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Separation of Individual Tocopherols from Soybean Distillate by Low Pressure Column Chromatography

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Abstract α-Tocopherol, γ-tocopherol and δ-tocopherol were successfully separated and purified from soybean vitamin E concentrate (53% purity, from soybean oil deodorizer distillate) using a low-pressure glass column (500 mm × 25 mm, I.D., packed with silica gel). The effects of eluent, flow-rate and sample loading on separation efficiency were investigated using product purity and recovery as evaluation indices. On the basis of the single factor experiment results, an orthogonal test was designed to optimize the chromatographic separation conditions. The optimum conditions obtained by using analyses of extreme difference and variance are as follows: cyclohexane-ethanol 99.7:0.3 (*v*/*v*), flow-rate at 25 ml/min and loading amount being 2 ml (concentration 1 g/ml). Under these optimum conditions, purity of the α-tocopherol, γ-tocopherol and δ-tocopherol were 92.35, 91.23, 89.95%, respectively; the recovery of those products were 35.21, 36.25, 61.25%, respectively. The advantage of this process is that high purity individual tocopherols can be obtained directly from soybean vitamin E concentrate at 53% purity, without additional purification steps.

Keywords Column chromatography · Tocopherol · Separation · Purification

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Introduction

Tocopherols (T) are the main composition of natural vitamin E (V_E). The basic chemical structure of T is composed of a chromanol head and an alkyl side chain. Due to differences in the number of methyl groups and position on the chromanol head, T can be divided into four individual tocopherals: αT, βT, γT and δT (Fig. 1). The bioactivity of αT has been extensively studied [1, 2]. Recently, researchers have discovered that some non-alpha forms of individual tocopherols have unique properties that are important to improving human health. Jiang et al. [3] claimed that prostate cancer cells have demonstrated inhibition of proliferation by γT, but not by αT. Cooney et al. [4] demonstrated that γT, unlike αT, was strongly nucleophilic and thus was more efficient than αT in trapping reactive nitrogen species. Yu et al. [5] declared that δT inhibits the proliferation of breast cancer cells but other individual tocopherols appear to have little effect on the growth of breast cancer cells.

Presently, only high purity αT is economically available and widely used in functional foods and pharmaceuticals. Owing to the unique biological action of non-alpha tocopherols, the need for these high purity tocopherols is increasing in the fields of functional foods, cosmetics and pharmaceuticals.

Soybean oil deodorizer distillate is one of the important sources for the preparation of natural tocopherols. Soybean V_E concentrate is usually prepared from deodorizer distillate by solvent extraction [6] and molecular distillation [7]. High purity αT can be obtained by methylation of non-alpha T [8]. Other non-alpha T are usually separated by chromatographic methods such as thin-layer chromatography (TLC) [9–11], high-performance liquid chromatography (HPLC) [12–15] etc. Abidi [16] and Ruperez [17] reviewed the published

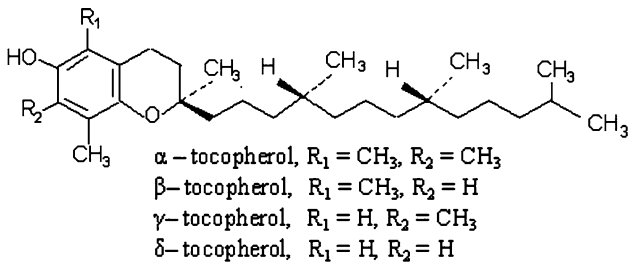

Fig. 1 The structure of the tocopherols

chromatographic methods for separation and purification of individual tocopherols. Among these methods, the separation efficiency of HPLC is the best. However, the equipment is expensive and the costs of operation and maintenance are high. The disadvantages of TLC are detection difficulties and product recoveries. Therefore, TLC and HPLC were not appropriate for the purification of αT, βT, γT and δT as industry processes with low costs. Compared with the HPLC and TLC methods, low-pressure column chromatography (LPCC) exhibits a great potentiality for industrial production due to its simple operation, low cost and high yield. In this paper, we attempt to separate individual tocopherols from soybean V_E concentrate using LPCC and explore the effects of eluent proportion, the flow-rate and loading amount on separation efficiency. These parameters were further optimized by orthogonal test and statistical analysis.

Owing to there being little βT in soybean [18], the objective of this paper is to establish an LPCC method to obtain αT, γT and δT from soybean V_E concentrate (53% purity).

Experimental

Materials and Chemicals

Natural soybean V_E concentrate (53% purity, 8.8% αT, 31.6% γT, 12.6% δT) was provided by Wuhan Kaidi Fine Chemical Industrial Co., Ltd (Wuhan, China). Standard αT (SIGMA, 95% purity), γT and δT (SUPELCO, 95% purity) were purchased from Superior-chemicals & Instrument Co., Ltd (Beijing, China).

Cyclohexane, ethanol and dichloromethane (analytical grade), acetonitrile and methanol (chromatography grade), were obtained from Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China). Silica gel (100–200 mesh, irregular) used for column chromatography and silica gel G used for TLC was obtained from Haiyang Chemical Group (Qingdao, China).

Water used in this work was re-distilled.

Apparatus

Rotary evaporator (RE-52CS, Shanghai Yarong Instrument Co., Ltd, Shanghai, China). Analytical balance (FA2104N, Shanghai Minqiao Precise Science Instrument Co., Ltd, Shanghai, China).

LPCC system (Tianjin Keqi High & New Technology Co., Ltd, Tianjin, China) included constant-flux pump (LB-80C), ultraviolet detector (LC-757), chromatography column (500 mm × 25 mm, I.D.) and chromatography workstation (N2000, Zhejiang University Star Information Technology Co., Ltd, Zhejiang, China).

The HPLC apparatus consisted of a Prostar-230 high-pressure pump, a Prostar-330 photodiode array detector (PDA), a half-auto injector valve with 100 μl sample loop and a 150 mm × 4.6 mm ODS column with 5 μm packings (Varian, CA, USA). The data was acquired with Prostar LC workstation Ver.6 software.

Analytical Methods

TLC Analytical Method

The silica TLC plate (75 mm × 25 mm) was prepared with a thickness of 0.5 mm. Dichloromethane was used as the developing solvent, and iodine vapor was used as the color reagent. Samples were spotted at 5 mm above the solvent level, the height of which was 10 mm from the bottom. The development was terminated when the solvent front moved to the location of 10 mm from the upper edge of the TLC plate. Then the plate was dried and colored with iodine vapor.

HPLC Analytical Method

Acetonitrile (0.1% formic acid, *v*/*v*) was used as the mobile phase, filtered and degassed before use. The flow-rate was set at 1 ml/min. The loading volume was 100 μl and the detection wavelength of UV was 210 nm. The quantitative analyses were performed by using an external standard based on the height of the peak. The concentrations of the standard solutions for calibration were 0.01, 0.02, 0.04, 0.06, 0.08, 0.17, 0.25 and 0.33 mg/ml in methanol.

Preparation of Sample

One hundred grams of soybean V_E concentrates were dissolved in cyclohexane for use (the concentration was 1 g/ml).

Preparation of Low-Pressure Chromatography Column

A 500 mm × 25 mm (I.D.) glass tube was used as the preparative chromatography column with a constant-flux pump to control the flow-rate of eluent. About 100-g silica gel (100–200 mesh) was activated in a drying oven at 120 °C for 60 min, and then slurry was made using cyclohexane as solvent and packed into the column.

Procedure of Column Chromatography

Separation of tocopherols was performed on a 500 mm × 25 mm (I.D.) chromatographic column packed with silica gel (100–200 mesh), and the wavelength of ultraviolet detector was adjusted at 290 nm. After the separation system was equilibrated, the sample was injected and data acquired.

At the initial peak response, the eluate were collected every 10 ml until it returned to base line. All fractions were analyzed by using TLC. The fractions containing the same individual tocopherols were collected together, respectively. Solvents of combined fractions were removed using a rotary evaporator at 30 °C under vacuum and the residue was weighed (W). An amount of residue was taken and weighed (w), and then dissolved in a measuring flask with methanol for HPLC quantitative analysis. The purity and recovery was calculated according to the following equation:

$$P = \frac{n \times v}{w} \times 100\% \quad (1)$$

$$R = \frac{W \times P}{N \times V \times m_i} \times 100\% \quad (2)$$

where P and R referred to purity and recovery, n was concentration acquired from quantitative analyses by HPLC; v was the volume of the measuring flask and w was the weight of substances in the measuring flask; W was the weight of residue; N was the concentration of the sample (1 g/ml); V was the injected volume; m_i referred to the content of αT, γT and δT in natural V_E concentrates (8.8% αT, 31.6% γT, 12.6% δT).

Purity and recovery were two of the indices for preparative separation. In this paper, three products of αT, γT and δT were obtained under a certain condition, and two indices (purity and recovery) needed to be evaluated for each product. Thus, valuation of the separation efficiency was very difficult. For solving this problem, the membership function was introduced [19]:

$$Y_i = [X_{\alpha i} + X_{\gamma i} + X_{\delta i}]/3 \quad (3)$$

where Y_i was the comprehensive value for evaluation of the separation efficiency of three individual tocopherols; i was the subscript representing different conditions; 1/3 was the average of separation efficiency for three products; $X_{\alpha i}$, $X_{\gamma i}$ and $X_{\delta i}$ were the membership function value of purity and recovery of αT, γT and δT, respectively, each X was calculated according to the following equation.

$$X = (X_P + X_R)/2 \quad (4)$$

where X_P and X_R referred to the membership function value of the purity (P) and recovery (R) of each individual tocopherol, which was normalized in the range of 0–1 according to the Eqs. (5) and (6); 1/2 referred to purity and recovery being equally important for evaluation.

$$X_{Pi} = (P_i - P_{min})/(P_{max} - P_{min}) \quad (5)$$

$$X_{Ri} = (R_i - R_{min})/(R_{max} - R_{min}) \quad (6)$$

where P_{min}, R_{min}, P_{max} and R_{max} referred to minimum and maximum of purity and recovery in all investigated conditions for a certain product.

Effect of Chromatographic Conditions on Separation Efficiency

The effect of eluent, flow-rate and loading amount on separation efficiency was investigated by changing the volume ratio of cyclohexane-ethanol (v/v) as 100:0, 99.9:0.1, 99.8:0.2, 99.7:0.3, 99.6:0.4 and 99.5:0.5; flow-rate of 10, 20, 30 and 40 ml/min and loading amount of 1, 2, 3 and 4 ml (sample concentration was 1 g/ml). Procedures were the same as above and the comprehensive value (Y) was calculated according to Eqs. (1)–(6).

Optimization of Parameters of LPCC for Separation of Soybean Individual Tocopherols

Eluent proportion, the flow-rate and loading amount were factors to be optimized by orthogonal test design. The investigated levels of each factor were selected depending on the experimental results. Purity and recovery were used as indices for evaluating separation efficiency.

Results and Discussions

The Result of TLC and HPLC

When using dichloromethane as developer, three individual tocopherols (αT, γT and δT) can be separated completely on the silica TLC plate. The analysis of tocopherols standards and soybean V_E concentrate are illustrated in Fig. 2. R_f value was used for evaluation of separation in TLC

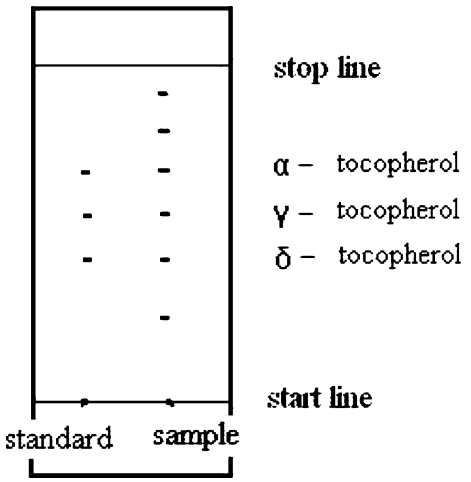

Fig. 2 The result of TLC analysis for mixed standard and soybean V_E concentrate. Adsorber, silica gel G; developer, dichloromethane; mixed standard, αT, γT and δT; sample, soybean V_E concentrate (53% purity)

analysis, which was the ratio of distance between sample dot and developer front and start line. The R_f values of αT, γT and δT were 0.67, 0.55 and 0.43, respectively.

The HPLC chromatogram of mixed standard tocopherols (αT, γT and δT) and soybean V_E concentrate is shown in Fig. 3 (A and B). There is a good linear relationship for each individual tocopherol when the concentration is in the range of 0.01–0.33 mg/ml ($R^2 > 0.999$), respectively.

Effect of LPCC Conditions on Separation Efficiency

The purity and recovery of αT, γT and δT product under different conditions of eluent proportion, the flow-rate and loading amount are listed in Tables 1, 2, 3. The comprehensive value (Y) was calculated according to Eqs. (1)–(6). The value of Y is used to evaluate the separation efficiency. The effects of LPCC conditions on separation efficiency are shown in Figs. 4, 5, 6.

By increasing the ethanol in cyclohexane-ethanol (v/v) from 0 to 0.5%, the separation efficiency increased from to a maximum 0.4%, and then dropped at 0.5% (see Fig. 4). At 0% ethanol only αT and γT were obtained, and δT was not eluted off even after 3.5 h. With increased proportions of ethanol in cyclohexane-ethanol, the strength of eluents increased, δT could be eluted off the column. When the volume ratio of ethanol in cyclohexane-ethanol (v/v) was 0.5%, the eluent was too strong to have enough time for complete separation of αT, γT and δT, resulting in overlapping chromatographic peaks and a decrease in separation efficiency.

By increasing the flow-rate of the eluent from 10 to 40 ml/min, the separation efficiency increased and then

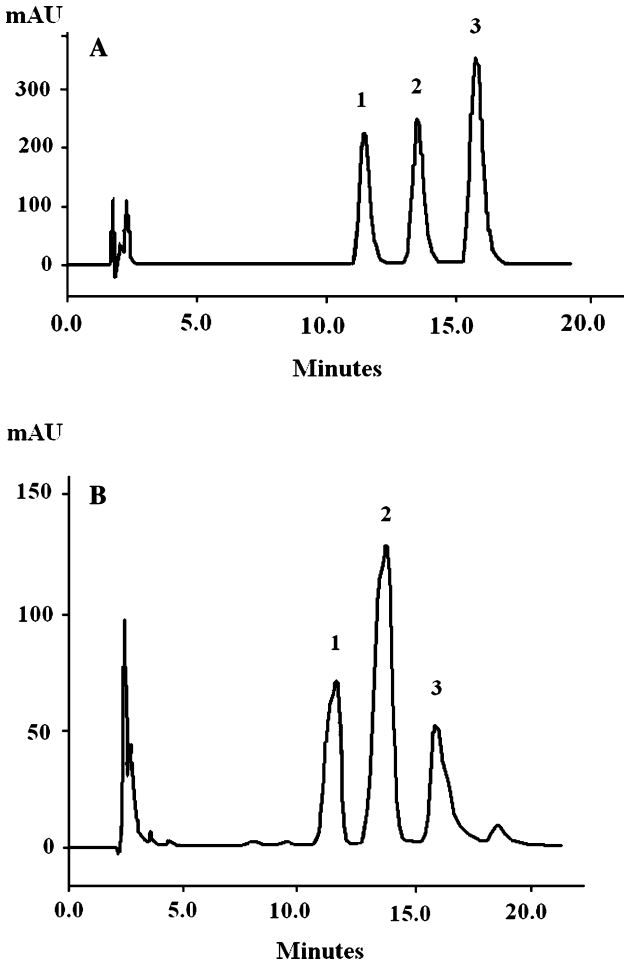

Fig. 3 The HPLC chromatogram of mixed standard tocopherols of αT, γT and δT (**a**) and soybean V_E concentrate (**b**). Column, 150 mm × 4.6 mm ODS column with 5-μm packings; mobile phase, acetonitrile (0.1% formic acid); detection wavelength, 210 nm; injection volume, 100 μl; flow-rate, 1 ml/min. Peaks: (1) δT; (2) γT; (3) αT

Table 1 Purity and recovery of individual tocopherols with different eluent proportion

Indices (%)	Cyclohexane-ethanol (v/v)					
	100:0	99.9:0.1	99.8:0.2	99.7:0.3	99.6:0.4	99.5:0.5
αT						
Purity	92.60	75.70	86.00	96.20	80.60	58.80
Recovery	30.48	30.42	30.00	33.41	49.83	22.09
γT						
Purity	92.90	90.10	81.00	90.20	91.50	84.10
Recovery	28.12	36.71	44.00	36.99	38.92	19.14
δT						
Purity	0.00	87.00	76.00	88.30	85.50	81.60
Recovery	0.00	28.82	60.00	60.64	57.86	52.09

The injected volume was 3 ml and sample concentration was 1 g/ml, the flow-rate was 20 ml/min. Purity and recovery were calculated according to Eqs. (1) and (2)

Table 2 Purity and recovery of individual tocopherols with different flow-rate of eluent

Indices (%)	Flow-rate (ml/min)			
	10	20	30	40
αT				
Purity	76.80	80.60	88.90	88.20
Recovery	26.18	49.83	35.32	38.92
γT				
Purity	84.50	91.50	93.30	92.50
Recovery	31.17	38.921	47.11	47.13
δT				
Purity	83.70	85.50	91.10	86.00
Recovery	44.44	57.86	58.13	55.74

The injected volume was 3 ml and sample concentration was 1 g/ml, the volume ratio of cyclohexane-ethanol (*v*/*v*) was 99.6:0.4. Purity and recovery were calculated according to Eqs. (1) and (2)

Table 3 Purity and recovery of individual tocopherols with different loading amounts

Indices (%)	Loading amount (ml)			
	1	2	3	4
αT				
Purity	84.50	89.70	80.60	0.00
Recovery	34.70	31.80	49.83	0.00
γT				
Purity	92.90	91.60	91.50	0.00
Recovery	51.20	47.50	38.92	0.00
δT				
Purity	88.10	89.50	85.50	90.40
Recovery	20.10	60.00	57.86	32.68

The volume ratio of cyclohexane-ethanol (*v*/*v*) was 99.6:0.4, the flow-rate was 20 ml/min and sample concentration was 1 g/ml. Purity and recovery were calculated according to Eqs. (1) and (2)

dropped. The maximum was at 30 ml/min (see Fig. 5). At a lower flow-rate, there was more separation time resulting in diffusion of substances in the column. Hence, the separation efficiency was the lowest with the flow-rate at 10 ml/min because of the long separation time. With the flow-rate increasing from 10 to 30 ml/min, the diffusion gradually decreased. Thus, the separation efficiency increased from 10 to 30 ml/min. When the flow-rate was at 40 ml/min, the flow-rate was too fast to have enough time for separation which led to lower separation efficiency.

There was a similar trend for the loading amount. With an increasing loading amount, the separation efficiency also increased and then dropped. In general, the separation efficiency should decrease as the loading amount increases because it gradually exceeds the capacity of the column. However, it was not observed when the loading amount

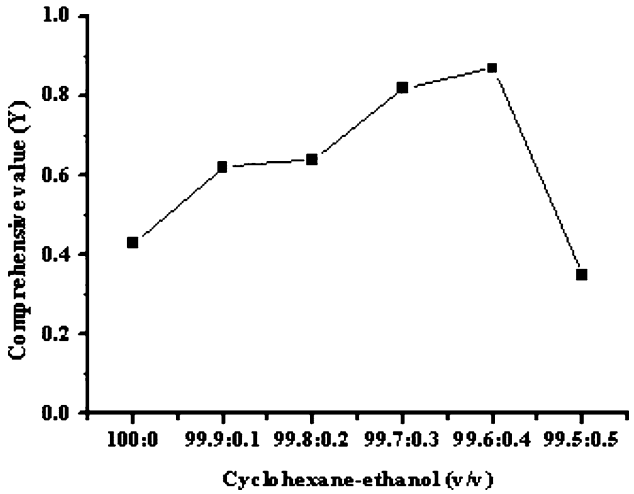

Fig. 4 Effect of eluent proportion on separation efficiency. Column, 500 mm × 25 mm (I.D.) glass column packed with silica (100–200 mesh); loading amount, 3 ml (sample concentration was 1 g/ml); flow-rate, 20 ml/min

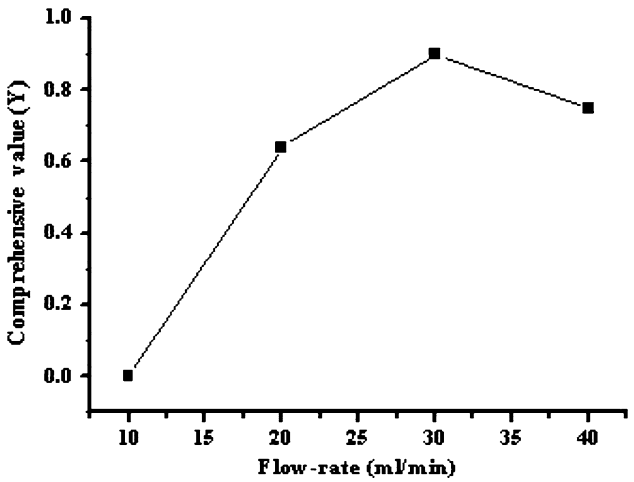

Fig. 5 Effect of flow-rate of eluent on separation efficiency. Column, 500 mm × 25 mm (I.D.) glass column packed with silica (100–200 mesh); loading amount, 3 ml (sample concentration was 1 g/ml); eluent proportion, cyclohexane-ethanol (99.6:0.4, *v*/*v*)

was 1 ml in our result (see Fig. 6). As the total waste was nearly constant regardless of the loading amount, relative waste would decrease as the load increased. Thus, the separation efficiency was improved when the loading amount increased from 1 to 2 ml. Nevertheless, it dropped when the loading amount was more than 2 ml, which was caused by overload resulting in an overlap of neighboring peaks and a decrease in separation efficiency. When the injected volume was 4 ml, only δT was obtained because of serious overload (see Table 3).

To sum up, the separation efficiency varied with changes in the eluent proportion, the flow-rate and the loading amount.

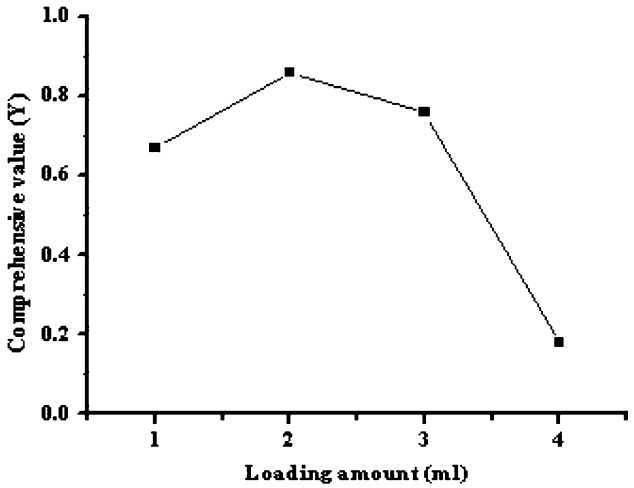

Fig. 6 Effect of loading amount on separation efficiency. Column, 500 mm × 25 mm (I.D.) glass column packed with silica (100–200 mesh); eluent proportion, cyclohexane-ethanol (99.6:0.4, *v*/*v*); flow-rate, 20 ml/min; sample concentration, 1 g/ml

Table 4 Factors and levels for orthogonal test

Levels	Factor *A* Cyclohexane-ethanol (*v*/*v*)	Factor *B* Loading amount (ml)	Factor *C* Flow-rate (ml/min)
1	99.7:0.3	2.5	25
2	99.6:0.4	3	30
3	99.5:0.5	2	35

Sample concentration was 1 g/ml

Result and Analysis of Orthogonal Test

We found that there should be an optimum condition leading to the highest separation efficiency. It was very important for preparative separation to look for these optimum parameters for reducing the cost. The effect of changing single factors on the separation efficiency was studied as above (see Figs. 4, 5, 6). However, it was not enough to judge what parameter was optimum because other factors were fixed under this condition. The optimum parameters should be obtained by using reasonable test design and mathematical analysis.

The orthogonal test was designed to optimize parameters on the basis of the experimental results. Three factors, eluent proportion (*A*), loading amount (*B*) and the flow-rate (*C*), were selected for optimization. Three levels of each factor were investigated. The levels were adjusted on the base of single factor experiment result. The selected factors and levels are listed in Table 4.

Purity and recovery were direct investigated indices. The comprehensive value (*Y*) calculated according to Eqs. (1)–(6) in the section on the procedure of column chromatography was used for analysis by statistical methods.

The results of the orthogonal test and extreme difference analysis are presented in Table 5. The analysis of variance was performed by statistical software SPSS 13.0 and the

Table 6 The results analysis of variance

Source	Sum of squares	*df*	Mean square	*F*	Sig. (*P*)
Model	3.454[a]	7	0.493	83.484	0.012
A[b]	0.116	2	0.058	9.780	0.093
B[b]	0.017	2	0.008	1.434	0.411
C[b]	0.130	2	0.065	10.964	0.084
Error	0.012	2	0.006		
Total	3.466	9			

[a] $R^2 = 0.997$ (adjusted $R^2 = 0.985$);

[b] *A*, *B* and *C* refer to factors of eluent proportion (*v*/*v*), injected volume(ml) and flow-rate (ml/min)

Table 5 Result of the orthogonal test and extreme difference analysis

	Factors			αT		γT		δT		*Y*[b]
	A[a]	*B*[a]	*C*[a]	Purity (%)	Recovery (%)	Purity (%)	Recovery (%)	Purity (%)	Recovery (%)	
1	1	1	1	92.20	38.14	89.60	47.74	87.20	61.07	0.79
2	1	2	2	92.60	34.90	90.90	43.08	86.80	54.70	0.70
3	1	3	3	85.80	32.52	90.00	42.51	86.00	60.61	0.59
4	2	1	2	76.80	39.27	89.50	40.84	87.40	51.97	0.49
5	2	2	3	75.30	23.25	87.80	30.15	88.50	51.86	0.17
6	2	3	1	86.50	34.60	89.90	47.04	86.20	59.45	0.65
7	3	1	3	92.30	27.82	90.70	32.74	89.70	46.87	0.55
8	3	2	1	87.40	31.45	90.70	37.48	89.80	59.87	0.74
9	3	3	2	81.80	34.11	90.80	33.04	89.50	61.87	0.68
I	2.08	1.83	2.18							
II	1.31	1.61	1.87							
III	1.97	1.92	1.31							
R[c]	0.77	0.31	0.87							

[a] *A*, *B* and *C* refer to factors of eluent proportion (*v*/*v*), injected volume(ml) and flow-rate (ml/min);

[b] *Y* is the comprehensive valuation for αT, γT and δT which is calculated according to Eqs. (1)–(6) in the text;

[c] *R* refers to the result of extreme difference analysis

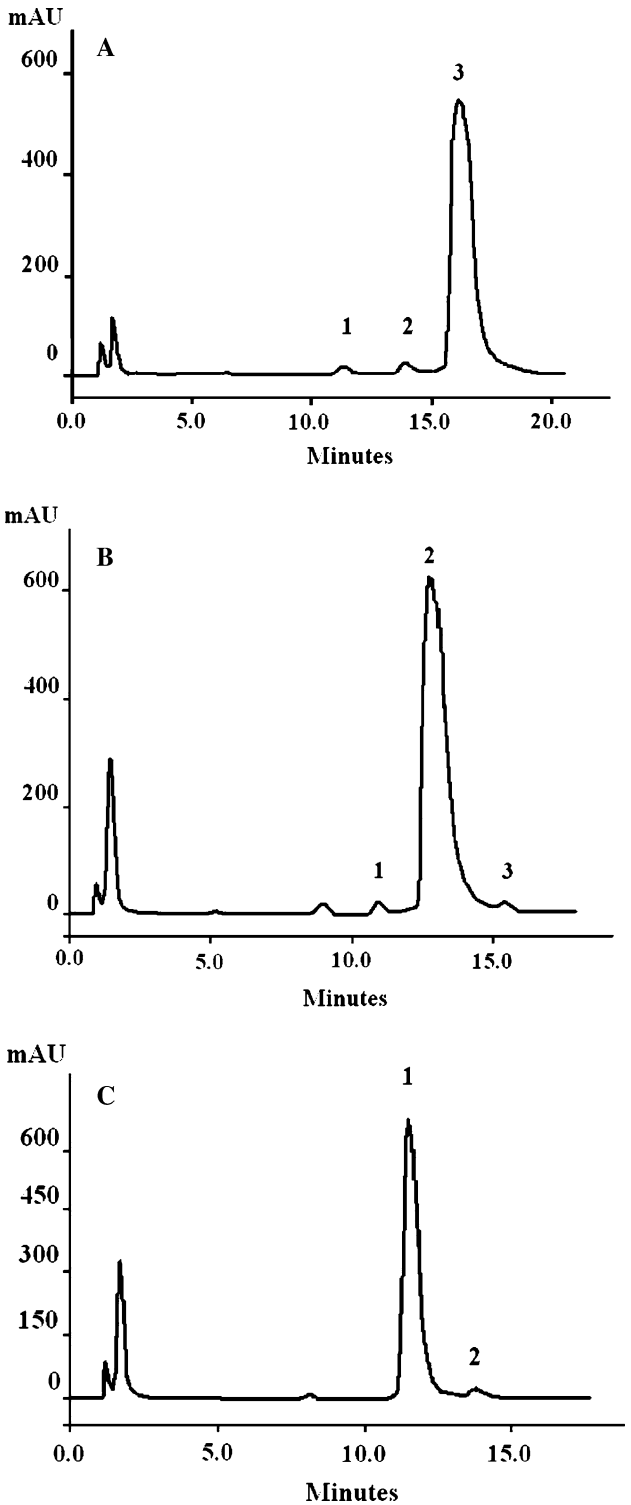

Fig. 7 The HPLC chromatogram of tocopherol individual products of αT (**a**), γT (**b**) and δT (**c**). Column, 150 mm × 4.6 mm ODS column with 5-μm packings; mobile phase, acetonitrile (0.1% formic acid); detection wavelength, 210 nm; injection volume, 100 μl; flow-rate, 1 ml/min. Peaks: (*1*) δT; (*2*) γT; (*3*) αT

results are listed in Table 6. From analysis of extreme differences, the influential order of three factors on separation efficiency was $C > A > B$, and the optimum condition was $A_1B_3C_1$ (see Table 5). The value of P (significance) for the model was 0.012 in analysis of variance, which illustrated that this model was available. Owing to the values of P for three factors being relatively high, it was shown that the contributions of three factors for separation efficiency were slightly different and no significant factor. From the values of P, C ($P = 0.084$) $< A$ ($P = 0.093$) $< B$ ($P = 0.411$), the parameters were different and the order was $C > A > B$ (see Table 6), which agreed with the result of extreme difference analysis.

Integrating the results of extreme difference and variance analysis, the optimum parameters were obtained as the followed: cyclohexane-ethanol (99.7:0.3, *v*/*v*), loading amount 2 ml, flow-rate 25 ml/min. Under the above optimum condition, purity of the αT, γT and δT products were 92.35, 91.23, 89.95%; the recovery of those products were 35.21, 36.25, 61.25%, respectively. The HPLC chromatograms of three individual tocopherols are shown in Fig. 7a–c.

The recoveries of αT and γT were rather low (only about 35%), which was a result of the effect of some impurities and the small scale (relative waste being high). For improving recovery, it will be necessary to further research pre-treatment methods to remove some impurities and carry out rather large-scale separation. High purity individual tocopherols (about 90%) could be obtained using this new method directly from soybean V_E concentrate (53% purity).

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References

1. Khanna S, Roy S, Slivka A, Craft KS, Chaki S, Rink C (2005) Neuroprotective properties of the natural vitamin E α-tocotrienol. Stroke 36:2258–2264
2. Anderson K, Simmons-Menchaca M, Lawson KA (2004) Deferential response of human ovarian cancer cells to induction of apoptosis by vitamin E succinate and vitamin E analogue, alpha-TEA. Cancer Res 64:4263–4269
3. Jiang Q, Wong J, Fyrst H, Saba JD, Ames BN (2004) γ-Tocopherol or combinations of vitamin E forms induce cell death in human prostate cancer cells by interrupting sphingolipid synthesis. Proc Natl Acad Sci USA 101:17825–17830
4. Cooney RV, Franke AA, Harwood PJ, Hatch-Pigott V, Custer LJ, Mordan LJ (1993) Gamma-tocopherol detoxification of nitrogen dioxide: superiority to alpha-tocopherol. Proc Natl Acad Sci USA 90:1771–1775

5. Yu W, Simmons-Menchaca M, Gapor A, Sanders BG, Kline K (1999) Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. Nutr Cancer 33:26–32
6. Zhou QX, Chen LP (2003) Study of the technology for isolating natural vitamin E from deodorizer distillate obtained from soybean oil. Cereal Food Ind 2:22–23
7. Moraes EB, Batistella CB, Torres Alvarez ME et al (2004) Evaluation of tocopherol recovery through simulation of molecular distillation process. Appl Biochem Biotechnol 114(3):689–711
8. Xian XF, Sun DW (2003) Vitamin E processed using methylation of non-α-tocopherol. Tsinghua Sci Technol 8(6):749–751
9. Janicki J, Olejnik D (1968) Thin-layer chromatography of tocopherols of oil seeds. Plant Foods Hum Nutr 16:230–236
10. Bunnell RH (1971) Modern procedures for the analysis of tocopherols. Lipids 6:245–253
11. Pyka A, Sliwiok J (2001) Chromatographic separation of tocopherols. J Chromatogr A 935:71–76
12. Shin TS, Godber JS (1994) Isolation of four tocopherols and four tocotrienols from a variety of natural sources by semi-preparative high-performance liquid chromatography. J Chromatogr A 678:49–58
13. Abidi SL, Mounts TL (1997) Reversed-phase high-performance liquid chromatographic separations of tocopherols. J Chromatogr A 782:25–32
14. Abidi SL (1999) Reversed-phase retention characteristics of tocotrienol antioxidants. J Chromatogr A 844:67–75
15. Kamal-Eldin A, Görgen S, Pettersson J, Anna-Maija L (2000) Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols: comparison of different chromatographic columns. J Chromatogr A 881:217–227
16. Abidi SL (2000) Chromatographic analysis of tocol-derived lipid antioxidants. J Chromatogr A 881:197–216
17. Ruperez FJ, Martın D, Herrera E, Barbas C (2001) Chromatographic analysis of a-tocopherol and related compounds in various matrices. J Chromatogr A 93:45–69
18. Chang L, Chang H, Sun S (2006) Cyclodextrin-modified microemulsion electrokinetic chromatography for separation of α-, γ-, δ-tocopherol and α-tocopherol acetate. J Chromatogr A 1110:227–234
19. Wu GS (1997) Experiment design and analysis. Metallurgical Industry Press, Beijing