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Short communication

Purification of D-*a*-tocopheryl polyethylene glycol 1000 succinate (TPGS) by a temperature-modulated silica gel column chromatography: Use of Taguchi method to optimize purification conditions

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ABSTRACT

The demand for high purity D-*a*-tocopheryl polyethylene glycol 1000 succinate (TPGS) is increasing with the exploitation of TPGS-related products. Previously, we synthesized a TPGS mixture by esterifying vitamin E succinate with polyethyleneglycol 1000. In this study, a temperature-modulated silica gel chromatographic column was used to purify the synthesized TPGS. Taguchi method was used to optimize purification conditions associated with column temperature, loading amount, feedstock concentration and flow rate of mobile phases. Purification efficacy under the Taguchi optimized conditions was predicted theoretically and the predicted results were verified experimentally. High-performance liquid chromatography was used to quantify the unpurified and purified TPGS. The Taguchi-based analysis separately produced an optimum combination of purification conditions for TPGS purity and recovery. Under the optimized conditions, both the theoretical prediction and the confirmatory experiment yielded TPGS purity and recovery approximating to 98% each. Impressively, the study also found that column temperature had a considerable effect on purification efficacy, in particular on TPGS purity, although it was a less influential factor compared to loading amount and feedstock concentration.

1. Introduction

D-*a*-Tocopheryl polyethylene glycol 1000 succinate (TPGS), which is an amphipathic derivative of vitamin E, is receiving increasing interest in various pharmaceutical and pharmacological applications. TPGS has been successfully used as enhancers of solubility, permeability, dispersibility and bioavailability for poorly water-soluble drugs [1–4]. Excellent potential has also been observed for TPGS as an emulsifier or additive in fabrication of nano-copolymer for promoting drug deliveries [5–7]. The versatile usefulness of TPGS indicates that TPGS-containing products will be widely marketed and available, and that there is a rising demand for TPGS material.

TPGS is commonly synthesized by catalytic esterification of vitamin E succinate (VES) with polyethyleneglycol 1000 (PEG). The synthetic crude TPGS contains several undesirable residues, including un-reacted parent components (PEG and VES) and esterification catalyst. The attachment of these residues in TPGS-containing products could weaken the expected effects (e.g. solubility and bioavailability enhancements) of TPGS introduction. In addition, manufacturing of pharmaceutical drug requires strict control of impurities. However, limited attention has been given to separation of impurities from TPGS mixture.

Column chromatography is widely recognized as an approach to separation of impurities [8–11]. Its purification performance is primarily dependent on operating conditions related to mobile phase, stationary phase, loading amount and chromatographic column. This raises a concern of how to optimize chromatographic conditions to achieve a desirable purification efficacy. Taguchi method has been successfully applied to various manufacturing industries and experimental designs [12–14]. Successful application of Taguchi method is able to allow operating conditions to be optimized with a minimized sensitivity to noise (a hard-to-control variable). In this study, we used a temperature-modulated column chromatographic procedure to purity TPGS that was synthesized previously. The primary focus was on the utilization of Taguchi method to optimize purification conditions to obtain favorable purification efficacy.

2. Materials and methods

2.1. Materials

The standard TPGS (99% purity) used as reference compound was purchased from Eastman Chemical Company (USA). Silica gel

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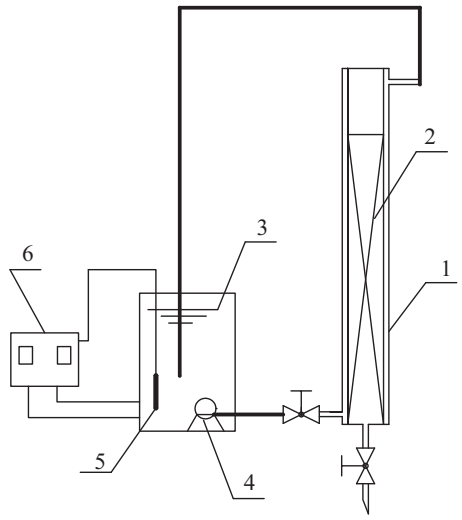

Fig. 1. Configuration of the temperature-modulated chromatographic column used to purify the TPGS mixture (1: chromatographic column with heating jacket; 2: silica gel; 3: water heater; 4: mini-pump; 5: thermocouple; 6: electrical heater controller).

(70–230 mesh) was obtained from Qingdao Haiyang Chemical Co., Ltd., Shandong, China. Ethanol, methanol, ethyl acetate, petroleum ether and chloroform were purchased from Shanghai Chemical Reagent Co., Ltd., Shanghai, China. The crude TPGS used in this study was previously synthesized in our laboratory, containing about 70% of target TPGS.

2.2. Configuration of chromatographic column

The configuration of the temperature-modulated chromatographic column is shown in Fig. 1. The column (inside dia. × ht. = 20 mm × 500 mm) was of double layer with heating jacket. A temperature-controllable electrical heating equipment was used to heat water and record water temperature simultaneously. The water with desired temperatures was pumped into the heating jacket by circulatory mode. The column (inside layer) was packed with approximately 50 g of silica gel that served as stationary phase. The silica gel was activated for 2 h at 150 °C and cooled to room temperature prior to use.

2.3. Loading and eluting

Samples of the crude TPGS were dissolved in several different amounts of methanol solvent. In a preliminary experiment, several different binary and ternary mixed mobile phases were tried and compared. The following mobile phases with a gradient elution mode were thereof determined: petroleum ether–chloroform (9:2, v/v), 550 mL → petroleum ether–chloroform–ethyl acetate (6:2:1, v/v), 600 mL → chloroform–ethyl acetate (7:2, v/v), 450 mL.

2.4. HPLC analysis

The TPGS quantification was conducted using a HPLC system (Waters, US) coupled with a UV detector. A YMC-carotenoid C30 column (4.6 mm × 250 mm i.d., 5 μm) was used for HPLC analysis. The column temperature remained 35 °C and 10 μL samples were injected into the column. Gradient elution was performed using the mobile phases consisting of acetonitrile (A) and isopropyl alcohol (B) at a flow rate of 1.5 mL/min. The elution program was: 65% A and 35% B for 10 min → 50% A and 50% B for 10 min → 35% A and 65% B for 10 min.

Table 1
Determination of factors and levels.

Factors	Level 1	Level 2	Level 3
A: Column temperature (°C)	30	40	50
B: Loading amount (g)	3.0	4.0	5.0
C: Feedstock concentration (g/mL)	0.2	0.3	0.4
D: Flow rate of mobile phases (mL/min)	2.0	3.0	4.0

2.5. Taguchi method

The first step in application of Taguchi method is to arrange a suitable orthogonal array, which depends on the number of factors and their levels to be investigated. In this study, four crucial factors (column temperature, loading amount, feedstock concentration and flow rate of mobile phases) each with three different levels, as shown in Table 1, were investigated as operating variables. Accordingly, a standardized L_9 (3^4) orthogonal array was selected for experimental set-up (Table 2). Each experimental run was performed at least twice to ensure the reproducibility of all the data within ±5% error limit.

Taguchi method recommends analysis of signal-to-noise (*S/N*) ratio that can at least present information on optimum process conditions and relative importance of process factors investigated. Typically, there are three options for *S/N* ratios analysis, including the larger-the-better, the smaller-the-better and the nominal-the better. In this study, the larger-the-better option was selected (the larger of *S/N* ratios, the better of TPGS purification efficacy). Accordingly, the values of *S/N* ratios were calculated using:

$$S/N = -10 \log\left(\frac{1}{n}\sum\frac{1}{y_i^2}\right) \quad (1)$$

where n is the number of replication for each experiment, y_i is the values of TPGS purity (%) or recovery (%) for each trial run.

Another benefit from Taguchi method is that its application allows process performance under optimized condition to be predicted. The prediction treatment is commonly conducted by:

$$\eta_{opt} = m_t + \sum_{i=1}^{q}(\eta_i - m_t) \quad (2)$$

where η_{opt} is the predicted *S/N* ratio, m_t is the overall mean of the *S/N* ratio, η_i is the mean *S/N* ratio at the optimal level, and q is the number of the factors considered.

3. Results and discussion

3.1. Determination of optimum purification conditions

The experimental measurements of the chromatographic purification efficacy indicated by TPGS purity and recovery are listed in Table 2, coupled with the corresponding *S/N* ratios calculated by Eq. (1). The data of *S/N* ratios at the same level for each factor were averaged to obtain level mean *S/N* ratios (Table 3). The maximum mean *S/N* ratio among the three levels of each factor and the difference between the maximum and minimum level mean *S/N* ratios were thereby determined (Table 3), which were used to determine optimum purification conditions and to compare relative importance among the factors, respectively.

It can be perceived from Table 3 that, loading amount (B) was the most important factor influencing TPGS purity, followed by feedstock concentration (C), column temperature (A) and flow rate (D). The optimum conditions for TPGS purity were A3–B2–C2–D2 (Table 3). For TPGS recovery, however, the order of influential importance of the factors was B > C > D > A, and the corresponding

Table 2
Taguchi experimental design and the response values.

Run no.	Factors				TPGS purity		TPGS recovery	
	A	B	C	D	Raw value (%)	*S/N* ratio (dB)	Raw value (%)	*S/N* ratio (dB)
1	1	1	1	1	89.36	39.02	92.48	39.32
2	1	2	2	2	93.72	39.44	97.31	39.76
3	1	3	3	3	78.96	37.95	85.86	38.68
4	2	1	2	3	90.79	39.16	91.59	39.24
5	2	2	3	1	91.28	39.21	89.76	39.06
6	2	3	1	2	88.52	38.94	91.33	39.21
7	3	1	3	2	89.65	39.05	88.61	38.95
8	3	2	1	3	92.84	39.35	94.55	39.51
9	3	3	2	1	90.15	39.10	89.42	39.03

Table 3
Response table of the level mean *S/N* ratios.

Factors	Mean *S/N* ratios of TPGS purity (dB)					Mean *S/N* ratios of TPGS recovery (dB)				
	Level 1	Level 2	Level 3	Max – Min[a]	Rank	Level 1	Level 2	Level 3	Max – Min[a]	Rank
A: Column temperature	38.80	39.10	39.17[b]	0.37	3	39.25[b]	39.17	39.16	0.09	4
B: Loading amount	39.08	39.33[b]	38.66	0.67	1	39.17	39.45[b]	38.97	0.47	1
C: Feedstock concentration	39.11	39.23[b]	38.74	0.49	2	39.35[b]	39.34	38.90	0.45	2
D: Flow rate	39.11	39.14[b]	38.82	0.32	4	39.14	39.31[b]	39.14	0.17	3

[a] The difference between the maximum and minimum level mean *S/N* ratios for each factor.
[b] The maximum level mean *S/N* ratio among three levels of each factor.

optimum conditions were A1–B2–C1–D2 (Table 3). Considering that the mean *S/N* ratio of the factor C at level 2 (39.34) extremely approximated to that at level 1 (39.35), while use of the level 2 instead of the level 1 can achieve a higher purification capacity, the optimum conditions (A1–B2–C1–D2) for recovering TPGS were thereby modified as A1–B2–C2–D2.

3.2. Performance prediction and confirmation

The maximum value of TPGS purity and of TPGS recovery under their individual optimum conditions was predicted by using a combination of Eqs. (1) and (2). A confirmatory experiment was subsequently carried out to verify the resulting prediction. The experimental data matched well with the predicted values for both TPGS purity and recovery (Table 4). This suggests that the optimization combination obtained and the predicted results could be valid.

The two optimized sets of operating conditions (A3–B2–C2–D2 for TPGS purity and A1–B2–C2–D2 for TPGS recovery) differing in column temperature (A) leaded to a perceptible difference in TPGS purification efficacy (Table 4). Although column temperature was less important than loading amount and feedstock concentration, its increase from A1 (30 °C) to A3 (50 °C) contributed to an impressive increase of TPGS purity (approximately 4%). Such increase appears to be important for obtaining high pure TPGS, in particular in the case that other factors (e.g. loading amount and feedstock concentration) have been optimized to exert their influential capacities for enhancing TPGS purification efficacy. Undesirably, the increase in column temperature had a slightly negative impact on TPGS recovery. This led to a dilemma to choose an appreciate column temperature for simultaneously reaching favorable TPGS purity and recovery. A compromise scheme is to use an intermediate temperature (e.g. 40 °C). The feasibility of this solution was discussed in Section 3.3. Another option is to apply a two-step separation procedure with different column temperature for each step, but this is beyond the scope of this work.

3.3. Effects of the operating factors

A further investigation onto the effects of the operating factors on the purification performance was performed by response graph analysis. The response graph (Fig. 2) of TPGS purity and recovery was plotted by using the data of the mean *S/N* ratios in Table 3. Increasing column temperature (A) from 30 °C to 40 °C leaded to a sharp increase in the mean *S/N* ratios of TPGS purity (Fig. 2a), and an appreciable decrease in the mean *S/N* ratios of TPGS recovery (Fig. 2b). However, these responses showed leveling-off tendencies when column temperature increased from 40 °C to 50 °C. It can be extrapolated from Fig. 2 that further increase of column temperature over 50 °C would yield insignificant response to TPGS purity and recovery. These findings could suggest that the appropriate column temperature for the TPGS purification is within the temperature range of 40–50 °C.

Table 4
Results of the Taguchi-based prediction and confirmatory experiment.

	Predicted values	Values from confirmatory experiment			
		Run 1	Run 2	Run 3	Mean ± S.D
Optimal conditions: A3–B2–C2–D2					
TPGS purity (%)	97.84	97.62	97.54	97.24	97.47 ± 0.20
TPGS recovery (%)	96.16	96.78	96.02	95.87	96.22 ± 0.49
Optimal conditions: A1–B2–C2–D2					
TPGS purity (%)	93.87	93.46	94.10	91.83	93.13 ± 1.17
TPGS recovery (%)	98.31	97.29	97.68	98.20	97.72 ± 0.46

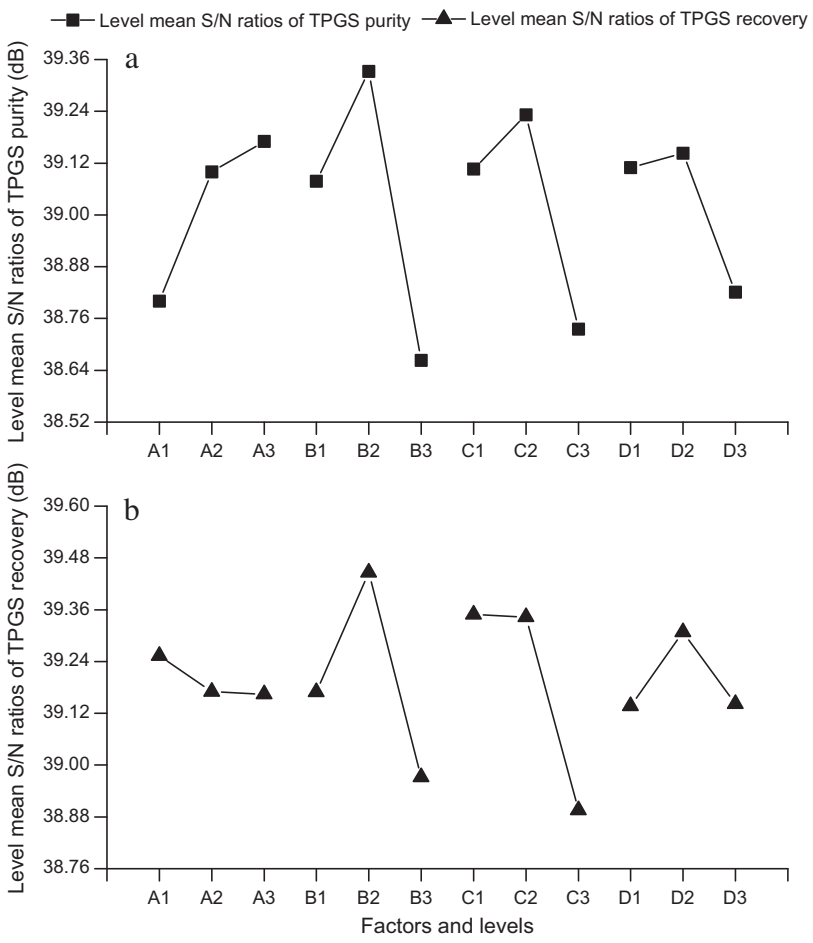

Fig. 2. Response graph for level mean *S/N* ratios of TPGS purity (a) and TPGS recovery (b). A = column temperature; B = loading amount; C = feedstock concentration; D = flow rate of mobile phases.

The dependences of the level mean *S/N* ratios of TPGS purity and of TPGS recovery on loading amount (B) showed a similar trend (Fig. 2). Significant increase in the mean *S/N* ratios was observed when loading amount increased from 3.0 to 4.0 g. However, excessive loading of feedstock resulted in decrease in the mean *S/N* ratios, indicating reduction of TPGS purity and recovery. Fig. 2 also shows that limited increase (from 0.2 to 0.3 mg/L) of feedstock concentration (C) was beneficial for the enhancement of TPGS purity. However, over-high feedstock concentration caused a steep decline for both TPGS purity and recovery. Similar results existed in the relationship of flow rate of mobile phases (D) with purification efficiency.

4. Conclusions

This work employed a temperature-modulated silica gel column chromatography to purify a previously synthesized TPGS mixture. Taguchi method was used to optimize the chromatographic factors including column temperature, loading amount, feedstock concentration and flow rate of mobile phases, and to investigate the factorial effects. The Taguchi-optimized chromatographic separation achieved as much as 98% of TPGS purity and recovery. Loading amount and feedstock concentration were found as the two most influential factors. The study also found that column temperature had a substantial influence on TGPS purification efficacy.

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