference between the migration times of two enantiomers in order to detect a minor enantiomer without overlapping by a major one. The adjustment of the enantiomer migration order can be very useful in this case, too [25]. For both, the optimization of a separation and the design of the enantiomer migration order CE offers certain advantages compared to HPLC. Thus, the challenges of chiral CE compared to HPLC from the viewpoint of detection sensitivity do not look as critical as one may think just based on the comparison of the standard size of the separation chamber and the path lengths of detection cells.

The fascination of CE is the remarkable simplicity of this technique. No pump, injector valves and detector cells are required. This not only simplifies the experiment markedly but also eliminates sources of additional peak broadening because the sample is injected and detected directly in the separation chamber. The single CE equipment can successfully be used not only to perform a separations in various CE modes (CZE, MEKC/cEKC, cITP, CGE, cIEF, etc.) but also capillary HPLC and CEC separations [26]. Taking care of the applied high voltage CE is quite a safe technique and at least not more difficult to learn than GC or HPLC.

CE is also a more rapid compared to chromatographic techniques from the viewpoint of method development. Changing and conditioning a column is very time consuming in chromatography. However, changing a capillary and/or chiral selector takes only few minutes in CE.

CE as microanalytical technique requires a minute amounts of solvents. This makes a technique especially environmentally friendly and inexpensive. Further saving of costs steams from the extremely low amount of chiral selectors and buffers. This allows to study a chiral recognition properties of rather expensive and/or exotic materials which are available in a small amounts. Fused-silica capillaries used in CE are rather cheap as well as most of the accessories. The basic CE equipment of a medium quality does not cost more than an HPLC or GC instrument. However, the costs from the use of the latter two fairly exceed those from CE.

At present, CE does not have any acceptance problems in academic laboratories. In the industry, HPLC still remains the dominating technique for chiral separations. However, taking in account the experience in academic institutions, it seems highly

probably that in the nearest future CE will become the technique of choice for analytical enantioseparations (at least) in pharmaceutical, food, chemical and agrochemical industries.

4. Recent developments in chiral electromigration techniques applied to bioanalytical problems

4.1. Sample pretreatment and injection in chiral capillary electrophoresis

The problems which may be associate the determination of enantiomers in biological samples are related to the complexity of the matrix, adsorption of high-molecular-mass sample components on the capillary wall, relatively high salt concentration and often, low concentrations of the analytes. Most of these problems must and in principle can be resolved in a sample preparation and injection steps.

Sample pretreatment may involve the isolation and preconcentration of components of interest. These two steps may be also integrated as one operation.

An interesting technique for on-line isotachophoretic sample pretreatment of enantiomers present in complex ionic matrices was recently described by Dankova et al. [27]. Tryptophan (Trp) was used as a model analyte and it was shown that 30 μl volume of a sample could be injected and the enantiomers quantified in a 300 μm I.D. fluorinated ethylene–propylene copolymer capillary. When using α-CD as a chiral selector it was possible to easily determine 0.5% of L(−)-Trp in D(+)-Trp at their total concentration 1.5 ng/ml (Fig. 3). The applicability of this technique for the determination of Trp enantiomers in a 90 component model mixture of organic acids, as well as in a urine sample was illustrated.

Palmarsdottir et al. also illustrated the enormous increase of sensitivity together with some improvement of system selectivity using the supported liquid membrane (SLM) technique coupled on-line with capillary zone electrophoresis (CZE) through a micro-column liquid chromatography (MCLC) interface [28] or just in the double stacking sequence [29]. The advantage of SLM technology is that it allows to clean up blood plasma samples from macromolecules that tend to adsorb strongly onto the capillary wall and simultaneously, to increase the analyte concentration. In the initial version of this technique [29] it was possible to inject in the capillary the content of 2–3 μl of plasma. A combination of the SLM technique and the double

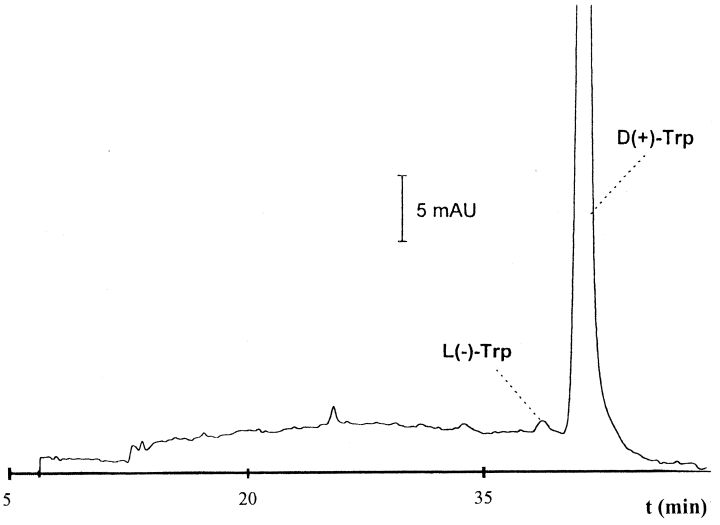

Fig. 3. An electropherogram from the separation of tryptophan enantiomers by the ITP-CZE combination. The injected sample (30 μl) contained L-(−)-Trp at a 25 nmol/l concentration and D(+)-Trp at a 5000 nmol/l concentration. UV-detection. (From Ref. [27] with permission).

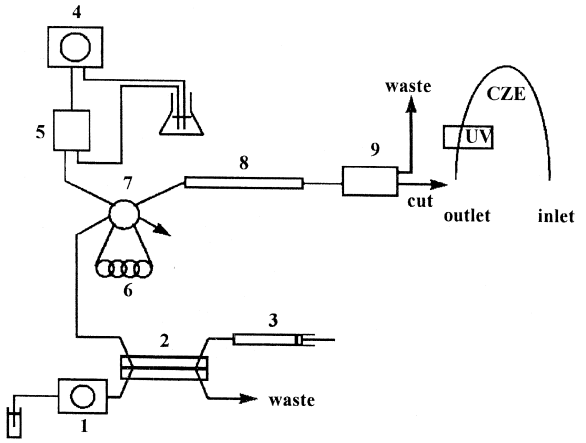

Fig. 4. The SLM-CLC-CZE experimental setup. (1) sample processor; (2) SLM-enrichment unit; (3) microinjection pump; (4) pump; (5) accurate microflow processor; (6) 50-μl injection loop; (7) valve; (8) MCLC column; (9) microdumper interface. (From Ref. [28] with permission).

stacking CZE procedure with a MCLC interface allowed to increase the injected volume to 50 μl (Fig. 4) [28]. This way it became possible to concentrate the enantiomers of bambuterol in human plasma about 40 000 times. As a result, subnanomolar concentrations of the enantiomers were detected despite the relatively weak UV absorbance of bambuterol (Fig. 5) [28].

Together with these techniques several others such as on-capillary solid-phase micro-extraction, different modifications of sample stacking [30–32] etc. may be successfully applied also for chiral CE. However, these techniques are not discussed in detail here because to the authors knowledge their application to chiral drugs have not yet been published.

As aforementioned, the amount of a minor enantiomer which can be quantified in the presence of a major component can be more critical issue rather the total sample concentration. This aspect is discussed below in Sections 5.1 and 5.2. However, just one example that illustrates the potential of chiral CE from this viewpoint is shown in Fig. 6. The concentration of the minor enantiomer was less than 0.05% in this experimental proprietary basic drug [33].

4.2. Sample separation in capillary electromigration techniques

4.2.1. Sample separation in chiral capillary electrophoresis

According to the authors concept the separation principle in chiral CE is chromatographic independent of the mode of operation. Therefore, below no difference is made between the enantioseparations in CZE, CGE, MEKC, cEKC, cIEF, cITP, etc. All chiral CE separations may be attributed to cEKC with different chiral selectors such as CDs, chiral

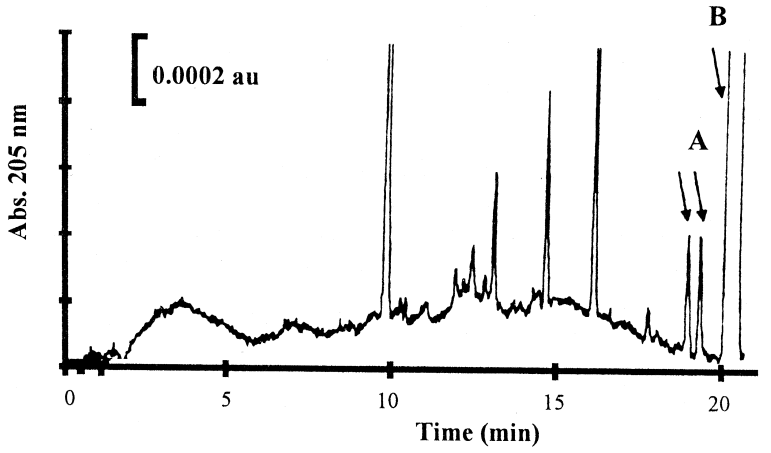

Fig. 5. Electropherogram showing plasma containing 10 μm physostigmine (B) and 0.5 n*M* of each bambuterol enantiomers (A). CE separation conditions were as follow: fused-silica capillary with 75 μm I.D.; 100 m*M* phosphate buffer, pH 2.5 containing 3.9 m*M* dimethyl-β-cyclodextrin. (From Ref. [28] with permission).

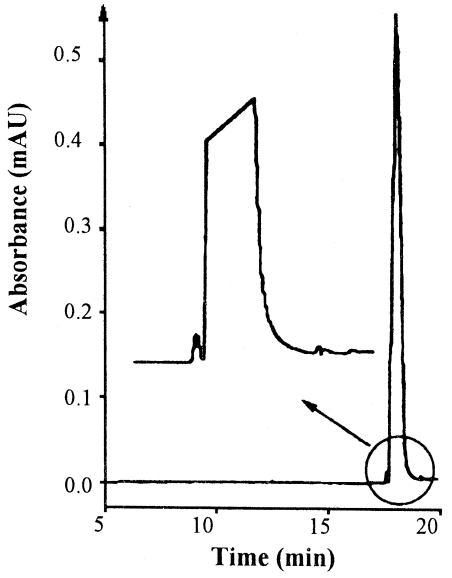

Fig. 6. Chiral excess analysis of a low-level enantiomeric impurity of an experimental proprietary basic drug. Capillary: 64 cm (56 cm effective length)×100 μm; buffer: 50 m*M* phosphate (pH 2.5) containing 5 m*M* γ-CD; sample concentration: 1 mg/ml. (From Ref. [33] with permission).

micelles, a combination of achiral micelles and chiral selectors, crown ethers, macrocyclic antibiotics, peptides, etc. To the other group of chiral separations using electromigration techniques belong those performed in capillary electrochromatography (CEC) were true stationary phase is used instead of pseudo-stationary phase.

Charged components of the sample may be resolved from each other according a true electrophoretic separation principle, i. e. without any additives to the background electrolyte. When a true electrophoretic separation is insufficient then additives may be used to enhance the resolving power of a system. This can be an achiral surfactant that may also allow to resolve uncharged sample components from each other. Neither a true electrophoretic principle nor an achiral additive may allow a separation of enantiomers in CE. For this purpose a chiral selector must be introduced into a separation system. In addition to enantioseparations, a chiral selector may also affect a true electrophoretic separation factor.

In order to understand the current trend in the use of chiral CE selectors for bioanalytical purposes the more recent literature (from January 1997 until August, 1999) was analyzed. In this time almost 85% of all chiral CE separations for biomedical purposes were performed using cyclodextrins (CDs). Among them native α-, β-, and γ-CD were used in 32% of cases and their derivatives in 68% of cases. Neutral derivatives of CDs are still more widely used (62%) than charged ones (38%). Among the neutral CD derivatives randomly and selectively substituted analogs were equally popular (49 and 51%, respectively). This trend is still very different in the case of charged CD derivatives where in 83% of cases randomly substituted derivatives were used.

The almost universal chiral recognition ability of CDs is certainly one of the major reasons for their wide acceptance as chiral selector in CE. Another reason seems to be the wide variety of commercially available CD derivatives. There is definitely no other family of chiral selectors which may compete with CDs from this point of view. As the analysis of the literature indicates currently the commercial availability (at low prices) of a chiral selector is directly reflected in its application in CE. For instance, it is obvious that a selectively substituted well characterized chiral selector is a absolute ''must'' for the development of a well-reproducible and validated chiral bioanalytical method. Despite this fact, randomly substituted CD derivatives, especially charged ones, are still intensively used. The reason for this does not seem to be ignorance by the authors of the clear advantages of well-characterized uniform chiral selectors, but a limited number of them on the market and the relatively high costs.

Among 90 cases of the use of single-isomer neutral CD derivatives in 84 cases these were either heptakis-(2,6-di-*O*-methyl)-β-CD (DM-β-CD) or heptakis (2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) that are commercially available from several suppliers. The similar derivatives of α- and γ-CD that are commercially available still only from one company (Cyclolab, Budapest, Hungary) were used only in the few cases. The reason for an intensive application of DM-β-CD and TM-β-CD as chiral selectors in CE does only not seem to be their availability from well-known chemical and biochemical companies with worldwide distribution service such as Sigma, Fluka, etc. More attractive for the researchers apparently is the often complementary chiral recognition

ability of these CDs compared to native β-CD. For instance, native β-CD is not a very effective chiral selector for enantioseparations of chiral arylpropionic acid derivatives such as the drugs ibuprofen, ketoprofen, etc. However, TM-β-CD exhibits excellent chiral recognition ability towards this group of chiral drugs [34–36]. From this point of view it seems interesting to mention the recently published data about the almost universal chiral recognition ability of heptakis-(2,3-dimethyl)-α-CD towards the enantiomers of chiral phenoxy acid herbicides [37].

The complementarity of selectively methylated and native β-CD is expressed not only in their different coverage of chiral analytes but also in the chiral recognition pattern (the affinity of the enantiomers towards a chiral selector) [38–40].

Complementary properties may be observed in the family of other chiral selectors too. For instance, for macrocyclic antibiotics it is known that vancomycin, ristocetin, teicoplanin, etc. are mainly useful for enantioseparations of chiral analytes containing carboxylic acid groups, whereas ansamycins (rifamycin B and rifamycin SV) are better suited for enantioseparations of basic compounds. The problem is that the latter are, in general, not very powerful chiral selectors even for basic analytes. In addition, although rare examples are described in the literature [41] macrocylic antibiotics do not seem to be well suitable chiral selectors for enantioseparation of neutral analytes.

In contrast to this, native CDs and their neutral derivatives are useful for enantioseparations of both, acidic and basic compounds. In addition, charged CD derivatives are applicable for a separation of uncharged enantiomers.

One of the most important development in the field of chiral selectors for CE seems to be the introduction of a single isomer charged CDs [42–46]. This creates a solid background for the development of validated chiral CE separations which may be used for bioanalysis [15,21].

New members appeared also in the family of macrocyclic antibiotics [47–49]. The future will show if avoparcin and tubocurarin may extend the applicability of macrocyclic antibiotics behind the well established vancomycin.

Besides the already known crown ether, 18-crown-6-tetracarboxylic acid (18C6H4) which was also studied in the combination with other chiral selectors [50] as well as in nonaqueous conditions [51] chiral calixarenes were also tested as CE selectors [52,53]. In principle, this approach may allow to extend the potential of synthetic macrocyclic compounds in chiral CE because chiral calixarenes in difference to crown ethers may be used not only for enantioseparation of chiral primary amines but in principle for any kind of chiral analyte. However, the more universal chiral recognition ability than reported to date will be required for success. In addition, the strong UV absorbance of calixarens and resorcarens may cause some inconveniences in their use as chiral buffer additives in CE.

The application of chiral surfactants in CE was scarcely published during the last years [54]. Together with the known members the application of purified saponin and digitonin were reported as chiral selectors in two studies. However, the results do not seem to be clearly competitive. The applications of new chiral selectors of other groups such as monosaccharides [55], ergot alkaloids [56] modified peptides [57], cyclopeptides [58,59], etc., seem to be valuable just from a mechanistic point of view or expanding the number of chiral selectors. However, their use does not seem to affect significantly the bioanalytical potential of chiral CE.

The application of a new chiral selector is just one way to optimize a chiral CE separation. At least, not less promising is to apply an alternative separation principle (for example, the designed combination of chiral selectors, chiral selector as a carrier, counterbalancing mobilities, etc.). As was shown in a number of studies, a combination of chiral selectors may allow to observe a significant enhancement of the separation selectivity in chiral CE [60]. However, a separation system needs to be very carefully designed in this case [5,6].

If required, a chiral selector may also be used as carrier of a chiral analyte. When appropriately designed, this may result a significant enhancement of the separation selectivity because the analyte will migrate to the detector mainly in the complexed form with a chiral selector. This means that it will participate in the chiral recognition during its whole residence time in a separation capillary. Alternative-

ly, a countercurrent analyte-selector migration principle can be realized more effectively when using chiral selector also as a (chiral) carrier.

One additional example of selectivity enhancement in chiral CE is shown in Fig. 7 [8]. In this case both enantiomers of chlorpheniramine are equally decelerated by applying a pressure-driven counter flow on the outlet side of the capillary. Significant (in principle, even unlimited) enhancement of separation factor may be observed by this operation. However, this approach seems to be of more interest for micropreparative rather than for analytical purposes.

Several examples of enantioseparations in nonaqueous BGEs were described during last years [51,61–65]. The significant disadvantage of these studies is that the chiral selectors already well studied in aqueous solutions are mainly used except of Ref. [64]. However, to the authors opinion the major driving force of the development of chiral CE in nonaqueous buffers will be the application of new chiral selectors which due to solubility or stability reasons are impossible to use or do not exhibit sufficient chiral recognition ability in the aqueous solutions.

4.2.2. Sample separation in chiral capillary electrochromatography

Capillary electrochromatography (CEC) offers some alternative mechanisms for solving a separation problem and, therefore, represents a very useful extension to CE. A pseudostationary chiral selector that is responsible for the high flexibility and versatility of CE sometimes may also cause problems. In particular, a dissolved chiral selector is not desirable in on-line coupling of chiral CE to polarimetric, circular dichroism, mass spectrometric, etc. detectors. In addition, it is impossible to find a suitable solvent for every chiral selector that may dissolve it without destroying or diminishing its

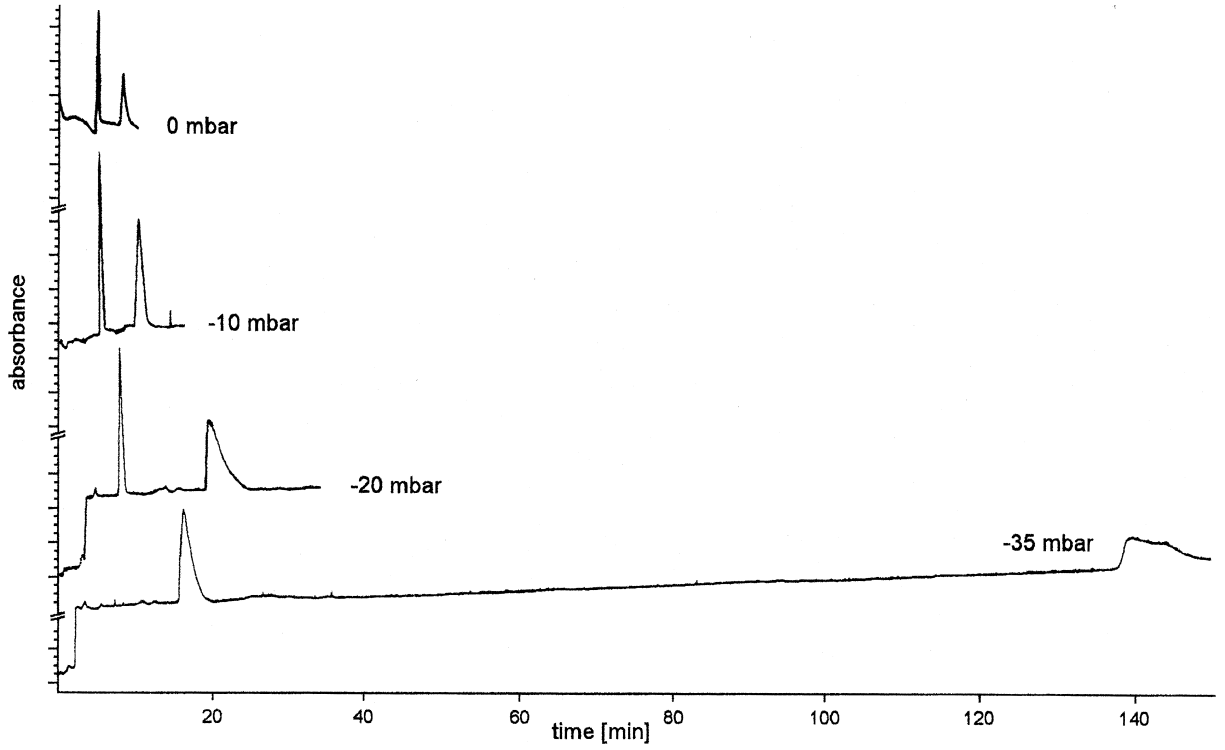

Fig. 7. Counterbalanced enantioseparation of racemic chlorpheniramine. (From Ref. [8] with permission).

chiral recognition properties, and at the same time, serve as a good buffer for electrophoretic separation. Mobile chiral selectors must be replaced after each analysis that is not optimal from an economic point of view and, in addition, may lead to certain problems for run to run reproducibility of analytical characteristics. These problems may be eliminated in chiral CEC.

The history of CEC apparently goes back to early 1950's when Mould and Synge noted the potential of the EOF as a driving force in separation techniques [66,67]. Later, Pretorius et al. [68] paid attention to some important advantages of the EOF such as (plug-like profile, independence of the EOF on the particle size and geometry, etc.). In 1980's Jorgenson and Lukacs [69] and Knox's group [70–72] contributed significantly to the development of the capillary format of electrochromatography. Many reviews appeared during last years on the general principles of this hybrid technique of HPLC and CE [73–77]. Only few selected works among those published since 1997 are summarized below. A summary of earlier studies on chiral CEC can be found in Ref. [75]. The updated treatment of cyclodextrin-type chiral selectors in CEC is given in the review by Schurig and Wistuba [76].

Enantioseparations may be performed in the both formats of CEC, in open tubular wall-coated capillaries [75,76,78–81], as well as in the capillaries packed with chiral stationary phases (CSPs) [75,82–93]. Both formats were developed during last 2–3 years but the achievements were more significant in the latter technique. Here, the main attempt has been made on the transfer of the successful CSPs for HPLC to CEC [82–89]. In rare cases CSPs were developed specially for the use in chiral CEC [90]. In two recent studies, in analogy to earlier work by Lelievre et al. [91], chiral modifiers of the buffer were used in combination with achiral stationary phases [92,93].

Several CEC studies report examples of enantioseparation of chiral drugs. However, in many cases separation efficiency is lower compared to CE. The examples of simultaneous enantioseparations of chiral drugs and their metabolites that may be applied to biomedical studies have almost not been published yet [88].

Currently, chiral CEC separations in nonaqueous (NAQ) buffers are developing with chiral stationary phases [86–88] as well as chiral buffer additives in combination with achiral mobile phases [92]. The Joule heating is lower in NAQ buffers that may allow the application of higher field-strengths. NAQ solvents are also more convenient for on-line CEC–MS coupling. However, the most exiting idea for developing nonaqueous chiral CEC was the fact that some CSPs (to these belong also the most successful CSPs in HPLC, polysaccharide derivatives) do not work as effectively in the aqueous medium as they do in the NAQ environment.

Some basic dependencies in NAQ CEC as well as the examples of enantioseparations of chiral drugs are described in Refs. [86–88]. Interestingly, CEC as well as capillary LC in NAQ medium seems to be effective also for separations of chiral drugs and their metabolites that were impossible to realize in aqueous or more convenient *n*-hexane/2-propanol mobile phases in HPLC mode (Fig. 8) [88].

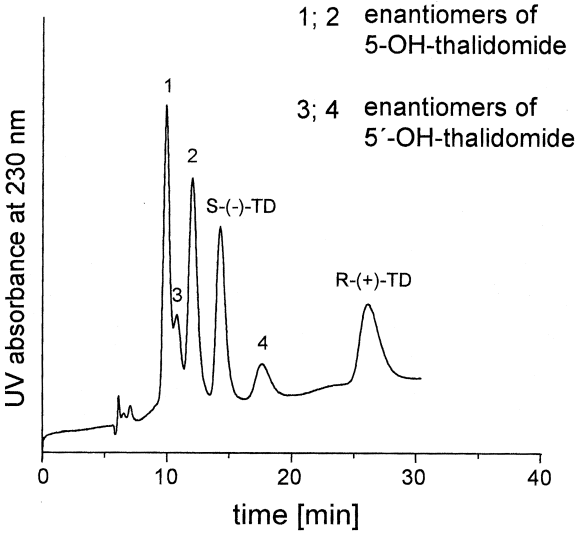

Fig. 8. Nonaqueos simultaneous CEC enantioseparation of thalidomide and its metabolites. Capillary: 31 cm (25 cm effective length×100 μm I.D., packed with aminopropylsilica coated with 16% (Chiralpak-AD)+4% (Chiralcel-OD) materials. Buffer: methanol–ethanol (75:25, v/v), containing 2.5 m*M* ammonium acetate. Applied voltage –25 kV. (From Ref. [88] with permission).

4.3. Sample detection

4.3.1. Various derivatizing reagents, detection cells and detectors

The main issue of any detection is the sensitivity and the specificity of the analysis. In general, all advancement for improving the detection sensitivity in achiral CE can be transferred to chiral CE. Two principal approaches for this involve the designs of detection cell and the new detection principles. Capillaries with an extended detection cells (z-cell, bubble-cell, etc.) are commercially available [94]. Bubble cells with 5–6 times extended optical path lengths can also easily be prepared in a laboratory. Recently, significant enhancement of detection sensitivity was illustrated using newly commercially available (Hewlett-Packard, Waldbronn, Germany) capillary flow-cell [94]. In the capillaries, in which a detection occurs in the extended or bent parts some decrease of resolution can occur. This may be critical for separations with low separation factors.

Several papers were published during the last years in which LOD at nanomolar level was achieved. Thorsen et al. [95] proposed new chiral precolumn reagents, (+)- and (−)-1-(9-anthryl)-2-propyl chloroformate which are useful for indirect chiral separations and laser-induced fluorescence (LIF) detection of amino acids and small peptides. The use of these reagents in combination with argon ion LIF allowed the determinations of enantiomers at ppm levels.

Liu et al. [96] used a fluorescent chiral reagent, 4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole and LIF with the Ar ion laser allowing a determination of amino acid enantiomers in nanomolar amounts in complex biological matrices (rabbit serum and homogenate of *Aplysia californica buccal ganglion*) without any interferences of matrix components.

The technique of on-column fluorescent labeling of D- and L-amino acids described by Tivesten and Folestad [97] is also of significant interest for chiral drugs. The idea of the method is the consecutive injection of a sample and the reagent onto the capillary as two discrete plugs. By utilizing their difference in mobility, the zones are mixed by the electrophoretic process in a designed way. Thus, the analytes are both, derivatized within a few seconds and subsequently separated in a single step. Compared with precolumn derivatization, dilution of the original sample is minimized which allows a labeling of nano- to picoliter samples. The mass limit of detection in the low amol level was achieved using a He-Cd laser at 325 nm.

In the methods described in references [95–97] a chiral selectors was not applied because chiral derivatizing reagents allowed both, to improve a detection limit and to transform the enantiomeric compounds into their corresponding diastereomers, which are resolvable also in buffers without a chiral selector. This so called indirect enantioseparation technique, bears also some disadvantages (requirements of chemical and enantiomeric purity of derivatizing reagents, completeness of chemical reactions, etc.) which shall be considered when using this technique.

Kaneta et al. [98] used FluoroLink Cy5 Mono Reactive Dye (Cy5) for achiral derivatization of amino acids which were further resolved using γ-CD as chiral selector. A diode laser emitting at 635 nm was used as an exciting light source and the detection limit $6 \cdot 10^{-8}$ *M* was achieved for tyrosine, alanine and valine.

Among other novelties in detection technology electrochemical detection represents a certain interest [99,100]. This is obviously not as universal detection principle as UV but can provide a very low LOD for appropriate compounds. Hadwiger et al. [99] developed a technique for a stereoselective study of the elimination profile of each enantiomer of isoproterenol (IP) administered as a racemic mixture to Sprague–Dawley rats. Resolution of the enantiomers of IP was performed using a running buffer containing methyl-β-cyclodextrin as a chiral selector. A LOD of 0.63 ng/ml achieved using a combination of electrokinetic sample preconcentration, pH-mediated sample stacking and electrochemical detection. This setup allowed monitoring of the elimination of IP for up to six half-lives. Microdyalysis sampling was capable of continuously monitoring the concentration of IP with 60 s time resolution. This was a very critical issue in this particular case due to the relatively rapid elimination kinetics of IP. The experimental setup allowed also to reduce the sample

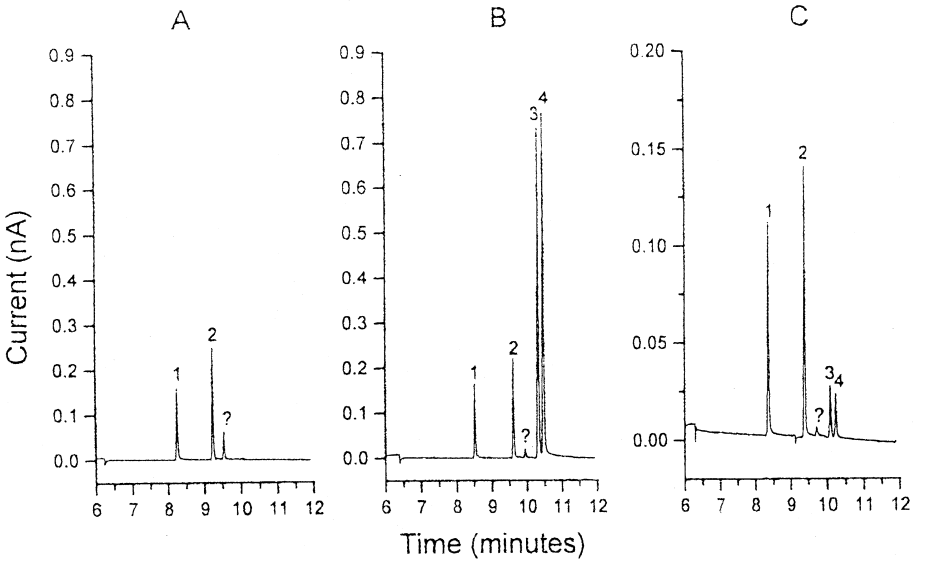

Fig. 9. Typical electropherograms of plasma microdyalisate samples. (A) prior to dosing; (B) 10 min after dosing; (C) 60 min after dosing. Peaks 3 and 4 belong to IP. (From Ref. [99] with permission.).

volume to 2–4 μl which is also significant when performing a pharmacokinetic experiment in small animals. The electropherograms of a plasma in a 0–60 min interval after dosing is shown in Fig. 9. Although not obvious from this figure, a careful treatment of the pharmacokinetic data revealed that the difference between the elimination half-lives of the IP enantiomers was significant at the 95% confidence level [99].

4.3.2. Chiral capillary electrophoresis–mass spectrometry coupling

Among the detection principles applied in chiral CE, the mass spectrometer (MS) is of special interest as a sensitive, universal and the same time selective detector [23,24,101–107]. In the very first study on chiral CE–MS coupling [23] a sensitivity 1000-fold better than with UV absorbance detection was reported with ion-spray MS detection in the single ion monitoring (SIM) mode. The sensitivity problems that may be caused due to the presence of a chiral selector in the ion-source of the MS were not addressed in this study. However, this problem was noted in several subsequent works [101–106]. Recently, Lu and Cole studied in detail the effect of the CD concentration in CE-separation buffers on the relative abundance of several chiral cationic drugs (terbutaline, ketamine, propranolol) [106]. For all of the compounds studied a significant decrease of relative abundance was observed with increasing concentrations of CD in the CE-separation buffer (Fig. 10) [106].

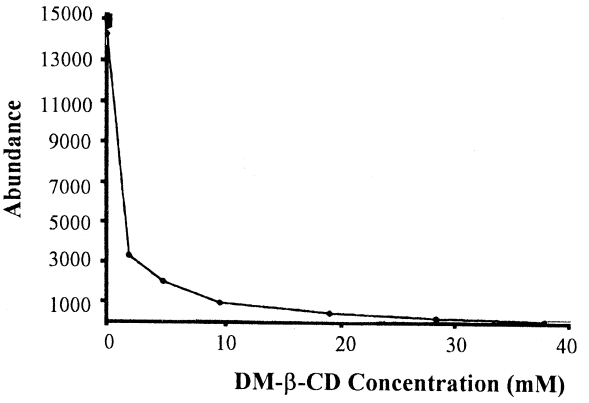

Fig. 10. Dependence of ESI-MS signal (arbitrary units) of protonated terbutaline ($m/z=226$) on the concentration of DM-β-CD in operating CE buffer consisting of 0.8 *M* acetic acid and 5 m*M* ammonium acetate in methanol–water (80:20, v/v). The concentration of terbutaline is 10^{-4} *M*. (From Ref. [106] with permission).

Counter-current migration [24,102] and so called ''partial filling'' [103,105] techniques were proposed as a solution for the aforementioned MS source contamination and sensitivity impairment problems. The counter-current migration principle is shown in Fig. 11 [6]. A chiral analyte and a chiral selector are oppositely charged and, therefore, migrate in opposite directions. The term ''partial filling'' does not exactly describe the technique used in Refs. [103,105] because the entire separation capillary is filled with the chiral selector. When neutral chiral selectors are used in this mode and the EOF in the direction of the MS is eliminated together with suction effects a (chiral) selector will remain almost immobile in the capillary.

The application of chiral CE–ESI-MS coupling has been described for the optical purity test of commercial *R*-(−)- and *S*-(+)-camphorsulfonic acids [103] as well as for an artificial mixture of the (*S*)- and (*R*)-enantiomers of the local anesthetic drug ropivacaine [105]. It has been determined that the commercial samples of (*R*)-(−) and (*S*)-(+)-camphorsulfomic acids contained ca. 2.0% and 0.9% of enantiomeric impurities, respectively [103]. An enantiomeric impurity of 0.25% of (*R*)-ropivacaine could be detected in (*S*)-ropivacaine [105].

The potential of chiral CE–MS coupling can be fully exploited in metabolic studies where peak identification, peak purity testing and overcoming matrix effects become issues of critical importance. Difficulties with micropreparative fraction collection in CE for their further off-line identification make direct chiral CE–MS even more significant for metabolic studies. The stereoselective CE–ESI-MS analysis of the phase-I and phase-II metabolites of non-steroidal anti-inflammatory drug etodolac in human urine is shown in Fig. 12 also illustrating the value of on-line chiral CE–MS coupling for peak identification and peak purity testing [102].

5. Applications of chiral electromigration techniques in drug analysis

5.1. Standard mixture of enantiomers

Artificial mixtures of enantiomers containing one of the components in a minor amount (0.05–1.0%) have been intensively analyzed using chiral CE (Table 1) [108–115]. Although these studies illustrate the potential of chiral CE, the case of artificial mixtures of enantiomers is the simplest one because no matrix effects are present and in addition, an overall sample concentration is not always limited.

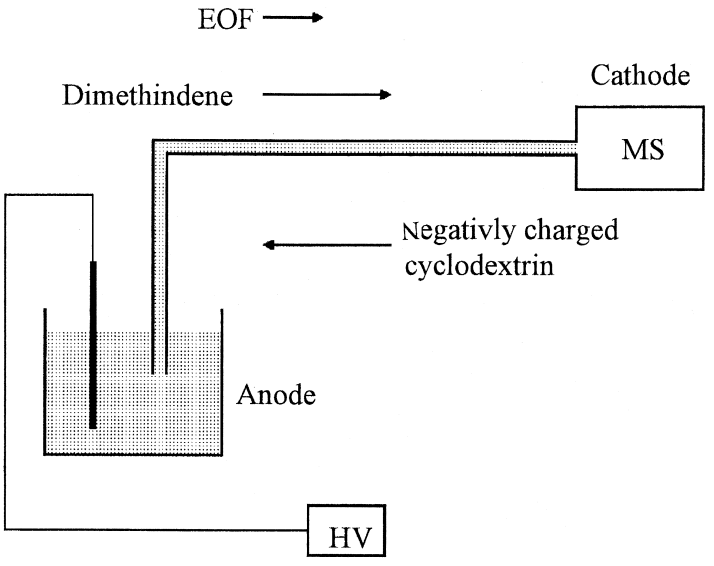

Fig. 11. Schematic representation of chiral CE–ESI-MS coupling based on the counter-migration principle. (From Ref. [6] with permission).

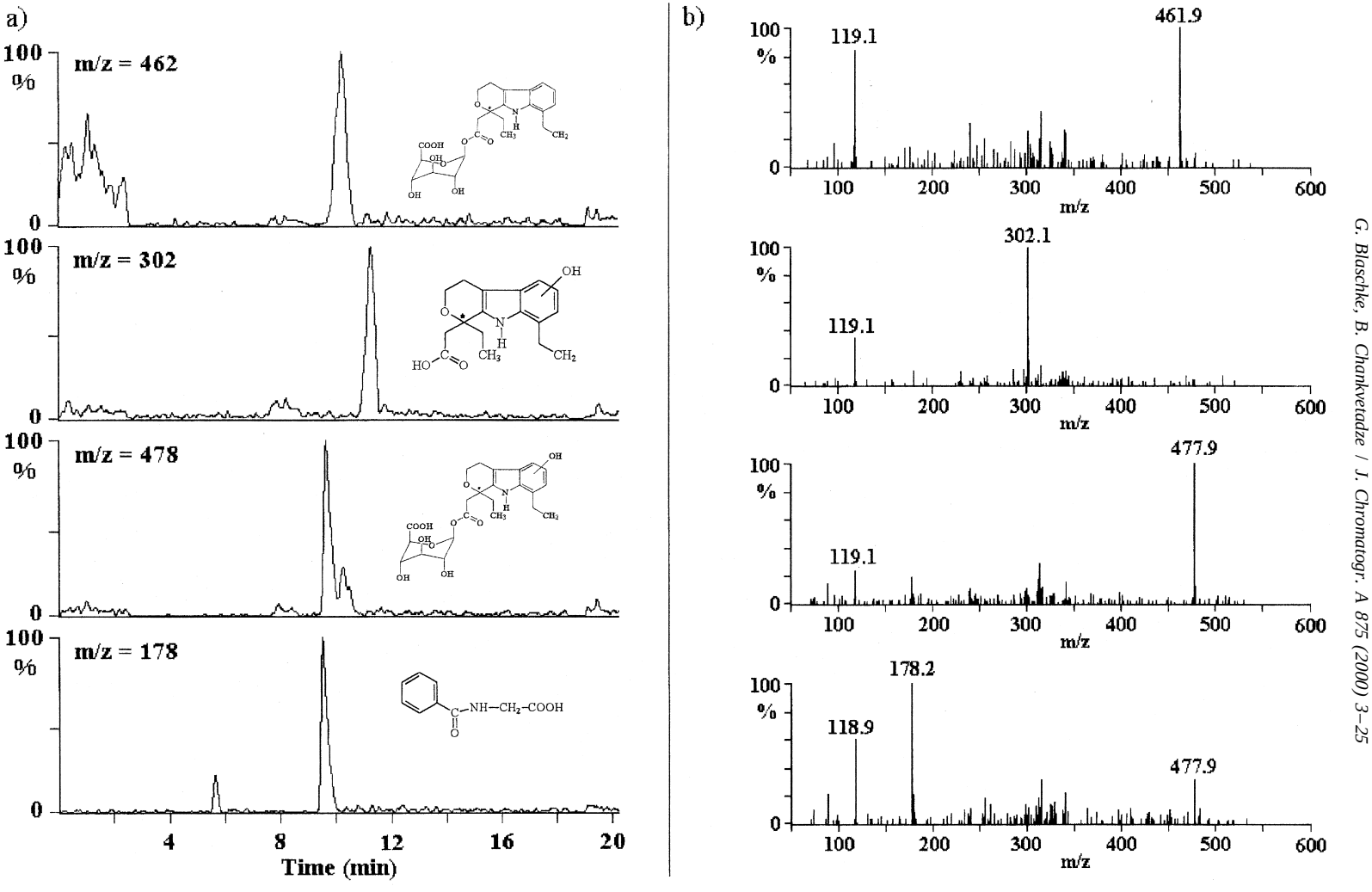

Fig. 12. CE–ESI-MS electropherograms (selected mass tracks) (a), and full scan ESI-MS spectra (b) corresponding to each peak of the cumulative urine sample (3–7 h after drug administration) of a volunteer receiving a 400 mg single oral dose of racemic etodolac. (From Ref. [102] with permission).

Table 1
Recent examples of determination of a minor impurity in standard enantiomeric mixtures using CE [108–115]

Chiral drug	Chiral selector	Limit of detection of minor enantiomer (%)	Ref.
Terbutaline	HE-β-CD	0.1	[108]
Carnitine	DM-β-CD	1	[109]
Tryptophan	α-CD	0.1	[110]
Tramadol	CM-β-CD	0.3	[111]
Methamphetamine	β-CD polymer	0.5	[112]
Selegiline	β-CD polymer	0.5	[112]
Ephedrine	β-CD polymer	0.5	[112]
Ropivacaine	DM-β-CD	0.1	[113]
Sotalol	HP-β-CD	0.2	[114]
DOPA	HDAS-β-CD	0.1	[115]
Carbidopa	CM-β-CD	0.1	[115]

Together with the above mentioned example (Fig. 6) where 0.05% of the minor enantiomer was determined the minor enantiomer of the 0.1% level was quantified in several studies (Table 1) [108–115].

5.2. Drug forms

Drug forms are relatively difficult to analyze compared to standard mixtures. Various cellulose derivatives used in drug formulations tend to adsorb to the capillary wall and may significantly affect the EOF. This can create significant problems for the reproducibility of migration times. Despite these problems the number of chiral CE studies performed on real samples increased during the last years. Tanaka and Terabe [116] examined the optical purity of commercial (*R*)-(+)- and (*S*)-(−)-sulpiride. They found 1.2% and 1.0% enantiomeric impurities in the (*R*)- and (*S*)-sulpiride, respectively. Commercial *S*-naproxen tablets also contain of 0.61% of *R*-naproxen according to Fillet et al. [117]. Blanco et al. studied the enantiomeric composition of the single enantiomer of ketoprofen pharmaceuticals [118]. The chiral CE separation method was developed and validated based on heptakis (2,3,6-tri-*O*-methyl)-β-CD as a chiral selector. The method was applied for Enantyum tablets, the active principle of which is *S*-(+)-ketoprofen (Fig. 13). The enantiomeric purity was determined to be 99.71% based on CE and 99.5% based on a comparative HPLC study. Thus, the authors showed that chiral CE provided results that are comparable to chiral HPLC from the viewpoint of analytical characteristics. In addition, they mentioned some advantages of CE such as flexibility in selectivity (sensitivity) adjustment, ease of operation, etc.

5.3. Drug enantiomers and their metabolites in biological fluids

In the early stage of the development of chiral CE examples of determinations of chiral drugs and their metabolites in biological fluids were rarely published [119–122]. This was apparently caused by possible disturbing effects of plasma proteins, high ionic strength of urine samples, complex matrix effects, etc. The picture significantly changed during the last few years [123–139]. Recently, this subject was summarized by Bojarski and Aboul-Enein [123] and the present state of the art is highlighted in a review by Zaugg and Thormann [124]. Therefore, just few aspects are discussed below.

Together with the above mentioned study by Hadwiger et al. [99] in which the enantiomers of isoproterenol were determined in microdialisates from the rat *vena cava*, a stereoselective determination of chiral drugs and, in selected cases, of their metabolites were reported in serum [125–128], plasma [129,130], liver homogenates [131,132], hair [128,133] and urine samples (Table 2) [128,133–139].

Solid-phase extraction [125–127] and liquid–liquid extraction [129,130] were used as sample pretreatment steps when serum and plasma samples were analyzed. In addition, a low amount of cationic detergent was added to the buffer in order to

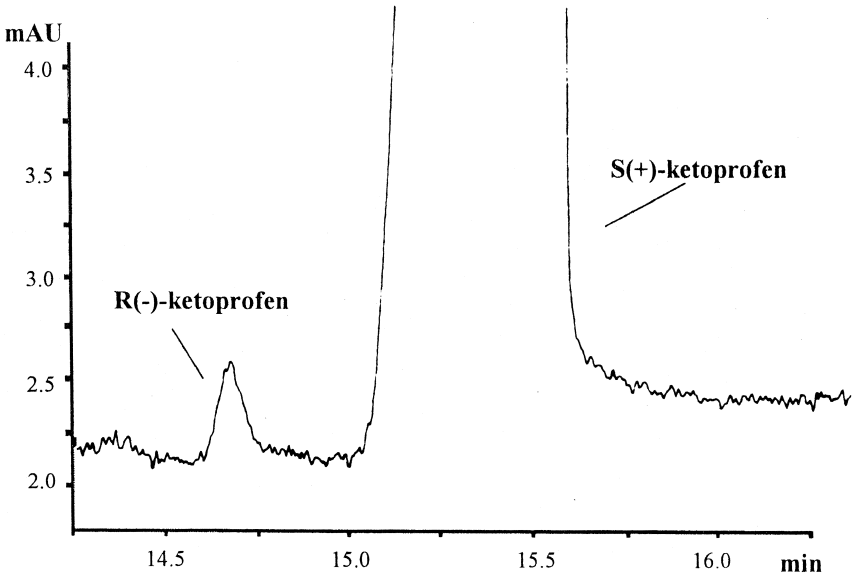

Fig. 13. Detection of *R*(−)-ketoprofen impurity contained in Enantyum tablets using chiral CE. (From Ref. [118] with permission).

diminish the adsorption of endogenous substances to the inner wall of a fused-silica capillary [125,126]. The LOD reported in the studies [125,126,130] were 10, 15 and 38 ng/ml for ondansetron, reduced haloperidol and prilocaine, respectively.

Zaugg et al. [129] quantitatively determined the enantiomers of the anesthetic agent thiopental and its oxybarbiturate metabolite, pentobarbital, in human plasma samples. The analytes from plasma were extracted at low pH using dichloromethane and enantioseparated by CE using phosphate buffer at pH 8.5 containing 5 m*M* hydroxypropyl-γ-CD as a chiral selector (Fig. 14). The total *S*-(−)-thiopental plasma concentration was on average about 24% higher compared to the concentration of *R*-(+)-thiopental, whereas the total *R*-(+)-pentobarbital concentration was 29% higher compared to the *S*-(−)-pentobarbital concentration. To the authors opin-

Table 2
Recent examples of determination of chiral drugs and their metabolites in human urine using CE

Chiral drug	Metabolites	Chiral selector	Ref.
Methadone	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	2 m*M* DM-β-CD, 4.3 m*M* 2-HP-β-CD	[128] [134]
Ephedrine; amphetamine; methamphetamine; 3,4-MDM; 3,4-MDA; 3,4-MDE	–	15 m*M* β-CD	[133]
3,4-MDM	4-Hydroxy-3-methoxymethamphetamine	30 m*M* 2-HP-β-CD	[135]
Clenbuterol	–	30 mg/ml 2-HP-β-CD	[136]
Ibuprofen	2^{I}-Hydroxyibuprofen, 2^{I}-carboxyibuprofen	10% (w/v) dextrin+ 20 m*M* TM-β-CD	[137]
Tramadol	*O*-Demethyltramadol, *N*,*O*-didemethyltramadol, *N*-demethyltramadol	30 mg/ml CM-β-CD, 5 m*M* CM-β-CD	[138] [139]

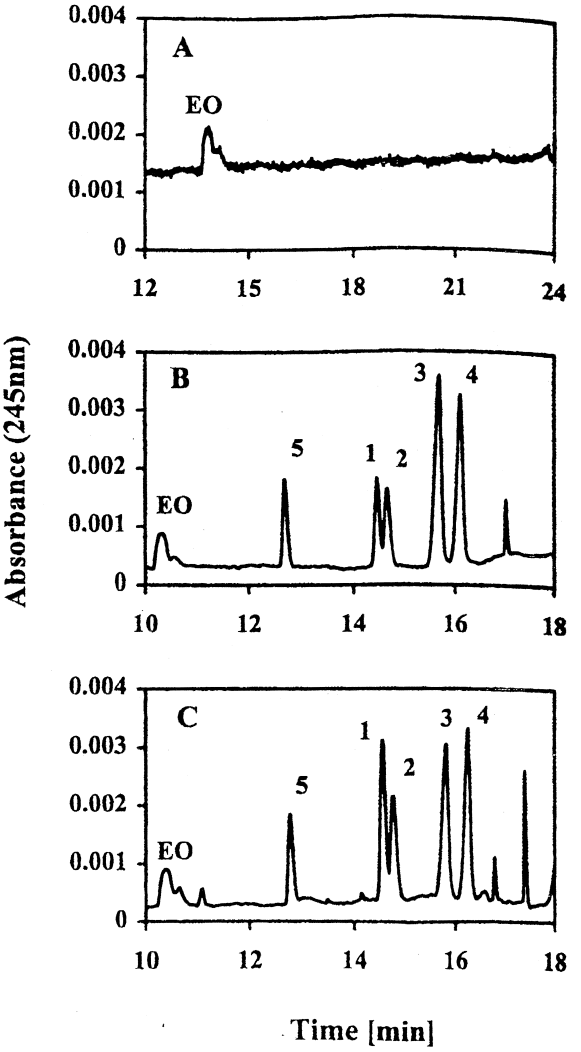

Fig. 14. Chiral CZE data of extracts prepared in 10-fold diluted running buffer obtained of (A) blank plasma, (B) a control sample containing 6.67 μg/ml I.S., 25 μg/ml racemic thiopental and 10 μg/ml racemic pentobarbital, and (C) a patient sample containing 10.3/11.6 μg/ml *R*-(+)-thiopental/*S*-(−)-thiopental and 8.1/6.0 μg/ml *R*-(+)-pentobarbital/*S*-(−)-pentobarbital. Key: (1) *R*-(+)-pentobarbital, (2) *S*-(−)-pentobarbital, (3) *R*-(+)-thiopental, (4) *S*-(−)-thiopental. (From Ref. [129] with permission).

ion, CZE represents ''a simple, attractive and inexpensive method for the enantiomeric separation of thiopental and its oxybarbiturate metabolite, pentobarbital. No expensive stereospecific separation column and no sample derivatization are required to separate the enantiomers'' [129].

The high separation efficiency of CE allowed Lerch and Blaschke [132] to detect together with the known *trans*-4-hydroxy- and *cis*-4-hydroxy metabolites of chiral anthelmenthic drug praziquantel also four unknown metabolites (peaks 4 to 7, Fig. 15) in liver homogenates. The unknown peaks were further identified using on-line HPLC–ESI-MS coupling.

As noted by Tagliaro et al. [133] hair analysis requires a higher sensitivity than other biological samples. However, using effective sample stacking in CE the authors could achieve a LOD of 0.1–0.2 ng/mg for the amphetamine type components of ''ecstasy'' drugs of abuse (Fig. 16) [133]. The data obtained by CE correlated well with those obtained by non-chiral GC–MS. In addition, equal concentrations of the enantiomers of 3,4-methylenedioxyamphetamine (3,4-MDA) and 3,4-methylenedioxyethylamphetamine (3,4-MDE) were found in human hair samples while one enantiomer of 3,4-methylenedioxymethamphetamine (3,4-MDM) exceed the other [133].

Several similar studies of biomedical application of chiral CE appeared by different groups, independently. Adequate principal results obtained in these studies once again show that chiral CE is an accurate technique for bioanalytical analyses. Thus, Frost et al. [128] and Lanz and Thormann [134] although using different chiral selectors in their studies on the enantioselective biotransformation of the chiral drug methadone they came to the same conclusions. Tagliaro et al. [133] and Lanz et al. [135] performed the enantioselective determination of amphetamine related chiral ''ecstasy'' drugs in different biomaterials and made again very similar conclusions. Kurth and Blaschke [138] observed the stereoselective excretion of the chiral centrally acting analgesic drug tramadol and its *O*-demethyl metabolite based on a stereoselective CE assay applying carboxymethyl-β-CD as chiral selector in basic buffers (Fig. 17). Parallel to this study, Rudaz et al. [139] published a chiral CE separation method also using carboxymethyl-β-CD as chiral selector but acidic buffers and relatively low CD concentrations. These authors also found that the urinary excretion of tramadol is stereoselective. Thus, these studies are good examples of ''non-designed'' cross-laboratory validation of chiral CE in bioanalysis. They definitely indicate that chiral CE has a future in this field.

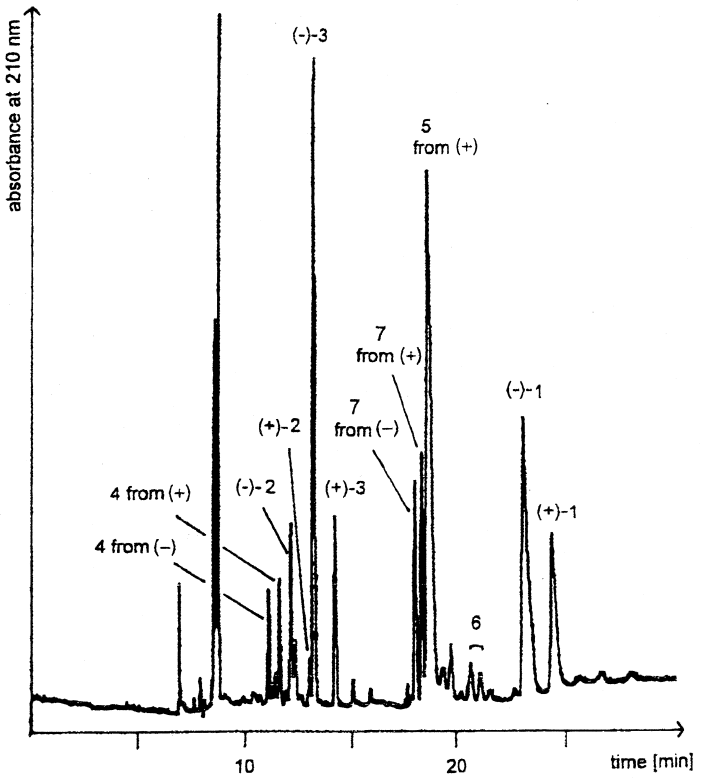

Fig. 15. Simultaneous enantioselective separation of praziquantel (1) and its metabolites after incubation with rat liver microsomes. (2) *trans*-4-hydroxypraziquantel, (3) *cis*-4-hydroxypraziquantel, (4) M1, (5) M2, (6) M3, (7) M4. (From Ref. [132] with permission).

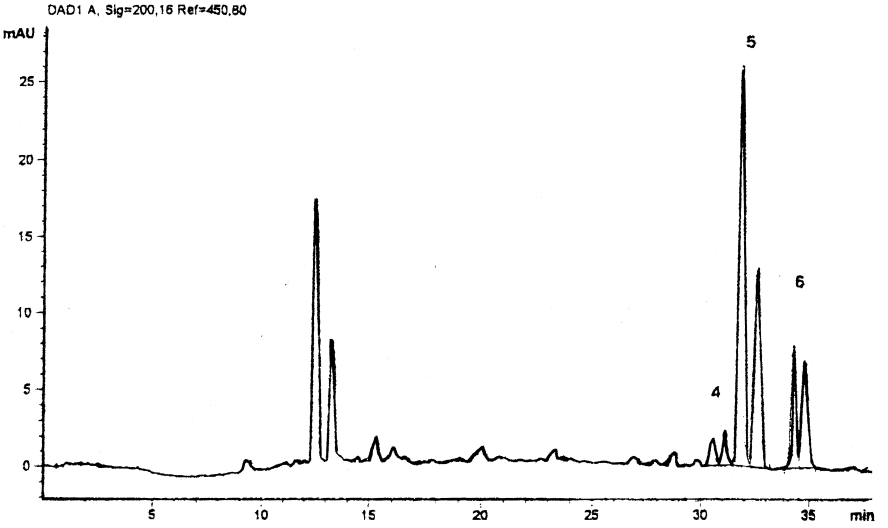

Fig. 16. Typical electropherogram of a hair extract from a real ''ecstasy'' user, injected by field-amplified sample stacking procedure. Concentrations of racemic analytes in the original sample (determined by achiral GC–MS) were as follow: (peak 5) MDMA, 11.2 ng/mg; (peak 4) MDA, 0.9 ng/mg; (peak 6) MDE, 4.3 ng/mg. (From Ref. [133] with permission).

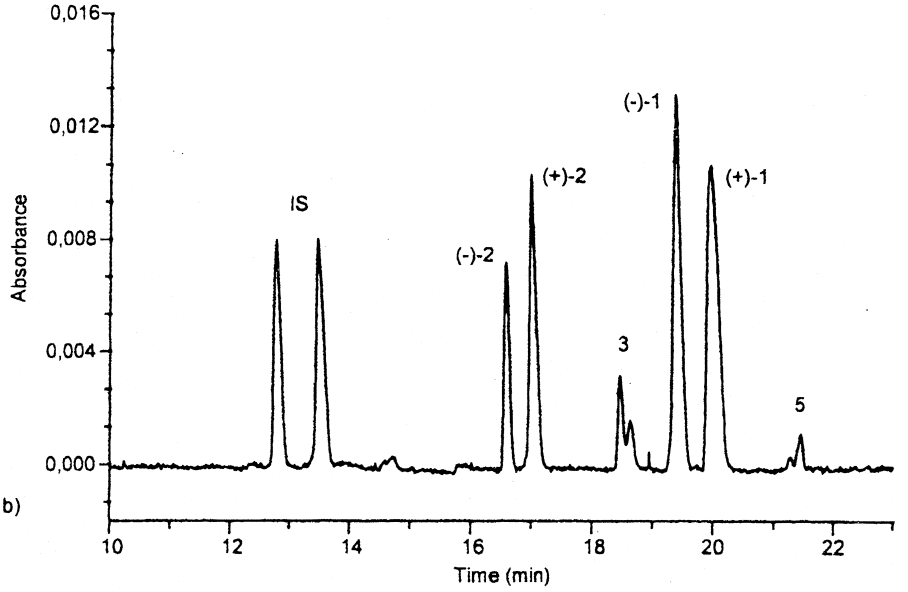

Fig. 17. Electropherogram of the human urine sample collected in the interval 6–8 h after receiving 100 mg single dose of tramadol hydrochloride. (1) Tramadol, (2) *O*-demethyltramadol, (3) *N,O*-didemethyltramadol, (5) *N*-demethyltramadol. (From Ref. [138] with permission).

6. Nonstandard abbreviations

BGE	Background electrolyte
CD	Cyclodextrin
CEC	Capillary electrochromatography
cEKC	Capillary electrokinetic chromatography
CGE	Capillary gel electrophoresis
18C6H4	18-Crown-6-tetracarboxylic acid
cIEF	Capillary isoelectric focusing
cITP	Capillary isotachophoresis
CM-β-CD	Carboxymethyl-β-CD
CS	Chiral selector
CSP	Chiral stationary phase
CZE	Capillary zone electrophoresis
DM	Dimethindene
DM-β-CD	Heptakis-(2,6-di-*O*-methyl)-β-CD
ESI-MS	Electrospray ionization mass spectrometry
HDAS-β-CD	Heptakis-(2,3-diacetyl-6-fulfato)-β-CD
HIV	Human immunodeficiency virus
HE-β-CD	2-Hydroxyethyl-β-CD
HP-β-CD	2-Hydroxypropyl-β-CD
FDA	The United States Food and Drug Administration
IP	Isoproternol
LIF	Laser-induced fluorescence
LOD	Limit of detection
LOQ	Limit of quantitation
MCLC	Microcolumn liquid chromatography
3,4-MDA	3,4-Methylenedioxyamphetamine
3,4-MDE	3,4-Methylenedioxyethylamphetamine
3,4-MDM	3,4-Methylenedioxymethylamphetamine
MEKC	Micellar electrokinetic chromatography
NAQ	Nonaqueous
SIM	Single ion monitoring
SLM	Supported liquid membrane
TD	Thalidomide
TM-β-CD	Heptakis-(2,3,6-tri-*O*-methyl)-β-CD
TNF	Tumor necrosis factor
Trp	Tryptophan

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