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Separation of the four stereoisomers of Z11556A, intermediate in the synthesis of triazole antimycotics, by capillary electrophoresis

Pierfrancesco Castelnovo*, Carlo Albanesi

Medicinal Chemistry Analytical Laboratory, Zambon Group, Bresso (Milan), Italy

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Abstract

Z11556A, a key intermediate in the synthesis of triazole antimycotics, was resolved into its four stereoisomers by high-performance capillary electrophoresis. It migrates retarded with respect to the electrosmotic flow since it is negatively charged at mild alkaline pH. The selectivity of a number of native and derivatized cyclodextrins was screened and β-cyclodextrin turned out to be the only effective. An extensive optimization of the cyclodextrin concentration, of the type and amount of the organic modifier, of the buffer pH and molarity, and of the applied voltage led to baseline separations (resolution factors from 2.0–3.0) among all of the four stereoisomers. The reproducibility of the assay was also evaluated.

Keywords: Enantiomer separations; Z11556A; Triazole antimycotics

1. Introduction

Z11556A is a key intermediate in the synthesis of triazole compounds having fungicidal activity. Since it has two asymmetric carbon atoms in its structure (Fig. 1), four stereoisomers can exist. A reliable and accurate assay is therefore necessary to monitor the separation processes used to isolate the stereoisomer of interest.

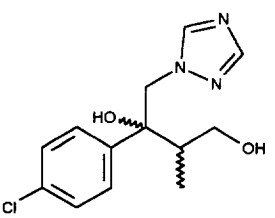

Fig. 1. Structure of Z11556A.

High-performance capillary electrophoresis (HPCE) is becoming a more and more popular technique for the separation of stereoisomers due to its very high separation efficiency and versatility. Some reviews have appeared in the past few years that provide overviews of the techniques and the applications available in the field of the enantioselective separations using capillary electrophoresis [1–4], the most recent and comprehensive being the excellent paper by Nishi and Terabe.

In particular, the resolution of the enantiomers of triazole and imidazole antifungal agents was recently reported. Furuta et al. employed cyclodextrin-modified micellar electrokinetic chromatography for the enantiomeric separation of diniconazole and uniconazole [5], which were previously separated also by chiral high-performance liquid chromatography (HPLC) [6,7], while Gooddall and co-workers

*Corresponding author.

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studied the separation and the binding of tioconazole to cyclodextrins [8,9] and Blaschke et al. the resolution of a number of imidazole derivatives [10].

The present paper describes the development of a high-performance capillary electrophoresis assay for the separation of the four stereoisomers of Z11556A using a cyclodextrin as chiral selector and an organic modifier to improve resolution. An alkaline pH of the background electrolyte was required to promote ionization. The type of cyclodextrin and of the organic modifier turned out to be the most critical parameters. A careful optimization of the pH and molarity of the running buffer and of the applied voltage were also required in order to obtain maximum resolution and a low detection limit.

This assay is, to our knowledge, the first example of the separation by capillary electrophoresis of all the stereoisomers of a compound with two chiral carbon atoms.

2. Experimental

2.1. Chemicals

α-, β- and γ-Cyclodextrin were from Fluka (Buchs, Switzerland). Heptakis(2,6-di-O-methyl)-β-cyclodextrin and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin were from Sigma (St. Louis, MO, USA). Hydroxyethyl-β-cyclodextrin, hydroxypropyl-α-cyclodextrin, hydroxypropyl-β-cyclodextrin, and hydroxypropyl-γ-cyclodextrin were from Aldrich (Steiheim, Germany). Sodium tetraborate decahydrate was from Carlo Erba (Milan, Italy). All solutions were prepared in Milli-Q water (Millipore, Bedford, MA, USA), filtered through a 0.22-μm cellulose filter from Hewlett-Packard (Cernusco S/N, Italy) and degassed by sonication.

2.2. Instrumentation

A Beckman P/ACE 2100 capillary electrophoresis system equipped with a filter UV detector set at 214 nm was used. Separations were performed in an unmodified silica capillary (57 cm×75 μm I.D., 50 cm to detector) mounted on a liquid-cooled cartridge, from Beckman. Data acquisition and processing was performed using the Beckman System Gold software installed on Hewlett-Packard Vectra QS/20 personal computer.

2.3. Running conditions

The samples were introduced hydrodynamically at the anodic site for 1 s. Separations were carried out at 25°C using the constant voltage mode.

Running buffer was prepared by dissolving sodium tetraborate (25–100 mM) in water, adjusting the pH to 9.0 with hydrochloric acid if necessary, adding urea (2 M) and the desired amount (2.5–30 mM) of cyclodextrin and finally the organic modifier. The sample solutions were prepared in sodium tetraborate at a concentration of approx. 0.5 mg/ml.

Conditioning for each experiment was done by rinsing the capillary with running buffer for 2 min while the daily conditioning before the beginning of a set of experiments was with 0.1 M sodium hydroxide for 2 min.

2.4. Calculation of the resolution parameters

The resolution parameters were calculated as follows:

$$\text{separation factor } (\alpha) = MT_i/MT_{i-1} \quad (1)$$

$$\text{resolution factor } (R_s) = 2(MT_i - MT_{i-1})/(W_i + W_{i-1}) \quad (2)$$

where MT_{i-1}, MT_i, W_i and W_{i-1} are the migration times and the bandwidths at baseline of the chromatographic peaks. The suffixes $i-1$ and i refer to the first and the last eluting stereoisomer of each adjacent pair.

2.5. Nomenclature

The individual stereoisomers of Z11556A were identified by a two-character code: a letter (A or B) that identifies the two diastereoisomeric pairs, and a digit (1 or 2) that identifies the two enantiomers of a diastereoisomer. Thus, for example, A1 is the first eluting enantiomer of diastereoisomer A and B2 is the late eluting enantiomer of diastereoisomer B.

3. Results and discussion

3.1. Migration properties

As expected from its chemical structure, Z11556A is not charged in the acidic pH range and hence migrates with the velocity of the electroendosmotic flow (EOF). At pH 7.0 Z11556A is slightly retarded from mesityl oxide, used as marker of the EOF, showing an effective mobility of -0.016×10^{-4} $cm^2/V\,s$. Raising the pH, the migration time of Z11556A continues to decrease and separation of the diastereoisomers eventually occurred. At pH 9.0 baseline separation ($R_s=2.4$) between the two diastereoisomers takes place, their effective mobilities and the EOF being -0.123×10^{-4}, -0.204×10^{-4} and 4.60×10^{-4} $cm^2/V\,s$ respectively. Thus Z11556A shows an electrophoretic mobility towards the anode at neutral and alkaline pH, whose origin is unclear.

3.2. Effect of the type of cyclodextrins on chiral recognition

The effect of the addition of a number of native and derivatized cyclodextrins on the separation of the four stereoisomers of Z11556A, with borate buffer pH 9.0 as background electrolyte, is shown in Table 1. Methanol was also added since preliminary experiments showed that the addition of an organic modifier was necessary for the enantioseparation. Chiral recognition was dependent on both the size of the cavity and the lipophilicity of the rim substituents on the cyclodextrin. In fact, among the native cyclodextrins, only the β type could effectively resolve the two diastereoisomers into their enantiomers and neither its polar hydroxyalkylated nor its apolar methylated derivatives shared this property, the apolar methylated cyclodextrins being even unable to separate the diastereoisomers.

3.3. Effect of β-cyclodextrin concentration on the resolution

The next step to maximize resolution after the choice of the most suitable cyclodextrin is to optimize its concentration. The effect of the amount of the chiral selector on resolution was studied by Wren and Rowe [12–14], who developed a mathematical model which predicted that no single CD concentration will be the best for all separations and that optimum CD concentration is different from analyte to analyte depending on the equilibrium constants of the complexation between the individual enantiomers and the CD.

An increase of β-CD concentration from 2.5 to 30 m*M* (see Table 2) only slightly influenced the migration time of the four stereoisomers, but had a profound and different effect on their resolutions. This behaviour is rather different from that showed by cationic analytes under acidic conditions where the EOF is strongly reduced. In fact, in a work on the

Table 1
Migration times of the four stereoisomers of Z11556A using different native and derivatized cyclodextrins

Cyclodextrin type	Migration time (min)			
	A1	A2	B1	B2
α	9.06		9.16	
Hydroxypropyl-α		8.80		
β[a]	9.36	9.88	9.49	10.03
di-*O*-Methyl-β		8.73		
tri-*O*-Methyl-β		9.10		
Hydroxyethyl-β		9.04		9.21
Hydroxypropyl-β	10.19		10.40	
γ	9.48		9.56	9.89
Hydroxypropyl-γ		9.46		9.56

Electrolyte: 50 m*M* borate buffer (pH 9.0) with 5% methanol; [CD] = 30 m*M*. Applied potential = 15 kV. See Section 2 for the other running conditions.

[a] 2 *M* urea added.

Table 2
Resolution parameters of the HPCE separation of the four stereoisomers of Z11556A at increasing β-cyclodextrin concentration

[β-CD] (m*M*)	A1/B1			B1/A2			A2/B2		
	R_s	α	*MT*	R_s	α	*MT*	R_s	α	*MT*
2.5	1.90	1.016	8.29	0.00	1.000	8.42	1.85	1.034	8.42
5.0	2.10	1.016	8.45	1.56	1.018	8.58	1.79	1.023	8.74
10	2.10	1.015	8.68	2.20	1.031	8.81	1.74	1.019	9.08
20	1.20	1.015	9.12	2.82	1.037	9.26	1.35	1.018	9.60
30	0.85	1.011	9.37	3.60	1.040	9.48	1.36	1.015	9.86

Electrolyte: 50 m*M* borate buffer (pH 9.0) with 5% methanol and 2 *M* urea added, applied potential = 15 kV. See Section 2 for the other running conditions. R_s = resolution factor, α = separation factor and *MT* = migration time (min) of the first eluting stereoisomer.

enantioseparation of aminotetralins [15], we found that a tenfold increase of hydroxyethyl- and hydroxypropyl-β-CD concentration in the range 10–200 m*M* shifted the migration time of 5,6- and 6,7-dihydroxy and dimethoxy-2-aminotetralins from about 20 to 55–65 min. This was due to the difference in the electrophoretic mobilities between the free and the CD-complexed analyte. In the case of Z11556A, both the free analyte and its β-CD complex migrate towards the anode, the former with a higher mobility because complexation increases the its size without altering the charge. Therefore, an increase in CD concentration is expected to reduce the migration time to the cathodic site, where detection occurs, due to the increased molar fraction of complexed Z11556. But, since the EOF is an order of magnitude greater than the mobility towards the anode, the effect of CD concentration on migration time is marginal (see Table 2 and Fig. 2) and the slight increase may be ascribed to the reduction of the EOF (from 4.60×10^{-4} to 4.17×10^{-4} $cm^2/V\,s$) caused by the increase in viscosity of the background analyte that occurs when the concentration of β-CD is increased [16] from 0- to 30 m*M* without the addition of the organic modifier.

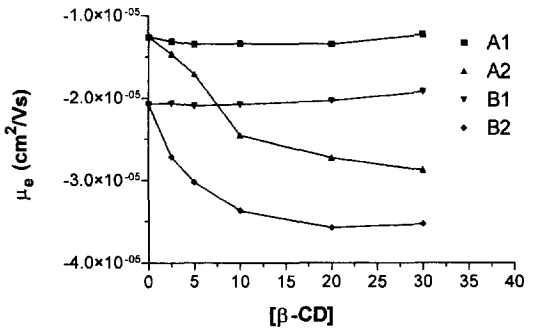

Fig. 2. Influence of β-cyclodextrin concentration on the electrophoretic mobility of the four stereoisomers of Z11556A. Conditions: 50 m*M* borate buffer (pH 9.0) + 2 *M* urea + 5% 2-propanol, voltage = 15 kV. See Section 2 for the other conditions.

The highest resolution between A1 and B1 was reached at 5.0–10 m*M*, while for the B1/A2 and the A2/B2 separations it was above 30 m*M* and below 2.5 m*M*, respectively. The best compromise was found at 10 m*M*, where resolution is ≥2.1 for the first two separations and baseline resolution is still maintained for the separation between the two last eluting stereoisomers.

3.4. Effect of the type of organic modifier on chiral recognition

The addition of an organic solvent to the background electrolyte may have a profound effect on the enantioseparation. An organic modifier is expected to reduce the equilibrium constant between the enantiomer and the chiral selector by increasing the lipophilicity of the bulk buffer [17] or by competing with the analyte in forming an inclusion complex with the CD [5]. Whether this modification of the equilibrium increases or decreases the resolution will depend on whether the amount of cyclodextrin is above or below its optimum concentration [17].

The effect of the addition of a fixed amount (5%) of an organic modifier to the background electrolyte, with β-CD added as chiral selector, is shown in Table 3. This addition slightly increases the migration time and, as stated in the previous paragraph to explain the effect of CD on the migration time, it is likely due to the decrease of the EOF associated with

Table 3
Migration times of the four stereoisomers of Z11556A using β-cyclodextrin with different organic modifier

Organic modifier	Migration time (min)				
	A1	A2		B1	B2
None	7.52	7.72		7.62	7.94
Methanol	8.59	8.85		8.72	9.10
Ethanol	9.46		9.64		10.02
1-Propanol	9.29	9.35		9.48	9.73
2-Propanol	9.19	9.24		9.38	9.63
n-Butanol	9.17	9.19		9.34	9.42
iso-Butanol	9.10	9.15		9.29	9.42
tert.-Butanol	9.32	9.34		9.52	9.58
Acetonitrile	8.00		8.13		8.48
Tetrahydrofurane	7.99	8.04		8.14	8.29

Electrolyte: 50 m*M* borate buffer (pH 9.0) with 2 *M* urea added, [β-cyclodextrin] = 5 m*M*, applied potential = 15 kV, [organic modifier] = 5%. See Section 2 for the other running conditions.

the addition of the organic modifier (from 4.60×10^{-4} to 4.02×10^{-4} $cm^2/V\,s$ when methanol is added). Since the EOF is an order of magnitude greater than the mobility towards the anode, its effect overwhelms any effect on the electrophoretic mobility of Z11556 caused by change in the equilibrium between CD and the analyte.

When no organic modifier is added to the background electrolyte overlapping occurs in the elution order of the enantiomers of the two diastereoisomeric pairs since A2, the late eluting enantiomer of A elutes after B1, the first eluting enantiomer of B, showing low resolution ($R_s = 0.94$) and a bad peak shape when present in low amount (<5%). The effect of the addition of methanol is only a increase of the migration time, with no effect on the migration order which is altered by higher molecular weight alcohols. In fact, using ethanol coelution between B2 and A1 occurs, while with propanols and butanols both the enantiomers of A migrates before the enantiomers of B, all the stereoisomers showing excellent peak shapes even at low amount. The best overall resolutions among the alcohols were obtained with the propanols, 1- and 2-propanol giving the about same results, while among the C_4 alcohols the best results were in the order iso-butanol > *n*-butanol > > *tert.*-butanol. Acetonitrile behaved in the same way as ethanol with coelution between the same stereoisomers, while very good results where obtained with tetrahydofurane.

3.5. Effect of other operating parameters on the resolution using 2-propanol as organic modifier

Following the choice of the cyclodextrin and of the organic modifier, which are the parameters that mostly influences the separation of the four stereoisomers, the effects of other operating parameters such as molarity and pH of the buffer, amount of the organic modifier and applied voltage were investigated for a fine tuning of the resolution. In addition, since the optimization of the resolutions as a function of the amount of β-CD was performed with methanol added to the electrolyte and this addition influences the equilibrium constant between the analyte and the chiral selector, it was repeated with 2-propanol added as organic modifier. The effect of the increasing amount of β-CD on the electrophoretic mobilities shown Fig. 2. As can be seen, it affects the electrophoretic mobility of only one enantiomer of each diestereoisomeric pair, thus enhancing the difference in the electrophoretic mobilities between the enantiomers. It must also be pointed out that above 10 m*M* inversion of the elution of A2 and B1 occurs. Again, we chose 5 m*M* as since it eluted both the enantiomers of diastereoisomer A before those of B still maintaining good resolutions.

Data on resolution, separation factor and migration time are summarized in Table 4. As can be seen, resolution is indeed greatly affected also by buffer concentration. Lowering molarity from 50 m*M* to 25 m*M* caused a complete loss of resolution for the first two separations, while raising it to 100 m*M* greatly improved the resolution of the first separation with little effect on the other two. This is explained by the effect of buffer concentration on the zeta potential of the capillary inner surface, and hence on the electro-osmotic flow. At low buffer concentration the electro-osmotic flow may be too rapid, resulting in elution of analyte before separation has occurred and, clearly, the analytes with lower mobility are mostly influenced. The use of concentration buffer higher than 100 m*M*, however, is constrained by the higher current generated which causes baseline instability and band broadening due to Joule effect.

The effect of 2-propanol concentration on resolution is different for the three separations. The separation of the enantiomer of diastereoisomer A shows a maximum at 5%, while the separation

Table 4
Effects of operating conditions on the resolution parameters of the HPCE separation of the four stereoisomers of Z11556A with β-cyclodextrin using 2-propanol (i-PrOH) as organic modifier

Buffer		i-PrOH	Voltage	A1/A2			A2/B1			B1/B2		
m*M*	pH	(%, v/v)	(kV)	R_s	α	*MT*	R_s	α	*MT*	R_s	α	*MT*
15	9.0	5.0	15	0.00	1.000	7.38	0.00	1.000	7.38	1.40	1.024	7.38
50	9.0	5.0	15	0.94	1.006	9.04	1.71	1.014	9.09	2.51	1.029	9.21
100	9.0	5.0	15	2.48	1.016	11.98	1.85	1.015	12.17	2.43	1.030	12.35
100	9.0	2.5	15	2.24	1.015	10.88	1.35	1.012	11.04	2.98	1.037	11.17
100	9.0	5.0	15	2.48	1.016	11.98	1.85	1.015	12.17	2.43	1.030	12.35
100	9.0	7.5	15	2.32	1.016	13.55	2.33	1.018	13.77	2.45	1.026	14.02
100	9.0	7.5	10	2.35	1.017	22.04	2.35	1.019	22.41	2.57	1.028	22.83
100	9.0	7.5	15	2.22	1.015	13.20	2.08	1.017	13.40	2.18	1.025	13.63
100	9.0	7.5	20	2.01	1.014	8.77	1.92	1.016	8.89	2.04	1.022	9.04

Electrolyte: borate buffer with 2 *M* urea added, [β-cyclodextrin] = 5 m*M*. See Section 2 for the other running conditions. R_s = resolution factor, α = separation factor and *MT* = migration time (min) of the first eluting stereoisomer.

between the second eluting enantiomer of A and the first eluting enantiomer of B increases, and the separation of the enantiomers of diastereoisomer B decreases as 2-propanol is increased from 2.5 to 7.5%. The best compromise was found at 7.5% where resolution was above 2.0 for all the three separations.

The effect of decreasing the applied voltage is a gain in resolution at the expense of the analysis time. We chose 15 kV because it gave the best compromise in terms of resolution (above 2.0 for all the three separations) and analysis time (below 15 min). An electropherogram of a sample of Z11556A showing the separation of the four stereoisomers is reported in Fig. 3.

3.6. Effect of other operating parameters on the resolution using tetrahydrofurane as organic modifier

Since the work on the choice of the best organic modifier showed that, in addition to 2-propanol, also tetrahydrofurane gave good results the optimization described in the previous paragraph was repeated for tetrahydrofurane. The results are shown in Table 5.

Both the enantioresolutions A1/A2 and B1/B2 are improved as the amount of tetrahydrofurane is decreased form 10 to 2.5%, while the resolution between A2 and B1 is only slightly reduced. We also found that if borate was used at its natural pH (9.3), an overall increase of resolution occurs and therefore

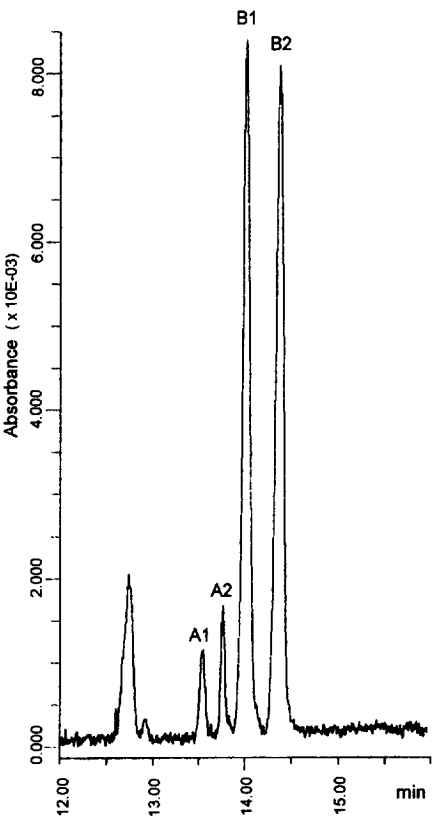

Fig. 3. Separation of the four stereoisomers of Z11556A using 2-propanol as an organic modifier. Conditions: 100 m*M* borate buffer (pH 9.0) + 5 m*M* β-cyclodextrin + 2 *M* urea + 7.5 % 2-propanol, voltage = 15 kV. See Section 2 for the other conditions.

Table 5
Effects of operating conditions on the resolution parameters of the HPCE separation of the four stereoisomers of Z11556A with β-cyclodextrin using tetrahydrofurane (THF) as organic modifier

THF (%)	Buffer		Voltage (kV)	A1/A2			A2/B1			B1/B2		
	pH	m*M*		R_s	α	*MT*	R_s	α	*MT*	R_s	α	*MT*
2.5	9.0	50	15	1.11	1.006	8.16	2.19	1.014	8.21	1.50	1.017	8.46
5.0	9.0	50	15	0.85	1.005	8.79	2.24	1.016	8.84	1.07	1.011	8.98
5.0	9.3	50	15	0.72	1.004	9.16	3.28	1.024	9.2	1.20	1.012	9.42
10.0	9.0	50	15	0.10	1.002	9.46	2.40	1.018	9.48	0.83	1.008	9.65
2.5	9.3	25	15	0.00	1.000	6.66	1.10	1.015	6.66	1.20	1.015	6.86
2.5	9.3	50	15	0.84	1.005	8.25	2.94	1.020	8.29	1.81	1.018	8.46
2.5	9.3	100	15	1.97	1.014	11.10	3.70	1.029	11.26	1.89	1.020	11.58
2.5	9.3	100	5	1.29	1.016	39.22	2.38	1.029	39.84	2.02	1.026	41.01
2.5	9.3	100	10	2.06	1.015	19.11	4.03	1.032	19.41	2.06	1.023	20.03
2.5	9.3	100	15	1.97	1.014	11.10	3.70	1.029	11.25	1.89	1.020	11.58
2.5	9.3	100	20	1.79	1.014	7.24	3.20	1.026	7.35	1.74	1.018	7.54

Electrolyte: borate buffer with 2 *M* urea added, [β-cyclodextrin] = 5 m*M*. See Section 2 for the other running conditions. R_s = resolution factor, α = separation factor and MT = migration time (min) of the first eluting stereoisomer.

a concentration of 2.5% of tetrahydrofurane and pH 9.3 were chosen for the rest of the optimization. Interestingly, the same pH gave excellent resolution for the enantioseparations A1/A2 and B1/B2 but caused a complete loss of the resolution between A2/ and B1 with 2-propanol as organic modifier. As seen above for 2-propanol, higher buffer concentrations greatly improved all the separations while again an applied voltage of 15 kV gave the best compromise in terms of resolution and analysis time. The separation of the four stereoisomers of Z11556A recorded using these optimized conditions is shown in Fig. 4.

3.7. Reproducibility data

The assay was evaluated in terms of reproducibility of migration time, quantitation (corrected area %) and efficiency of the electrophoretic peaks, and of the three separations. We employed the set of conditions optimized using tetrahydrofurane as organic modifier and the results are summarized in Table 6. The assay features an excellent reproducibility (<1%) of the migration times as well as good data for a quantitative determination of the relative amount of the individual stereoisomers. Peak efficiency was always in excess of 250 000 theoretical plates/m, the higher values obtained for the two early eluting peaks being due to their lower amount, as previously demonstrated [15,18]. It must also be

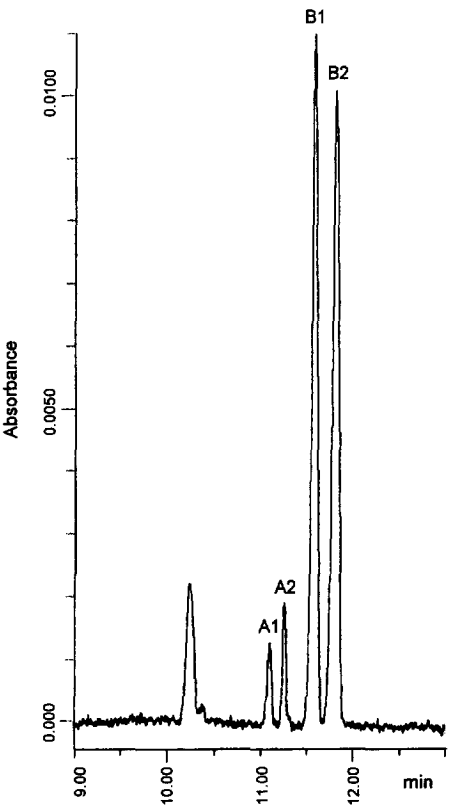

Fig. 4. Separation of the four stereoisomers of Z11556A using tetrahydrofurane as an organic modifier. Conditions: 100 m*M* borate buffer (pH 9.3) + 5 m*M* β-cyclodextrin + 2 *M* urea + 2.5% tetrahydrofurane, voltage = 15 kV. See Section 2 for the other conditions.

Table 6
Validation data ($n=6$) for the separation of the four stereoisomers of Z11556A

Stereoisomer	Migration time (min)		Peak area (%)		Peak efficiency (theor. plates/m)$\times 10^{-3}$		Resolution	
	Mean±S.D.	C.V. (%)	Mean±S.D.	C.V. (%)	Mean±S.D.	C.V. (%)	Mean±S.D.	C.V. (%)
A1	10.38±0.08	0.76	4.86±0.21	4.3	466±34	7.4		
A2	10.53±0.07	0.69	5.20±0.09	1.7	1060±168	14.5	2.19±0.18	8.1
B1	10.80±0.09	0.81	44.42±0.26	0.58	322±14	4.5	3.33±0.08	2.3
B2	11.03±0.08	0.69	45.53±0.08	0.17	256±6	5.0	2.02±0.18	9.0

Conditions: 100 m*M* borate buffer (pH 9.3)+5 m*M* β-cyclodextrin+2 m*M* urea+2.5% tetrahydrofurane, voltage=15 kV. See Section 2 for the other conditions.

highlighted that the resolutions turned out to be reproducible not only in this intra-assay evaluation, but also with time since we did not experienced neither the decrease in the resolutions nor the increase of the EOF found by Furuta [5].

References

[1] M.M. Rogan, K.D. Altria and D.M. Goodall, Chirality, 6 (1994) 25.
[2] M. Novotny, H. Soini and M. Stefansson, Anal. Chem., 66 (1994) 646A.
[3] T. Ward, Anal. Chem., 66 (1994) 633A.
[4] H. Nishi and S. Terabe, J. Chromatogr. A, 694 (1995) 245.
[5] R. Furuta and T. Doi, J. Chromatogr. A, 676 (1994) 431.
[6] R. Furuta and H. Nakazawa, J. Chromatogr., 625 (1992) 231.
[7] R. Furuta and H. Nakazawa, Chromatographia, 35 (1993) 555.
[8] S.G. Penn, D.M. Goodall and J.S. Loran, J. Chromatogr., 636 (1993) 149.
[9] S.G. Penn, E.T. Bergstrom, D.M. Goodall and J.S. Loran, Anal. Chem., 66 (1994) 2866.
[10] B. Chankvetadze, G. Endresz and G. Blaschke, J. Chromatogr. A, 700 (1995) 43.
[12] S.A.C. Wren and R.C. Rowe J. Chromatogr., 603 (1992) 235.
[13] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 635 (1993) 113.
[14] S.A.C. Wren, J. Chromatogr., 636 (1993) 57.
[15] P. Castelnovo and C. Albanesi, Chirality, 7 (1995) 459.
[16] Ph. Baumy, Ph. Morin, M. Dreux, M.C. Viaud, S. Boye and G. Guillaumet, J. Chromatogr., 707 (1995) 311.
[17] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 609 (1992) 363.
[18] E.C. Rickard and R.J. Bopp, J. Chromatogr. A, 680 (1994) 609.