Determination of α-Tocopherol in the Traditional Chinese Medicinal Preparation Sea Buckthorn Oil Capsule by Non-aqueous Reversed Phase-HPLC

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A non-aqueous reversed phase HPLC was developed for determining α-tocopherol in Sea buckthorn oil capsule without the need for saponification. A reversed phase column (Alltima C_{18}, 4.6×250 mm, 5 μm) was used with a mobile phase of methanol–acetonitrile (95 : 5, v/v) and flow rate of 1 ml/min. The contents in capsule were extracted with *n*-hexane. Detection wavelength was set at 292 nm. Each analysis requires no longer than 20 min. The linearity range for α-tocopherol was 9.4—47.0 μg/ml. The detection limit was 0.94 μg/ml. The mean recovery was 95.82 (RSD 2.3%). This method is suitable for quantitative analysis of α-tocopherol in Sea buckthorn oil or its Traditional Chinese Medicinal preparation.

Key words α-tocopherol; sea buckthorn oil capsule; reversed phase-HPLC

Sea buckthorn oil capsule is a Traditional Chinese Medicinal (TCM) preparation, which is mainly composed of Sea buckthorn oil and two plant drugs, *Radixet Caulis Acanthopanacis Senticosi* and *Radix Astragali*, according to classical formula. It is commonly used as medicine for strengthening self immunity function, resisting aging and reducing blood fat. Sea buckthorn oil is main ingredient in the formulation, which is a plant oil extracted from dried fruits or seeds of *Hippophae rhamnoides* L. It contains many biological active components. The α-tocopherol is one of major active constituents in the plant oil. The analysis of α-tocopherol in oil has been examined by reversed phase (RP)-HPLC,[1] normal-phase (NP)-HPLC[2] and gas chromatography (GC)[3] respectively. Many of these methods were very complicated and time-consuming procedures for sample preparation and purification. The Sea buckthorn oil TCM capsule contains many complicated chemical constituents, the determination of α-tocopherol in capsule is more difficult than of single plant oil because of the interference caused by other ingredients in the formulation. Therefore, in this paper, we have developed a non-aqueous reversed phase HPLC (NARP-HPLC) method for the quality analysis of α-tocopherol in Sea buckthorn oil capsule. The results indicate that the method is specific, accurate as compared to NP-HPLC and GC. The procedure was simple without need for saponification and purification. The structure of α-tocopherol is shown in Fig. 1.

Experimental

Materials and Reagents α-tocopherol was purchased from Sigma, U.S.A. Sea buckthorn oil was obtained from Bengjing Xing-Long Associate Inc. (China). Plant material of *Radixet Caulis Acanthopanacis Senticosi* was grown in the experimental farm of Dongbei area (China), *Radix Astragaliseu Hedysari* comes from Shanxi province. Methanol, ethanol and acetonitrile (HPLC grade) were used. The *n*-hexane was of analytical-reagent grade. Sea buckthorn oil capsule was developed by department of Pharmaceutics, China Pharmaceutical University.

HPLC Systems The HPLC system consisted of two delivery pumps (Shimadzu LC-10AD, Japan), a UV detector (Shimadzu, LC-10A vp, Japan), a model 7725i manual injector valve with a 20 μl sample loop. The signals from the detector were connected and analyzed with a computer equipped with a software of N-2000 system (Zhejiang University).

An alltima C_{18} column (4.6×250 mm, 5 μm, Alltech Associates Inc.) was used. The mobile phase was composed of methanol (A)–acetonitrile (B) (95 : 5, v/v). The solvents were filtered through a 0.45 μm Millipore filter and degassed prior to use. A constant flow-rate of 1 ml/min was used during analysis. The detector wavelength was set at 292 nm and column temperature was maintained at 30 °C.

Standard Curve Preparation Freshly preparated solution of α-tocopherol in ethanol (0.2 mg/ml) was used as standard stock solution. Then, different volume of above solution were taken into 5 ml volumetric flasks respectively and diluted with non-aqueous ethanol. Linearity of the responses was determined for five concentrations with three injections for each level. The standard curve was based on the concentration (*C*, μg/ml) to peak area (*A*) of α-tocopherol.

Sample Preparation The contents of six capsules were transferred into a flask and dissolved with 40 ml *n*-hexane in an ultrasonic bath for 30 min at 30 °C, and filtered into 50 ml volumetric flask. The residue was washed using suitable amount of *n*-hexane and filtered into the same flask as stock solution. A 5.0 ml of the above solution was transferred into a 5 ml volumetric flask and was dried in an atmosphere of pure nitrogen. The residue was dissolved and diluted to mark with non-aqueous ethanol as sample solution.

Twenty microliters of the sample solution was injected to HPLC column and analyzed under above chromatographic condition. The contents of α-tocopherol was calculated by external standard.

Interference Tests In order to check in the interference from other drugs and excipients used in the formulation, *Radixet Caulis Acanthopanacis Senticosi* and *Radix Astragali* were weighted and prepared according to the formula to the negative control solution.

Results and Discussion

Selection of α-Tocopherol for Quality Control There are many active constituents, such as Vitamin E, in Sea buchhorh oil. Vitamin E occurs in eight different forms (α-, β-, γ- and δ-tocopherols and α-, β-, γ- and δ-tocotrienols) with varying biologic activities. Of these eight compounds, α-tocopherol was reported to have the highest biological activity and the highest contents. It was also reported that the existence of α-tocopherol related to pharmacology effects of the plant oil for resisting aging, reducing blood fat and restoration to health *et al.*[4,5]

α-tocopherol was selected for quality control, because: (1)

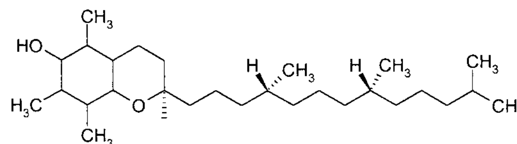

Fig. 1. Structure of α-Tocopherol

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β-tocopherol and γ-tocopherol have the similar structure, their retention time were draw near and the two peaks were overlapped. Further research work on separation of the two constituents are in processing. (2) The main effect of δ-tocopherol was the antioxidative effect and the content of it in plant oil was low.

Optimization of Chromatographic Condition The composition of the mobile phase was optimized using different proportions of methanol, methanol–acetonitrile, methanol–water, and the final result was methanol–acetonitrile (95 : 5, v/v). Because tocopherol is nearly insoluble in water, aqueous elution solvent, such as methanol–water (95 : 5, v/v) result in higher column pressure, baseline drift and bad resolution. The final result was methanol–acetonitrile (95 : 5, v/v). Figure 2 illustrates the separation of α-tocopherol in sample extract (A), negative control extract (B) and standard (C). Experimental results showed that the peak at 10 min was δ-tocopherol, the peak at 12 min was the mixture of β-tocopherol and γ-tocopherol in addition to α-tocopherol in Fig. 2(A). The peak corresponding to α-tocopherol was symmetrical. The peak purity tested by using a photodiode array detector showed the result was satisfactory (purity factor 999).

Linearities and Sensitivities of the Method Linearity was determined by using five concentrations in the range of 9.4—47.0 μg/ml. Linear regression equation: $A=1.4514\times10^3C+1.5948\times10^3$, correlation coefficient (r) was 0.9998. These values indicated good linearity in the examined range. Limit of detection (LOD, Signal/Noise=3) and limit of quantitative (LOQ, Signal/Noise=10) of α-tocopherol from a standard solution was 0.94 μg/ml, 3.10 μg/ml respectively.

In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed. Various amounts of α-tocopherol was added to the known amounts capsule contents, and the mixture was analyzed by the described method. From the amount of standard found, the percentage recovery was calculated. The results were shown in Table 1. High percentage recovery data indicated that the method is deemed to be accurate.

Six assay preparations from one batch were analyzed. The RSD of the six results was 2.1%.

The stability of the analytical solution was determined at 0, 4, 6 and 8 h. The RSD of peak area of α-tocopherol was 1.95%. The solution was considered to be stable for 8 h (store at 5 °C).

Assay in Capsule Dosage indicated that the proposed method can be used for quantitative analysis of α-tocopherol. The results are shown in Table 2.

Comparision of Three Analytical Methods As compared to NP-HPLC and GC, the NARP-HPLC method was considered a relative convenient and was free from the interferences of the other constituents in the sample.

Chromatographic separations of α-tocopherol in sample by NP-HPLC were shown in Fig. 3. NP-HPLC conditions were as follows: column: Hypersil Silica (4.6×250 mm, 5 μm), mobile phase: n-hexane–amyl alcohol (98 : 2, v/v).

Figure 3 indicated that separation of α-tocopherol was effected from negative control.

Chromatogram of α-tocopherol by GC was shown in Fig. 4.

GC conditions were as follows: Shamadzu GC-14A, column: DB-5 (30 m×0.32 mm×0.25 μm); column temperature 260 °C, 5 °C/min to 280 °C; injector temperature 300 °C; carries gas: N_2, flow rate 40 ml/min; tail flow 20 ml/min; split ratio 1 : 70; injector volume 2 μl. Figure 4 shown that high temperature was needed to separate of α-tocopherol. Each assay was finished within 40 min by GC.

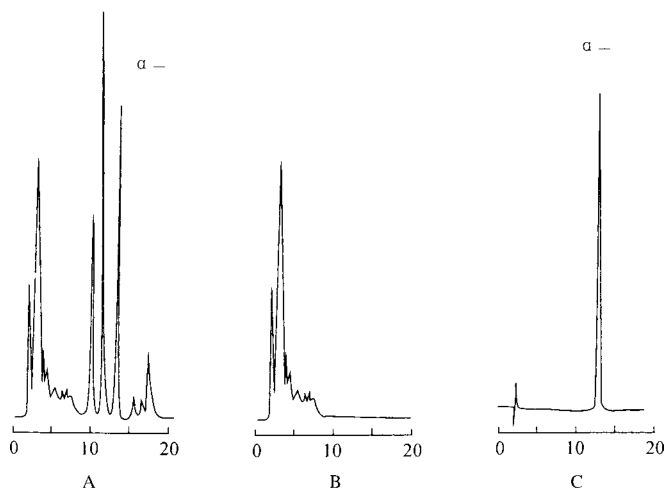

Fig. 2. RP-HPLC Chromatograms of α-Tocopherol in Sample (A), Negative (B) and Standard (C)

Table 1. Recovery of α-Tocopherol in Sample

Sample	No. (n=6)	Added (mg)	Found (mg)	Recovery (%)	Average recovery (%)	RSD (%)
α-Tocopherol	1	0.6110	0.6120	100.2		
	2	0.6110	0.5860	95.91		
	3	0.6110	0.5803	94.98	95.82	2.3
	4	0.6110	0.5740	93.94		
	5	0.6110	0.5781	94.62		
	6	0.6110	0.5821	95.27		

Table 2. Contents (mg/Capsule) of α-Tocopherol in Sample

Batch No.	α-Tocopherol			Mean
010505	0.2245	0.2241	0.2239	0.2242
010507	0.2210	0.2209	0.2207	0.2209
010509	0.2205	0.2210	0.2205	0.2207

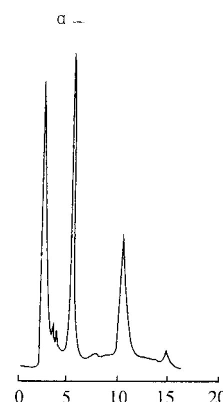

Fig. 3. NP-HPLC Chromatogram of α-Tocopherol in Sample

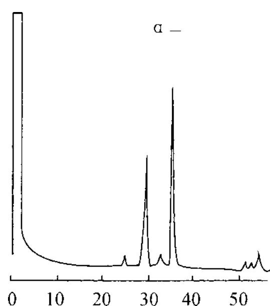

Fig. 4. GC Chromatogram of α-Tocopherol in Sample

Conclusion

The non-aqueous RP-HPLC method gives a good resolution between α-tocopherol and other constituents within a short analysis time (<20 min). The sample preparation is very simple without need for saponification. The persent method can be used as quality control of α-tocopherol in Sea buckthorn oil and TCM preparation.

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