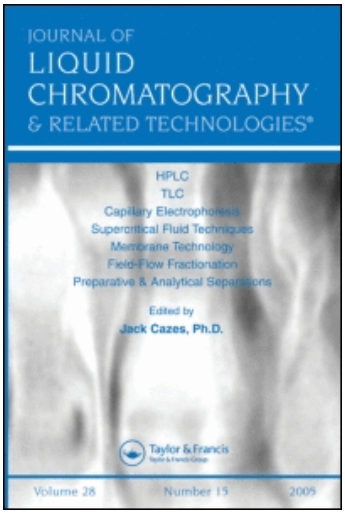

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 02 March 2003

To cite this Article Diagone, C. A. , Ogawa, C. A. and Lanças, F. M.(2003) 'Evaluation of Cyclodextrins as Chiral Selectors in the Separation of Selected Monoterpenes by Capillary Liquid Chromatography and Capillary Electrophoresis', Journal of Liquid Chromatography & Related Technologies, 26: 4, 505 — 516
To link to this Article: DOI: 10.1081/JLC-120017895
URL: http://dx.doi.org/10.1081/JLC-120017895

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JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 4, pp. 505–516, 2003

Evaluation of Cyclodextrins as Chiral Selectors in the Separation of Selected Monoterpenes by Capillary Liquid Chromatography and Capillary Electrophoresis

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ABSTRACT

Two techniques are evaluated in this paper for the separation of chiral monoterpenes commonly present in *citrus*: capillary liquid chromatography (C-LC) and capillary electrophoresis (CE). The utilization of cyclodextrins, as additive in the mobile phase (C-LC), and in the buffer (CE) was verified. It was observed, that the analytes interacted with methyl-β-cyclodextrin (met-β-CD), since the addition of cyclodextrin promoted alteration in the retention and migration times. Separation of stereoisomers of citral was also obtained with both techniques. However, the separation of the optical isomers of carvone, utilizing cyclodextrin in the mobile phase, was not observed. From the obtained results, the possibility of optical isomers and stereoisomers separation by C-LC and CE, using

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DOI: 10.1081/JLC-120017895 1082-6076 (Print); 1520-572X (Online)

cyclodextrins as chiral additive, is discussed. Both techniques show samples and chiral additives economy being a potential tool to be further evaluated as alternative options for routine analysis.

Key Words: Capillary liquid chromatography; Capillary electrophoresis; Monoterpenes; Cyclodextrins; Citrus.

INTRODUCTION

The volatile fraction of *citrus* essential oil is a complex mixture of terpenes, most of which are chiral terpenes with the (+) and (−) enantiomers often having different biological properties. In *citrus* essential oils, the monoterpenes are responsible by flavor and off flavor of the fruits. For some monoterpenes, just one enantiomeric form (D or L) is important to the flavor. Cotroneo[1] related the enantiomeric separation of linalool by GC to verify the authenticity of bergamot oil, using a fused-silica column coated with a mixture of (2,3,6-tri-*O*-methyl)-*β*-cyclodextrin and OV 1701 in a ratio of 30% and 70% by weight, respectively. In recent years, several papers have described the separation and determination of terpenes and monoterpenes mainly by gas chromatography (GC)[2–6] and liquid chromatography (LC).[7–9] Some papers also related the separation of monoterpenes constituents of essential oils by GLC (gas–liquid chromatography)[10] LC-GC[11] and CZE (capillary zone electrophoresis).[12] However, to the best of our knowledge, the analysis of monoterpenes by capillary liquid chromatography (C-LC) (using a fused-silica column with 0.53 mm I.D. or less) and by micellar electrokinetic chromatography (MEKC) have not been related. In this work, C-LC and capillary electrophoresis (CE) will be utilized to separate monoterpenes commonly found in *citrus.* Methyl-*β*-cyclodextrin (met-*β*-CD) is used in the mobile phase for C-LC, and in the buffer for CE (CZE and MEKC), to verify its potential to separate the optically active monoterpenes (Fig. 1).

EXPERIMENTAL

Apparatus

Capillary electrophoresis was performed at room temperature using a Waters Quanta 4000 CE System (MA) equipped with an on-column UV detector. The chromatographic system used a Carlo Erba (Milan, Italy) C-LC instrument designed for isocratic elution operations with packed capillary columns. A Phoenix 20 syringe pump with a capacity of 20 mL was used for mobile phase delivery. The samples were injected through a 60 nL internal

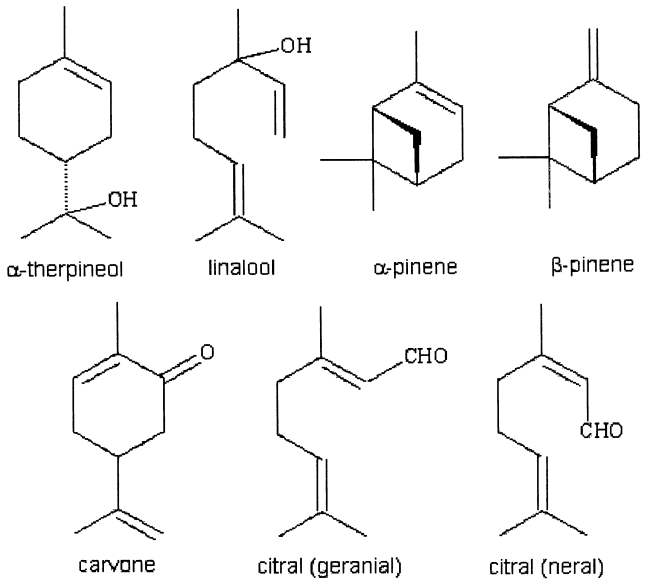

Figure 1. Structures of monoterpenes investigated in this work.

loop valve from Valco Instruments (TX). A programmable variable wavelength UV-Vis detector, Micro UVIS 20 from Carlo Erba (Milan, Italy), equipped with a "U" shape flow cell from LC-Packings (CA), having 35 nL illuminated volume and 8 mm path length, was employed. Data were collected and analyzed with a Chrom Card data acquisition system from Carlo Erba (Milan, Italy). The packed capillary column was made of fused-silica tubing from Siemens, Germany. The column was packed in our laboratory by the slurry-packing technique using C18 (Alltech, IL) as the stationary phase.

Chemicals

Monoterpenes [citral, linalool, α-therpineol, α-pinene, β-pinene, (+) carvone, and (−) carvone] were supplied by local *citrus* industries and dissolved in methanol or acetonitrile (analyte concentration between 25 and 1000 mg/L for CE and between 1 and 1000 mg/mL for C-LC). Cyclodextrins (α-, methyl-β-, and γ-cyclodextrins) were purchased from Sigma (Deisenhofen, Germany). Methanol and acetonitrile were from E. M. Merck (Rio de Janeiro, Brazil). Monobasic potassium phosphate and sodium hydro-

xide were obtained from Reagen (Rio de Janeiro, Brazil). Sodium dodecyl sulfate (SDS) was purchased from Polyscience (IL). Water was purified in a Waters Millipore Milli-Q System (Eschborn, Germany).

Procedures

Separations in CE were performed in 75 μm I.D. fused-silica capillary of 52.5 cm effective length and 60 cm total length using a 20 mM phosphate buffer (pH 3.4, 4.0, 6.0, and 8.1). The capillary was washed with water for 5 min, then, rinsed with 0.5 N NaOH and buffer for 10 min each. The experiments were carried out with applied voltages of 15, 20, and 25 kV and by hydrodynamic injections (between 3 and 10 s). Linalool and α-therpineol were detected at 214 nm. Carvone and citral were detected at 254 nm. It utilized met-β-CD at 5, 15, and 25 mM for pH 4.0 and 5, 12.5, 15, 25, and 40 mM for pH 6.0 in CZE. Concentrations of 0.13 and 10 mM of α-CD were utilized in CZE for pHs 6.0 and 8.1, respectively. In MEKC it utilized 50 mM SDS as surfactant and 20 mM met-β-CD in the buffer at pH 8.1.

The separations in C-LC were performed in a 0.53 mm i.d. fused-silica capillary of 31.5 cm length using acetonitrile–water (80/20, 70/30) and methanol–water (55/45) as mobile phase. The flow rate used was 5 and 7 μL/min. Additions of 25 mM met-β-CD in acetonitrile–water and 10 mM of α-CD in methanol-water in the mobile phases, was done to study the chiral discrimination. Detection was performed by monitoring ultraviolet absorption at 210 nm for the terpenes hydrocarbons (α-pinene, β-pinene); alcohols (α-therpineol, linalool) and 254 nm for the aldehides terpenes citral and carvone.

RESULTS AND DISCUSSION

Capillary Zone Electrophoresis and Micellar Electrokinetic Chromatography

The preliminary study of the applied voltage over the separations showed that 20 kV gives good efficiency with short analysis time. Then, this was selected to be the applied potential to test the other experimental conditions, such as concentration of cyclodextrin. Using phosphate buffer at different pHs, we observed that the addition of met-β-CD does not change, significantly, the migration times of linalool and α-therpineol, and neither promoted separation between the analytes, since we noticed only one peak in the electropherograms (not shown). The migration times obtained at pH 6.0 are presented in Table 1.

Table 1. Data obtained in CZE for linalool and α-therpineol using different concentrations of CD (20 kV, pH 6.0).

Met-β-CD concentration (mM)	Migration time (min)	Efficiency (N/m)
0	4.02	4.92×10^4
5	3.61	7.07×10^4
15	3.56	6.87×10^4
25	3.62	6.24×10^4

A small increasing of the column efficiency using CD as additive, was observed in Table 1. Also, a small trend in decreasing the migration time by increasing the CD concentration up to 15 mM is observed. The fact that the compounds migrated all together, suggest that they are in the neutral form under the used conditions and that they migrated with the electroosmotic flow. At lower pH values the migration times were higher, because of the lower electroosmotic flow (Table 2). Again, increasing the CD concentration will decrease the retention time of the analyte.

The analysis of rac-carvone in CZE using different CDs at different concentrations, show us only one peak, suggesting that this type and concentration of CD are not adequate to separate the optical isomers of carvone. It also indicates that carvone remains in the neutral form under the used conditions. The migration times obtained are given in the Table 3.

The analytes, linalool, α-therpineol, carvone, and citral were separated by MEKC. Linalool and α-therpineol were detected at 214 nm and carvone and citral at 254 nm. The data obtained are shown in Table 4. In the analysis of linalool and α-therpineol, the addition of 2 mM of met-β-CD did not promote alteration of migration times, but by increasing the concentration of met-β-CD a decreasing of their migration times is observed. This indicates that the analytes interact with the met-β-CD by either hydrophobic cavity or by hydrophilic rim, under the tested conditions. Carvone and citral interacted more strongly with met-β-CD than linalool and α-therpineol, since the

Table 2. Data obtained in CZE for linalool and α-therpineol at 20 kV (met-β-CD in phosphate buffer, pH 4.0).

Concentration of met-β-CD (mM)	0	5	15	25
Migration times (min)	22.9	22.7	22.5	18.2

Table 3. Migration times obtained for *rac*-carvone in CZE at 20 kV using CD in the buffer at pH 6.0.

	Concentration of CD in the buffer (mM)				
	0	0.13	5	12.5	25
Met-β-CD	4.16	—	4.79	5.04	4.31
α-CD	4.16	5.81	—	—	—

migration times decreased markedly. Both Figs. 2 and 3 show electropherograms obtained for carvone and citral under different conditions. The migration times obtained in the analysis with met-β-CD are shown in Table 5. There are two migration times for citral because it was possible to separate geranial and neral, two stereoisomers that compose the citral. As shown in Fig. 3, the addition of 20 mM of met-β-CD promoted peak splitting[13] for carvone and citral, which can be due to complexation between analytes and cyclodextrin and micelle, or can be due to degradation of the analytes. Maybe, cyclodextrin would have promoted separation between analytes and impurities present in the sample.

Capillary Liquid Chromatography

Figure 4(a) shows an excellent resolution for the α-pinene and β-pinene isomers, without the need of CD addition to the mobile phase. Under these conditions, there was no separation of linalool and α-therpineol (peak $1 + 2$).

Figure 4(b) shows the chromatogram of linalool, α-therpineol, β-pinene, α-pinene using a chiral selector in the mobile phase. In this case, met-β-CD promoted the separation of linalool and α-therpineol. The addition of met-β-CD in the mobile phase also decreased the retention time owing

Table 4. Data obtained in the analysis of monoterpenes by MEKC (20 mM phosphate buffer, pH 8.1, 50 mM SDS, 20 kV).

Wavelength	α-Therpineol	Linalool	Rac-carvone	Citral	Citral	Efficiency (N/m)
214 nm	9.30	9.51	—	—	—	3.93×10^4
254 nm	—	—	13.47	15.89	17.84	9.55×10^4

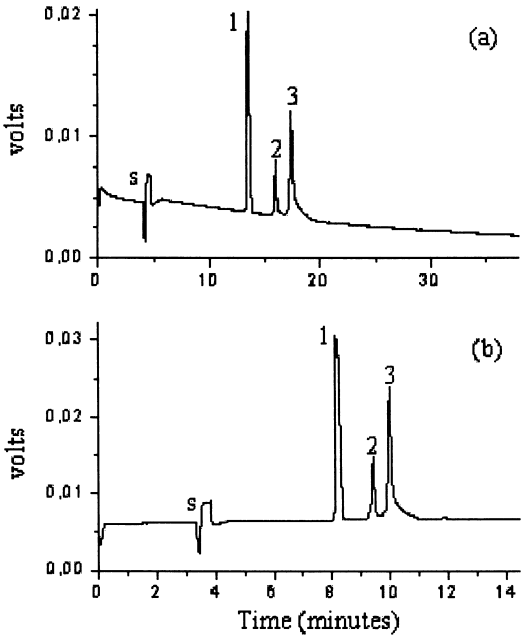

Figure 2. Electropherograms obtained by MEKC at different concentrations of met-β-CD. Applied voltage: 20 kV, 20 mM phosphate buffer pH 8.1, 50 mM SDS. (a) without met-β-CD. (b) 2 mM met-β-CD. (1) rac-carvone; (2) citral (geranial or neral); (3) citral (geranial or neral); (s) solvent.

to complex formation. α-pinene and β-pinene form a stable complex with met-β-CD, as demonstrated by the faster elution.

Chiral recognition between met-β-CD and monoterpenes (α- and β-pinene) was not significant in acetonitrile : water (ACN/H_2O) (80/20) as mobile phase, but it promoted separation of α-therpineol and linalool. As can be seen from the results presented in Fig. 4(b), linalool formed a less stable complex with this chiral selector, which is reflected by the faster elution of this compound.

Optimum experimental conditions were established for separation of aldehides terpenes. The chromatographic parameters of the solutes studied are listed in Table 6.

The addition of the chiral selectors met-β-CD and α-CD to the aqueous-organic mobile phase consisting of a methanol–water (55/45), did not promote the enantioseparation of rac-carvone. However, there were interactions of these analytes, rac-carvone and citral, with the selectors, as

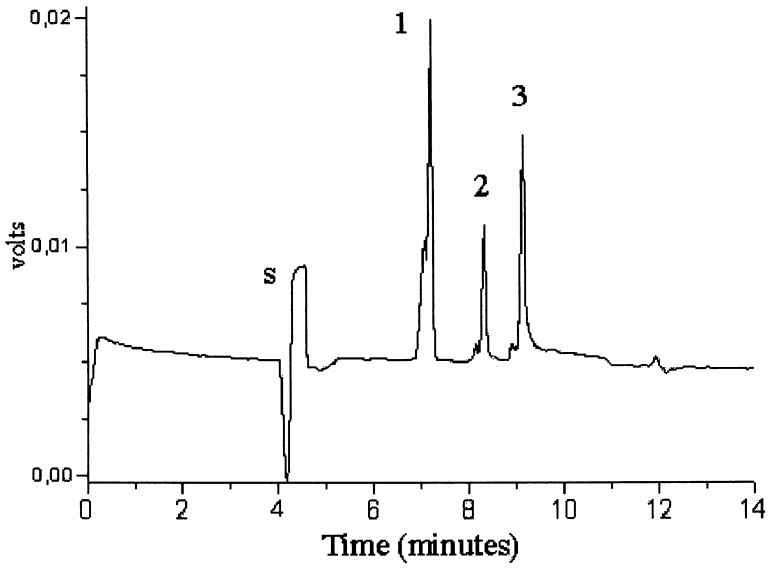

Figure 3. Electropherogram obtained by MEKC at 20 mM met-β-CD. Applied voltage: 20 kV, 20 mM phosphate buffer pH 8.1, 50 mM SDS. (1) rac-carvone; (2) citral (geranial or neral); (3) citral (geranial or neral); (s) solvent.

demonstrated by the faster elution. The chromatograms with and without CDs in mobile phase are shown in Fig. 5.

The retention factor (k) decreased with the addition of the chiral selectors α-CD and met-β-CD (Table 6), showing that the compounds studied produced interaction with the mobile phase. The decreasing of k with the addition of the chiral selectors, together with alteration of the retention time of the compounds, makes the complex formation clear. The decreasing of the retention

Table 5. Migration times of monoterpenes obtained by MEKC after addition of met-β-CD. Phosphate buffer 20 mM, pH 8.1, 50 mM SDS. Applied voltage: 20 kV.

Concentration of met-β-CD (mM)	Detection at 214 nm		Detection at 254 nm		
	α-Therpineol	Linalool	Carvone	Citral	Citral
2	9.23	9.49	8.22	9.39	10.37
20	7.35	7.55	7.20	8.35	9.21

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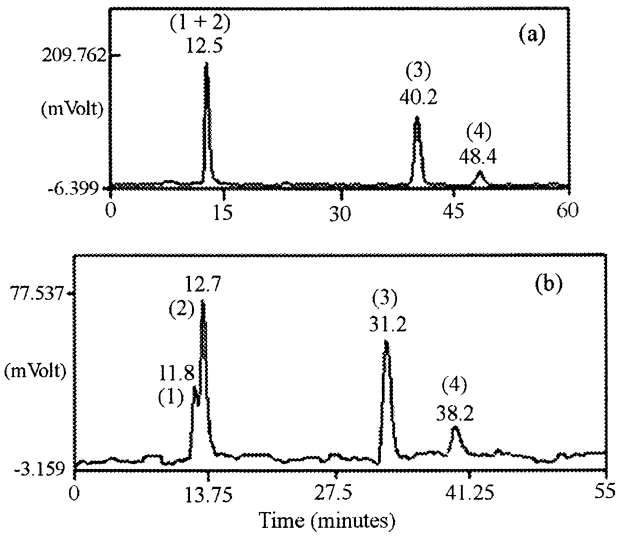

Figure 4. Chromatograms obtained in C-LC; column RP-18 (315 mm × 0.53 mm I.D. × 5 μm); mobile phase, ACN/H_2O (80/20); flow rate: 7 μL/min; $\lambda =$ 210 nm. (a) without CD; (b) with 25 mM of met-β-CD. (1) α-therpineol, (2) linalool, (3) α-pinene, (4) β-pinene.

times of the rac-carvone and citral with met-β-CD suggest a wider complexation than with α-CD.

The separation factor (α) decreased with the addition of α-CD, showing that the analyzed compounds interact with this selector. It was confirmed due to the decrease of retention times. After addition of met-β-CD [Fig. 5(c)], a larger $\alpha_{2/1}$

Table 6. Evaluation of the parameters (separation factor and retention factor) that influence in the separation of analytes using the column RP-18 and utilizing MeOH/H_2O (55/45) as mobile phase.

[CD] (mM)	t_M	t_{R1}	t_{R2}	t_{R3}	$\alpha_{2/1}$	$\alpha_{3/2}$	k_1	k_2	k_3
0	9.56	89.06	125.37	154.41	1.46	1.25	8.32	12.11	15.15
10 (α-CD)	9.42	63.24	84.93	102.52	1.40	1.23	5.71	8.02	9.88
10 (met-β-CD)	8.85	55.23	78.57	89.79	1.50	1.16	5.24	7.88	9.14

Note: t_M, migration of solvent peak (methanol) in minutes; t_{R1}, rac-carvone; t_{R2} and t_{R3}, citral (neral and geranial); ($\alpha_{2/1}$), t_{R2} (stereoisomers of citral) and rac-carvone; $\alpha_{3/2}$, citral (neral and geranial); k_1, rac-carvone; k_2, citral (t_{R2}); k_3, citral (t_{R3}). Column (RP-18): 315 mm (L) × 0.53 mm (I.D.) × 5 μm (p.d.). Flow rate: 5 μL/min.

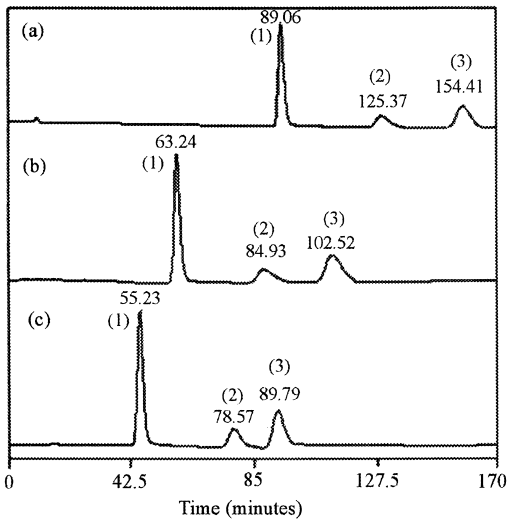

Figure 5. Chromatograms obtained in C-LC, column RP-18 (315 mm × 0.53 mm I.D. × 5 μm), MeOH/H_2O (55/45), flow rate: 5 μL/min, λ: 240 nm. (1) rac-carvone, (2) and (3) citral (neral and geranial). (a) without chiral selector; (b) with 10 mM of α-CD; and (c) with 10 mM of met-β-CD.

value between the rac-carvone and citral was obtained, while, a smaller $\alpha_{3/2}$ value was obtained between neral and geranial stereoisomers. These compounds showed a higher affinity for met-β-CD than rac-carvone. For the aldehydes, smaller values for the resolution (R_S) were obtained after the addition of chiral selectors. In this case, decreasing efficiency was also observed (Table 7).

Table 7. Evaluation of parameters (resolution and efficiency) that influence in the separation of analytes using the column RP-18 and utilizing mobile phase MeOH/H_2O (55/45).

Concentration of CD	$R_{S2/1}$	$R_{S3/2}$	N_1	N_2	N_3
0	12.75	9.07	9.45×10^3	3.61×10^3	4.75×10^3
10 mM (α-CD)	11.56	7.02	8.80×10^3	2.38×10^3	3.15×10^3
10 mM (methyl-β-CD)	10.28	8.33	6.66×10^3	3.63×10^3	4.70×10^3

Note: $R_{S2/1}$, t_{R2} (stereoisomers of citral) and rac-carvone; $R_{S3/2}$, citral (neral and geranial). Value of $R_{S2/1}$ was calculated related N_1 and k_1. Value of $R_{S3/2}$ was calculated related N_3 and k_3. Column (RP-18): 315 mm (L) × 0.53 mm (I.D.) × 5 μm (p.d.). Flow rate: 5 μL/min.

In conclusion, both investigated techniques, MEKC and C-LC, are good alternative for the analysis of monoterpenes, when economy of samples and solvents and/or short analysis time are required.

ACKNOWLEDGMENTS

CNPq and FAPESP for financial support to our laboratory.

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Received September 22, 2002
Accepted October 19, 2002
Manuscript 5971