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Separation of tamsulosin enantiomers by capillary electrophoresis with tandem mass spectrometry and online stacking preconcentration

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The aim of our work was to develop a new method for the analysis of tamsulosin enantiomers by capillary electrophoresis connected with tandem mass spectrometry. The pharmacologically active (*R*)-enantiomer of tamsulosin, is used to treat benign prostatic hyperplasia and chronic prostatitis. Under the optimal conditions, background electrolyte consisting of 200 mM acetic acid titrated with NH_4OH to pH 4.0 containing 4.0 mg mL^{-1} sulfated β-cyclodextrin, an injection time of 40 s at 50 mbar, a voltage of 20 kV and an optimized MS set-up (as *e.g.*, sheath liquid containing 75 : 24.9 : 0.1 MeOH, H_2O, and formic acid, v/v), a limit of detection of 1.6 nmol L^{-1} was achieved. The method was validated in terms of linearity, detection and quantification limits, precision, recovery, and selectivity. The results showed that the method can be used for the analysis of tamsulosin enantiomers in environmental samples, but generally, it can be applied to many different analytical tasks.

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1. Introduction

Chirality is a well-known phenomenon in nature. It represents an intrinsic property of the "building blocks of life" such as amino acids and sugars, and, therefore, of peptides, proteins, polysaccharides, and their composites (glycoproteins, glycolipids, *etc.*). Consequently, metabolic and regulatory processes mediated by biological systems are sensitive to stereochemistry. Therefore, stereochemistry must be considered when studying xenobiotics, such as drugs, agrochemicals, food additives, flavours, or fragrances. There is a broad range of examples where the stereoisomers of drugs show differences in terms of their bioavailability, distribution, metabolic behaviour and where stereochemical parameters have a fundamental significance in their action and disposition in biological systems.[1,2]

This phenomenon as well as the scientific and economic relevance of chiral substances has favoured developments in separation techniques during the last three decades. There is not only a need for the separation of enantiomers in the production process, but there is also a necessity to control the enantiomeric purity of products or to study the effects of single enantiomers in bodies as well as in the environment. Nowadays, plenty of techniques have been introduced for such tasks including gas and liquid chromatography, nuclear magnetic resonance, or electrochemical sensing.[3–9] A special place, in the list, is given to capillary electrophoresis (CE). This technique allows a very fast change of the separation medium (chiral selectors are added to the background electrolyte) and hence it can be used for finding the right interactions (conditions) for the separation of enantiomers. Moreover, since CE is traditionally performed in very thin capillaries, only a few microliters of background electrolytes are needed (50 μm i.d. capillary with a length of 50 cm has a volume of approx. 4 μL; plus the volume in inlet and outlet vials). This is of huge economic and environmental ("low-waste") relevance.[10–13]

CE can also be connected with mass spectrometry (MS) to gain the next dimension of the analysis and to have identification power. CE-MS is again a well-known tool; however, it suffers from the necessity of using volatile background electrolyte (BGE) components. Here, most of the chiral selectors used as BGE additives (*e.g.*, cyclodextrins and their derivatives, macrocyclic antibiotics, or proteins) are non-volatile compounds. Traditionally, three approaches are used: (i) a partial-filling technique, (ii) counter-migration, and (iii) physical bonding. In the partial-filling technique, the capillary is partially filled with a neutral or charged chiral selector. The selector movement is regulated so as not to enter the MS detector and not to suppress ionization processes. Counter-migration utilizes an oppositely charged chiral selector moving opposite to the analyte and EOF. Hence, the selector does not enter the MS, too. Finally, the chemical modification of a capillary inner surface using chiral selectors can also be a solution to the problems of volatility. Nevertheless, one can also use special selectors (*e.g.*, molecular micelles) which do not interfere with the ionization processes in CE-MS.[14–16]

In this work, we focused on tamsulosin which is used to treat symptomatic benign prostatic hyperplasia and chronic prostatitis. Tamsulosin (Fig. 1) has a molecular weight of 408.51 g

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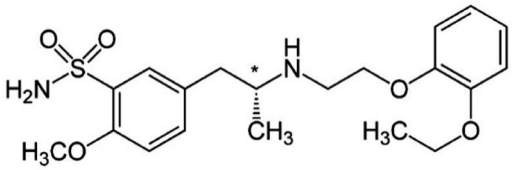

Fig. 1 Tamsulosin structure.

mol^{-1} and is administered at 0.4 mg once a day (0.8 mg for those who fail to respond to 0.4 mg). The pharmacologically active form is the (*R*)-enantiomer. Tamsulosin exhibits high plasma-protein binding. It is metabolized, mainly by cytochrome P450, to compounds with low abundance, and 8–15% of an oral dose is excreted renally as the parent compound.[17]

Tamsulosin was analysed under achiral conditions by *e.g.*, HPLC-UV,[18–20] HPLC-MS,[21–23] electrochemical methods,[24,25] or fluorescence.[26,27] Separation of tamsulosin enantiomers was performed by HPLC[28–30] and CE.[31–34] Sulfated cyclodextrins and sulfated cyclofructans were used in CE under acidic conditions. Under these conditions, tamsulosin migrates as a cation and the chiral selector migrates in the opposite direction to tamsulosin. Interestingly, Petr *et al.*[32] studied the effect of the presence of a chiral selector in different CE compartments (inlet vial, outlet vial, and capillary) to decrease the amount of the chiral selector necessary for chiral separation. The presence of a selector in a BGE only in the capillary led to the successful separation.

To the best of our knowledge, there is no publication regarding the separation of tamsulosin enantiomers in connection with mass spectrometry. Hence, we decided to develop a method for their separation by CE-MS using a discontinuous and counter-current migration system, as presented by Petr *et al.* in 2006.[32]

2. Experimental

2.1. Chemicals and materials

Chemicals, mainly acetic acid, formic acid, phosphoric acid, sodium hydroxide solution (0.1 mol L^{-1}), methanol, isopropanol, sulfated β-cyclodextrin (S-β-CD; substitution of 12-15 mol per β-CD; cat. no. 389153), water, and tamsulosin standards were bought from Sigma-Aldrich (St. Louis, MO, USA), all of analytical grade or higher (solvents of HPLC grade) purity. Deionized water with a resistivity of 18.2 MΩ cm was prepared using a MilliQ system from Millipore (Molsheim, France). Background electrolytes (BGEs) were prepared by dissolving the corresponding volumes of acids in HPLC-grade water. The pH was measured using a pH-meter inoLab (WTW, Weilheim, Germany). The additives (organic solvents or S-β-CD) were placed into the electrolytes after measurement of pH. All the BGEs were filtered using 0.45 μm nylon syringe filters (Labicom, Czech Republic).

2.2. Instrumentation

All the experiments were performed using the capillary electrophoresis instrument Agilent 7100 connected with an Agilent 6460 triple quadrupole mass spectrometer (Waldbronn, Germany). The sheath liquid was delivered into the electrospray interface using an isocratic LC pump Agilent 1260 with a 1 : 100 flow splitter. Separation was performed in fused silica capillaries of 85 cm length (the effective length was the same) and 50 μm ID, from Molex (Lisle, IL, USA). Prior to the first use, the capillaries were initially conditioned by rinsing them with 0.1 mol L^{-1} NaOH for 20 min and deionized water for 30 min, out of the MS. Between each sample run, the capillary was flushed with 0.1 mol L^{-1} NaOH for 5 s, HPLC-grade water for 3 min, and BGE for 3 min. All the rinsing was performed at a pressure drop of 935 mbar. The capillary cassette was thermostatted at 25 °C except for the part of the cassette leading to the MS interface. Each experiment was conducted in triplicate, unless stated otherwise.

2.3. Validation

The method was validated in terms of the following parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability of migration time and peak height, recovery, and selectivity. Linearity was tested using calibration within the concentration range of 1×10^{-7} to 1×10^{-5} mol L^{-1}. LODs and LOQs were calculated as 3.3 SD/s and LOQ = 10 SD/s, respectively. SD is the standard deviation of the signal intensity and s is the slope of the calibration curve. The reproducibility of migration times and peak areas was calculated from the repeated analyses at 5×10^{-7} mol L^{-1}, 1×10^{-6} mol L^{-1}, and 5×10^{-6} mol L^{-1} levels; the intraday repeatability was calculated from three repetitions within one day; the interday repeatability was calculated from repetitive analyses over three consecutive days (each day with three repetitions). The recovery was calculated from the addition of 5×10^{-7} mol L^{-1} tamsulosin ($n = 3$) as the ratio of concentration determined and concentration added. The selectivity was determined by comparing analyses of blank samples without addition of tamsulosin with the analyses of tamsulosin at a 5×10^{-7} mol L^{-1} level. Here, wastewater was used as the sample; only filtration *via* a nylon syringe filter (0.22 μm) was used as the pretreatment step.

3. Results and discussion

3.1. Method development

As discussed before, the connection of CE and MS for chiral separation needs to deal with selector movement. In our previous studies,[31,32] we showed that tamsulosin enantiomers can be separated at acidic pH with sulfated β-cyclodextrin as a chiral selector. Under these conditions, tamsulosin is positively charged and migrates in the opposite direction to the selector. Hence, we decided to use the counter current migration mode. In this mode, the capillary is flushed with BGE containing S-β-CD. After the injection, the inlet vial contains just BGE without S-β-CD (the "outlet" is represented by a direct connection to the electrospray ionization MS interface). In this mode, S-β-CD migrates out of the MS. The analytes are separated and can be detected by MS because there is no interference of S-β-CD with the electrospray ionization (Fig. 2).

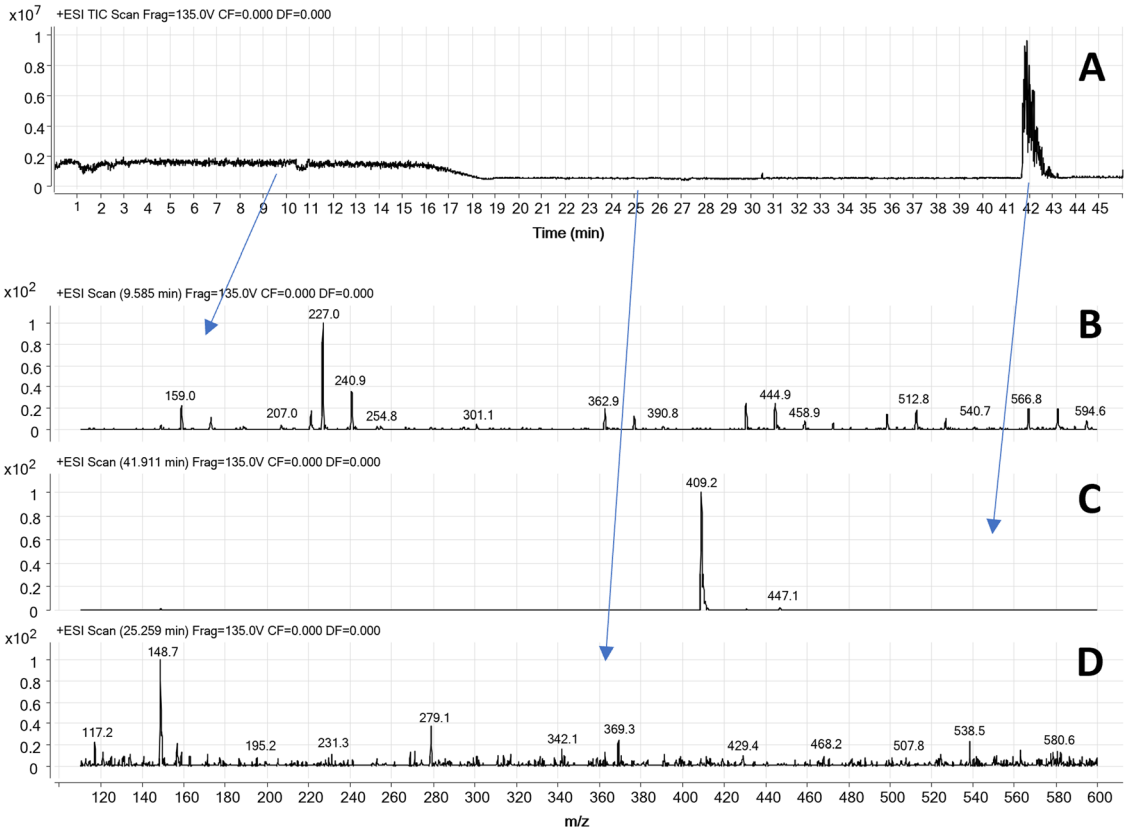

Fig. 2 CE-MS of tamsulosin in the counter-current mode (A) with the MS spectra of appropriate regions (B–D); migration regions: 0–18 min: BGE with S-β-CD (MS spectrum in (B) shows the signals of S-β-CD and adducts with Na^+ and NH_4^+, respectively; the signals were confirmed also by the negative ionization); 18+ min: BGE without S-β-CD (MS spectrum in (D) does not show any specific signal); approx. 42 min: non-separated tamsulosin enantiomers (MS spectrum in (C) shows one major signal *m/z* 409 referring to the $[M + H]^+$ ion of cetirizine); BGE: 200 mM acetic acid titrated with ammonium hydroxide to pH 4.0; 10 mg mL^{-1} S-β-CD.

As the first step of method development, MS conditions were optimized. The effect of the sheath liquid composition was studied. Initially, water–methanol mixtures at ratios of 25 : 75, 50 : 50 and 75 : 25 (v/v) were evaluated (without the addition of formic acid). The highest tamsulosin signal as well as the signal/noise ratio were observed for the ratio 25 : 75 (water–methanol, v/v), Fig. 3A. Then, the effect of formic acid presence (0%, 0.1%, and 0.5%; v/v) was analyzed (75% methanol, 25 or 24.9 or 24.5% water; v/v). The addition of 0.1% (v/v) had a positive impact on the tamsulosin signal (Fig. 3B). Hence, the addition of 0.1% (v/v) formic acid was used for further optimization. The next parameters included MS operational parameters as electrospray voltage (3.0–4.5 kV) and drying gas temperature (200–300 °C). As can be seen (Fig. 3C and D), the use of 4.0 kV and a drying gas temperature of 300 °C led to the highest signal intensities. Finally, the nebulizing gas flow rate (5–13 L min^{-1}), nebulizing gas pressure (10–20 psi), and sheath

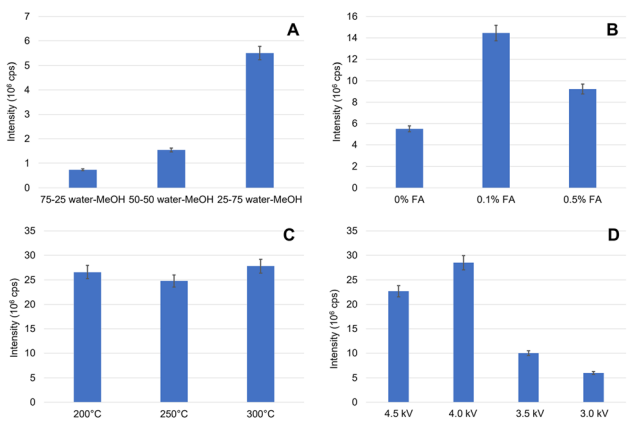

Fig. 3 Optimization of MS conditions; (A) the effect of methanol content in the sheath liquid; (B) the effect of formic acid content in the sheath liquid; (C) the effect of drying gas temperature; and (D) the effect of electrospray voltage; all on the intensity of the tamsulosin signal at *m/z* 409.0; see the text for conditions.

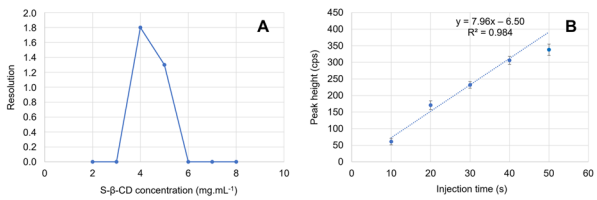

Fig. 4 The effect of S-β-CD concentration on tamsulosin enantiomers' resolution (A) and the effect of the injection time on peak height of tamsulosin under the stacking conditions (B); BGE: 200 mM acetic acid titrated with NH_4OH to pH 4.0 with addition of S-β-CD.

Table 1 Summary of method validation

Parameter	(*R*)-Tamsulosin	(*S*)-Tamsulosin
Linearity (mol L^{-1})	1×10^{-7} to 1×10^{-5}	1×10^{-7} to 1×10^{-5}
Calibration equation[a]	1.96×10^{9} $(4.6 \times 10^{7})x - 115$ (193)	1.99×10^{9} $(3.0 \times 10^{7})x - 129$ (127)
LOD (mol L^{-1})	1.6×10^{-9}	1.6×10^{-9}
LOQ (mol L^{-1})	4.8×10^{-9}	4.8×10^{-9}
Intraday repeatability of migration time (%)	0.34	0.36
Interday repeatability of migration time (%)	2.64	3.17
Intraday repeatability of peak heights (%)	1.1	2.2
Interday repeatability of peak heights (%)	5.8	7.4
Recovery (%) at 5×10^{-7} mol L^{-1}	92.0	87.9
1×10^{-6} mol L^{-1}	94.6	90.1
5×10^{-6} mol L^{-1}	94.9	92.5

[a] SD values are given in parentheses.

liquid flow rate (0.4–1.0 μL min^{-1}) were optimized. The highest signals were observed at nebulizing gas flow rate 5 L min^{-1}, nebulizing gas pressure 15 psi, and sheath liquid flow rate 0.6 μL min^{-1}.

To obtain the correct single reaction monitoring (SRM) transitions for both determination and identification, tamsulosin was fragmented by increasing collision energy from 0 eV to 50 eV. The following SRM transitions at 20 eV were used: *m*/*z* 409.0 → *m*/*z* 228.1 (quantitation), *m*/*z* 409.0 → *m*/*z* 271.1, and *m*/*z* 409.0 → *m*/*z* 200.1 (both for identification). The transitions are in accordance with those reported in previously published papers.[35,36]

In the next step, CE conditions were optimized using the MS conditions previously developed. Here, the effects of pH, buffer concentration (ionic strength), and S-β-CD concentration were studied. In our previous studies,[31,32] sodium or tris phosphate and acetate buffers were used. These are fully incompatible with the MS; hence, we decided to use acetic acid-based buffers titrated with ammonium hydroxide to the desired pH. Concentrations of 25 mM to 250 mM and pH of 3.0 to 5.0 were studied (with 5 mg mL^{-1} S-β-CD). 200 mM acetic acid titrated with ammonium hydroxide to pH 4.0 was found to be the best (highest intensities and resolution) for the separation of tamsulosin enantiomers. Interestingly, it has an approximately two-fold higher ionic strength (40 mM) than that in our previous study (20 mM).[32] However, here the length of the capillary is doubled, in comparison with that of CE-UV, so it does not have much impact on electric currents. As the final step, the concentration of S-β-CD was optimized in the range of 0.5–10 mg mL^{-1}. The highest resolution (1.8) was obtained at 4.0 mg mL^{-1} S-β-CD (Fig. 4A).

An increase in injection time with dilution of a sample in 50% (v/v) methanol–BGE was applied to decrease the LOD values *via* so-called stacking online preconcentration.[37,38] Here, the differences in electric field strength in adjoining zones lead to the slowdown of analytes and their preconcentration. To obtain the lowest LOD values, the injection time was varied between 10 and 60 s (Fig. 4B). A linear increase in the peak

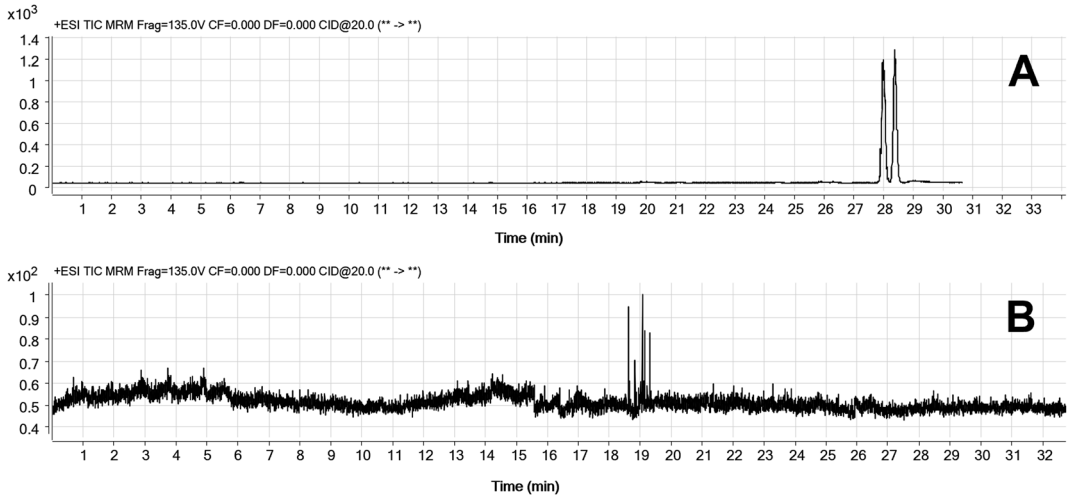

Fig. 5 Separation of tamsulosin enantiomers *via* CE-MS; (A) 5×10^{-7} mol L^{-1} tamsulosin in a wastewater sample; (B) blank (wastewater); BGE: 200 mM acetic acid titrated with NH_4OH to pH 4.0 with addition of 4.0 mg mL^{-1} S-β-CD; an injection time of 40 s at 50 mbar; a voltage of 20 kV; MS conditions: $U_{ESI} = 4.0$ kV, $T = 300$ °C, 5 L min^{-1}, 15 psi, sheath liquid: 75 : 24.9 : 0.1 MeOH, H_2O, formic acid (v/v), flow rate 0.6 μL min^{-1}; SRM for quantitation: *m*/*z* 409.0 → *m*/*z* 228.1, 20 eV.

height was observed at 40 s of injection; hence this was chosen as the optimal value for the validation and application of the method. The higher injection times led to peak broadening (not efficient preconcentration).

3.2. Validation

The developed CE-MS/MS method was finally validated in terms of linearity, LOD, LOQ, repeatability, selectivity, and recovery (Table 1). Calculations were carried out for both enantiomers, separately. All the calibrations were linear with correlation coefficients higher than 0.998. The LOD and LOQ values were 1.6 nmol L^{-1} and 4.8 nmol L^{-1}, respectively. Interestingly, the values are the same for both enantiomers (although the migration times of the enantiomers are different). The intraday and interday repeatability (precision) of migration times was less than 0.36% and 3.17%, respectively. The intraday and interday repeatability (precision) of peak heights was less than 2.2% and 7.4%, respectively. The trueness, expressed as the recovery was obtained by analysis of wastewater spiked with tamsulosin and ranged from 88% to 95%. The sample analysis including the blank (from the study of selectivity) is depicted in Fig. 5. As can be seen, the method allows determination of tamsulosin enantiomers in wastewater samples. Moreover, it can be supposed that it can be applied for analysis of tamsulosin enantiomers also in other matrices, including biofluids.

4. Conclusions

In our work, a new method for analysis of tamsulosin enantiomers by capillary electrophoresis connected with tandem mass spectrometry was developed. Under the optimal conditions (200 mM acetic acid titrated with NH_4OH to pH 4.0 with addition of 4.0 mg mL^{-1} S-β-CD and an injection time of 40 s at 50 mbar), nanomolar limits of detection were achieved. In combination with mass spectrometric identification, we developed a powerful tool for analyzing tamsulosin enantiomers in different samples. Our study was focused on the application of the method for analysis of wastewater samples with just a simple filtration pretreatment step. In this view, the CE-MS method is capable of determining the fate of tamsulosin enantiomers in the environment. Moreover, we believe that the excellent selectivity of MS detection can lead to the next possible application including analysis of biofluids.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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