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Chiral capillary electrophoresis–electrospray mass spectrometry coupling using vancomycin as chiral selector

Salvatore Fanali[a], Claudia Desiderio[a], Georg Schulte[b], Stefan Heitmeier[b], Dirk Strickmann[b], Bezhan Chankvetadze[b,1], Gottfried Blaschke[b,*]

[a]*Istituto di Cromatografia del C.N.R., Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome, Italy*
[b]*Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstrasse 58-62, 48149 Münster, Germany*

Abstract

Capillary electrophoresis–electrospray mass spectrometry coupling (CE–ESI-MS) using vancomycin (VC) as a chiral selector is described in this study. The self electrophoretic mobility of VC, as a positively charged chiral selector at low pH, allows us to avoid the contamination of the ion source of the ESI-MS with the chiral selector in a simple way. The usefulness of this technique is illustrated for the analysis of chiral anionic compounds of pharmaceutical importance. Additionally, examples of a stereospecific determination of ibuprofen and its phase I metabolites as well as etodolac and its metabolites in urine samples are described. The advantages of MS for peak identification, peak purity testing and for selective monitoring of overlapping peaks are demonstrated. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Chiral selectors; Chiral CE–ESI-MS; Vancomycin; Arylpropionic acids; Etodolac; Ibuprofen

1. Introduction

The separation of enantiomeric compounds can be easily achieved by capillary electrophoresis (CE) using the direct separation method, e.g., by addition of the chiral selector to the background electrolyte (BGE).

A wide number of chiral selectors have been used in CE [1–3] such as cyclodextrins and their derivatives, chiral surfactants, proteins, metal–amino acid complexes, crown ethers, and recently antibiotics [4–11].

Chiral CE–mass spectrometry (MS) coupling is developing within the last few years [12–14]. MS is sensitive, specific and at the same time a universal detection system. Additionally, MS provides important information about the structure of analytes which is especially useful for the identification of metabolites in clinical and biopharmaceutical analyses. On-line MS coupling is advantageous in CE because fraction collection for further analysis is very time-consuming and more difficult in this technique compared with high-performance liquid chromatography (HPLC).

In chiral CE, the presence of chiral selectors in the BGE may cause some problems due to the interference with analytes (UV) or contamination of the detector (MS). The appearance of the chiral selector in the ion source of the MS may decrease the sensitivity due to the competition with an analyte for available charge as well as due to the increase of the background noise [12,13]. To avoid the appearance

*Corresponding author.

[1]Permanent address: Department of Chemistry, Tbilisi State University, Chavchavadze Ave 1, 380028, Tbilisi, Georgia.

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of chiral selector in the ion source the use of column-coupling and multiple voltage switching technique has been proposed recently [13]. An alternative approach can be based on the opposite migration direction of an analyte and a chiral selector. This principle was proposed for UV-adsorbing chiral selectors by Valtcheva et al. [15] and involved filling only part of the separation capillary with the chiral selector. A slightly modified version of this technique allowing the filling of the whole capillary with a chiral selector was described in Ref. [16]. The usefulness of the partial filling technique in chiral CE with UV detection has been demonstrated in a number of studies [8,9,11,17]. The technique proposed in Ref. [16] has been recently used also in chiral CE–electrospray (ESI)-MS coupling in order to avoid the appearance of charged cyclodextrins in the ion source [14].

In this paper a chiral CE–ESI-MS coupling for the separation of several racemic arylpropionic acids (APAs) employing vancomycin (VC) as a chiral selector is reported.

2. Experimental

2.1. Chemicals and reagents

Acetic acid, ammonium acetate, ammonium hydroxide and methanol were purchased from Merck (Darmstadt, Germany). Racemic flurbiprofen (FP), ketoprofen (KP) and carprofen (CP) were from Sigma (Daisenhofen, Germany). (+)- and (−)-Naproxen (NP) were kindly provided by Dr. Cecilia Bartolucci, Istituto di Strutturistica Chimica, C.N.R. (Montelibretti, Rome, Italy).

Racemic etodolac (ET) and its phase I metabolites, 7-hydroxyetodolac (7-HET) and 8-(1′-hydroxyethyl)etodolac (8-HET) were gifts from Wyeth Pharma (Münster, Germany). Racemic ibuprofen

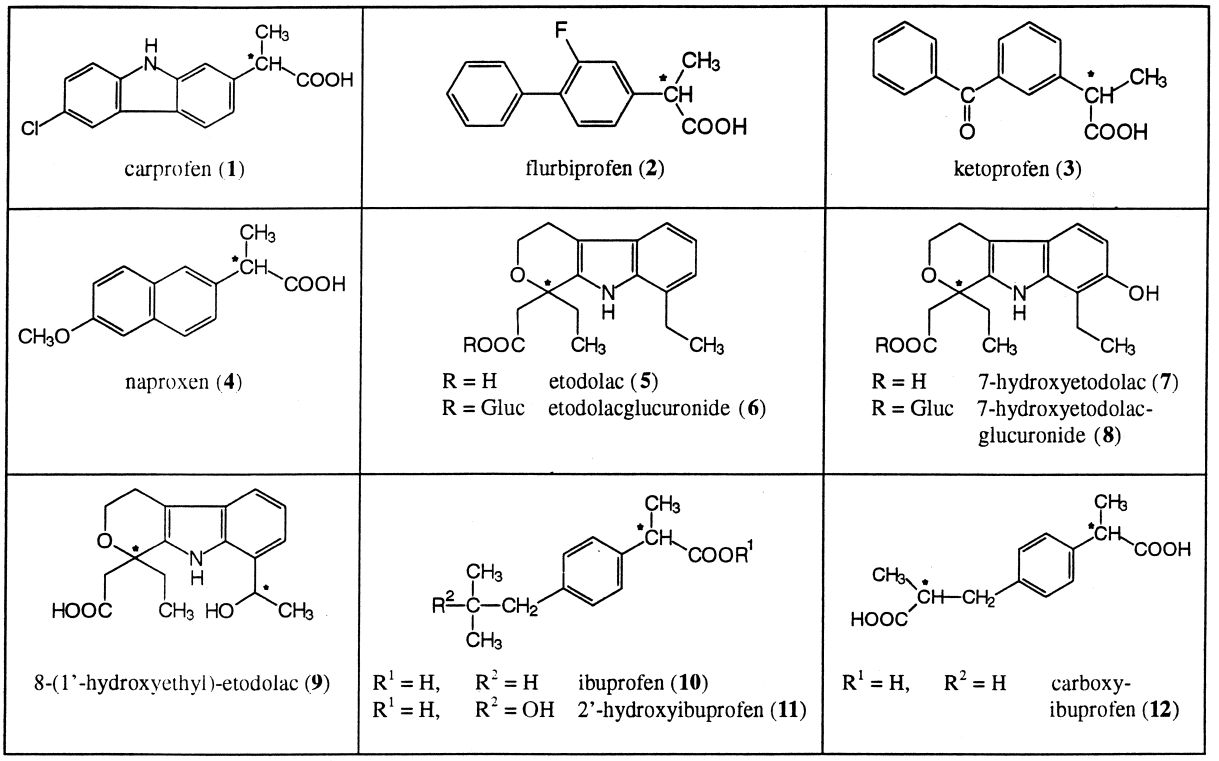

Fig. 1. Chemical structures of the compounds.

(IB) and its phase I metabolites, 2-hydroxyibuprofen (HIB) and carboxyibuprofen (CIB) were gifts from Kanoldt (Höchstadt/Donau, Germany). The structures of the chiral compounds used in this study are shown in Fig. 1.

VC, methacrylic acid 3-trimethoxysilylpropyl ester, ethylenediaminetetraacetic acid, acrylamide, sodium persulfate and N,N,N′,N′-tetramethylethylenediamine were obtained from Fluka (Buchs, Switzerland).

Stock solutions of racemic samples (1 mg/ml) were prepared in methanol, stored at 4°C and diluted to 50–100 μg/ml in acetate buffer pH 4.8 before the analysis. 50 m*M* Acetic acid–ammonium acetate, pH 4.8, containing various amounts of VC was the BGE used for the enantioseparation of the studied APAs, ET or their metabolites; the BGEs containing the chiral selector were freshly prepared daily.

2.2. Apparatus

A Grom capillary electrophoresis system 100 high voltage power supply (Herrenberg, Germany) was operated in a constant voltage mode and the electrophoretic runs were performed in a polyacrylamide coated capillary [18] of 44 cm×50 μm I.D. filled either with 50 m*M* acetic acid–ammonium acetate buffer pH 4.8 or the same buffer supplemented with the appropriate amount of VC. Hydrostatic injection (10 cm) for 5–10 s at the cathodic end of the capillary was used in all experiments. The other end of the separation capillary was protruded to the ion spray tip where a voltage of 2.6 kV was maintained. A LCQ ion trap mass spectrometer (Finnigan, Branford, CT, USA) equipped with an electrospray interface was used in the negative ion mode for the detection of analytes [12–14]. The sheath liquid consisting of methanol–water–ammonia (50:48:2) was delivered at a flow-rate of 6 μl/min using a syringe pump.

3. Results and discussion

VC is one of the most powerful chiral selectors for the separation of compounds containing free car-

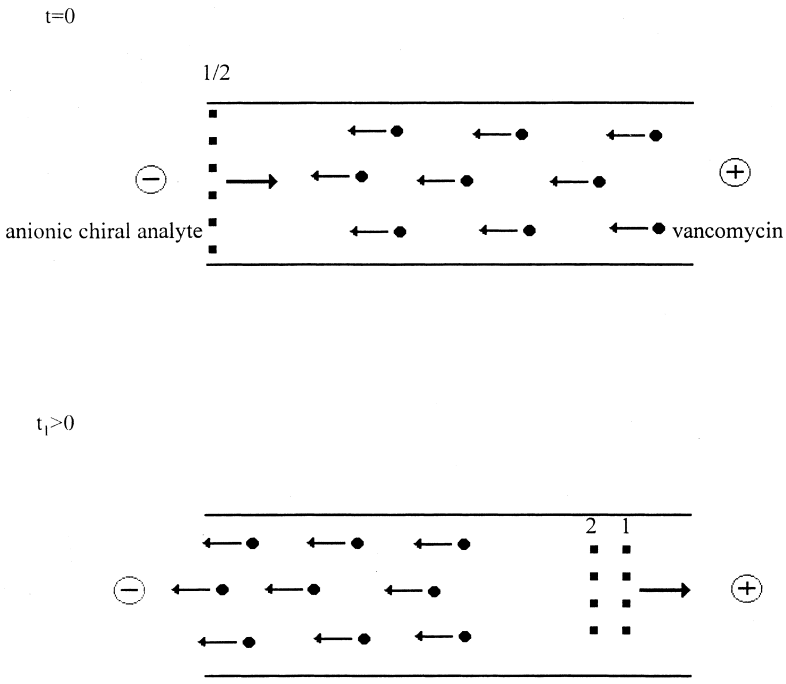

Fig. 2. Scheme of the counterflow electrophoretic separation of the anionic analytes using vancomycin as the chiral selector.

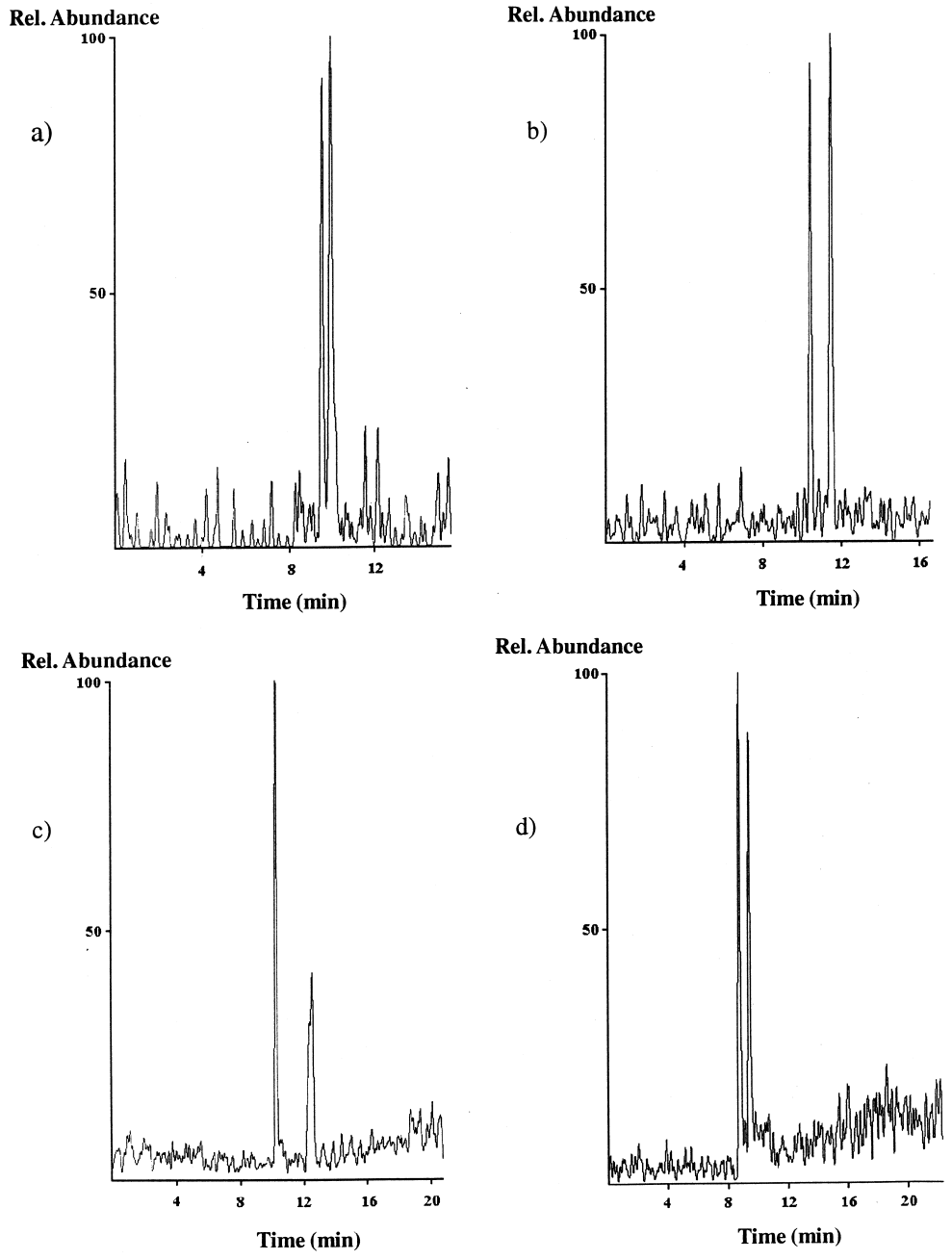

Fig. 3. CE–ESI-MS electropherograms of racemic non-steroidal-antiinflammatory drugs: (a) (±)-carprofen (CP), (b) (±)-flurbiprofen (FP), (c) (±)-ketoprofen (KP) and (d) (±)-naproxen (NP) in the presence of 5 m*M* of vancomycin in the BGE. Capillary (polyacrylamide coated) 44 cm×50 μm I.D.; applied voltage, 20 kV; BGE, 50 m*M* acetic acid–ammonium acetate pH 4.8; injection, hydrostatic (10 cm)×10 s at the cathodic end of the capillary.

boxylic groups [4,8–11]. However, the high absorbance in the UV range may cause signal interference with the analyte and consequently some sensitivity related problems in CE with UV detection. Recently, the partial filling technique has been proposed as the solution to this problem [8–11,17].

An interference between the signals of VC and the analytes is not the problem in CE with MS detection. However, the appearance of the chiral selector in the MS ion source may cause a decrease of detection sensitivity by competition with the analyte for an available charge as well as by an increase of the baseline noise due to the contamination of the ion source [12,13]. Therefore, it is desirable to avoid the penetration of nonvolatile buffer components in the MS.

As represented in Fig. 2 the technique similar to partial filling technique may also be useful in chiral CE with MS detection [14]. This technique allows us to avoid in a simple way a contamination of the ion source with the chiral selector and consequently improves the sensitivity and stability of the response. This has been demonstrated recently for chiral CE–ESI-MS coupling using charged cyclodextrins [14].

Acetic acid–ammonium acetate buffer at pH 4.8 was selected for the experiments. This buffer meets several important requirements for chiral CE–MS coupling, namely: (i) it is volatile and thus compatible with the MS detector, (ii) the studied analytes move as anions in a relatively short time and (iii) VC, which is positively charged at this pH, migrates in the opposite direction of analytes away from the ESI-MS source.

In order to confirm that the chiral selector does not appear in the MS interface the following experiment was performed. The separation capillary was filled with the BGE containing 12.5 m*M* VC and high voltage was applied so that the anode was on the MS side of the capillary. The detection was performed alternatively in both in the positive and in the negative ion mode. Only the minor peaks with m/z ranging from 700.6 to 760.5 were detected in both modes. It seems worth noting that although VC is positively charged in the separation capillary the sheath liquid used for the MS detection of the anionic analytes is alkaline which may transform the chiral selector to its anionic form. Thus, this experiment confirmed undoubtedly that the chiral selector does not continuously enter the MS. The traces found are probably due to the penetration into the MS during filling the separation capillary with the BGE containing VC.

The CE–ESI-MS electropherograms of racemic CP, FP, KP and NP using the BGE with 5 m*M* VC

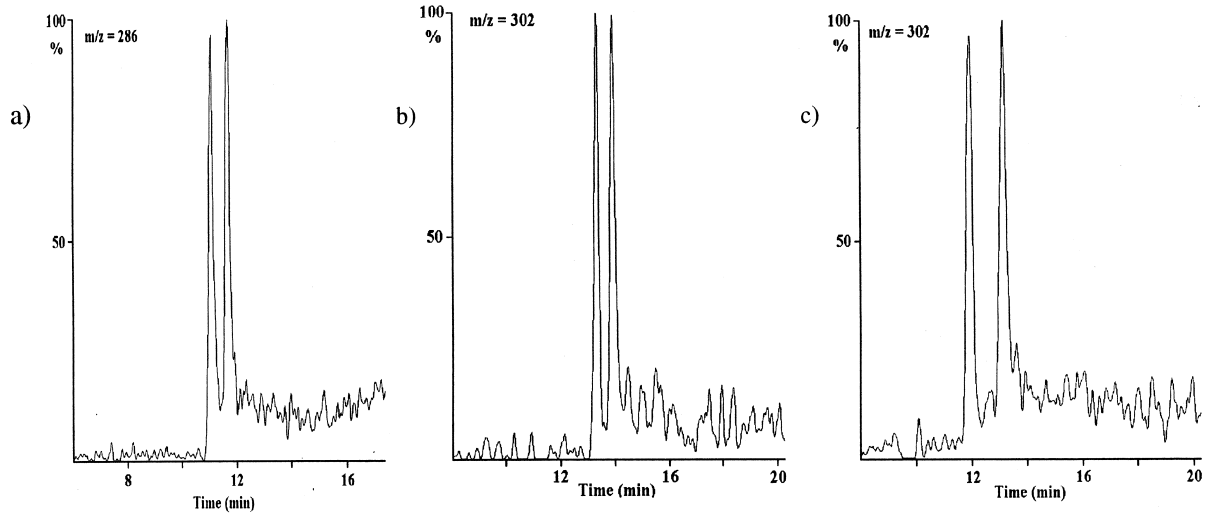

Fig. 4. CE–ESI-MS electropherograms of racemic (a) etodolac (ET) and its metabolites, (b) 7-hydroxyetodolac (7-HET) and (c) 8-(1′-hydroxyethyl)etodolac (8-HET) in the presence of vancomycin in the BGE. Experimental conditions: 5 m*M* vancomycin in a and b and 12 m*M* in c; applied voltage 15 kV in a and b and 12 kV in c. For other experimental conditions see Fig. 3.

are shown in Fig. 3. Very good enantiomeric resolution was achieved for all arylpropionic acids.

Similar results have been obtained for the enantiomeric separation of racemic ET and two of its phase I metabolites (Fig. 4). The analysis time of the separated enantiomers was relatively short (less than 15 min) and the peak efficiency high (90 000–110 000 plates/m). Only two among four possible 8-HET stereoisomers were resolved.

The mass tracks corresponding to molecular masses of IB (m/z 204.5–205.5), and its phase I metabolites, 2-HIB (m/z 220.5–221.5) and CIBs (m/z 234.5–235.5), from the full-scan CE–ESI-MS spectra of their mixture are shown in Fig. 5. The addition of 5 m*M* of VC in the BGE allowed the enantioseparation of IB, 2-HIB and all four stereoisomers of CIB. However, peak overlapping has been observed for IB and 2-HIB. In spite of the severe overlapping of the migration zones the selection of corresponding mass tracks allows us to perform peak identification of the compound possessing different molecular masses. This is one of the important advantages of MS as a detection system when samples of complex origin are analyzed. An additional advantage of this

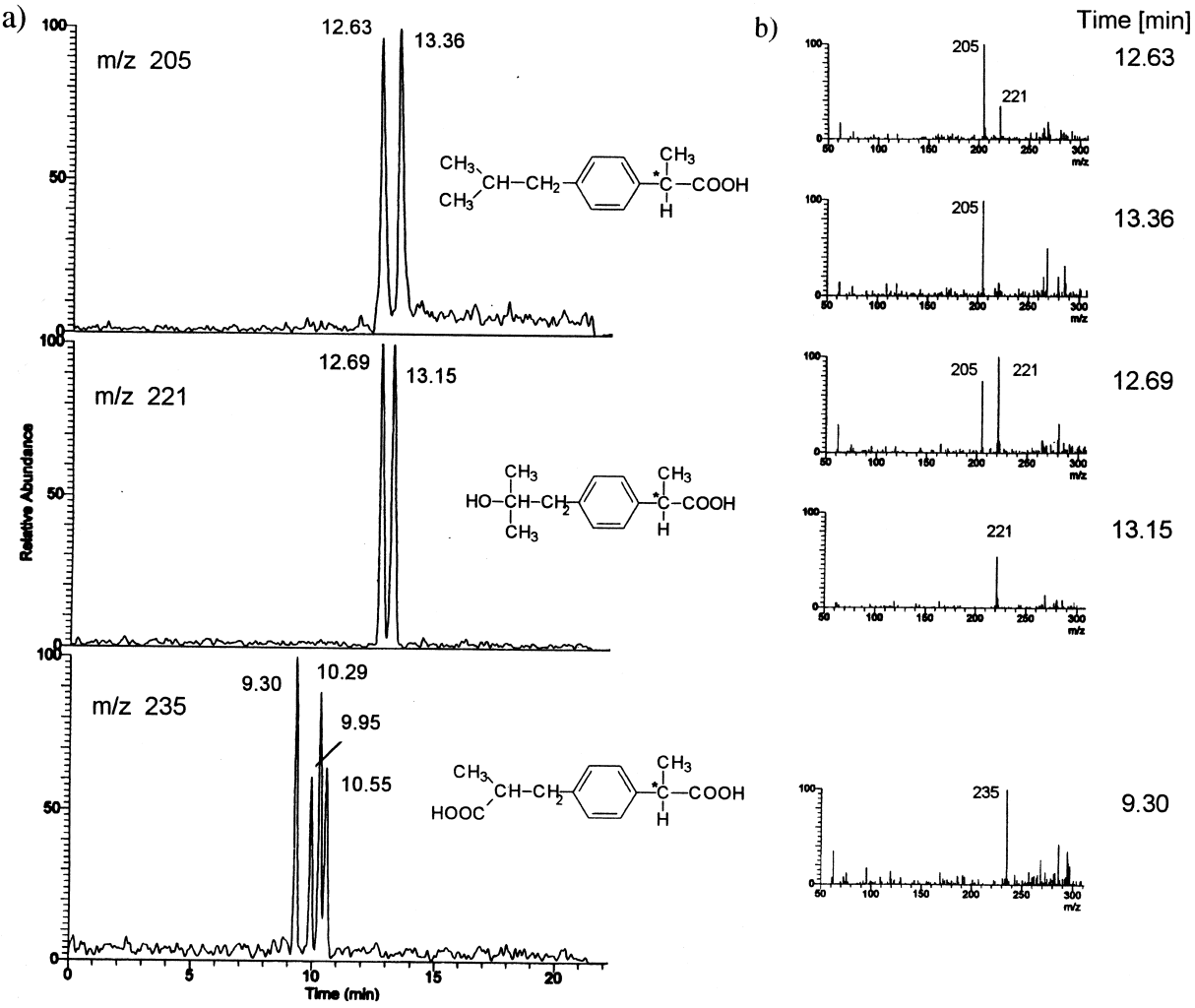

Fig. 5. CE–ESI-MS electropherograms (selected mass tracks) and full scan ESI-MS spectra (b) of selected peaks of racemic ibuprofen (IB), 2-hydroxyibuprofen (2-HIB) and carboxyibuprofens (CIB). Experimental conditions as in Fig. 3.

detection principle is the information about peak purity. This can be done by scanning the full MS spectra of each peak. As indicated by the MS spectra (Fig. 5b) the peak of IB enantiomer with the migration time 12.63 contains the impurity of HIB (m/z=221) and the first peak of the latter one (t= 12.69) is markedly contaminated with IB (m/z= 205). The second peaks of both compounds (t=13.36 and 13.15) seem to be relatively pure.

Another advantage of CE–MS coupling is the on-line identification of resolved peaks without any sample collection. For instance, it is obvious that all four peaks with m/z=234.5–235.5 belong to the stereoisomers of CIB.

Becker-Scharfenkamp and Blaschke [19] studied the phase I and the phase II metabolism of the non steroidal antiinflammatory drug ET. Evidence for a stereoselective elimination of ET-acylglucuronide and 7-hydroxyetodolac-acylglucuronide (7-HETG) were found by indirect determination of the differences of the concentrations of ET and 7-HET before and after alkaline hydrolysis of the conjugates using HPLC. Later, Berendes and Blaschke [20] developed a HPLC method which involves the acetylation of hydroxy groups and methylation of carboxy groups in order to increase the lipophilicity of the polar acylglucuronides of ET and 7-HET. Both of these techniques involve a tedious and time-consuming sample extraction and pretreatment. Therefore, the direct analysis of the urine sample without any sample extraction and pretreatment was attempted.

As shown in the chiral CE–ESI-MS electropherogram of a urine sample of a volunteer receiving a single 400 mg dose of racemic ET (Fig. 6) the single peaks of ETG (m/z=461.9), one of the isomeric HETs (m/z=302.1) and one of the glucuro-

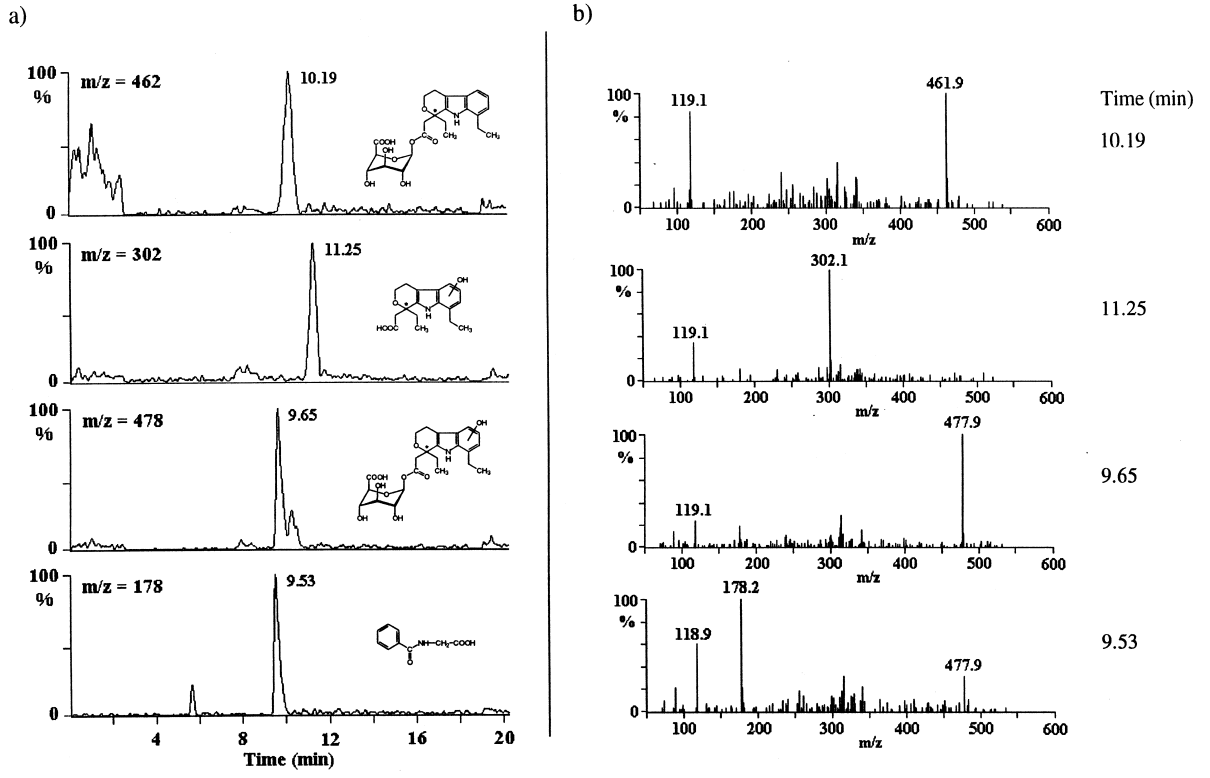

Fig. 6. CE–ESI-MS electropherograms (selected mass-tracks) (a) and full scan ESI-MS spectra (b) corresponding to each peak of a urine sample of a volunteer receiving a 400 mg single oral dose of racemic etodolac. The cumulative urine sample was collected in 3–7 h after drug administration and was directly introduced into the separation capillary by hydrodynamic injection (10 cm) for 10 s. The concentration of vancomycin was 12.5 m*M*; the applied voltage 15 kV; other experimental conditions were as in Fig. 3.

nides of the isomeric HETs ($m/z=477.9$) could be observed in the urine together with endogenous hippuric acid ($m/z=178.2$). According to previous results [19,20] the peak with $m/z=302.1$ most likely belongs to one of the enantiomers of 7-HET and the peak with $m/z=477.9$ to the corresponding glucuronide. Further studies on this subject are in progress now.

Thus, as shown in this preliminary study chiral CE–ESI-MS coupling represents a very effective alternative for expensive and time-consuming HPLC methods for the separation, identification and probably also for the quantification of chiral drugs and their phase I and phase II metabolites in biological matrices. In some cases the biological fluids can be directly injected into the separation capillary without any sample pretreatment.

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