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Journal of Chromatography A, 755 (1996) 251–259

JOURNAL OF CHROMATOGRAPHY A

Ergot alkaloids as chiral selectors in capillary electrophoresis Determination of the separation mechanism

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Received 24 April 1996; revised 25 June 1996; accepted 25 June 1996

Abstract

Recently ergot alkaloids were introduced as novel chiral selectors in capillary electrophoresis. In the present study, stereoselectivities of several ergot alkaloids, added to the background electrolyte (BGE), towards some racemic hydroxy organic acids are compared. The 1-allyl derivative of (5*R*,8*S*,10*R*)-terguride (allyl-TER) proved to be the best chiral selector for these analytes. Only the capillary was filled with BGE containing the chiral selector. The in- and outlet vial did not contain any ergot alkaloid. The effects of pH, and MeOH added to the BGE were investigated. Low pH proved to have an adverse effect on enantioseparation. Good separation for the enantiomers of some α-hydroxy acids was obtained at pH 4.2, and 25 m*M* allyl-TER. The addition of 50% MeOH to the BGE altered stereoselectivity and increased the solubility of the chiral selector. Using a BGE containing 50% MeOH, and 62.5 m*M* allyl-TER at pH* 5.5, the optical isomers of all test compounds, including tropic acid and other organic acids, were baseline resolved.

Keywords: Background electrolyte composition; Chiral selectors; Enantiomer separation; Alkaloids; Organic acids; Amino acids

1. Introduction

Capillary electrophoresis (CE) has shown to be a very useful analysis technique for the separation of optical isomers, as surveyed in some recent reviews [1,2]. Short migration times together with high separation efficiencies and rapid method development makes the method a powerful alternative to chiral chromatography methods. Chiral selectors, such as crown ethers, cyclodextrins, proteins, or chiral micelles can simply be added to the separation buffer. Much effort is taken to broaden the range of applications for CE in chiral analysis. For this purpose new chiral selectors have been introduced like macrocyclic antibiotics [3,4], or synthetic chiral micelles [5,6].

Recently, we introduced ergot alkaloids as novel chiral selectors in CE [7]. Ergot alkaloids are a large group of natural compounds that are derivatives of (5*R*)-lysergic acid [8]. The optical purity of some semi-synthetic ergot pharmaceutical preparations was determined in a previous study [9] using cyclodextrins as chiral selectors. These alkaloids have been used previously as chiral stationary phase in high-performance liquid chromatography (HPLC) [10–13]. An NMR study on the diastereoisomer

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0021-9673/96/$15.00
PII S0021-9673(96)00597-3

complexes formed between the 1-aminopropyl derivative of terguride and naproxen was performed to study the mechanism of chiral interaction [14]. This study shows that electrostatic interaction between the positively charged nitrogen in position N(6) and the acidic function of naproxen, as well as $\pi-\pi$ stabilizing interactions are the most significant bonding interactions concurring to the formation of diastereoisomer complexes.

In our earlier study [7], we reported baseline separation of some α-hydroxy organic acid enantiomers, α-methoxyphenylacetic acid enantiomers and partial separation of the optical isomers of tropic acid using the 1-allyl derivative of (5*R*,8*S*,10*R*)-terguride (allyl-TER). In the present study, a wider range of ergot alkaloids is applied as chiral buffer additive. We characterized these alkaloids in terms of pK_a values and mobilities. Also we studied the separation mechanism and determined the equilibrium constants of complex formation for several diastereomeric complexes.

The main drawback of the use of ergot alkaloids as chiral selector in CE was the limited solubility of the chiral selector in water. In the present study, we increased the solubility of the ergot alkaloids by the use of methanol as organic buffer modifier. The effect of methanol on complex formation and stereoselectivity is discussed.

2. Experimental

2.1. Equipment

All analyses were performed on a P/ACE 2200 CE system (Beckman, Fullerton, CA, USA). The Beckman instrument used PVA-coated capillaries (Hewlett-Packard, Palo Alto, CA, USA), with a total length of 37 cm and an effective length of 30 cm. The internal diameter was 50 μm. The capillary temperature was kept constant at 22°C. Before every electrophoretic run, the capillary was first flashed with background electrolyte (BGE) and then flushed with BGE containing allyl-TER with concentrations varying between 0–62.5 m*M*. In this way, allyl-TER was only present in the capillary and not in the in- or outlet vial, as described earlier [7,15,16]. Samples were introduced by pressure injection (10 s at $3.3\cdot10^3$ Pa). The detection wavelength was 200 nm in BGEs containing 100%, and 214 nm in BGEs containing 50% MeOH. The applied voltage was 20 kV or 25 kV.

2.2. Chemicals and sample preparation

The 1-allyl derivatives of ergot alkaloids (lisuride [18 016-80-3], terguride [37 686-84-3], luol [35 121-60-9], lysergol [602-85-7], festuclavine [569-26-6], ergotamine [113-15-5] and dihydroergotamine [511-12-6]) were synthesized by the method published earlier [13]. Dihydroergocristine [17 479-19-5] was a gift of Academy of Sciences of the Czech Republic (Prague, Czech Republic). The chemical structure of allyl-TER is shown in Fig. 1. Racemic mandelic acid and L-(+)-mandelic acid, and racemates of *p*-hydroxymandelic acid, 3,4-dihydroxymandelic acid, vanilmandelic acid, and tropic acid, were purchased from Sigma (St. Louis, MO, USA). Racemates of N-acetylphenylalanine, N-formylphenylalanine, 2-phenylglycine, and α-methoxyphenylacetic acid were a gift of DSM Research (Geleen, Netherlands). These samples were dissolved in demineralized water. The concentration of the analytes was 25 μg/ml. Samples of ergot alkaloids were dissolved in glacial acetic acid and diluted with 50 parts of water to a final concentration of 10^{-4} *M*.

2.3. Methods and electrophoretic systems

The pK_a values of the ergot alkaloids were determined by measuring mobilities from pH 2 up to

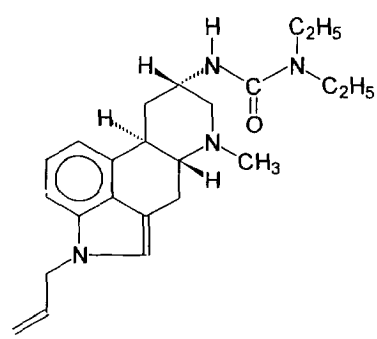

Fig. 1. Chemical structure of 1-allylterguride.

Table 1
BGEs used for the pK_a determination of ergot alkaloids

pH	Cations	Anion
2.0	10 m*M* H^+	Phosphate
3.0	10 m*M* Na^+	Formate
3.8	10 m*M* Na^+	Formate
5.0	10 m*M* Na^+	Acetate
6.0	10 m*M* Na^+	MES
7.0	10 m*M* Na^+	MOPS
8.0	25 m*M* $Tris^+$	Chloride
9.0	10 m*M* Na^+	Borate

MES = 2-(N-morpholino)ethanesulfonate, MOPS = morpholinopropanesulfonate, Tris = Tris(hydroxymethyl)aminomethane.

pH 9. For this purpose, BGEs were prepared with constant ionic strength *I* (10 m*M*). The composition of these BGEs is listed in Table 1. The pK_a values of some of the analytes were determined by measuring the (apparent) pH of a solution containing 10 m*M* NaOH and 20 m*M* of the organic acid. The BGE for the chiral analysis of the racemic organic acids was prepared by adjusting a 200 m*M* β-alanine solution with acetic acid up to pH 4.2. The ionic strength of the BGE was 50 m*M*. Ergot alkaloids were dissolved in glacial acetic acid and diluted with demineralized water. The pH was adjusted with a β-alanine solution up to pH 4.2. The ionic strength of the BGE with chiral selector was also 50 m*M*. A similar approach was used to prepare BGEs at pH 3.2 using formate instead acetate as background anion.

Ergot alkaloids were also soluble in MeOH. Solutions of allyl-TER in MeOH were diluted with NaAc solutions up to pH* 5.3 and *I*=50 m*M*, or with a 200 m*M* β-alanine–acetate solution up to pH* 5.5, both with a final MeOH concentration of 50% (v/v).

3. Results and discussion

3.1. Characterization of the ergot alkaloids

Fig. 1 shows the chemical structures of the ergot alkaloids, applied in this study. These natural compounds possess two or three asymmetric carbons, a π-acceptor represented by the indole ring, and a basic nitrogen on the N(6) position as electrostatic interaction site. This electrostatic interaction between the acidic function of a sample molecule, as well as π–π stabilizing interactions are the most significant bonding interactions concurring to the formation of diastereomeric complexes [14]. Furthermore, these compounds have strong UV absorbance between 200 and 300 nm. In order to estimate the charge of the chiral selectors, we determined the pK_a values. For this purpose, effective mobilities were measured as a function of the pH of the BGE. The ionic strength of these BGEs with varying pH, was kept constant at 10 m*M*. The degree of dissociation (α) was calculated from the measured mobility, and the mobility of the ergot alkaloid at pH 2. At this pH, the basic alkaloids were assumed to be fully protonated. These mobilities at pH 2 are tabulated in Table 2. The pK_a values could be determined graphically, according to the equation of Henderson–Hasselbalch:

$$pK_a = pH - \log\left[\frac{\alpha}{1-\alpha}\right] \quad (1)$$

This is shown in Fig. 2 for allyl-TER and 1-allylergotamine. The pK_a value is determined by the intersection of the linear curve with the pH axis. As listed in Table 2, the pK_a values varied between 6.1 and 8.9. Therefore, the assumption of full protonation at pH 2 was justified. The mass versus charge relation of these alkaloids indicates monovalent cations.

At higher pH (pH 5.0), is was not possible to dissolve allyl-TER in 100% water. For this reason, experiments were performed using methanol as organic buffer modifier.

Table 2
Mobilities and pK_a values of the applied ergot alkaloids

Ergot alkaloid	Mobility[a]	pK_a
1-Allylterguride	18.1	7.1
1-Allylfestuclavine	22.0	8.4
1-Allylluol	19.9	8.9
1-Allyllysergol	21.0	7.6
Dihydroergocristine	14.2	6.7
1-Allyllisuride	17.9	7.0
1-Allyldihydroergotamine	13.9	6.4
1-Allylergotamine	14.2	6.1

[a] Mobility $\times 10^{-9}$ [m^2/V s].

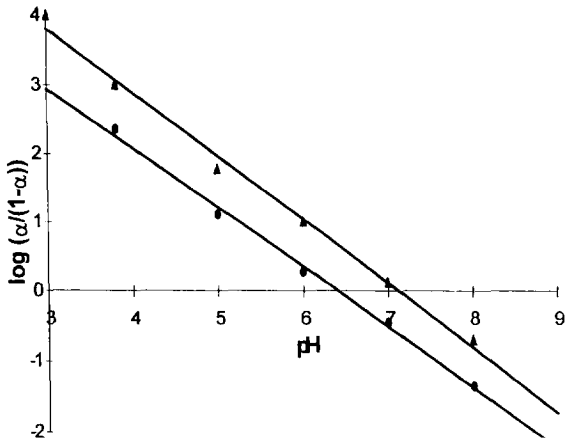

Fig. 2. Determination of pK_a values of 1-allylterguride (▲) and 1-allylergotamine (○).

3.2. Comparison of stereoselectivity of different ergot alkaloids towards some racemic organic acids

To determine the quality of a chiral separation, we used two different definitions. The enantioseparation factor (SF) is defined as the ratio of the equilibrium constants of complex formation of the two optical isomers. Selectivity (S) is defined as the ratio of the difference and the mean effective mobility of the two optical isomers. Mandelic acid, mandelic acid derivatives (3-hydroxymandelic acid, 3,4-dihydroxymandelic acid, vanilmandelic acid), and tropic acid were chosen as test compounds. These experiments were performed at pH 4.2. At their highest possible concentration in water, we found that the 1-allyl derivatives of luol, lisuride, and lysergol showed no selectivity towards the optical isomers of the selected compounds. Mandelic acid and mandelic acid derivatives were partly resolved using 90 mM 1-allylfestuclavine as chiral selector ($S<0.008$). Slightly higher selectivities were obtained at 40 mM dihydroergocristine. This ergot alkaloid showed also selectivity towards the tropic acid enantiomers ($S=0.005$), but no selectivity towards the optical isomers of mandelic acid. The best results were obtained with allyl-TER as shown in Fig. 3. Already at 25 mM, this chiral selector showed the highest selectivities towards all the selected racemic compounds ($S=0.01$ for mandelic acid up to 0.02 for vanilmandelic acid enantiomers), except tropic acid ($S=0.002$). For this reason, we focused our study on allyl-TER.

3.3. Determination of the mobility of the analyte interacting with allyl-TER

All analytes were moving as anions in the direction of the anode. To suppress electroosmotic flow (EOF), coated capillaries were used. The measured EOF was negligible ($m_{eof}<1\cdot10^{-9}$ m^2/V s). Interaction with the chiral selector occurred only in the first part of the capillary. Due to this interaction, the mobility of the analytes in this part of the capillary

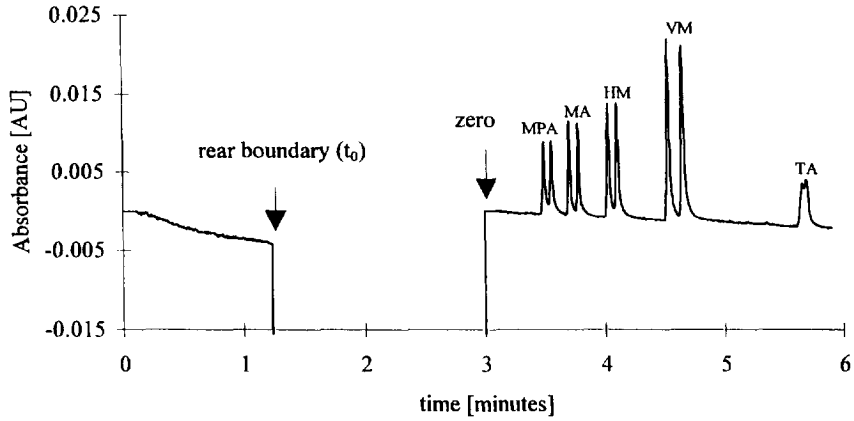

Fig. 3. Chiral separation of some racemic organic acids. MPA = α-methylphenylacetic acid, MA = mandelic acid, HM = 3-hydroxymandelic acid, VM = vanilmandelic acid, TA = tropic acid. An autozero was executed after 3 min. BGE: 25 mM 1-allylterguride + β-alanine–acetate, pH 4.2, ionic strength 50 mM. Capillary: 37 cm (30 cm)×50 μm. Separation: 25 kV.

(μ_i) will be lower than the mobility of the free analyte (μ_f).

Since all the experiments were performed at pH 3.2 and pH 4.2 (in BGE consisting of 100% water), the chiral selectors were fully charged. The mobility of the ergot alkaloids is higher than the mobility of the other counterion, β-alanine. Therefore, a self-sharpening rear boundary between BGE containing ergot alkaloid and pure BGE will be formed. This rear boundary will migrate in the direction of the inlet vial, opposite to the migration of the acidic analytes. Because of the strong UV absorbance of the ergot alkaloids, this rear boundary should pass the detection window before the analytes do. Detection of the analytes would not have been possible on this high background absorption. After the analytes have passed the rear boundary, but before detection, they will migrate in a BGE with an adjusted concentration of β-alanine and acetate, following the Kohlrausch regulation function [17]. Accordingly, the pH will decrease from 4.2 to 4.07, thus slightly decreasing the degree of dissociation and therefore the effective mobility of the acidic analytes. The electric field strength (E) in this zone however, was calculated to be approximately 6% higher. Consequently, the effects of the pH and of E on the mobility of the analytes are counter-productive. Therefore, for the determination of μ_i both pH and E are assumed to be homogeneous throughout the capillary.

First, after about 1 min, the rear boundary will pass the detection window (see Fig. 3). This time (t_0) could be accurately determined due to the sharp drop in absorbance. From this, the velocity of the rear boundary (v_{RB}) could be calculated, according to Eq. (2).

$$v_{RB} = (l_t - l_d)/t_0 \tag{2}$$

In this equation, l_d is the effective capillary length, and l_t is the total capillary length. (In all these equations, the velocity's have positive values).

At $t = t_i$ the analyte will pass the rear boundary, which is migrating in the opposite direction. This time is dependent of the analyte velocity (v_i) and will therefore be different for each sample.

$$t_i = \frac{l_t}{v_{RB} + v_i} \tag{3}$$

During this time the analyte will cover a distance equaling $\mu_i E t_i$. The remaining part of the capillary up to the detection window should equal $\mu_f E(t_m - t_i)$ and therefore:

$$l_d = \mu_i E t_i + \mu_f E(t_m - t_i) \tag{4}$$

Rearrangement of Eqs. (2–4) gives:

$$\mu_i = \frac{(l_t - l_d)(l_d - \mu_f E t_m) + (t_0 \mu_f E l_t)}{E t_0 (l_t - l_d + E \mu_f t_m)} \tag{5}$$

The interaction time of the analytes with the chiral selector measured 60–75% of the analysis time, depending on μ_i. As an example, the time of interaction of the mandelic acid enantiomers in Fig. 3 was 155 s (72% of the analysis time), and 180 s (64%) for vanilmandelic acid enantiomers.

3.4. Influence of pH on stereoselectivity

According to Rawjee et al. [18] the pH can have major influence on chiral selectivity in CE. In order to study the influence of the pH on the separation of the optical isomers of the selected organic acids, we studied complex formation and enantioselectivity at pH 3.2 and pH 4.2. Since the pK_a values of the analytes used in these experiments were about 3.5 for the mandelic acid derivatives and for α-methoxyphenylacetic acid, we could expect the pH to have a major influence on the degree of dissociation, and consequently on the stereoselectivity.

As illustrated in Fig. 4, the analytes had a lower migration velocity at pH 3.2 than at pH 4.2. This is due to the difference in the degree of dissociation of the weak acids. According to our results, the chiral selector had only little interaction with the analytes, at pH 3.2. This corresponds to a weak "ion pairing" effect exerted by the selector, due to the low dissociation of the carboxylic function of the analytes at this pH. Also the selectivity of allyl-TER towards the analytes was very limited (Fig. 4). At pH 4.2, the analytes were clearly retarded due to interaction with the chiral selector. At this pH, good enantioselectivities were obtained towards the optical isomers of the acidic compounds at this pH. As well as for the compounds shown in this figure, we also obtained baseline resolution for the enantiomers of 3,4-dihydroxymandelic acid and 2-phenylglycine.

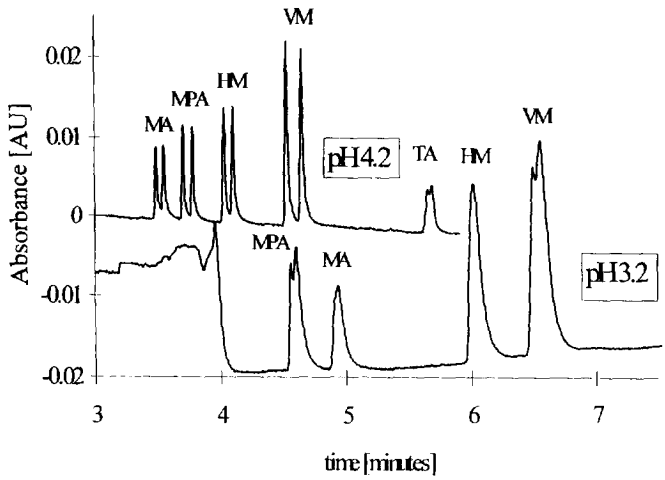

Fig. 4. Influence of pH on chiral separation of racemic organic acids. BGE of top electropherogram: 25 m*M* 1-allylterguride + β-alanine–formate, pH 3.2, $I = 50$ m*M*. BGE of bottom electropherogram: 25 m*M* 1-allylterguride + β-alanine–acetate, pH 4.2, $I = 50$ m*M*. Symbols and other experimental conditions as in Fig. 3.

This is in good agreement with HPLC experiments, using terguride as a chiral stationary phase [13]. From these results we concluded that only the dissociated acid interacts with the ergot alkaloid. The non-dissociated acid is not retarded due to interaction with the chiral selector.

3.5. Determination of equilibrium constants of complex formation

To study the interaction between analyte and chiral selector, the BGE in the capillary was supported with 0, 5, 10, 15, 20, and 25 m*M* ergot. Mobility (μ_i) of the racemic compounds decreased with increasing concentration of allyl-TER. The theoretical model of Wren and Rowe [19,20] was used to determine the equilibrium constants (K_c). According to this model, the electrophoretic mobility of the analytes (μ_i) will be a function of the mobility of the free enantiomer (μ_f), the mobility of the analyte–chiral selector complex (μ_c), the concentration of the chiral selector ([C]), and the equilibrium constant:

$$\mu_i = \frac{\mu_f + \mu_c K_c[C]}{1 + K_c[C]} \tag{6}$$

As mentioned earlier, only the dissociated acid interacts with the ergot alkaloid. Since the dissociated acid has a single negative charge, and the chiral selector has a single positive charge, the complex will be uncharged. Consequently, the complex mobility (μ_c) will be negligible, and Eq. (6) can be simplified:

$$\mu_i = \frac{\mu_f}{1 + K_c[C]} \tag{7}$$

This rearranges to:

$$\frac{\mu_f}{\mu_i} = 1 + K_c[C] \tag{8}$$

Consequently, a plot of (μ_f/μ_i) versus [C], should result in a linear relation, with a slope equaling K_c. This is demonstrated in Fig. 5 for the determination of the K_c values of the vanilmandelic acid enantiomers, using allyl-TER as chiral selector. The two solid lines show the best linear fit for these two optical isomers. The upper solid line represents the isomer which has the strongest interaction with the chiral selector (steepest slope).

Table 3 lists K_c values of all the compounds studied, in BGEs without, and with 50% MeOH as organic buffer modifier. Also the SF (as defined by the ratio of the K_c values of both optical isomers) is tabulated. Vanilmandelic acid and tropic acid have the highest affinity towards the chiral selector.

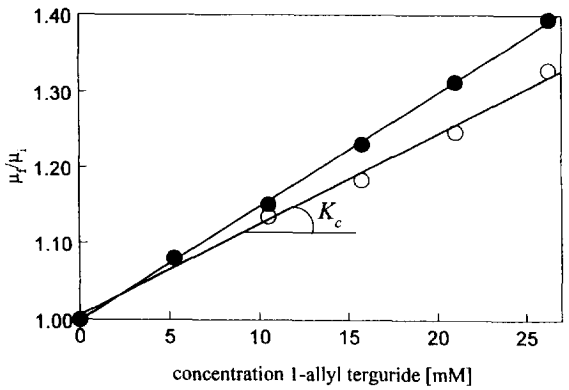

Fig. 5. Determination of K_c values of the vanilmandelic acid enantiomers. Solid lines represent best linear fits for the stronger (●) and weaker (○) interacting isomer. For further explanation see Section 3.5. BGE: 1-allylterguride + β-alanine, pH 4.2, $I = 50$ mM. Other experimental conditions as in Fig. 3.

Enantioselectivity however, towards the tropic acid enantiomers is very limited, under these conditions (0% MeOH, pH 4.2). The pK_a value of tropic acid is relatively high, resulting in a relatively low degree of dissociation, at pH 4.2 (Table 4). Despite the lower degree of dissociation, tropic has a high K_c value compared to the other organic acids. The SF however, is very low. A difference between tropic acid and the other analytes is that tropic acid is β-substituted, while the other analytes are α-substituted, counting both from the carboxylic acid functional group, and from the aromatic group. This could be a possible explanation for the low selectivity obtained for this analyte.

3.6. The influence of MeOH on chiral separation

We also investigated the effect of the addition of MeOH to the BGE on the interaction between the chiral selector and the racemic analytes. The chiral selector is well soluble in MeOH, thus making it very advantageous to use this organic buffer modifier. Upon addition of 50% MeOH to the BGE at pH 4.2 (200 mM β-alanine–acetate) the apparent pH shifted up to pH* 5.3. Since we did not know the pK_a value of β-alanine under these conditions, we used the strong counterion sodium, in order to make a proper calculation of the ionic strength of the BGE. Sodium however migrates faster than allyl-TER. For this reason, the rear boundary between BGE with and without chiral selector, will not have self-sharpening properties.

Table 4
Influence of MeOH on the pK_a values and degree of dissolution (α) for mandelic and tropic acid

	Mandelic acid		Tropic acid	
BGE	pK_a	α	pK_a	α
0% MeOH, pH 4.2	3.3	0.89	4.0	0.60
50% MeOH, pH* 5.3	4.2	0.90	5.1	0.62

The addition of MeOH to the BGE caused a shift in the apparent pH of the buffer. At the same time the pK_a value of the analytes was shifted in the same direction. The change in the degree of dissociation (α) was calculated for mandelic acid and for tropic acid by the determination of the pK_a values in BGEs with and without MeOH. As shown in Table 4, no significant change of the α value of these two analytes was observed between BGEs with and without MeOH.

Fig. 6 shows the influence of MeOH on the chiral separation of the racemic analytes. First, as expected, a longer migration time was observed in BGEs containing MeOH.

More interesting, an increase in resolution is observed for some of the racemic analytes. The

Table 3
K_c values and enantioseparation factors (SF) of 1-allylterguride towards some racemic organic analytes. Symbols as in Fig. 3

	100% Water, pH 4.2			50% MeOH, pH* 5.3		
	K_{c1}	K_{c2}	SF (K_{c1}/K_{c2})	K_{c1}	K_{c2}	SF (K_{c1}/K_{c2})
MA	5.5	6.3	1.15	11.2	13.2	1.18
MPA	7.3	8.7	1.19	10.6	13.2	1.25
HM	7.8	8.9	1.14	12.1	13.2	1.09
VM	12.0	14.9	1.24	13.5	14.9	1.10
TA	14.1	14.3	1.01	6.7	7.2	1.07

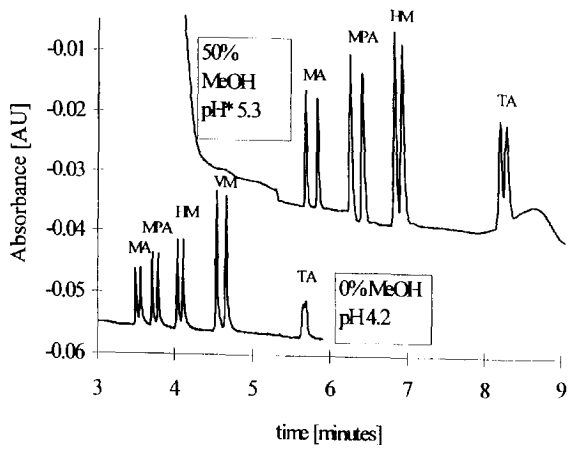

Fig. 6. Influence of MeOH on the chiral separation of some racemic organic acids. BGE bottom electropherogram: 25 m*M* 1-allylterguride + β-alanine–acetate, pH 4.2, $I = 50$ m*M*. BGE top electropherogram: 25 m*M* 1-allylterguride + sodium–acetate, pH* 5.3, $I = 50$ m*M*. Symbols and experimental conditions as in Fig. 3.

increased resolution is very pronounced for the tropic acid enantiomers. As mentioned earlier, this effect could not be attributed to a change in α value of this analyte. Therefore, we can conclude that MeOH can have a positive influence on the enantioselectivity of allyl-TER towards some racemic organic acids. This is also shown in Table 3. The SF is increased for mandelic acid, α-methylphenylacetic acid, and for tropic acid. On the other hand, a decrease of the K_c value is observed for *p*-hydroxymandelic acid and for vanilmandelic acid. At the same time however, the addition of MeOH to the BGE caused an increase in the K_c value for all analytes, except for tropic acid. According to the model of Wren and Rowe [19,20] an increased K_c value results in a lower optimum concentration of the chiral selector. This is of course beneficial for the separation of the racemic samples, since solubility of the chiral selector is a limiting factor under these circumstances. Another main advantage of MeOH is the increased solubility of the ergot alkaloid. Fig. 7 shows a high-resolution separation of some racemic organic acids using a BGE supported with 62.5 m*M* allyl-TER at pH* 5.5. This was the maximum pH at which the chiral selector was still soluble, under these conditions. Using 100% water as solvent, it was not possible to dissolve such a high concentration of this ergot alkaloid.

4. Conclusions

It is shown that ergot alkaloids have great potential as chiral selectors in CE. Baseline resolutions, are obtained for all the racemic acidic test compounds used in this study. The analysis times are short and the method is relatively cheap due to the low consumption of the chiral selector. Results have shown that a high degree of dissociation of the analytes is beneficial for the formation of diastereomeric complexes.

Solubility of allyl-TER in 100% water is limited,

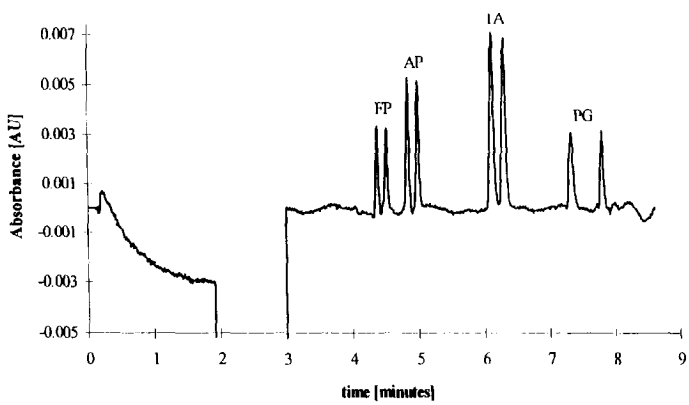

Fig. 7. Chiral separation of racemic N-formylphenylalanine (FP), N-acetylphenylalanine (AP), tropic acid (TA) and 2-phenylglycine (PG). BGE: 100 m*M* β-alanine–acetate, 62.5 m*M* 1-allylterguride, 50% MeOH, pH* 5.5. PVA coated capillary: 27 cm (20 cm)×50 μm I.D.. Voltage: 25 kV.

but can be increased by the addition of 50% MeOH to the BGE. The addition of MeOH also alters the stereoselectivity and the degree of complex formation.

Acknowledgments

Dr. J.C. Reijenga (Eindhoven University of Technology, Netherlands) is greatly appreciated for valuable discussion.

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