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ANALYSIS

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF α-TOCOPHEROL IN INCLUSION COMPLEXES WITH CYCLODEXTRINS

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Abstract: A new high performance liquid chromatography (HPLC)-based method is proposed for the determination of vitamin E, free and in inclusion complexes with natural ß-cyclodextrin and its 2-hydroxy derivative. The method has been validated on the basis of the following parameters: specificity, selectivity, linearity, precision, range and recovery.

Key words: vitamin E, cyclodextrin, HPLC, validation

Generation of free radicals accompanies many biochemical transformations taking place in human organism. Moreover, some environmental factors such as chemical compounds, irradiation of different types, in particular ionizing radiation, can provoke generation of free radicals (1, 2). Living cells have developed mechanisms protecting against the toxic effect of free radicals, such as the antioxidative enzymatic barrier and exogenic and endogenic antioxidants (3). Very effective inhibitor of lipid peroxidation *in vivo* is vitamin E, however, its therapeutic use is limited because of the limited absorption in the alimentary tract (4). Therefore, attempts have been made at transforming the lipophilic molecule of tocopherol in the amphiphilic derivatives that are much easier soluble in body fluids. One of the methods of increasing solubility of therapeutic drugs is to make inclusion complexes with solubility excipients such as cyclodextrins (CD). In these complexes the hydrophobic substance captured inside cyclodextrin is in the state of molecular dispersion in hydrophilic support, which facilitates its solubility (5–8). Cyclodextrins are cyclic oligosaccharides composed of six, seven or eight units of glucopyranose linked by α-1,4-glucoside bonds. The polar cavity of cyclodextrin permits formation of inclusion complexes with hydrophobic substances of different types and in this way it is possible to achieve an increase in solubility in water, reduce the sensitivity to light, oxygen or temperature (5, 8). Such properties have the hydrophilic (methylated or hydroxyalkylated) derivatives of cyclodextrins. The hydrophobic derivatives of cyclodextrin (ethylated or acylated) are used as carriers that slowly release therapeutic drugs of short half-lifetime period (9). In this paper, we propose a new HPLC method for the determination of the content of vitamin E in the inclusion complexes with natural ß-cyclodextrin (ß-CD) and its 2-hydroxypropyl derivative (2-HP-ß-CD).

EXPERIMENTAL

Chemicals

Vitamin E 975 was purchased from Sigma-Aldrich Chemie (Germany), ß-cyclodextrin from Fluka Sigma-Aldrich Chemie (Japan), 2-hydroxypropyl-ß-cyclodextrin from Fluka Sigma–Aldrich Chemie (USA) and methanol Lichrosolv for liquid chromatography was from Merck KGaA (Germany). All the reagents were analytically and chromatographically evaluated.

Chromatographic conditions

Chromatographic process was conducted using a high-performance liquid chromatograph (Agilent Technologies series 1200 with DAD detector). Chromatographic separation was performed in the reversed phase mode with UV detection on a column (LiChroCART 250-4) packed with LiChrospher 100 RP-18 (5 μm). The results were collected by com-

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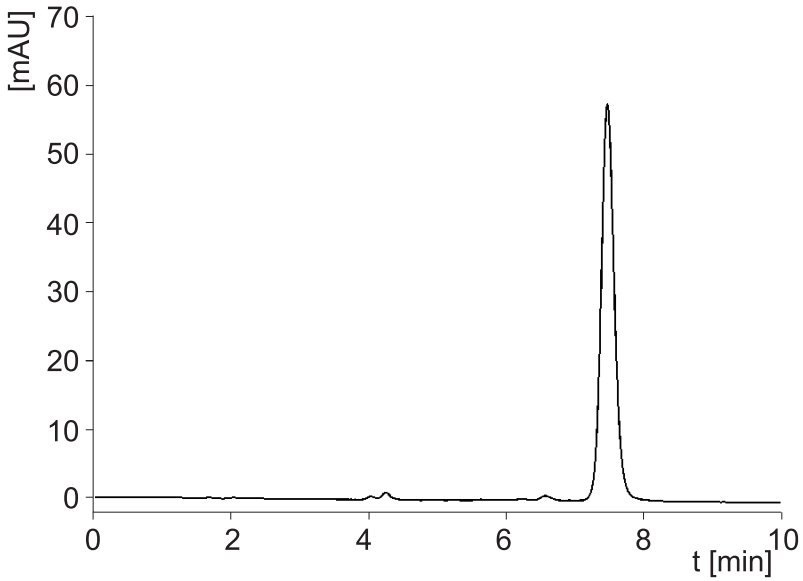

Figure 1. An example of vitamin E chromatogram

Table 1. Statistical evaluation of the linear relations y = ax + b, y = ax.

No.	C [mg/mL]	P	Statistical evaluation of	
			y = ax + b	y = ax
1	0.15	813.27509	a = 5422 ± 204.16 b = 1.7842 ± 49.7 r = 0.9994 Es = 18.67	a = 5428.385 ± 89.26 r = 0.999 Es = 16.73
2	0.2	1086.34619		
3	0.25	1381.99723		
4	0.3	1601.14688		
5	0.375	2040.97494		

C –concentration, P – peak area, r – correlation coefficient, Es – standard error.

Table 2. Precision of the determination of vitamin E by HPLC method.

No.	Volume of 0.25 mg/mL vitamin E solution [mL]	Peak area [mAU·s]	Vitamin E content [mg/mL]	Statistical evaluation [mg/mL]
1	1.0	1260.64771	0.23	c_{av} = 0.2286 $E_s = 1.709\times10^{-3}$ RSD = 7.477×10^{-3}
2		1243.25720		
3		1234.90845		
4		1237.40662		
5		1238.74255		
6		1245.94653		
7		1243.54590		
8		1226.67041		
9		1240.02222		

c_{av} – average concentration, E_s – standard error of simple result; RSD – relative standard deviation.

puter connected to the chromatograph. Free and complexed (ß-CD and 2-HP-ß-CD) vitamin E was analyzed at the following parameters of the chromatographic system: mobile phase: 100% methanol; flow rate of the mobile phase of 1.3 mL/min; detector wavelength: 292 nm; thermostated column temperature: 25°C; injection volume: 20 µL.

Sample preparation

Samples of vitamin E were prepared by weighing a 25 mg portion of vitamin E and placing it in a measuring flask of 10.0 mL in capacity. This portion of vitamin E was made to 10.0 mL with methanol. Then, 1.0 mL of so prepared solution was collected and diluted to 10.0 mL. To prepare samples of inclusion complexes with cyclodextrins, portions of 10 mg of each complex were weighed and made to 10.0 mL with methanol. The solutions were filtered prior to injection onto the column.

Validation of HPLC method

The following parameters of the method were subjected to validation: specificity, selectivity, linearity, precision, range of method and recovery.

Specificity

Chromatograms of the samples studied were recorded using the following wavelengths of the detector operation: 254, 280, 292, 294 and 296 nm. Taking into regard the clarity of the chromatographic image for free vitamin E and for cyclodextrin-complexed vitamin E, we have chosen the detector operation wavelength of 292 nm. At this wavelength no signal from cyclodextrins was observed.

Selectivity

In the time range from 0 to 12 min the chromatograms revealed only the signal assigned to vitamin E. The chromatogram is presented in Figure 1.

Linearity

The standard curve for vitamin E was determined for the concentration range from 0.15 to 0.375 mg/mL, since in this range the detector response was linear. The plot of P = f(c) was made, where: P is the area under the peak assigned to vitamin E and c = concentration of vitamin E [mg/mL]. The parameters of equations y = ax + b and y = ax, were calculated and together with results of statistical evaluation are given in Table 1.

Precision

The method precision expressed by repeatability of peak area and retention time was determined as the relative standard deviation (RSD) calculated form the obtained data (10). In order to establish the precision of the method, a 2.50 mg/mL solution of vitamin E was made. Portions of 1.0 mL were col-

Table 3. Recovery of vitamin E.

No.	Volume of stock solution [mL diluted in 10 mL]	Peak area [mAU·s]	Vitamin E content [mg/mL]	Recovery [%]	Mean recovery [%]
1	0.6	794.09320	0.15	100.68	
2	0.6	809.41431	0.15	102.13	
3	0.6	798.38647	0.15	100.75	100.85
4	0.6	797.71564	0.15	100.69	
5	0.6	801.12449	0.15	100.00	
1	1.0	1293.48303	0.24	98.02	
2	1.0	1303.52051	0.24	98.80	
3	1.0	1297.13745	0.24	98.31	98.49
4	1.0	1298.94653	0.24	98.44	
5	1.0	1304.77295	0.24	98.89	
1	1.5	1941.74890	0.36	98.16	
2	1.5	1942.77539	0.36	98.22	
3	1.5	1939.59302	0.36	98.05	98.15
4	1.5	1940.31885	0.36	98.08	
5	1.5	1943.49927	0.36	98.24	

lected from this solution and placed in 9 flasks of 10.0 mL in capacity and made to 10.0 mL with methanol. The solutions obtained were injected onto a chromatographic column and the chromatograms were recorded. The results are given in Table 2.

Range of applicability

The range of applicability is that between the lowest and highest concentrations of the substance to be determined for which the determination can be performed with linearity and preset precision (11). For this method, the range is from $1.50 \cdot 10^{-1}$ to $3.75 \cdot 10^{-1}$ mg/mL. The detection limit (DL) of vitamin E, found from the formula 3.3 Sy/a, was 4.55×10^{-6} mg/mL and the limit of quantification (QL), calculated from the formula 10 Sy/a, was = 1.38×10^{-5} mg/mL (12).

Recovery

Recovery of pure vitamin E and that bound in inclusion complexes with cyclodextrins was investigated. The accuracy of the method was determined for the vitamin E solution of the concentration of 2.5 mg/mL. Portions of 0.6, 1.0 and 1.5 mL were collected from the stock solution, placed in vials and made to 10.0 mL with methanol. Five determinations were made from each diluted solution. The 97 percent of content pure vitamin E in reagent was taken into consideration in the calculations. Results are presented in Table 3.

At the next step, the inclusion complexes were made of vitamin E and ß-CD or 2-HP-ßCD at the 1:1 molar ratio and the recovery of vitamin E from the complexes was determined. Portions about 10 mg for each inclusion complex were diluted in 10 mL of methanol. Results are given in Tables 4 and 5.

RESULTS AND DISCUSSION

To the best of our knowledge, in the literature there are no reports on inclusion complexes of vitamin E with natural cyclodextrin and its derivatives; in particular, no methods on the determination of the content of this vitamin in the inclusion complexes. Therefore, to perform this, it was necessary to choose and optimize an analytical method that would permit determination of vitamin E content in complexes with cyclodextrins. Having analyzed the available GC and HPLC methods for the determination of free vitamin E, the HPLC method was selected and modified to adapt for determination of vitamin E in inclusion complexes. This choice was supported by the fact that the HPLC method can be applied for the determination of the vitamin in a mixture, without a preliminary purification thus protecting it against the losses (13).

Validation of the method has shown that it is:
– linear in the concentration range from $1.50 \cdot 10^{-1}$ mg/mL to $3.75 \cdot 10^{-1}$ mg/mL; the correlation coefficient characterizing the linearity is 0.999;
– precise – low variability (coefficient of variation: 0.75%);
– sensitive – an increase in the measured value is detectable for a very small change in the determined value.

Analysis of concentration changes as a function of time has shown that the HPLC method is selective. In the analyzed spectral range, cyclodextrins give no signal. Under conditions applied, vitamin E was separated from ß-CD and 2-HP-ß-CD. The recovery of pure vitamin E from the inclusion complex with natural ß-CD is in agreement with FDA (Food and Drug Administration) recommenda-

Table 4. Recovery of vitamin E from the inclusion complex with β-CD.

No.	Portion of complex [mg/10 mL]	Peak area [mAU·s]	Vitamin E content [mg/mL]	Recovery [%]	Mean recovery [%]
1	10.41	1511.38220	0.28	100.18	99.53
2	10.37	1486.26440	0.27	98.88	

Table 5. Recovery of vitamin E from the inclusion complex with 2-HP-β-CD.

No.	Portion of complex [mg/10 mL]	Peak area [mAU·s]	Vitamin E content [mg/mL]	Recovery [%]	Mean recovery [%]
1	10.78	1309.59497	0.24	95.75	92.81
2	10.52	1199.7356	0.22	89.87	

tions admitting error in the range 100 ± 2%. The earlier described HPLC method for the determination of free uncomplexed vitamin E (14, 15) was characterized by a long retention time (30 min), which significantly extended the time of determination, especially when many determinations were needed. According to the ICH (International Conference on Harmonization) directives and recommendations, the kinetic evaluation of the stability of drugs (so also vitamin E) involves multiple determinations of the therapeutic substance (16). In the HPLC method proposed, the composition of the mobile phase was changed: water and acetonitrile was eliminated to the advantage of methanol. This change brought about a considerable shortening of the retention time of vitamin E to 7 min, after water elimination. Furthermore, elimination of acetonitrile resulted in higher percentage of recovery and had a good economical aspect (17). In the proposed method, 100% of the mobile phase is methanol which is relatively safe for the natural environment, profitable for economical reasons and it also allows to shorten the time and reduce the facilities of preparing the uncomplicated mobile phase.

CONCLUSION

The HPLC method proposed and described above can be used for the determination of vitamin E, both free and complexed with cyclodextrins. The presence of cyclodextrins does not interfere with the determination of vitamin E as the former do not give any signals in the spectral range analyzed.

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