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METHYLATED CYCLODEXTRINS AS MOBILE PHASE ADDITIVES IN LIQUID CHROMATOGRAPHY

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SUMMARY

Both 2,3,6- and 2,6-methylated derivatives of α- and β-cyclodextrins were added to the mobile phases in liquid chromatography using an octadecylsilyl stationary phase. The 2,3,6-methyl-substituted derivative of α-cyclodextrin improves the separation of the *ortho, meta* and *para* isomers of disubstituted benzenes and also decreases their retention compared with the unmodified α-cyclodextrin. By the addition of 2,3,6-methylated β-cyclodextrin, the three isomers of nitrobenzoic acid can be completely separated, and the separation of *trans*-nitrocinnamic acid is greatly improved. In contrast, 2,6-dimethylation of both α- and β-cyclodextrins did not bring about such significant improvements in the retention of the solutes investigated.

INTRODUCTION

Cyclodextrins and their chemically modified derivatives have been extensively investigated and used for various purposes. Many functional groups have been introduced on to their rim[1]. It is well known that the chemical modification brings about changes in the shape and size of the cyclodextrin cavities, in the hydrogen-bonding ability and in other physical properties. Methylation of cyclodextrins is a case in point. X-ray structural investigations have shown that the host–guest interactions (inclusion geometries) in complexes of 2,3,6-tri-O-methylated cyclodextrins are different from those of unmodified cyclodextrins. These changes are ascribed to the differences in the shape and size of the host cavities[2–4].

In previous papers[5–9], we reported the specific liquid chromatographic separations of some aromatic compounds on unmodified, acylated and methylated cyclodextrin stationary phases prepared by treating succinamidopropyl-silica with monosubstituted amino-cyclodextrin derivatives. The acylation or methylation resulted in selectivity changes in solute retention, as expected.

Unmodified cyclodextrins have been used as mobile phase additives in reversed-phase high-performance liquid chromatography. They have been applied for the resolution of mandelic acid[10] and its derivatives[11] and an analgesic[12] into enantiomers, and for the separation of positional isomers of aromatic compounds[13–16].

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Methylated cyclodextrins dissolve not only in water at room temperature, but also in organic solvents more readily than unmodified cyclodextrins, in addition to the above-mentioned changes in the inclusion. Therefore, the use of methylated cyclodextrins as mobile phase additives is more feasible and of great interest.

In this work, 2,3,6-tri-O-methylated and 2,6-di-O-methylated derivatives of α- and β-cyclodextrins were prepared. Each of these cyclodextrin derivatives was added to the mobile phases on an octadecylsilyl (ODS) stationary phase. The retention behaviour of some aromatic compounds, especially disubstituted benzene derivatives, in these mobile phases was studied and compared with that in the unmodified cyclodextrin mobile phases.

EXPERIMENTAL

Materials

α- and β-cyclodextrins (α- and β-CD) were purchased from Hayashibara Biochemical Labs. (Okayama, Japan) and other chemicals from Wako (Osaka, Japan).

Chromatography

All chromatographic studies were carried out with a system consisting of a Model KHD-W-52 pump, a Type KD-1 pulse damper, a Model KHP-UI-130A injector (Kyowa Seimitsu, Tokyo, Japan) and a Uvilog-7 variable-wavelength UV detector (Oyo Bunko, Tokyo, Japan).

The ODS-packed columns, column A (15 cm × 4 mm I.D.) and column B (15 cm × 6 mm I.D.), were obtained from Gasukuro Kogyo (Tokyo, Japan) and Yamamura Kagaku (Kyoto, Japan), respectively. The flow-rate of mobile phase (methanol–water or methanol–0.01 *M* phosphate buffer) was 1.0 ml/min. The concentration of unmodified and methylated cyclodextrins in mobile phases was $5 \cdot 10^{-3}$ *M* unless specified otherwise. All the mobile phases were filtered through a 0.45-μm membrane filter prior to use. The solutes were dissolved in water, methanol or methanol–water [0.2 m*M*, except for benzene (2 m*M*)], and a volume of 10 μl was injected.

All solutes were detected at 254 nm except for xylenol and aminobenzoic and chlorobenzoic acids, which were detected at 205 nm.

Preparation of methylated cyclodextrin derivatives

Hexakis(2,3,6-tri-O-methyl)-α-cyclodextrin (TM-α-CD), hexakis(2,6-di-O-methyl)-α-cyclodextrin (DM-α-CD), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) were synthesized and purified according to a similar method published by Boger *et al.*[17] and Szejtli *et al.*[18].

Analysis. Calculated for $(C_9H_{16}O_5)_6$ (TM-α-CD), C 52.93, H 7.90; found, C 52.62, H 7.91%. Calculated for $(C_8H_{14}O_5)_6$ (DM-α-CD), C 50.52, H, 7.42; found, C 49.84, H 7.52%. Calculated for $(C_9H_{16}O_5)_7$ (TM-β-CD): C 52.93, H 7.90; found, C 53.01, H 8.05%. Calculated for $(C_8H_{14}O_5)_7$ (DM-β-CD): C 50.52, H 7.42; found, C 50.72, H 7.48%.

RESULTS AND DISCUSSION

Retention behaviour of disubstituted benzene derivatives

Fig. 1 shows the retention times of six disubstituted benzene derivatives on the ODS column in mobile phases containing unmodified or methylated cyclodextrins (open symbols), together with those in mobile phases without the cyclodextrin additives (closed symbols). The addition of α-CD to the mobile phase of methanol–water (30:70 or 40:60) did not bring about considerable changes in the retention of the solutes, except for iodoaniline. The retention of *p*-nitrophenol, which had been fairly strongly retained on the chemically bonded α-CD stationary phase[6], was not affected by the addition of α-CD. On the other hand, the presence of β-CD or TM-α-CD resulted in a decrease in the retention of each solute and an increase in the separation of the *ortho, meta* and *para* isomers (except for that of *o*- and *m*-dinitrobenzene in β-CD). A complete separation of the three cresol isomers could be obtained in the presence of β-CD or TM-α-CD (see Fig. 4). TM-β-CD did not improve the separation of the three isomers, but resulted in their poor resolution for dinitrobenzene and iodoaniline. DM-β-CD was in an intermediate position in retention behaviour between β-CD and TM-β-CD. The addition of DM-α-CD, however, increased the retention of the solutes.

A decrease in retention was observed for all the solutes with increasing methanol:water ratio in the mobile phase from 30:70 to 60:40, regardless of the presence of the cyclodextrin additives.

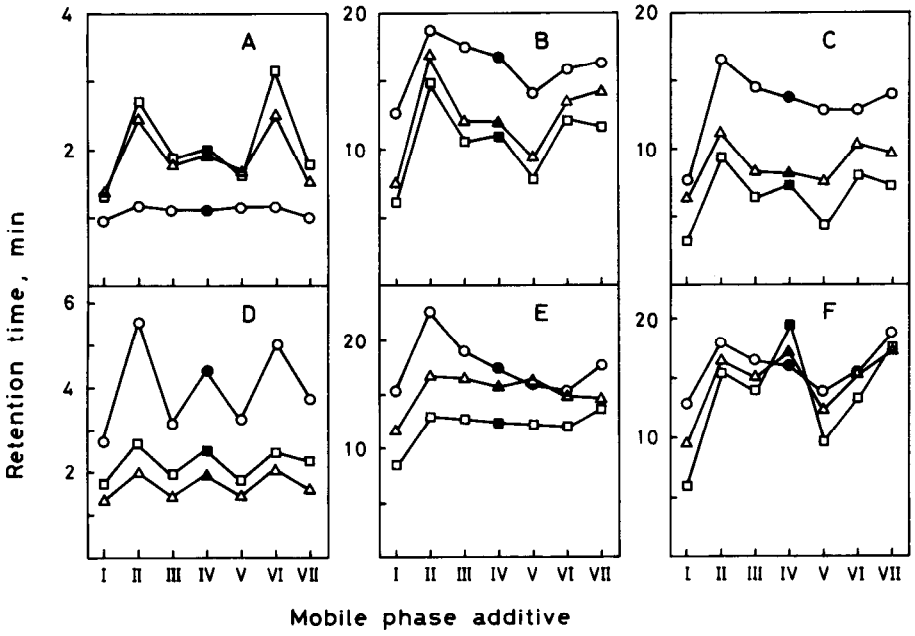

Fig. 1. Retention times of disubstituted benzene isomers (○, *ortho*; △, *meta*: □, *para*) on column A in methanol–water (30:70), except for F [methanol–water (40:60)]. Mobile phase additives: I, TM-α-CD; II, DM-α-CD; III, α-CD;IV, none; V, β-CD; VI, DM-β-CD; VII, TM-β-CD. Solutes: A, nitrobenzoic acid; B, nitrophenol; C, nitroaniline; D, aminobenzoic acid; E, dinitrobenzene; F, iodoaniline.

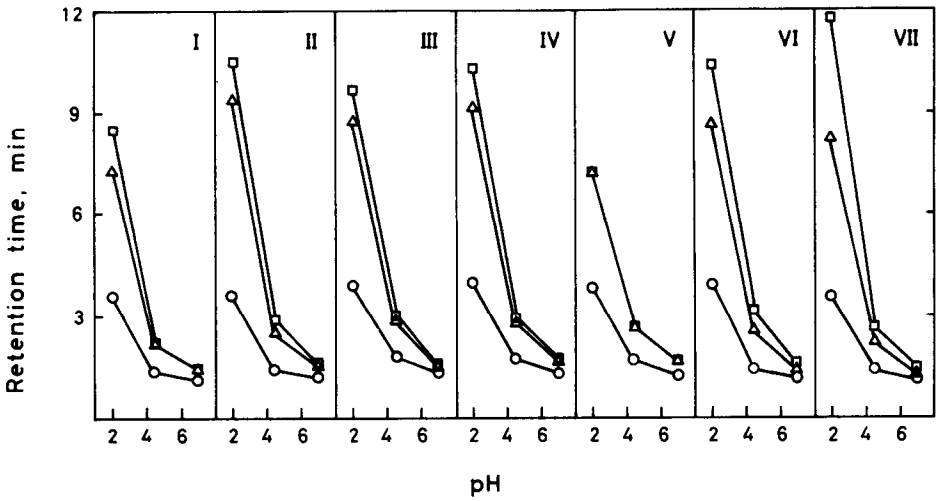

Fig. 2. Effect of pH of the mobile phases on retention times of nitrobenzoic acid isomers (○, *ortho*; △, *meta*; □, *para*) on column A in methanol–0.01 *M* phosphate buffer (40:60). Mobile phase additives as in Fig. 1.

Methanol–0.01 *M* phosphate buffer at pH 2.0, 4.5 or 7.0 was used as the mobile phase in order to investigate the effect of pH on the separation of the isomers. The retention of nitrobenzoic acid, in particular, increased with decreasing pH of the mobile phase (Fig. 2). This is reasonably explained by a decrease in the anionic forms of the acid, which are less strongly retained on the ODS stationary phase. Moreover, only when TM-β-CD was used as the additive could the three isomers of nitrobenzoic

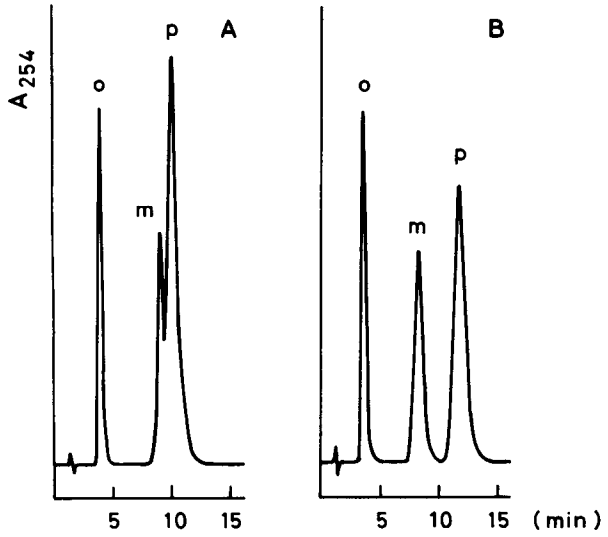

Fig. 3. Liquid chromatograms of nitrobenzoic acid isomers on column A in methanol–0.01 *M* phosphate buffer (pH 2.0) (40:60). Mobile phase additives: A, none; B, TM-β-CD. *o*, *m* and *p* denote *ortho*, *meta* and *para* isomers, respectively.

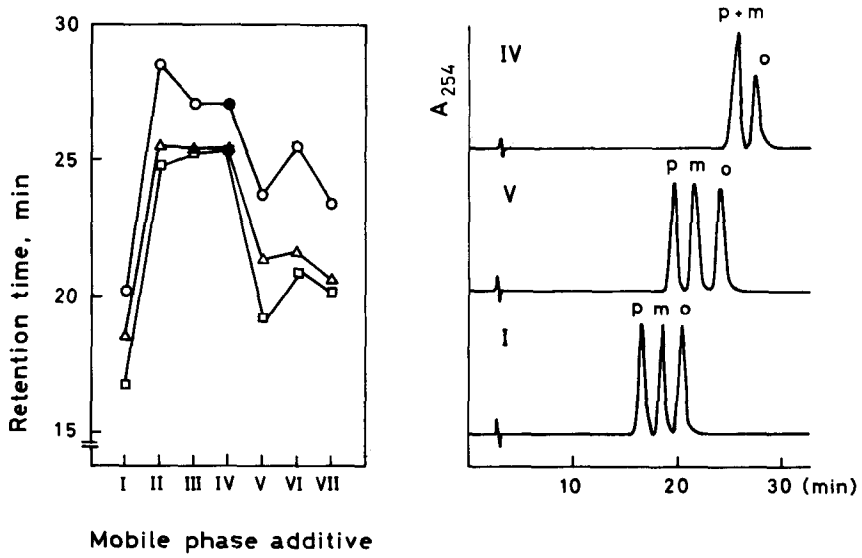

Fig. 4. Separation of cresol isomers (○, *ortho*; △, *meta*; □, *para*) on column B in methanol–water (40:60). Mobile phase additives as in Fig. 1. *o*, *m* and *p* denote *ortho*, *meta* and *para* isomers, respectively.

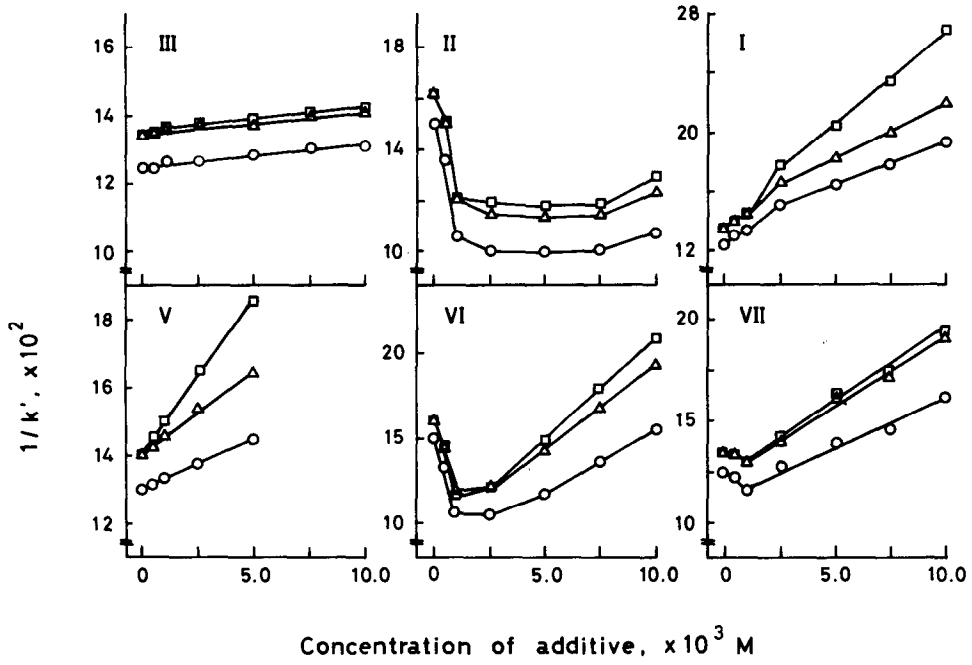

Fig. 5. Effect of concentration of mobile phase additives in methanol–water (40:60) on retention of cresol isomers (○, *ortho*; △, *meta*; □, *para*) on column B. Mobile phase additives as in Fig. 1.

TABLE I

R_F VALUES OF CYCLODEXTRIN ADDITIVES ON ODS HPTLC PLATES DEVELOPED WITH METHANOL–WATER

Cyclodextrin additive	*Methanol content (%)*		
	80	*60*	*50*
α-CD	0.96	0.94	0.92
DM-α-CD	0.46	0.13	0.04
TM-α-CD	0.18	0.02	0
β-CD	0.96	0.94	0.92
DM-β-CD	0.32	0.06	0.03
TM-β-CD	0.18	0.02	0

acid be completely separated. This can be seen from Fig. 3B. The retentions of aminobenzoic acid and nitrophenol also increased on decreasing the pH from 7.0 to 4.5; a substantial change in their retention or selectivity was not observed on reducing the pH from 4.5 to 2.0.

Effect of concentration of cyclodextrin additives on retention of cresol

It has been reported that the pair *m*- and *p*-cresol could not be resolved using reversed-phase liquid chromatography[19]. By adding β-CD or TM-α-CD ($5 \cdot 10^{-3}$ *M*) to methanol–water (40:60), they can be completely separated, as shown in Fig. 4 (V and I). The other cyclodextrin additives did not effect a complete baseline separation of the two isomers.

Assuming that the retentions of cyclodextrins and their 1:1 inclusion complexes on the ODS stationary phase are negligible, a linear relationship can be obtained

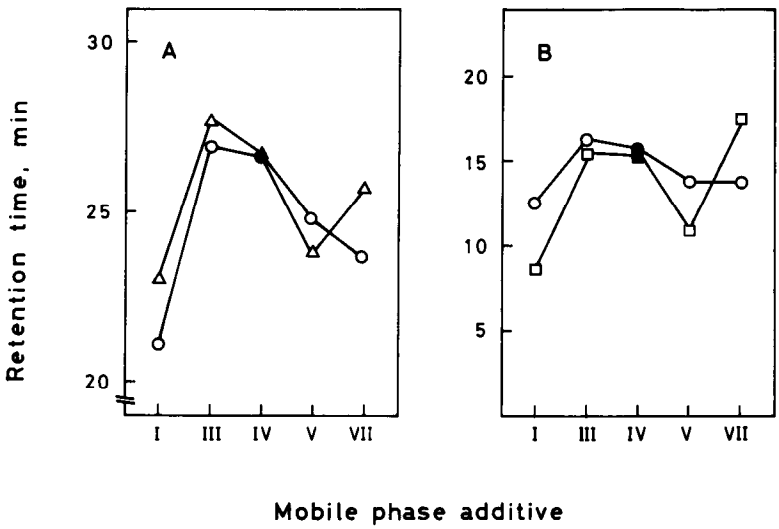

Fig. 6. Separation of (A) 2,3- (△) and 2,6-xylenol (○) on column B and (B) *m*-chlorophenol (□) and 2,6-xylenol (○) on column A. Mobile phase, methanol–water: A, 45:55; B, 40:60. Mobile phase additives as in Fig. 1.

between the reciprocal of the capacity factor of the solutes ($1/k'$) and the concentration of cyclodextrins in the mobile phase[16]. Fig. 5 shows the effect of the concentration of the cyclodextrin additives on the retention of the cresol isomers in methanol–water (40:60). Such linear relationships were found with the unmodified α- and β-CD additives; this is not the case with the methylated cyclodextrin derivatives, as shown in Fig. 5. This suggests that the above simplified assumptions are not valid in the latter instance: the equilibria of adsorption and/or complexation are more complicated. We speculate that the adsorption of the methylated cyclodextrin derivatives on the ODS stationary phase plays an important role. Each cyclodextrin additive was spotted on ODS HPTLC plates (RP-18 F_{254-s}, Merck) and developed with methanol–water. Table I gives the R_F values as a function of the methanol content in the mobile phase. Unmodified α- and β-CD exhibited large R_F values, whereas the methylated cyclodextrin derivatives gave much smaller values. This is ascribed to an increase in hydrophobicity of the cyclodextrin derivatives as a result of methylation of the hydroxy groups in unmodified cyclodextrins. Therefore, the above speculation of adsorption of the methylated cyclodextrin additives on the stationary phase can be reasonably accepted, although further work is needed for a more convincing, quantitative explanation.

Separation of other compounds

Fig. 6. shows the separation of 2,3- and 2,6-xylenol (A) and *m*-chlorophenol and 2,6-xylenol (B). These two pairs could not be resolved by reversed-phase liquid chromatography without additives. However, the components of each pair could be separated on the ODS stationary phase in the presence of TM-α-CD, β-CD or

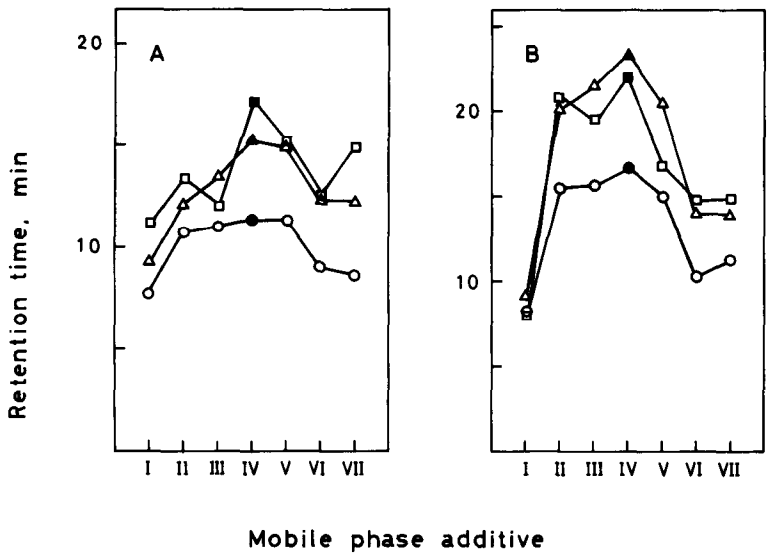

Fig. 7. Retention times of (A) *trans*- and (B) *cis*-nitrocinnamic acid isomers (○, *ortho*; △, *meta*; □, *para*) on column A. Mobile phase, methanol–0.01 *M* phosphate buffer (pH 2.0): A, 40:60; B, 30:70. Mobile phase additives as in Fig. 1.

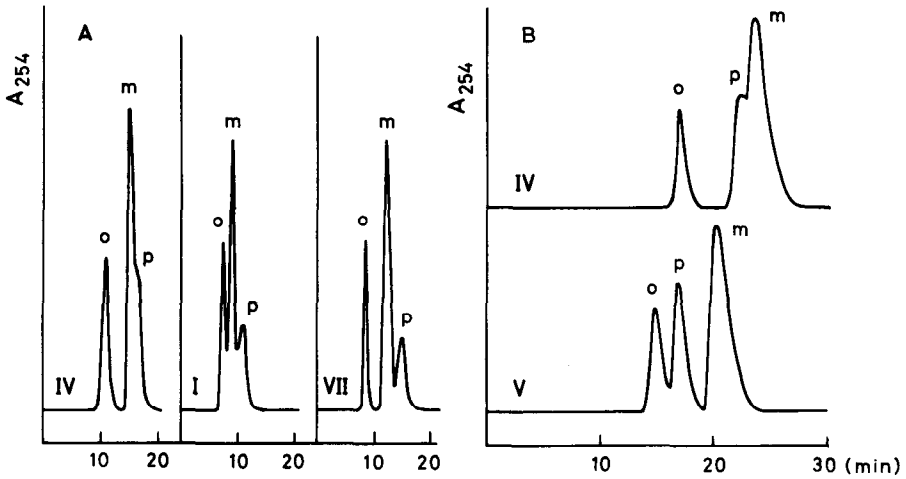

Fig. 8. Liquid chromatograms of (A) *trans*- and (B) *cis*-nitrocinnamic acid isomers. *o*, *m* and *p* denote *ortho*, *meta* and *para* isomers, respectively. Others as in Fig. 7.

TM-β-CD in the mobile phase. The other cyclodextrin additives studied could not completely separate these mixtures.

Figs. 7 and 8 give the retention times of the *ortho*, *meta* and *para* isomers of *trans*- and *cis*-nitrocinnamic acids and their liquid chromatograms, respectively. The addition of TM-β-CD greatly improved the separation of the three isomers of *trans*-nitrocinnamic acid; a similar improvement for *cis*-nitrocinnamic acid was given by the addition of β-CD. It has been reported that the presence of β-CD in the mobile phase is accompanied by enhancement of selectivity for *m*- and *p*-nitrocinnamic acids whether in the *cis* or *trans* configuration[15]. Compared with β-CD, TM-β-CD is much more effective in their separation in the *trans* configuration.

In conclusion, α- or β-CD was completely or partially methylated and used as a mobile phase additive. It was found that 2,3,6-tri-O-methylation in particular effects an improvement in the separations of the aromatic compounds studied. On the other hand, 2,6-di-O-methylation did not result in such a significant improvement.

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