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Journal of Chromatography A, 676 (1994) 431–436

JOURNAL OF CHROMATOGRAPHY A

Enantiomeric separation of diniconazole and uniconazole by cyclodextrin-modified micellar electrokinetic chromatography

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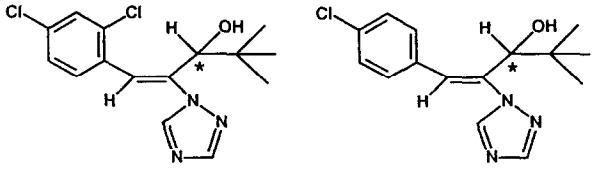
First received 30 December 1993; revised manuscript received 11 March 1994

Abstract

Enantiomeric separation of the structural analogues diniconazole and uniconazole by cyclodextrin (CD)-modified micellar electrokinetic chromatography was studied. The effects of the type of CDs, CD concentration and other operating parameters on the resolution of the enantiomers were clarified and the addition of an organic modifier to the separation solution was demonstrated to improve significantly the enantiomeric separation of both compounds. Enantiomeric separation of uniconazole was, in fact, achieved only with the addition of organic modifiers.

1. Introduction

Diniconazole and uniconazole (Fig. 1), which are vinyl triazoles, have fungicidal and plant growth-regulating activities. They each have an asymmetric carbon, and their enantiomers are known to differ significantly in their biological properties. In both instances, the *R*-enantiomer demonstrates stronger fungicidal activity than the *S*-enantiomer, whereas the *S*-enantiomer is more active than the *R*-enantiomer with regard to plant growth-regulating activity. In addition, uniconazole has a higher plant growth-regulating activity than diniconazole but is less active as a fungicide. Consequently, diniconazole-M, containing a high proportion of the *R*-enantiomer, and uniconazole-P, containing a high proportion of the *S*-enantiomer, have been developed as a high-activity fungicide and an effective plant growth regulator, respectively [1,2]. Reliable and efficient methods for separating the enantiomers are therefore necessary and we reported previously a reversed-phase liquid chromatographic approach using cyclodextrin (CD)-bonded columns and discussed the possible mechanisms of chiral recognition [3,4].



Diniconazole Uniconazole

Fig. 1. Structures of diniconazole and uniconazole.

Micellar electrokinetic chromatography (MEKC) is a high-resolution separation method [5–7] that is somewhat similar to reversed-phase chromatography. Solutes are separated due to differences in their hydrophobic interaction with

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0021-9673/94/$07.00
SSDI 0021-9673(94)00257-A

the stationary phase bonded to the surfaces of packing materials. Separation in MEKC results from the distribution of solutes between micelles, which act as a pseudo-stationary phase, and the aqueous phase in the presence of electroosmotic flow [8–10]. For enantiomeric separation in MEKC, CDs have been successfully used as chiral additives [11,12], as well as for HPLC.

We also found that the enantiomers of diniconazole and uniconazole can be separated by CD-modified MEKC (CD-MEKC) and, further, that organic modifiers offer an effective approach to improving the resolution.

2. Experimental

2.1. Apparatus

The MEKC experiments were performed with a P/ACE 2100 capillary electrophoresis system (Beckman, Palo Alto, CA, USA). The capillary cartridge (Beckman) contained a 75-μm I.D. capillary with a total length of 57 cm and 50 cm to the detector.

2.2. Chemicals

Diniconazole-M (S:R = 16:84) and uniconazole-P (S:R = 79:21) were synthesized by Sumitomo Chemical (Osaka, Japan) and used in all experiments as diniconazole and uniconazole, respectively. The α- and β-CDs were purchased from Kanto (Tokyo, Japan), γ-CD from Wako (Osaka, Japan), heptakis (2,6-di-O-methyl)-β-CD (DM-β-CD) from Aldrich (Milwaukee, WI, USA) and heptakis (2,3,6-tri-O-methyl)-β-CD from Sigma (St. Louis, MO, USA). Sudan IV, organic solvents and other reagents were of analytical-reagent grade from Kanto or Wako. Water was processed through an RO/NANOpureII system (Barnstead, Dubuque, IA, USA).

2.3. Procedures

Standard operating conditions, unless stated otherwise in the text or figure legends, were as follows: applied voltage, 15 kV; temperature, 25°C; detection, UV at 254 nm; and sample introduction, 1-s pressure. The separation solution was 100 mM sodium dodecyl sulphate (SDS) and 2 M urea in 100 mM borate buffer (pH 9.0) containing 50 mM γ-CD. Organic modifiers were added to the separation solution, where appropriate. Sample solutions (0.2 mg/ml) were prepared by dissolving each compound in methanol followed by mixing with the separation solution (Sudan IV was added to the aqueous solution in order to measure the migration time of the micelle, where appropriate) in the ratio of 1:4.

3. Results and discussion

3.1. Chiral recognition of CDs

The effect of the type of CDs on the chiral recognition of diniconazole and uniconazole was investigated by using α-, β-, γ-, DM-β- and TM-β-CDs as chiral additives.

Chiral recognition was dependent on the type of CDs, that is, the cavity diameter and lipophilic nature of the external portion of the CD molecule. The enantiomers of diniconazole were separated when γ-CD or DM-β-CD were used and the S-enantiomer eluted first in both instances. Because γ-CD is not solubilized by the micelle and migrates with the same velocity as the electroosmotic flow, the stable inclusion complex formation of the solute with the CD provides a faster migration under experimental conditions where the electroosmotic flow is stronger than the electrophoretic mobility of the micelle [12]. This is also the case for DM-β-CD, although this latter may be somewhat solubilized by the micelle. The findings indicate that the S-enantiomer forms a more stable inclusion complex than the R-enantiomer with γ-CD or DM-β-CD. With uniconazole, separation occurred only with γ-CD and the R-enantiomer was followed by the S-enantiomer (see Table 1). With HPLC using CD-bonded columns (α-, β- and γ-types were used), the enantiomers of diniconazole were separated completely on the β-CD column (retention order R, S) and partly

Table 1
Enantiomeric separation of uniconazole and diniconazole with CDs.

CD	Uniconazole			Diniconazole		
	t_R [a] (min)	t_S [b] (min)	R_s	t_S (min)	t_R (min)	R_s
α	61.72	61.72	0.00	68.45	68.45	0.00
β	43.84	43.84	0.00	54.11	54.11	0.00
γ	30.47	30.70	0.63	38.49	39.33	1.91
DM-β	23.79	23.79	0.00	27.14	27.59	2.21
TM-β	21.00	21.00	0.00	22.42	22.42	0.00

Separation solution, 100 m*M* SDS and 2 *M* urea in 100 m*M* borate buffer (pH 9.0) containing 50 m*M* CD–acetonitrile (95:5, v/v).
[a] t_R = Migration time of the *R*-enantiomer.
[b] t_S = Migration time of the *S*-enantiomer.

separated on the γ-CD column (retention order *S*, *R*), and those of uniconazole were partly separated on the γ-CD column (retention order *S*, *R*) as reported in previous papers [3,4]. The observed differences in enantioselectivity between the two methods could be explained by the presence of an SDS monomer, which can be included in the CD and can influence inclusion complex formation in MEKC [12]. An investigation to clarify these differences is in progress.

γ-CD was used in all subsequent experiments as it was the only form with which all enantiomers were separated.

3.2. Effect of CD concentration

The effects of γ-CD concentration on retention and resolution were examined by varying the concentration in steps from 20 to 70 m*M*. Organic modifiers were not added in this experiment. Fig. 2 shows plots of the capacity factor for the *R*-enantiomers, calculated according to the equation derived by Terabe *et al.* [5], the separation factor and the resolution, against the CD concentrations for diniconazole. The capacity factor decreased with increase in the CD concentration, because a more stable inclusion complex of the solute with the CD is formed at higher CD concentration and it therefore migrates faster, as described in the above section. The separation factor and resolution increased with increase in the CD concentration up to 50 or 60 m*M*. The peaks obtained with 60 and 70 m*M* CD were not symmetrical and 50 m*M* CD was the optimum concentration for the enantiomeric separation of diniconazole. Enantiomeric separation of uniconazole was not achieved at any concentration.

3.3. Effects of other operating parameters

The effects of operating parameters, other than the CD concentration described above, on retention and resolution were investigated to establish the optimum conditions without organic modifiers. The parameters investigated were

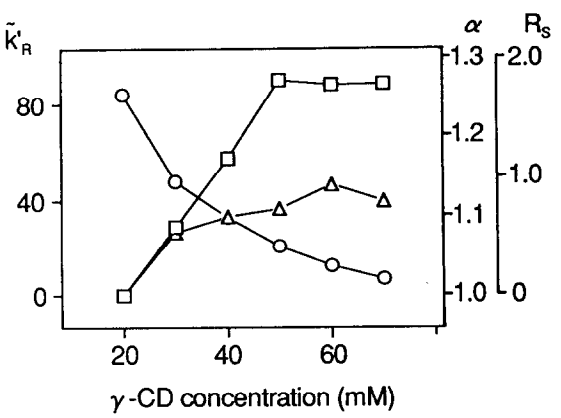

Fig. 2. Effect of γ-CD concentration in the separation solution on the (○) capacity factor, $\tilde{k}'_R$ (capacity factor of the *R*-enantiomers), (△) the separation factor, α, and (□) the resolution, R_s. Separation solution, 100 m*M* SDS and 2 *M* urea in 100 m*M* borate buffer (pH 9.0) containing γ-CD; for other analytical conditions, see the text.

concentration of SDS, urea or borate buffer in the separation solution, pH, temperature and applied voltage.

Data on the capacity factor, separation factor and resolution for diniconazole are summarized in Table 2. None of the parameters significantly influenced the chiral recognition at a constant CD concentration, as shown by the fact that the separation factors hardly changed. The resolutions obtained under the same operating conditions, that is, 100 m*M* SDS, 2 *M* urea, 100 m*M* borate buffer, pH 9.0, 15 kV at 25°C, decreased gradually with time (from 1.71 to 1.42). This is probably due to change in the capillary surface condition caused by, *e.g.*, corrosion by alkaline solution, because it is accompanied by a decrease in the migration time of methanol, which shows electroosmotic flow. A different capillary was used when the effect of the applied voltage was studied and less resolution was obtained. These phenomena represent problems which require further investigation to improve reproducibility.

As optimum conditions, 100 m*M* SDS, 2 *M* urea, 100 m*M* borate buffer and pH 9.0 were chosen because these gave the best resolution. With regard to temperature and voltage, 25°C and 15 kV were used in further experiments because of the appropriate analysis time and ease of control, although they did not provide the best resolution. Uniconazole could not be optically resolved under any of the conditions.

3.4. Effects of organic modifiers

It has been reported that adding an organic solvent to the separation solution is effective for

Table 2
Effects of operating parameters on the enantiomeric separation of diniconazole

Separation solution[a]				Applied voltage (kV)	Temperature (°C)	$\tilde{k}'_R$[b]	α	R_s
SDS (m*M*)	Urea (*M*)	Buffer (m*M*)	pH					
70	2	100	9.0	15	25	4.7	1.08	1.40
100	2	100	9.0	15	25	20.1	1.12	1.70
100	0	100	9.0	15	25	32.0	1.14	1.29
100	2	100	9.0	15	25	20.9	1.12	1.71
100	4	100	9.0	15	25	17.0	1.09	1.70
100	2	50	9.0	15	25	25.1	1.11	1.08
100	2	100	9.0	15	25	20.8	1.11	1.49
100	2	100	8.0	15	25	24.0	1.11	1.25
100	2	100	8.5	15	25	24.4	1.11	1.20
100	2	100	9.0	15	25	20.8	1.11	1.49
100	2	100	9.0	15	22	21.7	1.12	1.60
100	2	100	9.0	15	25	20.9	1.11	1.42
100	2	100	9.0	15	30	19.1	1.10	1.20
100	2	100	9.0	10	25	24.2	1.12	1.13[c]
100	2	100	9.0	15	25	22.7	1.11	1.00[c]
100	2	100	9.0	20	25	21.5	1.10	0.95[c]

[a] Concentration of γ-CD was 50 m*M*.
[b] $\tilde{k}'_R$ = Capacity factor of the *R*-enantiomer.
[c] A different capillary was used in these three experiments.

improving the enantioselectivity for some compounds in MEKC [13–15]. The effects of addition of organic solvents on the enantiomeric separation were therefore investigated under the optimum conditions determined as described above. The organic solvents added were acetonitrile, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol and 2-methyl-2-propanol. The concentrations of each organic modifier tested were 2% and 5%, if miscible.

Table 3 shows the resulting data for migration times and resolutions of the enantiomers of diniconazole and uniconazole. The migration times are given instead of capacity factor values, which may be unreliable when an organic solvent is present because the marker for the micelle, Sudan IV, can distribute between the micelle and the aqueous phase containing the organic solvent [9]. Organic modifiers effectively improved the resolution of both compounds, and in fact the enantiomers of uniconazole were only resolved with separation solutions containing an organic modifier. The higher the content of the organic modifier, the better was the resolution, except with 2-butanol and 2-methyl-2-propanol for diniconazole. Hence there might be an optimum content of organic modifiers for each compound.

The organic modifier added to the separation solution not only increases the hydrophobicity of the solution and influences the distribution of the solute among the micelle, the aqueous phase and the CD, but can also compete with the solute in forming an inclusion complex with the CD. This indicates that the enantioselectivity by CD would decrease when bulkier and more hydrophobic

Table 3
Effects of organic modifiers on the enantiomeric separation of uniconazole and diniconazole

Organic modifier	Content (%)	Uniconazole			Diniconazole		
		t_R [a] (min)	t_S [b] (min)	R_s	t_S (min)	t_R (min)	R_s
None		27.35	27.35	0.00	31.17	31.56	1.24
Acetonitrile	2	27.60	27.60	0.00	32.22	32.69	1.54
	5	30.47	30.70	0.63	38.49	39.33	1.91
Methanol	2	29.23	29.23	0.00	34.50	35.08	1.42
	5	32.54	32.54	0.00	40.06	40.97	1.97
Ethanol	2	31.16	31.16	0.00	36.82	37.47	2.08
	5	34.58	34.87	0.75	43.77	44.73	2.25
1-Propanol	2	29.42	29.51	[c]	35.06	35.78	2.53
	5	31.99	32.47	1.40	41.70	42.96	3.10
2-Propanol	2	29.98	30.07	[c]	35.61	36.28	2.34
	5	32.33	32.78	1.38	41.63	42.68	2.64
1-Butanol	2	26.88	27.22	1.22	32.81	33.62	3.00
2-Butanol	2	28.21	28.43	0.73	33.91	34.69	2.62
	5	27.67	28.23	2.16	36.01	37.07	2.09
2-Methyl-1-propanol	2	28.62	29.03	1.32	35.50	36.57	3.59
2-Methyl-2-propanol	2	28.33	28.53	0.69	33.82	34.51	2.52
	5	29.28	29.99	2.52	38.61	39.62	2.13

[a] t_R = Migration time of the *R*-enantiomer.
[b] t_S = Migration time of the *S*-enantiomer.
[c] First peak eluted as a shoulder.

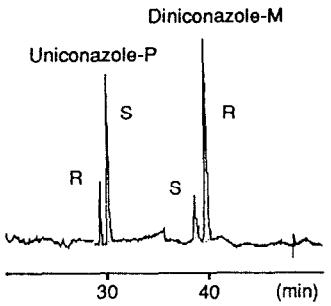

Fig. 3. Enantiomeric separation of diniconazole-M and uniconazole-P. Separation solution, 100 m*M* SDS and 2 *M* urea in 100 m*M* borate buffer (pH 9.0) containing 50 m*M* γ-CD–2-methyl-2-propanol (95:5, v/v); for other analytical conditions, see the text.

organic modifiers are used, because their molecules would be expected to form stable inclusion complexes. However, bulkier and more hydrophobic modifiers in the present experiments gave a better resolution. From this, we can hypothesize that the modifier molecule might be included together with the solute in the CD cavity and play a positive role in filling the space. It has been reported that tight fitting of a molecule to be complexed to a CD cavity is one of the important factors for chiral recognition by CD [16]. A typical chromatogram of uniconazole and diniconazole is shown in Fig. 3.

4. Conclusions

The pairs of enantiomers of both diniconazole and uniconazole can be successfully separated by CD-MEKC. Chiral recognition is dependent on the type of CDs and the CD concentration is the most important operating parameter determining the capacity factor and the enantiomeric separation. Addition of an organic modifier to the separation solution can significantly improve the enantiomeric separation, as shown for both compounds, even in cases where no resolution is observed in their absence.

5. References

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