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Separations of tocopherols and methylated tocols on cyclodextrin-bonded silica

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Abstract

α-, β-, γ-, and δ-tocopherols (methyl-substituted tocols) and 5,7-dimethyltocol were separated by normal-phase HPLC on β- or γ-cyclodextrin-bonded silica (CDS) with fluorescence detection. The HPLC behavior of the tocol components was studied under various mobile phase conditions. Hexane or cyclohexane was used in combination with oxygen-containing solvents (alcohol, ether, and esters) in binary and ternary mobile phases. Capacity factors (k') and separation factors (α) for adjacent tocol components were determined. Incorporation of non-polar ethers in the hydrocarbon (hexane or cyclohexane) mobile phases favored the separation of β- and γ-tocopherols with improved α values, which enabled trace analysis of the β-isomer present in soybean oil. Analyte solutes tended to be more strongly adsorbed in mobile phases containing branched-chain alcohols and ethers than in those containing the corresponding straight-chain solvents. Generally, the k' values obtained with hexane mobile phases or with the β-CDS phase were greater than those observed in HPLC with cyclohexane mobile phases or with the γ-CDS phase.

1. Introduction

During the course of another study on the HPLC evaluation of minor non-triglyceride constituents in genetically modified soybean oil, it was necessary to solve analytical problems associated with the separation of tocopherol components in the oil samples. In HPLC with hexane–2-propanol mobile phases, β-tocopherol eluted first from a commercial amino column (Waters μBondapak NH_2, 10 μm) followed very closely by the γ-isomer. Separation factors (α) for these β- and γ-isomers were much lower than those for adjacent α–β- and γ–δ-pairs of tocopherol isomers. An efficient separation of the β-γ pair was highly desirable because of small amounts of the β-isomer normally present in soybean oil. Therefore, a comprehensive HPLC study was undertaken to achieve adequate separations of all isomeric components and to obtain accurate analytical results.

Earlier attempts at separating α-, β-, γ-, and δ-isomers of both the parent and methyl ether derivatives of tocopherols in soybean oil by reversed-phase HPLC were unsuccessful because the β- and γ-tocopherols remained unresolved under all HPLC conditions employed [1]. An extensive literature search indicated that little

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progress had been made for the past decade in the reversed-phase HPLC separation of all four soybean tocopherols. However, the first report of the separation of β-, and γ-homologues by normal-phase HPLC appeared in 1973 [2].

Since the normal-phase HPLC technique has proven to give better separations of tocopherol components than the reversed-phase method, many researchers have used the normal-phase technique for their tocopherol analyses with various sample matrices [3–14]. Most reported studies employed silica columns of various particle sizes and dimensions. There are only two publications in the literature describing the use of amino and cyano polar phases in tocopherol analyses [15,16]. Despite the vast volume of published work on tocopherol analyses, HPLC methods for the separation of the title compounds on cyclodextrin-bonded silica (CDS) columns have remained unexplored.

β- and γ-cyclodextrins (CD) are macrocyclic molecules containing several glucopyranose units arranged in the shape of hollow truncated cones with relatively hydrophobic interior cavities of 7.5 and 9.5 Å in diameter, respectively. Because of the presence of hydroxy groups, the CD exterior surfaces are hydrophilic. When commercially available β- and γ-CDS columns are used in the normal-phase mode, the chromatographic characteristics of analytes resemble those obtained with other silica-based polar phases. Normal-phase HPLC studies of a variety of compounds on CDS phases have been reported [17–21]. In view of the scarcity of published information concerning HPLC of tocopherols on polar phases, we report the results of a systematic HPLC study of the chromatographic behavior of tocopherol isomers including the 5,7-dimethyltocol analogue on the β- and γ-CDS phases under various HPLC solvent conditions.

2. Experimental

2.1. Chemicals and reagents

Analytical reference standard compounds α-tocopherol (5,7,8-trimethyltocol) (TMT), β-tocopherol (5,8-dimethyltocol) (DBT), γ-tocopherol (7,8-dimethyltocol) (DGT) and 5,7-dimethyltocol (DMT) were obtained from Matreya (Pleasant Gap, PA, USA). Analytical reference standard samples of δ-tocopherol (8-methyltocol) (MDT) were obtained from Eastman Kodak (Rochester, NY, USA). Chromatography-grade HPLC solvents hexane (HX), cyclohexane (CHX), dioxane (DIOX), tetrahydrofuran (THF), and ethyl acetate (EtOAc) were purchased from Fisher (Fair Lawn, NJ, USA). Other HPLC solvents, including diisopropyl ether (IPIP), *tert.*-butyl methyl ether (TBM), *n*-butyl methyl ether (NBM), tetrahydropyran (THP), ethanol (EtOH), 1-propanol (1-PR), 2-propanol (2-PR), *n*-butanol (*n*-BU), and *tert.*-butanol (*t*-BU) were high-purity products of Aldrich (Milwaukee, WI, USA).

2.2. High-performance liquid chromatography

A Spectra-Physics (San Jose, CA, USA) Model SP8700 liquid chromatograph interfaced with an Applied Biosystems (Foster City, CA, USA) Model 980 programmable fluorescence detector was used throughout the HPLC studies. Tocopherols and 5,7-dimethyltocol were detected at an excitation wavelength of 298 nm and an emission wavelength of 345 nm. Mobile phases employed binary solvent systems each consisting of a non-polar hydrocarbon solvent (hexane or cyclohexane) and a slightly polar oxygen-containing solvent such as an alcohol, ether, or ester. In some HPLC experiments, ternary solvent systems were also used. Mobile phase eluents were prepared by mixing binary of ternary solvents at various proportions.

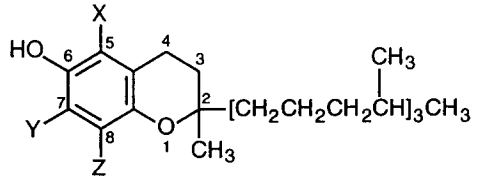
Two different CDS stationary phases were obtained from Advanced Separation Technologies (Whippany, NJ, USA). These columns included (1) Cyclobond I (β-CDS), 5-μm spherical particles, 250 × 4.6 mm I.D., and (2) Cyclobond II (γ-CDS), 5-μm spherical particles, 250 × 4.6 mm I.D. The mobile phase eluents were degassed with helium sparge, filtered through a 0.02-μm filter, and pumped at a flow-rate of 1 ml/min. Aliquots (5–10 μl) of analyte samples in hexane (50–100 μg/ml) were injected

onto the column via a Rheodyne (Cotati, CA, USA) Model 7125 injector equipped with a 10-μl loop. Samples were stored in amber vials at −30°C for protection from light. Throughout the HPLC analyses, three replicate injections were made for each analysis of a tocol mixture under well-equilibrated HPLC conditions. Normally it required about 2–3 h between mobile phase variations for the HPLC column to reach equilibrium. Retention times (t) were mean values of three determinations with coefficients of variation ranging 2–5%. Capacity factors (k') were computed as $k' = t - t_o/t_o$ where t and t_o represent retention times for an analyte and an unretained solute, respectively. Separation factors (α) were determined for adjacent tocopherol components as $\alpha = k'_{c+1}/k'_c$ where subscript "c" represents an analyte component.

3. Results and discussion

Structures of the five methyl-substituted tocols (four tocopherols and 5,7-dimethyltocol) are shown in Fig. 1. These isomeric compounds were found to elute from a CDS column in the following sequence: 5,7,8-trimethyltocol (TMT) < 5,7-dimethyltocol (DMT) < 5,8-dimethyltocol (DBT) < 7,8-dimethyltocol (DGT) < 8-methyltocol (MDT). As expected, the elution order parallels the decreasing number of methyl substituents on the tocol molecules which is also indicative of an increasing order of polarity of the compounds. In normal-phase HPLC of the tocol compounds on CDS, analyte solute retention is believed to be due to solute adsorption to the outside of the CD molecule, while the CD cavity is occupied by a non-polar hydrocarbonaceous solvent [22]. The elution–polarity relationship observed in this normal-phase HPLC study can be interpreted in terms of the adsorption rationale described.

Figs. 2 and 3 show the effect of mobile phase compositions of hexane binary systems on the retention of tocol components on γ-CDS. HPLC with a small amount of alcohol in hexane produced a typical k' *vs.* % hexane profile in which



(A) X = CH_3, Y = CH_3, Z = CH_3
(B) X = CH_3, Y = CH_3, Z = H
(C) X = CH_3, Y = H, Z = CH_3
(D) X = H, Y = CH_3, Z = CH_3
(E) X = H, Y = H, Z = CH_3

Fig. 1. Structures of investigated tocol compounds. (A) 5,7,8-trimethyltocol (TMT) (α-tocopherol), (B) 5,7-dimethyltocol (DMT), (C) 5,8-dimethyltocol (DBT) (β-tocopherol), (D) 7,8-dimethyltocol (DGT) (γ-tocopherol), and (E) 8-methyltocol (MDT) (δ-tocopherol).

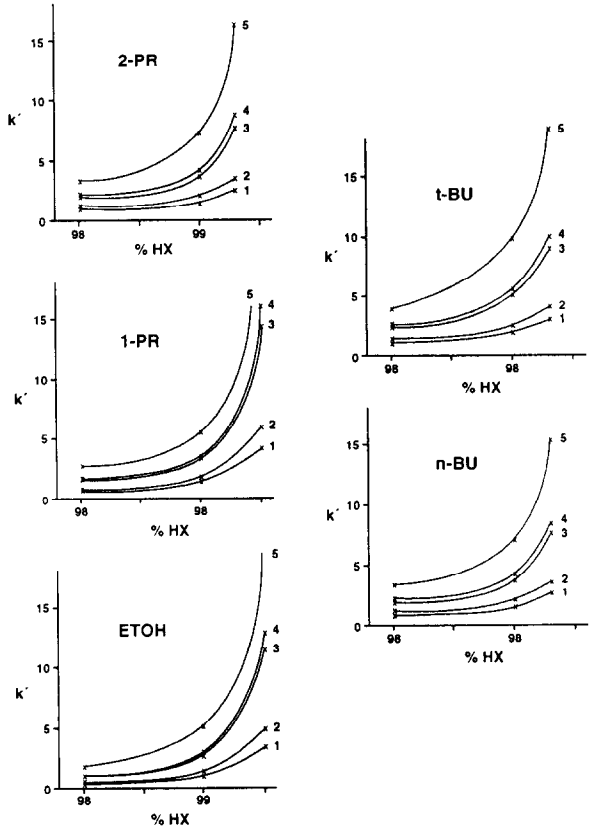


Fig. 2. Variation of capacity factor, k', with alcohol composition in hexane binary mobile phase. Stationary phase: γ-CDS. Component identification: (1) TMT, (2) DMT, (3) DBT, (4) DGT, and (5) MDT. For component abbreviations, see Fig. 1.

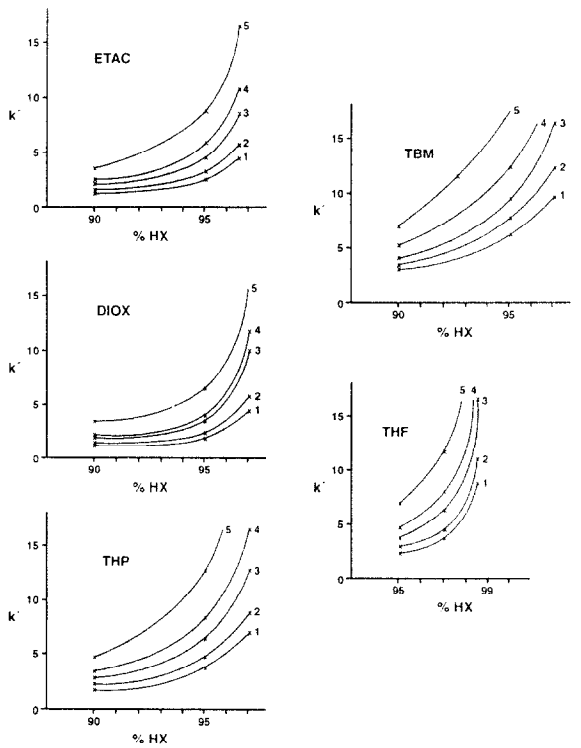


Fig. 3. Variation of capacity factor, k', with ether or ester composition in hexane binary mobile phase. Stationary phase: γ-CDS. For component identification, see Fig. 2.

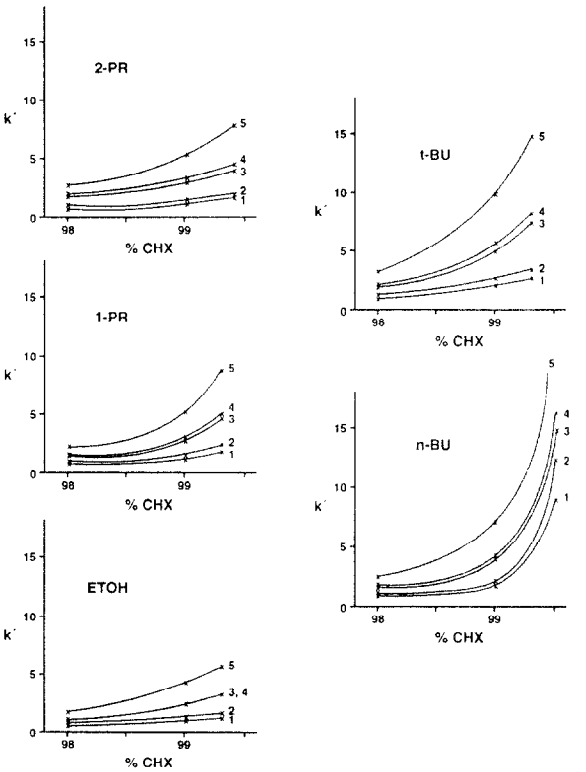


Fig. 4. Variation of capacity factor, k', with alcohol composition in cyclohexane binary mobile phase. Stationary phase: γ-CDS. For component identification, see Fig. 2.

the differences in k' values between components 1 and 2 or components 3 and 4 were much smaller in comparison with those between components 2 and 3 or components 4 and 5 (Fig. 2). On the other hand, inspection of the k' *vs.* % hexane plots in Fig. 3 indicated that differential k' values among adjacent pairs [$\Delta k'$ (1–2), $\Delta k'$ (2–3), $\Delta k'$ (3–4), and $\Delta k'$ (4–5)] observed in HPLC with mobile phases containing ethers (or esters) became close in magnitude, particularly in analyses where ethers of low polarity were used. In other words, the five tocols tended to be more equally dispersed among the components in the latter solvent systems than in hexane–alcohol mobile phases.

In addition to the hexane binary mobile phases used in the study, the corresponding cyclohexane binary solvent systems were also used to ascertain the effect of the cyclic hydrocarbon structure of cyclohexane on the HPLC separation of the compounds of interest. As shown in Figs. 4 and 5, the k' *vs.* % cyclohexane profiles are strikingly similar to the corresponding k' *vs.* % hexane profiles in Fig. 2 and 3. Generally the curvature of k' *vs.* % hexane curves appeared to be somewhat greater than that of the cyclohexane counterparts, especially in the region of higher hydrocarbon contents. These results suggest that adsorptions of tocol solutes in HPLC with hexane binary solvents are more susceptible to solvent effects than with cyclohexane mobile phases. Further, differential k' values [$\Delta k'$ (1–2) and $\Delta k'$ (3–4)] for the two pairs of tocol components observed in experiments using cyclohexane–ethers (or esters) (Fig. 5) seemed to be slightly smaller than those found in HPLC with hexane containing the same ether or ester solvents (Fig. 3).

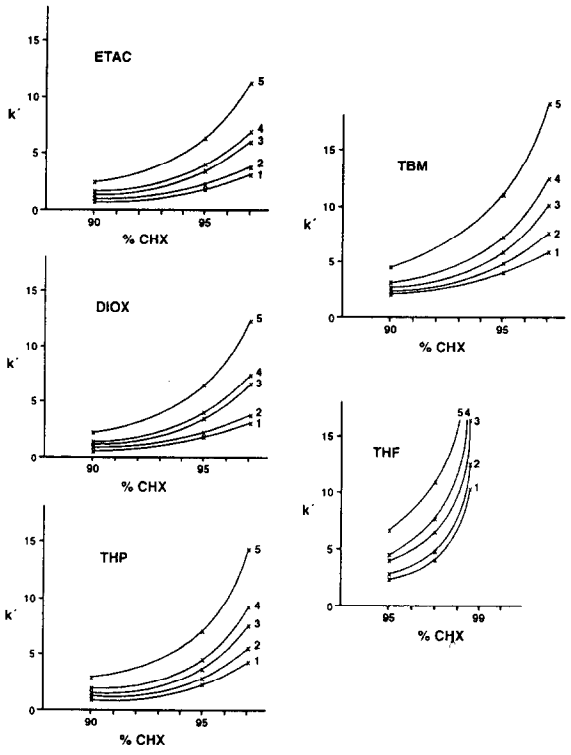


Fig. 5. Variation of capacity factor, k', with ether or ester composition in cyclohexane binary mobile phase. Stationary phase: γ-CDS. For component identification, see Fig. 2.

Typical examples of chromatograms showing normal-phase separations of the five tocols on β-CDS are given in Fig. 6. Regardless of the type of oxygen-containing solvents used, solvent polarity plays an important role in controlling the separation of components 1 and 2 from components 3 and 4. Thus, in relation to separations of other adjacent pairs, the separation between components 1 and 2 or between components 3 and 4 is apparently smaller when relatively more polar solvents were used, as demonstrated in Fig. 6D (ether), Fig. 6F (alcohol) and Fig. 6G (ester). With ethyl acetate in the mobile phase (Fig. 6G), components 3, 4, and 5 emerged as weak peaks on the chromatogram probably due to solubility problems of the analyte components in the solvent systems employed.

Normal-phase retention and separation characteristics of the methylated tocols examined

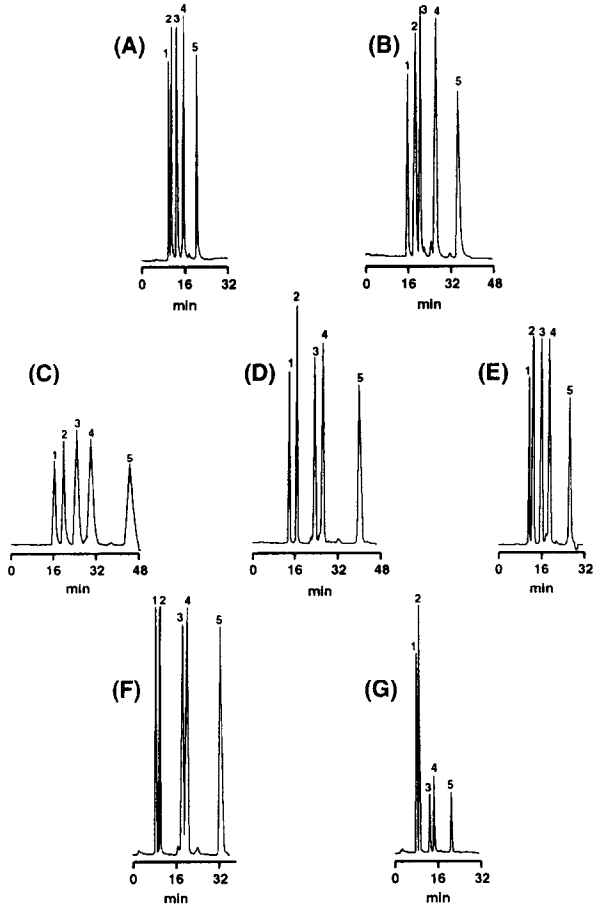


Fig. 6. Typical chromatograms showing normal-phase separations of tocol components on β-CDS. Mobile phases (A) hexane–tetrahydropyran (90:10), (B) hexane–*tert.*-butyl methyl ether (90:10), (C) cyclohexane–*tert.*-butyl methyl ether (95:5), (D) cyclohexane–diisopropyl ether (95:5), (E) cyclohexane–tetrahydropyran (95:5), (F) cyclohexane–*tert.*-butanol (99:1), and (G) cyclohexane–ethyl acetate (95:5). For component identification, see Fig. 2.

under various HPLC conditions are compiled in Tables 1–5. Of the four different HPLC systems studied, highest k' values of tocols were invariably obtained from HPLC experiments where hexane binary mobile phases and a β-CDS stationary phase were used (Table 1). The analyte k' values normally decreased with the increasing polar nature of given HPLC solvent systems including stationary phases. If the mobile phase and stationary phase variables specified in Tables 1, 2, 3, and 4 are designated

Table 1
Normal-phase HPLC of methyl-substituted tocols on β-cyclodextrin-bonded silica with hexane binary mobile phases

Mobile phase	k' TMT	α	k' DMT	α	k' DBT	α	k' DGT	α	k' MDT
HX–2-PR (99:1)	1.70	1.34	2.28	1.80	4.11	1.17	4.81	1.58	7.60
HX–1-PR (99:1)	1.48	1.34	1.99	1.73	3.44	1.15	3.96	1.54	6.10
HX–*t*-BU (99:1)	2.93	1.23	3.62	1.82	6.60	1.16	7.67	1.54	11.8
HX–*n*-BU (99:1)	2.61	1.20	3.14	1.63	5.11	1.13	5.77	1.42	8.20
HX–EtOH (99:1)	1.38	1.36	1.88	1.80	3.38	1.12	3.79	1.42	5.84
HX–THF (95:5)	3.09	1.20	3.71	1.31	4.86	1.30	6.32	1.37	8.66
HX–DIOX (95:5)	2.33	1.23	2.87	1.50	4.32	1.16	5.00	1.53	7.65
HX–EtAc (95:5)	3.30	1.23	4.06	1.38	5.60	1.30	7.29	1.40	10.2
HX–THP (95:5)	5.20	1.22	6.34	1.31	8.32	1.31	10.9	1.44	15.7
HX–TBM (95:5)	8.60	1.28	11.0	1.25	13.8	1.35	18.6	1.51	28.0

For abbreviations see Experimental.

as (A), (B), (C), and (D), respectively, the general trends for the variation of k' values with polarity of HPLC systems are observed as follows: $k'(A) > k'(B) > k'(D)$; $k'(A) > k'(C) > k'(D)$. Since β- and γ-CD have respective seven and eight glucopyranose units in the molecules, the γ-CDS phase would be expected to be more polar than the β-CDS phase. However, comparisons of retention data obtained with the β-CDS phase (Tables 1 and 2) to those obtained with the γ-CDS phase (Table 3 and 4) indicate otherwise. The relatively less polar nature of the γ-CDS phase indicated by the observed lower k' values is presumably due to its lower CD loading. Although retention of analyte solutes on CDS appears to proceed largely by adsorption, the exact nature of interactions between tocol solutes and the CDS phase is not clear at the present time.

Structural effects of oxygen-containing solvents in hexane or cyclohexane binary mobile phases on retention of tocols on β- or γ-CDS are demonstrated in Tables 1–4. Evidently, tocol compounds tended to be adsorbed more strongly on CDS in mobile phases containing branched-chain alcohols or ethers than those containing the straight-chain analogues: for example, (1) in the alcohol series, $k'(t\text{-BU}) > k'(n\text{-BU}) > k'(2\text{-}$

Table 2
Normal-phase HPLC of methyl-substituted tocols on β-cyclodextrin-bonded silica with cyclohexane binary mobile phases

Mobile phase	k' TMT	α	k' DMT	α	k' DBT	α	k' DGT	α	k' MDT
CHX–2-PR (99:1)	1.46	1.25	1.82	1.79	3.26	1.12	3.65	1.63	5.95
CHX–1-PR (99:1)	1.40	1.29	1.80	1.81	3.26	1.08	3.53	1.64	5.80
CHX–*t*-BU (99:1)	2.33	1.23	2.86	1.98	5.65	1.10	6.20	1.71	10.6
CHX–*n*-BU (99:1)	1.52	1.27	1.93	1.90	3.67	1.06	3.89	1.68	6.53
CHX–EtOH (99:1)	1.38	1.27	1.76	1.81	3.19	1.09	3.48	1.60	5.56
CHX–THF (95:5)	2.79	1.18	3.29	1.31	4.31	1.21	5.22	1.44	7.51
CHX–DIOX (95:5)	1.96	1.25	2.45	1.57	3.85	1.10	4.24	1.57	6.66
CHX–EtAc (95:5)	2.13	1.22	2.60	1.46	3.80	1.16	4.40	1.50	6.60
CHX–THP (95:5)	3.00	1.15	3.46	1.31	4.53	1.22	5.54	1.45	8.06
CHX–TBM (95:5)	5.40	1.22	6.60	1.25	8.26	1.22	10.1	1.50	15.1
CHX–IPIP (95:5)	3.73	1.27	4.73	1.48	7.00	1.15	8.06	1.58	12.7

Table 3
Normal-phase HPLC of methyl-substituted tocols on γ-cyclodextrin-bonded silica with hexane binary mobile phases

Mobile phase	k' TMT	α	k' DMT	α	k' DBT	α	k' DGT	α	k' MDT
HX–2-PR (99:1)	1.31	1.35	1.77	1.84	3.26	1.11	3.62	1.75	6.33
HX–1-PR (99:1)	1.20	1.38	1.66	1.91	3.00	1.09	3.26	1.67	5.46
HX–*t*-BU (99:1)	2.06	1.29	2.66	1.92	5.10	1.13	5.76	1.70	9.80
HX–*n*-BU (99:1)	1.73	1.27	2.20	1.75	3.86	1.10	4.26	1.69	7.20
HX–EtOH (99:1)	1.01	1.42	1.42	1.96	2.78	1.11	3.09	1.73	5.34
HX–THF (95:5)	2.46	1.22	3.00	1.29	3.86	1.26	4.86	1.44	7.00
HX–DIOX (95:5)	1.97	1.23	2.42	1.51	3.66	1.16	4.24	1.54	6.53
HX–EtAc (95:5)	2.67	1.21	3.23	1.45	4.68	1.27	5.95	1.49	8.86
HX–THP (95:5)	3.86	1.26	4.87	1.47	6.65	1.28	8.53	1.49	12.7
HX–TBM (95:5)	6.26	1.23	7.67	1.24	9.53	1.30	12.4	1.42	17.6
HX–NBM (95:5)	3.98	1.25	4.98	1.49	7.42	1.20	8.90	1.55	13.8
HX–NBM (90:10)	2.80	1.21	3.40	1.27	4.32	1.24	5.34	1.47	7.86

PR) > k'(1-PR) > k'(EtOH), (2) in the ether series, k'(TBM) > k'(NBM) > k'(THP) > k'(THF) > k'(DIOX). It is apparent that such structural effects on tocol retention seem to contradict, in part, conventional observations on the solvent polarity–retention relationship. A similar structural effect of alcohols on solute retention has been reported previously in reversed-phase HPLC of aromatic compounds on CDS [23].

Examination of the methyl substitution pattern in tocol molecules (Fig. 1) reveals that the 6-hydroxy group is flanked by two methyls at the 5- and 7-positions in TMT and DMT molecules, but there is only one methyl adjacent to the hydroxy group in DBT and DGT structures. Correlation of the effect of methyl substitution in the three dimethyltocols DMT, DBT, and DGT with their k' values obtained in HPLC with polar solvents enabled us to understand why the k' values of DMT are very close to those of TMT and are much farther apart from those of DBT or DGT. The 6-hydroxy group in the latter pair of dimethyltocols appears to be more easily

Table 4
Normal-phase HPLC of methyl-substituted tocols on γ-cyclodextrin-bonded silica with cyclohexane binary mobile phases

Mobile phase	k' TMT	α	k' DMT	α	k' DBT	α	k' DGT	α	k' MDT
CHX–2-PR (99:1)	1.39	1.25	1.74	1.77	3.08	1.12	3.45	1.61	5.55
CHX–1-PR (99:1)	1.20	1.26	1.51	1.91	2.89	1.05	3.03	1.65	5.00
CHX–*t*-BU (99:1)	2.04	1.29	2.64	1.89	4.99	1.13	5.64	1.71	9.65
CHX–1-BU (99:1)	1.65	1.25	2.06	1.87	3.86	1.09	4.20	1.67	7.00
CHX–EtOH (99:1)	1.00	1.40	1.40	1.86	2.60	1.00	2.60	1.63	4.25
CHX–THF (95:5)	2.51	1.20	3.01	1.33	4.01	1.17	4.69	1.42	6.66
CHX–DIOX (95:5)	1.93	1.21	2.33	1.57	3.66	1.09	4.00	1.62	6.46
CHX–EtAc (95:5)	1.86	1.25	2.33	1.52	3.53	1.15	4.06	1.56	6.33
CHX–THP (95:5)	2.46	1.22	3.00	1.31	3.93	1.22	4.80	1.50	7.20
CHX–TBM (95:5	4.00	1.25	5.00	1.20	6.00	1.22	7.32	1.50	11.0
CHX–NBM (95:5)	3.29	1.25	4.12	1.30	5.35	1.14	6.10	1.59	9.70
CHX–NBM (90:10)	2.00	1.23	2.46	1.27	3.13	1.17	3.66	1.55	5.67

Table 5
Normal-phase HPLC of methyl-substituted tocols on β-cyclodextrin-bonded silica with hexane ternary mobile phases

Mobile phase	k' TMT	α	k' DBT	α	k' DGT	α	k' MDT
HX–2-PR–DIOX (99:0.5:0.5)	3.13	2.28	7.13	1.19	8.46	1.74	14.7
HX–2-PR–DIOX (99:0.7:0.3)	3.15	1.63	5.13	1.11	5.67	1.52	8.60
HX–2-PR–DIOX (98:1:1)	1.00	1.67	1.67	1.08	1.80	1.52	2.73
HX–2-PR–DIOX (98.2:0.9:0.9)	2.33	2.03	4.73	1.15	5.46	1.60	8.73
HX–2-PR–DIOX (98.7:0.3:1)	5.34	2.51	13.4	1.17	15.7	1.77	27.8
HX–EtOH–DIOX (98:0.5:1.5)	3.73	2.20	8.20	1.28	10.5	1.46	15.3
HX–2-PR–THF (99:0.5:0.5)	9.00	1.07	9.67	1.10	10.6	1.44	15.3
HX–2-PR–THF (98.5:0.5:1)	1.66	2.05	3.40	1.19	4.06	1.63	6.53
HX–2-PR–THF (98.7:0.5:0.8)	2.06	1.97	4.06	1.15	4.66	1.59	7.40
HX–2-PR–THF (98.6:0.7:0.7)	1.86	2.00	3.73	1.19	4.45	1.47	6.53
HX–2-PR–THF (96.7:0.3:3)	3.06	1.59	4.86	1.25	6.06	1.40	8.46
HX–2-PR–EtAc (96.1:0.4:3.5)	0.86	1.47	1.26	1.31	1.65	1.41	2.32
HX–2-PR–EtAc (96.3:0.2:3.5)	1.93	1.79	3.46	1.17	4.06	1.49	6.06

accessible for interactions with the CDS phase than that in the TMT–DMT pair. Strong interactions of the polar mobile phase solvents (*e.g.* alcohol or polar ether) with the CDS phase had adverse effects on column selectivity for component separation. This rationalization can be used to account for the following elution sequences observed in experiments with polar mobile phases: k'DBT (or DGT) > k'TMT (or DMT); $\Delta k'$(MDT–DGT) or $\Delta k'$(DBT–DMT) >> $\Delta k'$(DGT–DBT) or $\Delta k'$(DMT–TMT). Because—of weak interactions of mobile phase solvents with the CDS stationary phase, HPLC with non-polar ether mobile phases provided superior selectivity for components yielding uniformly separated component peaks on individual chromatograms of tocol samples analyzed (Fig. 6).

Separation factors, α, for adjacent tocol component peaks on individual chromatograms were measured from normal-phase HPLC retention data (Tables 1–5). These α values were found to vary with the type and polarity of the oxygen-containing solvents in hexane (Tables 1 and 2) or in cyclohexane (Tables 3 and 4). In HPLC experiments with alcohols and polar ethers (*e.g.* dioxane) in hexane or cyclohexane mobile phases, the α values for DBT and DGT are lower than those observed for other adjacent pairs of tocols. The same observations were also true in experiments conducted under similar conditions with ethyl acetate in cyclohexane mobile phases. The specific solvent effects described above are apparently independent of the type of CDS phases used. Separations of the DBT–DGT pair improved considerably with increased α-values (1.21–1.35) by the use of non-polar ethers (*e.g. tert.*-butyl methyl ether, tetrahydropyran, tetrahydrofuran) in hexane as mobile phases and a β-CDS column as the stationary phase.

Normal-phase HPLC data for the separation of soybean tocopherols on β-CDS with hexane–2-propanol-based ternary mobile phases are presented in Table 5. As demonstrated in Table 5, manipulation of solvent ratios in the hexane–2-propanol ternary systems brought about variable degrees of separations (variable α values) for the DBT–DGT pair. Thus, fairly small changes in the α values (1.08–1.19) were noted by adjusting the ratios of hexane–2-propanol–dioxane solvents. With THF in the hexane–2-propanol ternary systems, the α values were between 1.10–1.25, whereas with EtOAc they were between 1.17–1.31 in response to the change in the ratio of the three solvent components in the mobile phases. HPLC with a combination of hexane–ethanol–dioxane led to a favorable separation of the DBT–DGT pair (α = 1.28). In the ternary

mobile phase systems studied, k' values obtained with tetrahydrofuran were higher than those with dioxane, in agreement with the earlier findings in HPLC with binary mobile phases.

In connection with another study on the HPLC analysis of soybean tocopherols using hexane–2-propanol mobile phases, problems associated with the separation of β- and γ-tocopherols (DBT and DGT) were often encountered during the analysis of samples with low levels of the β-isomer. Under the routine HPLC conditions (hexane–2-propanol) used, separations of β- and γ-isomers (DBT–DGT pair) were too close to reveal any interfering peaks for the precise quantitation of the β-tocopherol in soybean oil samples. To solve such analytical problems, ethers are highly recommended for use as co-solvents in hexane or cyclohexane mobile phases for practical tocopherol analysis. This is one of the reasons why discussion has focused on the separation of the DBT–DGT pair of tocopherol isomers throughout this study. Since a tocopherol-specific fluorescence detector was used in this study, the HPLC method developed can be applied to the direct analysis of tocopherols in soybean oil samples without sample cleanup.

In conclusion, the results of the present study represent the first report on the use of CDS columns for the separation of tocol compounds. Excellent separations of five methylated tocol compounds were achieved in most cases and can be extended to other tocols of similar structures by optimization of normal-phase HPLC conditions. The comprehensive approach taken to delineate the chromatographic behavior of the tocols of interest led to a better understanding of the mode of interactions between analyte solutes and CDS phases. HPLC data from comparative studies of mobile phase effects on retention and separation characteristics of tocols provide useful information regarding solvent selection to obtain optimal separations of the analyte components within reasonable retention times.

4. References

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