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Short communication

Enantiomeric separation of acidic compounds of pharmaceutical interest by capillary electrochromatography employing glycopeptide antibiotic stationary phases

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Abstract

Enantiomeric separation of some selected acidic compounds of pharmaceutical interest belonging to the group of non-steroidal anti-inflammatory drugs were separated by capillary electrochromatography employing silica based glycopeptide antibiotic stationary phases, namely vancomycin or a teicoplanin derivatives (Hepta-Tyr). The vancomycin stationary phase allowed to achieve the chiral resolution of some racemic studied compounds only using mobile phases containing ammonium formate at a relatively low pH 2.5–3.5 and acetonitrile. Employing the teicoplanin derivative stationary phase, good enantiomeric resolution was achieved eluting with mobile phases containing sodium phosphate pH 6–acetonitrile. Enantiomers were moved to the detector because a relatively high reversed electroosmotic flow (due to the positive charge of the stationary phase) and to the electrophoretic mobility of analytes.

Keywords: Enantiomer separation; Pharmaceutical analysis; Stationary phases, electrochromatography; Nonsteroidal anti-inflammatory drugs

1. Introduction

Capillary electrochromatography (CEC) is a modern electromigration method possessing great potentiality for the separation and analysis of a wide number of compounds either charged/chargeable or neutral. Analytes and mobile phase are moved to the detector by a relatively strong electroosmotic flow (EOF) generated by the applied high electric field and due to the presence of charged/chargeable groups mainly present on the stationary phase surface. Therefore CEC can be considered an hybrid of two separation techniques, namely high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) because it combines the high selectivity and the high efficiency belonging from the two methods, respectively [1–4].

CEC was used for the separation of a wide number of compounds belonging to different classes including enantiomers that, due to their physical properties, require the presence of a chiral selector in the environmental separation system. The chiral selector was immobilized either on the capillary wall (open-CEC) or on the stationary phase (packed-CEC or

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monolithic) or added to the mobile phase [3,5]. Among the large number of chiral selectors used in CEC, glycopeptide antibiotics exhibited a high enantiorecognition capability towards a wide number of both basic (vancomycin and teicoplanin) and acidic compounds (Hepta-Tyr antibiotic) [6–13]. To our knowledge, only a few acidic compounds were enantioresolved using vancomycin or teicoplanin chiral stationary phases (CSPs) by CEC. Thalidomide enantiomers were separated by Wikstrom et al. with vancomycin CSP [9] while ibuprofen and two amino acids were resolved by Karlsson et al. [10] and Carter-Finch and Smith [7], respectively. Recently we successfully resolved mandelic acid and several derivatives using a silica based Hepta-Tyr antibiotic CSP [13].

In this communication we extended our previous studies employing CEC with capillaries packed with stationary phases containing vancomycin or Hepta-Tyr antibiotic to the enantioseparation of acidic compounds of pharmaceutical interest such as non steroidal anti-inflammatory or candidate drugs.

2. Experimental

2.1. Instrumentation

The CSPs containing diol-silica modified with vancomycin and Hepta-Tyr antibiotic (MDL 63 246) were synthesized using previously described methods [11,13]

Fused silica capillaries of 75 μm I.D., purchased from Composite Metal Services (Hallow, UK), were packed using a LC series 10 HPLC pump (Perkin-Elmer, Palo Alto, CA, USA). The vancomycin packed capillary was 33.4 cm long full packed with two frits at the ends of the tube. The chiral stationary phase mixed with silica (3:1, w/w) was 23.0 cm long and the rest was packed with a mixture of diol silica/silica particles (3:1, w/w); the detection window was done with a razor at 25 cm in the sector of the capillary that did not contain vancomycin. The selection of 3:1 ratio diol/silica was done with the aim to have similar amount of silanol groups along the column. In this way, because the silanol are responsible for the increase of EOF, we obtained the following advantages: (i) more stable current; (ii) more easy preparation of frits; and (iii) bubble formation disturbing the detection was not observed. Besides; the detection through the packed bed allowed acceptable sensitivity: this was not the optimum solution, however, the above mentioned advantages have to be considered.

The Hepta-Tyr chiral stationary phase mixed with aminopropyl silica was packed after the preparation of the first frit on silica-diol silica packed particles for 23.4 cm followed by a short zone with silica-diol silica packing mixture for the end frit preparation. The end frit was protected by an epoxy resin covering the outside of the capillary burned by the heated wire obtaining a robust capillary. Finally the detection window was prepared at 24.5 cm.

Electrochromatographic experiments were done by using an Agilent ^{3D}CE automatic system (Agilent, Waldbronn, Germany) equipped with an UV-diode array detector operated at 195 nm.

2.2. Chemicals

Vancomycin hydrochloride, racemic, carprofen, cicloprofen, fenoprofen, ketoprofen, indoprofen and suprofen were purchased from Sigma (St. Louis, MO, USA). 2-[(5′-Benzoyl-2′hydroxy)phenyl]-propionic acid (DF 1738Y), 2-[(4′-benzoyloxy-2′-hydroxy)phenyl]propionic acid (DF 1770Y); 2-(4′-isobutylphenyl)-3-methylbutanoic acid (DF 1902Y), 2-(4′-isobutylphenyl)-butanoic acid (DF 1903Y) and 2-(4′-isobutylphenyl)ciclopentylacetic acid (DF 1927) are candidate drugs under evaluation and kindly provided by Dompè (L'Aquila, Italy).

LiChrospher diol silica phase and LiChrospher Si-60, both 5 μm particle diameter, sodium cyanoborohydride and sodium periodate were from Merck (Darmstadt, Germany). Ammonium acetate, methanol (MeOH), acetonitrile (MeCN), all of HPLC analytical grade, were purchased from BDH (Poole, UK).

Hepta-Tyr antibiotic (MDL 63 246), a modified teicoplanin, was a gift of Professor P.G. Righetti, University of Verona, Italy; for its chemical structure, see Fig. 1. Phosphoric acid and sodium hydroxide were from Carlo Erba (Milan, Italy).

Mobile phases were prepared by mixing the appropriate volumes of aqueous buffer at the desired pH with the organic modifier; the final concentration

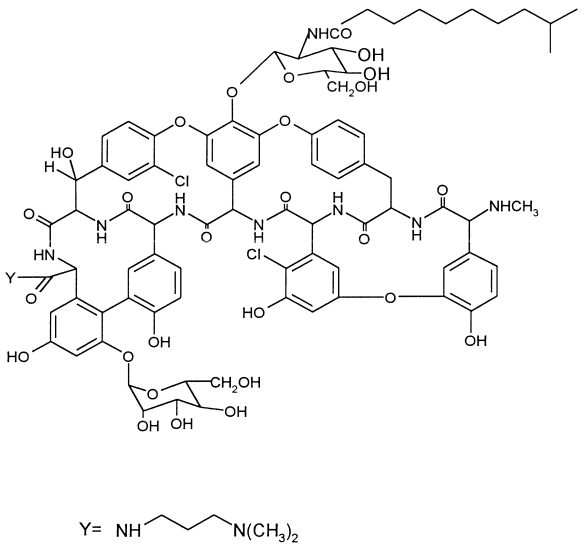


Fig. 1. Chemical structure of Hepta-Tyr antibiotic.

of the aqueous buffer was 5 m*M* in all experiments. Standard sample solutions were prepared in methanol (1 mg/ml) and daily diluted to the desired concentrations with the mobile phase (0.3–0.5 mg/ml).

3. Results and discussion

Vancomycin stationary phase was firstly studied for the CEC enantiomeric separation by Wikstrom et al. achieving good chiral resolution of several basic compounds employing both eluting modes, namely reversed-phase and polar organic [9]. Additionally, in this study, the authors mentioned that the only acidic compound enantioresolved was thalidomide. Recently we also employed capillaries packed with a vancomycin stationary phase separating by CEC several basic compounds and therefore confirming the results reported in literature [11,12].

Based on these results we tried the enantiomeric separation of some selected acidic compounds of pharmaceutical interest employing vancomycin based silica stationary phase. Experiments were carried out using a mobile phase containing relative high concentrations of acetonitrile (70–90%, v/v) and aqueous buffers at different pH values in the range 2.5–6 (ammonium formate or ammonium acetate). At the highest pH no peaks were detected probably due to the charge of studied acidic compounds. Better results were obtained decreasing the pH at 2.5–3.5 and dissolving the aqueous buffer in 90% MeCN.

Table 1 shows the data obtained analyzing selected nonsteroidal anti-inflammatory drugs (NSAIDs).

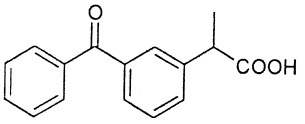
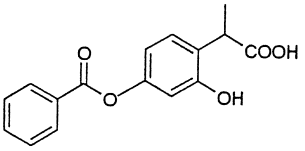
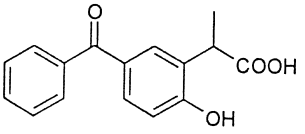
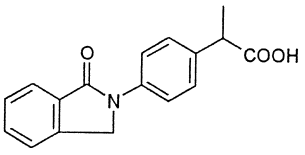
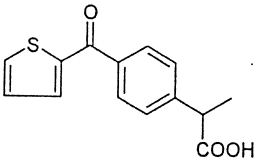
As can be observed in Table 1 the highest enantioresolution was achieved for DF 1738Y differing from ketoprofen by one hydroxyl group at position 2′ which clearly shows, the importance of this substituent group in the enantiorecognition mechanism. On the other hand the benzoyl group at position 5′ was also playing an important role; in fact in the case of DF 1770Y where the benzoyloxy group is present at position 4′, the resolution was lower than that observed for DF 1738Y but higher than that of ketoprofen.

From the above reported results we can conclude that: (i) the enantiorecognition capability of vancomycin stationary phase in CEC towards acidic compounds is limited (only a few NSAIDs were resolved); and (ii) can be achieved only with buffers at a relatively low pH (2.5–3.5).

Therefore considering the interactions involved in the affinity resolution mechanism using glycopeptide antibiotics in CEC (electrostatic, hydrogen, π–π, etc.) and based on our experience using a modified teicoplanin stationary phase, we employed the Hepta-Tyr packed column for our experiments. This packed capillary column, containing positively charged particles, allowed the chiral resolution of several hydroxyacid derivatives by reversing the polarity (EOF and analytes to the anode) [13]. Consequently the acidic samples were analyzed as anions eluting with mobile phases containing MeCN (40 or 50 or 60%, v/v) and 60–40% of ammonium acetate or sodium phosphate at pH 6 (final concentration, 5 m*M*). Although good enantiomeric resolution was achieved for the majority of the studied acidic compounds by using mobile phases containing ammonium acetate–MeCN, the results were not satisfactory due to the peak broadening probably due to the low kinetic interaction between studied enantiomers and stationary phase (results not shown).

Replacing ammonium acetate with sodium phosphate solution at the same pH greatly improved the results, giving higher efficiencies and better peak shapes when analyzing the acidic compounds at 50

Table 1
Enantiomeric resolution of acidic compounds of pharmaceutical interest by CEC using a vancomycin stationary phase

Compound	Chemical structure	pH	t_{R2} (min)	k_1 k_2	α	R_s
Ketoprofen		3.5	9.14	0.440 0.486	1.105	0.91
DF1770Y		3.5	9.70	0.446 0.589	1.321	1.48
DF1738Y		2.5	15.04	0.904 1.374	1.519	2.55
Indoprofen		3.5	7.56	0.417 0.491	1.178	0.69
Suprofen		3.5	10.20	0.644 0.744	1.202	1.25

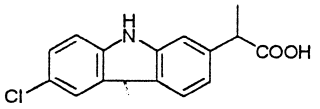
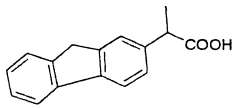
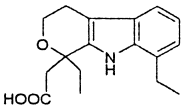
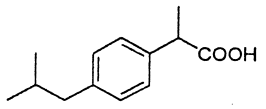
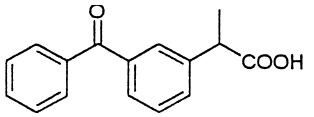
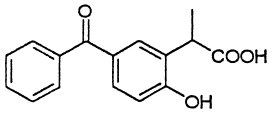
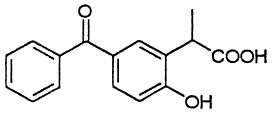
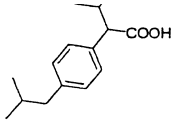
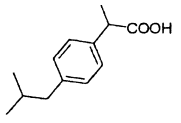
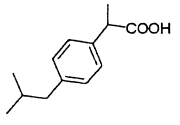
Capillary 75 μm I.D. full packed with the two frits at the ends (23 cm vancomycin-diol mixed with bare silica 3:1, w/w; 10.5 cm diol mixed with silica 3:1, w/w); effective length, 25 cm with the window in the zone where vancomycin was not present. Applied voltage, 25 kV, 20 °C; mobile phase ammonium formate (5 m*M* final concentration)–90% acetonitrile. Injection by pressure at 12 bar, 0.5 min of 0.3–0.5 mg/ml of racemic analyte followed by a mobile phase plug 12 bar, 0.2 min; both ends pressurized at 6 bar.

and 60% of sodium phosphate; however the best performance of the used capillary column was observed using 50% of aqueous buffer in the presence of MeCN. The results are reported in Table 2.

The highest enantiomeric resolution was achieved for DF 1738Y, a compound with a structure related to that of ketoprofen. The enantioresolution factors of ketoprofen and related compounds (DF 1738Y and DF 1770Y) were higher than those measured when using vancomycin. From these data we can also observe that, at least for the three acidic analytes, the two chiral packed capillaries exhibited similar enantiorecognition capability (DF 1738Y> DF 1770Y>ketoprofen). This is also shown in Fig. 2 where the enantioresolution of the three related compounds is reported.

The elution order was verified analyzing mixtures of ketoprofen, ibuprofen and suprofen enantiomers

Table 2
Racemic acidic compounds of pharmaceutical interest enantioresolved by CEC with Hepta-Tyr antibiotic chiral stationary phase

Compound		t_{R1} t_{R2}	k_1 k_2	N_1/m	α	R_s
Carprofen		14.41 16.89	1.411 1.825	109908	1.293	2.59
Cicloprofen		12.02 12.46	1.012 1.085	22908	1.072	0.55
Etodolac		9.40 9.68	0.587 0.634	39917	1.081	0.69
Ibuprofen		9.14 9.45	0.529 0.580	53650	1.096	0.76
Ketoprofen		12.02 13.86	1.012 1.318	22846	1.303	1.99
DF 1738Y		10.11 12.44	0.691 1.081	44858	1.565	4.15
DF 1770Y		11.50 13.99	0.924 1.340	16771	1.451	3.35
DF 1902Y		10.97 11.48	0.851 0.938	54029	1.102	0.98
DF 1903Y		10.35 10.96	0.747 0.850	61788	1.138	1.33
DF 1927		14.57 15.80	1.43 1.64	50621	1.144	1.60

Capillary 32.4 cm (effective length 24.0 cm)×75 μm I.D. Mobile phase, sodium phosphate pH 6 (final concentration 5 m*M*)–MeCN (1:1, v/v). Applied voltage, −25 kV, 3.8 μA, both sides pressurized at 6 bar; capillary temperature, 20 °C. Injection at 12 bar, 0.5 min of 0.5 mg/ml of racemic samples followed by a mobile phase plug 12 bar, 0.2 min.

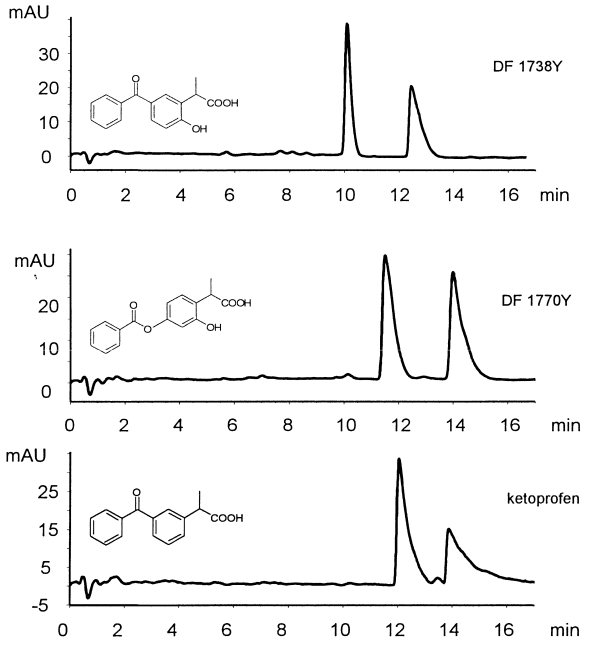


Fig. 2. Electrochromatograms of the enantiomeric separation of three structurally related drugs in the Hepta-Tyr silica stationary phase. Experimental conditions as reported in Table 2.

[R(−)/S(+), v/v] finding that the S(+) isomer was faster than its antipode while for ibuprofen the opposite was observed.

In conclusion, the two capillaries packed with vancomycin or modified teicoplanin CSPs can be both used for the chiral resolution of acidic compounds of pharmaceutical interest. However the Hepta-Tyr silica stationary phase allows to achieve higher enantioresolutions factors than those observed with the vancomycin CSP. Furthermore with the Hepta-Tyr packed capillary we resolved several acidic compounds that could not be separated with the vancomycin CSP.

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