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Short communication

Enantioseparation of omeprazole and its metabolite 5-hydroxyomeprazole using open tubular capillary electrochromatography with immobilized avidin as chiral selector☆

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ABSTRACT

The present paper demonstrates the enantiomeric separation of omeprazole and its metabolite 5-hydroxyomeprazole performed with open tubular capillary electrochromatography (OT-CEC). The protein avidin was used as the chiral selector. Avidin was immobilized by a Schiffs base type of reaction where the protein was via glutaraldehyde covalently bonded to the amino-modified wall of a fused-silica capillary, 50 μm i.d. Both racemates were baseline resolved. Resolution was 1.9 and 2.3, respectively, using ammonium acetate buffer, pH 5.8, 5% methanol, with UV-detection. These values of resolution using OT-CEC are higher than earlier published results regarding chiral separation of omeprazole and 5-hydroxyomeprazole on packed CEC. The number of theoretical plates also indicated good separation efficiency.

1. Introduction

Omeprazole, and the single enantiomeric form *S*-omeprazole, are used as antiulcer agents, with the brand names Prilosec or Losec, and Nexium, respectively. The metabolism of omeprazole results in several metabolites where 5-hydroxyomeprazole is one of the major ones. *R*-Omeprazole is stereoselectively hydroxylated by the Cytochrome P450 CYP2C19 enzyme, resulting in an almost twofold higher plasma concentration if the *S*-isomer is administrated as compared to if the racemate is administrated in equivalent [1]. By studying the metabolism of omeprazole and the major metabolite 5-hydroxyomeprazole in the human liver the drug can be used as a probe when searching for mutations of CYP2C19. Hence, these mutations affect its activity in the liver, and thus the metabolic and pharmacokinetic profiles of omeprazole [2].

Due to its rapid analysis time, low sample requirement and high separation efficiency CE is a very effective technique for chiral separations. One particular advantage is the simplicity of altering the enantioselectivity by adding different chiral selectors, to the buffer. Cyclodextrins (CDs) are commonly used as chiral selectors. They are compatible with the UV-detector usually used with CE because they are, in general, not UV-absorptive. A major drawback with the UV-detector is its relatively high limit of detection (L.O.D.). MS would be a viable alternative for detection. This is because MS can give high sensitivity as well as positive identification.

Omeprazole is a relatively instable compound, especially at acidic and neutral pHs [3–5], and monitoring of the decomposition products by MS can be of interest. When using for example cyclodextrins as chiral selector in the running buffer of CE connected to MS, the MS signal becomes suppressed why the entrance of the CD into the ionization section of the detector should be minimized. A commonly used method to achieve this is partial filling [6–8]. When an electric field is applied over the capillary the analyte and CD, possessing opposite charges, starts to migrate in opposite directions, the analyte toward the detector and the CD toward the inlet end of the capillary, away from the MS. In earlier work it was found that the difference in mobility between the basic analyte, uncharged at the running buffer employed [9], and the negatively charged heptakis-(6-sulfo)-β-CD (HS-β-CD), was not great enough to allow resolution of the enantiomers. According to Mol et al. [10], the counter-ion of the CD is still entering the ion source and causes ionization suppression when using partial filling. A method where CD is used but where the entrance of it into the MS is minimized was described in our earlier paper on omeprazole [11]. In the present work we attempt to circumvent the described problem by immobilizing the chiral selector avidin on the inner surface of the capillary. This open tubular capillary electrochromatography

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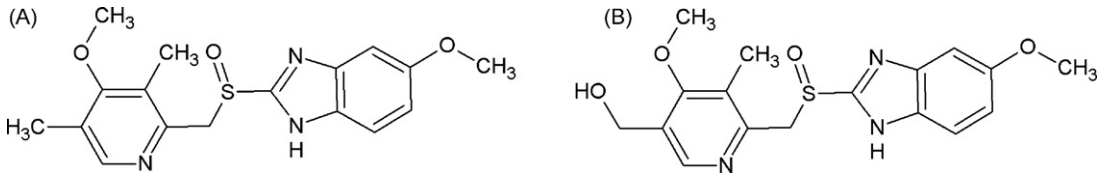

Fig. 1. Structures of (A) omeprazole and (B) 5-hydroxyomeprazole.

(OT-CEC) method would be into line with MS detection in further studies.

Enantiomeric separation by CEC has been given increased attention during the last 15 years; this has been because of the relatively high separation efficiency and high chiral resolution capacity, which has resulted in many publications in this field, Ref. [12] and references therein. Omeprazole has been chirally separated using CEC before [13,14]. Hebenstreit et al. [13] used packed CEC with strong cation exchange (SCX)-type chiral stationary phases based on β-amino sulfonic acid-terminated dipeptide derivatives as chiral selectors, immobilized on thiol-modified silica particles. The chiral stationary phase was suspended in acetone and slurry packed into a fused-silica capillary of 100 μm i.d. The best resolution of omeprazole was 1.36 when using *N*-[*N*-(4-allyloxy-3,5-dichlorobenzoyl)-leucyl]-2-amino-3,3-dimethylbutane sulfonic acid (LA-(*R*)-Leu-(*S*)-β-Tle-SO_3H) as chiral selector. Phenylcarbamate derivatives of cellulose and amylose are known to exhibit high chiral recognition capabilities [15]. For an evaluation of amylose tris(3,5-dimethylphenylcarbamate) as chiral selector, chiral separations of a number of analytes *inter alia* omeprazole were attempted on a fused-silica column, 100 μm i.d., packed with silica particles coated with the selector [14]. A resolution of 1.06 of omeprazole was reported for separation in CEC mode, using 5 mM ammonium acetate in acetonitrile/water 60/40 (v/v) as the mobile phase. On the other hand, resolution of omeprazole in HPLC on amylase tris(3,5-dichlorophenylcarbamate) was 2.5 when using MeOH as mobile phase and 0.7 with ACN [16].

Compared to packed or monolithic CEC, open tubular columns have a lower phase ratio. Oftentimes, OT-CEC provides shorter analysis times and higher separation efficiencies which makes the technique attractive. The small amount of stationary phase results in a low sample capacity. When the same coated film-thickness is used the low phase ratio can be improved if narrower i.d. of the capillaries is employed. For on-column UV detection narrower i.d. will however affect the L.O.D. since the detection path-length becomes shorter according to Lambert–Beers law. By using MS detection this drawback can be avoided making the OT-CEC-MS technique quite powerful. To the best of our knowledge the present work is the first result using OT-CEC for the enantiomeric separation of omeprazole and 5-hydroxyomeprazole enantiomers.

Proteins have been extensively used as chiral selectors in LC, CE and CEC [17–19]. A major advantage of such selectors is that they provide multiple chiral recognition sites, therefore these phases have the ability to separate a wide range of chiral compounds. Proteins have been especially useful for the separation of pharmaceutically active compounds [17–19]. Avidin is a protein that is suitable for this purpose. Physically adsorbed avidin on a monolithic silica capillary [20] and on open tubular fused-silica capillaries [21,22] have been used for electrochromatography. However, these types of columns suffered from leakage of avidin leading to reduced reproducibility and stability. These problems could be solved by means of covalent bonding of the avidin to the supporting surface as described by Kitagawa et al. [19]. Here, a Schiffs base type of reaction was employed to covalently bond the protein via glutaraldehyde to the inside of the amino-modified fused-silica capillary wall. In the present work, using CE-UV with the avidin-coated capillary, both omeprazole and its metabolite 5-hydroxyomeprazole were successfully separated with no additives needed in the electrolyte buffer.

2. Experimental

2.1. Equipment

OT-CEC separations were performed using a Hewlett-Packard HP^{3D} instrument (Waldbronn, Germany) equipped with a diode array UV-detector and temperature control system. Untreated fused-silica capillaries (50 μm i.d., 375 μm o.d.), were obtained from Polymicro Technologies (Phoenix, AZ, USA). Total capillary length was 50 cm (effective length 41 cm).

2.2. Chemicals and reagents

Racemic omeprazole and 5-hydroxyomeprazole were supplied by AstraZeneca R&D (Mölndal, Sweden). The analyte structures are shown in Fig. 1. Formic acid (FA), acetone, acetic acid, 4% glutaraldehyde solution, pH 7.6, in 50 mM borate buffer, potassium *tert*-butoxide, 3-aminopropyltrimethoxysilane (APTS), racemic ibuprofen, boric acid and avidin were from Sigma–Aldrich (Stockholm, Sweden). Heptakis-(2,3,6-tri-*O*-methyl)-β-CD (PM-β-CD) was purchased from Cyclolab (Budapest, Hungary). Deionized Milli-Q water (Millipore, Billerica, MA, USA) was used, with a resistivity of 18.2 MΩ/cm. Methanol and sodium hydroxide (NaOH) were from Merck (Darmstadt, Germany). Ammonium acetate was from Fluka Biochemika (Buchs, Switzerland) and dimethylsulfoxid (DMSO) was from VWR (Stockholm, Sweden). The pH of the stock solution of ammonium acetate buffer (300 mM) was adjusted to 5.8 with acetic acid, and NaOH was added to get a pH of 9.0 in the stock solution of borate buffer (200 mM). The PM-β-CD, 30 mM, was prepared by weighing directly into a CE vial and then dissolving it using the buffer electrolyte.

2.3. Coating of capillary

The untreated fused-silica capillary was cut to 55 cm. The immobilization of avidin was performed according to [19]: (i) activation of the capillary surface, (ii) introduction of amino group, (iii) introduction of aldehyde group and (iv) immobilization of avidin. First, the capillary was rinsed with 12.5 mM potassium *tert*-butoxide, dissolved in DMSO, for 60 min, followed by a flush with Milli-Q water for 15 min. After this 2% APTS, dissolved in acetone, was flushed for 15 min. To remove all unreacted APTS the capillary was rinsed with water followed by methanol for 15 min, respectively. A solution of 4% glutaraldehyde in boric acid, pH 7.6, was flushed through the capillary. Kitagawa et al. used 10% glutaraldehyde dissolved in 50 mM borate buffer, pH 9, for 60 min. Because of the lower concentration of glutaraldehyde that we used the reaction time was increased to 90 min. This was followed by 2 mg/L avidin in 50 mM borate buffer, pH 9, for 60 min. In the final step the capillary was rinsed with Milli-Q water for 15 min. Burning of detection-window

for UV-detection was performed after the coating to locally displace the UV absorbing avidin [23] from the inner surface of the capillary.

2.4. Sample preparation

Stock solutions of racemic omeprazole and 5-hydroxyomeprazole were prepared by dissolving the solid sample in methanol, and the solutions were stored for a maximum of 1 month at −20 °C, without any sign of discoloration. Final sample concentrations were made by diluting with buffer directly before injection.

2.5. Electrophoretic technique

Before the enantiomeric separation was preformed, the capillary with immobilized avidin was conditioned with running buffer for 30 min. Hydrodynamic injection, 50 mbar 3 s, was performed. The capillary and vial tray were thermostated to a constant temperature of 16 °C. UV detection was performed at 301 nm. All solutions were degassed and filtered using 0.45 μm pore size filters. Reversed polarity was used. The applied CE voltage was −25 kV or −30 kV.

3. Results and discussion

3.1. Method development

Ammonium acetate buffer, 10 mM, with a pH of 5.8 was used as in our earlier publication [11]. As mentioned above, omeprazole has been reported to be somewhat instable under acidic and neutral pHs [3–5] why a maximum ammonium acetate pH was employed here (buffering capacity 3.8–5.8) to afford the best possible stability of omeprazole while still using an MS compatible buffer.

A run temperature of 16 °C was used as in our earlier publications about omeprazole. This was because of the increase in resolution observed at lower temperatures [11,24]. In addition, this has been reported by Berzas Nevado et al. [3]. Also in the present work the lower temperature resulted in an improved resolution.

3.2. Enantiomeric separation of omeprazole and 5-hydroxyomeprazole

Omeprazole and 5-hydroxyomeprazole were separated on the capillary with immobilized avidin. No additives to the buffer had to be used, which is preferable if MS detection is going to be used. A study of the optimal methanol concentration in the buffer was performed to improve the resolution between the enantiomers of omeprazole and also the number of theoretical plates. The best resolution was found with 5% methanol, Figs. 2 and 3 and Table 1. The numbers of theoretical plates per meter, measured on the capillary were 51,700 and 34,800 for the two enantiomers of omeprazole, 109,300 and 41,200 for the two enantiomers of 5-hydroxyomeprazole.

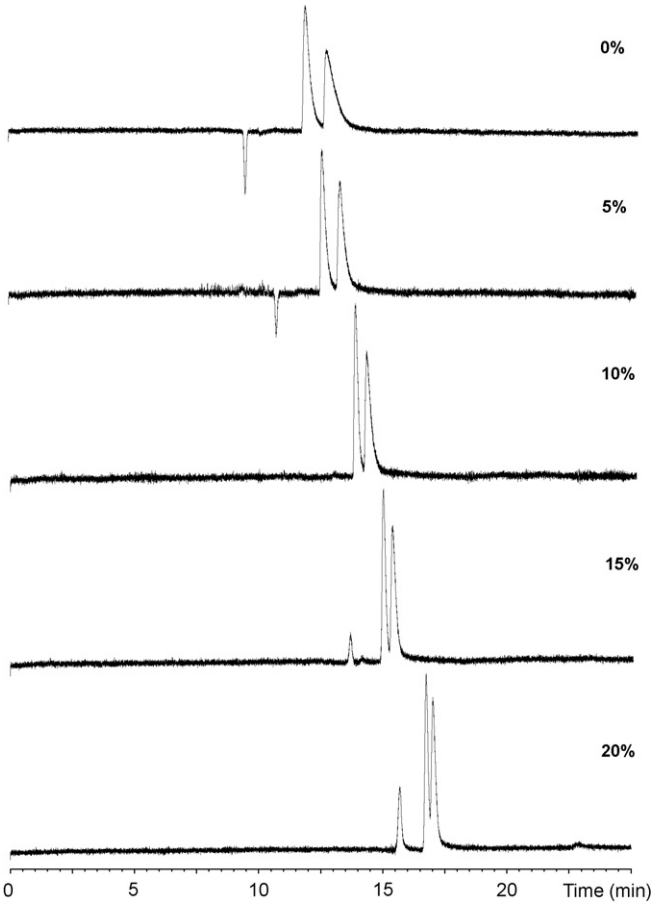

Fig. 3. Influence of methanol concentration in the electrolyte buffer on resolution. Conditions as in Fig. 2.

The number of theoretical plates (N) and the peak shapes improved with increasing amount of methanol, while the resolution decreased, this is in accordance with Kitagawa et al. [19]. However, we found an optimal resolution at 5% methanol (5% methanol was not tested in Ref. [19]). A further difference was that in our case the migration times increased with increasing concentration of methanol but the opposite effect was reported in [19].

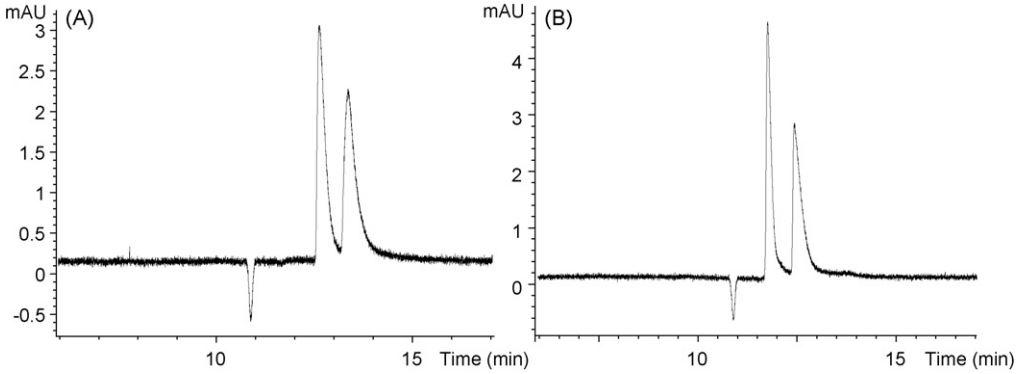

Fig. 2. Enantiomeric separation of (A) omeprazole at a concentration of 0.5 mM, R_S = 1.9 and (B) 5-hydroxyomeprazole at a concentration of 0.5 mM, R_S = 2.3. Capillary 50 cm (effective length 41 cm), 50 μm i.d., coated with 2 mg/mL avidin. 10 mM ammonium acetate buffer, pH 5.8, 5% methanol. Injection 3 s at 50 mbar; capillary and tray temperature 16 °C; voltage −30 kV; detection 301 nm.

Table 1
Determination of optimal concentration of methanol for chiral resolution of omeprazole

%MeOH	t_r (min)	N		Resolution
	Peak 1	Peak 1	Peak 2	
0	11.8	26,800	11,200	1.4
5	12.5	51,700	34,800	1.9
10	13.8	93,000	53,700	1.4
15	15	139,000	80,500	1.3
20	16.8	185,000	124,000	1

N is the number of theoretical plates/m.

In that case tailing was more severe, indicating strong adsorption of the anlyte flurbiprofen at the positively charged surface. At the pH used, 5.0, this analyte is partially protonated which led to much increased N compared to runs at pH 7.0, where the analyte is deprotonated. In our case, the analyte is omeprazole and the pH 5.8. As we indicated in an earlier article, omeprazole is largely non-charged at this pH [9,11]. This may explain the higher N in our case. The EOF was -1.0×10^{-4} cm^2 V^{-1} s^{-1}.

The single *S*-enantiomer was injected using the same method as for the racemate. In this way the first eluted enantiomer was shown to be *S*-omeprazole.

3.3. Repeatability

The run-to-run repeatability for the migration time and area of the first peak is described with the relative standard deviation, R.S.D., of 2.9% and 5.3%, respectively and for the second peak, R.S.D. was 2.5% and 8%, respectively. The R.S.D. for resolution was 5.0% ($n = 6$).

The day-to-day variations were 2.6% and 3.4% for the migration time and area, 2.7% for resolution ($n = 4$).

4. Conclusions

The use of open tubular columns immobilized avidin at the inner surface of the capillary is an effective approach for the enantiomeric separation of omeprazole and 5-hydroxyomeprazole. The chiral OT-CEC-technique was developed as a prerequisite for successful interfacing with MS detection, avoiding the use of non-volatile buffer or additives such as CD. Baseline separation of the two enantiomers of both omeprazole and 5-hydroxyomeprazole was achieved with values of resolution better than earlier published results using packed CEC. A relatively high value of the number of theoretical plates also indicated good separation efficiency.

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