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Short communication

Simple high-performance liquid chromatographic method for determination of α-tocopherol in human plasma

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Abstract

A simple high-performance liquid chromatographic method using UV detection was developed for the determination of α-tocopherol in human plasma. The method entailed direct injection of the plasma sample after deproteinization using acetonitrile–tetrahydrofuran (3:2). The mobile phase comprised methanol–tetrahydrofuran (94:6) and analysis was run at a flow-rate of 1.5 ml/min with the detector operating at 292 nm. A Crestpak C18S (5 μm, 250 mm×4.6 mm ID) was used for the chromatographic separation. The method had a mean recovery of 93%, while the within-day and between-day coefficients of variation and percentage errors were all less than 7%. The speed, specificity, sensitivity and reproducibility of this method make it particularly suitable for routine determination of α-tocopherol in human plasma. Moreover, only a small sample plasma volume (100 μl) is required for the analysis.

Keywords: α-Tocopherol; Vitamin E

1. Introduction

Vitamin E, and particularly α-tocopherol, is an important natural antioxidant in biological systems and is probably one of the most important fat-soluble chain breaking antioxidants in vivo [1]. Several papers have assessed the effect of Vitamin E administered with the aim of reducing the incidence of severe diseases, such as retrolental fibroplasia, intra ventricular haemorrhage, bronchopulmonary dysplasia and haemolytic anaemia [2–4].

Various high-performance liquid chromatographic methods for the determination of α-tocopherol in biological fluids have been described in the literature [5–13]. Many of these methods utilized ultraviolet detection [5–9], while others used fluorescence [10] and mass spectroscopy [11]. Gonzales et al. [12] and Sommerburg et al. [13] have also reported the use of a photo-diode array detector. However, all the above methods involved an extraction procedure in their sample preparation by first deproteinizing the plasma sample followed by extraction using a suitable organic solvent. In some cases, a double extraction was employed to improve the drug recovery [12,13]. Moreover, in the method of Gonzales et al. [12] the column was required to be heated to 50°C, to enable baseline separation of interfering substances during isocratic elution. Direct injection of the plasma sample after deproteinization with methanol has been reported by Cooper et al. [14]. However, this method requires the plasma samples to be pre-treated with magnesium chloride and sodium tungstate to enhance the recovery of α-tocopherol. Moreover, a relatively

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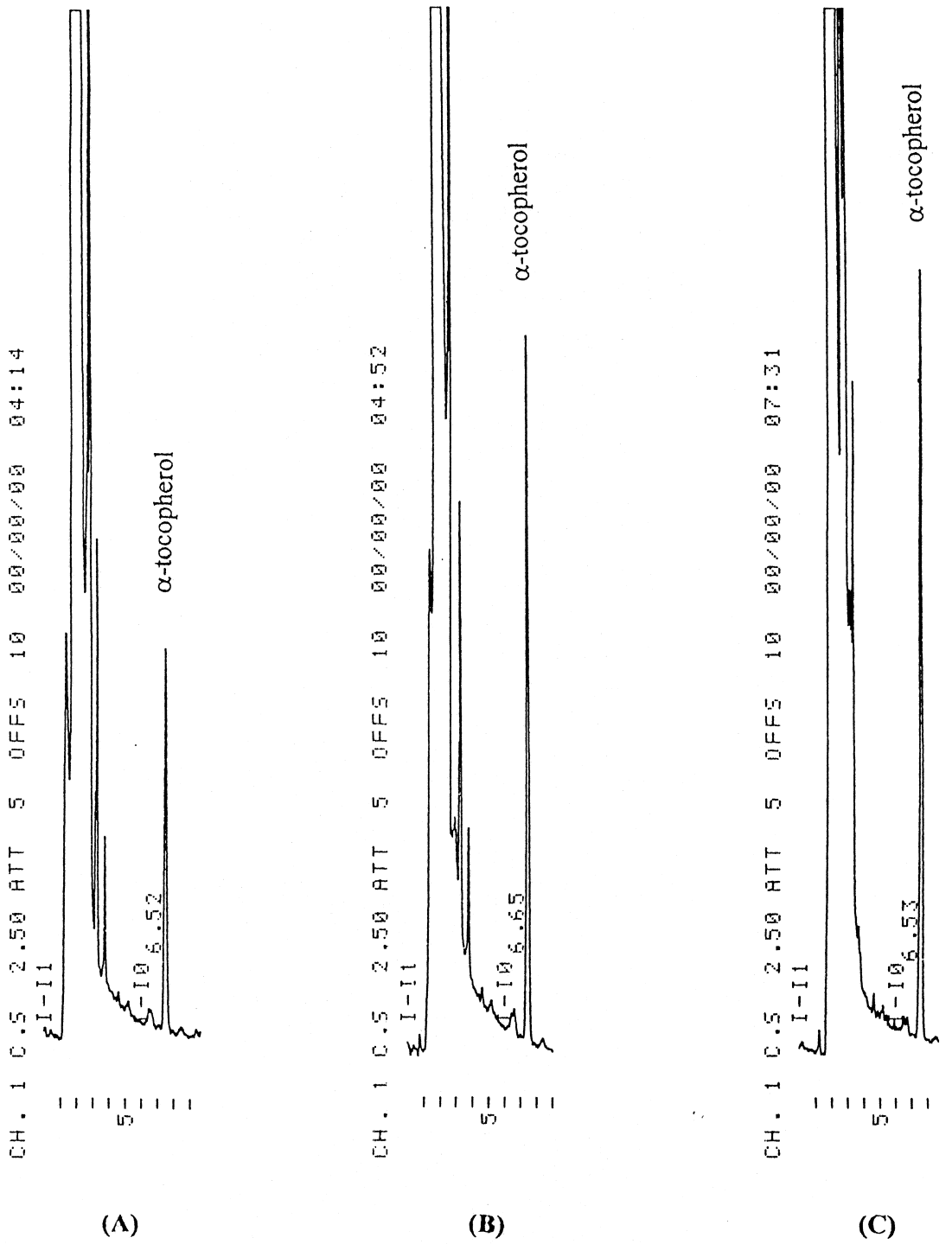

Fig. 1. Chromatograms for the analysis of α-tocopherol in plasma. (A) Blank plasma. (B) Plasma spiked with 6.75 μg/ml α-tocopherol. (C) A volunteer plasma obtained 5 h after oral administration of 400 IU of α-tocopherol. (*y*-axis: attenuation, 5; *x*-axis: chart speed=2.5 mm/min).

large volume (1 ml) of methanol was used to deproteinize 100 μl of plasma resulting in a decrease in the sensitivity of the assay method.

In this paper, we report a rapid, specific and sensitive HPLC method which requires a simple sample preparation step for the determination of α-tocopherol in human plasma using UV detection at room temperature. We also demonstrated the applicability of this method in a bioavailability study.

2. Experimental

2.1. Materials

d-α Tocopherol was purchased from Sigma (St. Louis, MO, USA), methanol (analytical grade) from Mallinkrodt Baker Inc. (KY, USA), tetrahydrofuran (HPLC grade) from Merck (Darmstadt, Germany) and acetonitrile (analytical grade) from R&M Chemicals (Essex, UK).

2.2. Instrumentation

The HPLC system comprised a Jasco PU-980 pump (Jasco, Tokyo, Japan) and a Gilson 119 UV/Vis detector (Gilson Medical Electronics, Villiers-le-Bel, France) equipped with a Hitachi D-2500 integrator (Hitachi, Tokyo, Japan) and Rheodyne 7161 sample injector fitted with a 50 μl sample loop. The detector was operated using a sensitivity range of 0.005 AUFS, output 15 mV and wavelength 292 nm. A Crestpak C18S column from Biosains, Kuala Lumpur, Malaysia (5 μm, 250 mm×4.6 mm I.D.) fitted with Perisorb RP-18, 30–40 μm pellicular stationary phase guard column (Upchurch Scientific, Oak Harbour, WA, USA) was used for the chromatographic separation. The mobile phase consisted of 6% tetrahydrofuran in methanol. Analysis was run a flow rate of 1.5 ml/min and the samples quantified using peak height.

2.3. Sample preparation

Prior to analysis, the plasma samples were treated using the following procedure: 100 μl plasma was measured into a eppendorf microcentrifuge tube and deproteinized using 200 μl of a mixture of acetonitrile and tetrahydrofuran (3:2). The mixture was vortexed for 2 min using a vortex mixer (Stuart Scientific, UK), and centrifuged at 12 800 *g* for 20 min (Eppendorf, USA). 50 μl of the clear supernatant was then injected onto the column.

2.4. Assay validation

Stock solution of α-tocopherol was prepared in methanol and a standard calibration curve was constructed by spiking pooled plasma with a known amount of α-tocopherol at a concentration range of 0.42–13.50 μg/ml. The plasma standards were found to be stable after one month with no significant change in concentration when stored at −20°C in ambered bottles filled with nitrogen. These spiked plasma samples were also used to determine the within-day and between-day accuracy and precision ($n=6$) of the method. In addition, the absolute recovery ($n=6$) of the method was estimated by direct comparison with solutions of the drug at the same corresponding concentrations after similar treatment with the deproteinizing mixture.

Table 1
Absolute recovery, between-day and within-day precision and accuracy values of the assay methods ($n=6$)

Concentration (μg/ml)	Recovery		Between-day		Within-day	
	Mean (%)	C.V. (%)	Accuracy (%)	Precision (C.V. %)	Accuracy (%)	Precision (C.V. %)
0.42	80.7	5.3	96.6	2.6	95.9	6.3
0.84	91.4	7.9	98.5	2.9	104.3	4.1
1.68	91.9	7.9	101.9	2.8	105.5	3.5
3.37	96.6	2.3	102.9	3.1	103.9	3.4
6.75	97.9	2.0	101.0	1.4	99.2	1.5
13.50	96.6	1.6	100.6	2.7	101.6	1.8

3. Results and discussion

Chromatograms obtained with blank plasma, plasma spiked with α-tocopherol and plasma taken from a volunteer 5 h after an oral dose of 400 IU α-tocopherol are shown in Fig. 1A–C respectively. Referring to Fig. 1A, it can be seen that endogenous α-tocopherol was present in the pooled blank plasma and has a concentration of approximately 7 μg/ml. This value was estimated by extrapolation of the standard curve according to the procedure of the standard addition method [15] and is within the range reported by Cooper et al. [14]. The α-tocopherol peak has a retention time of about 7.5

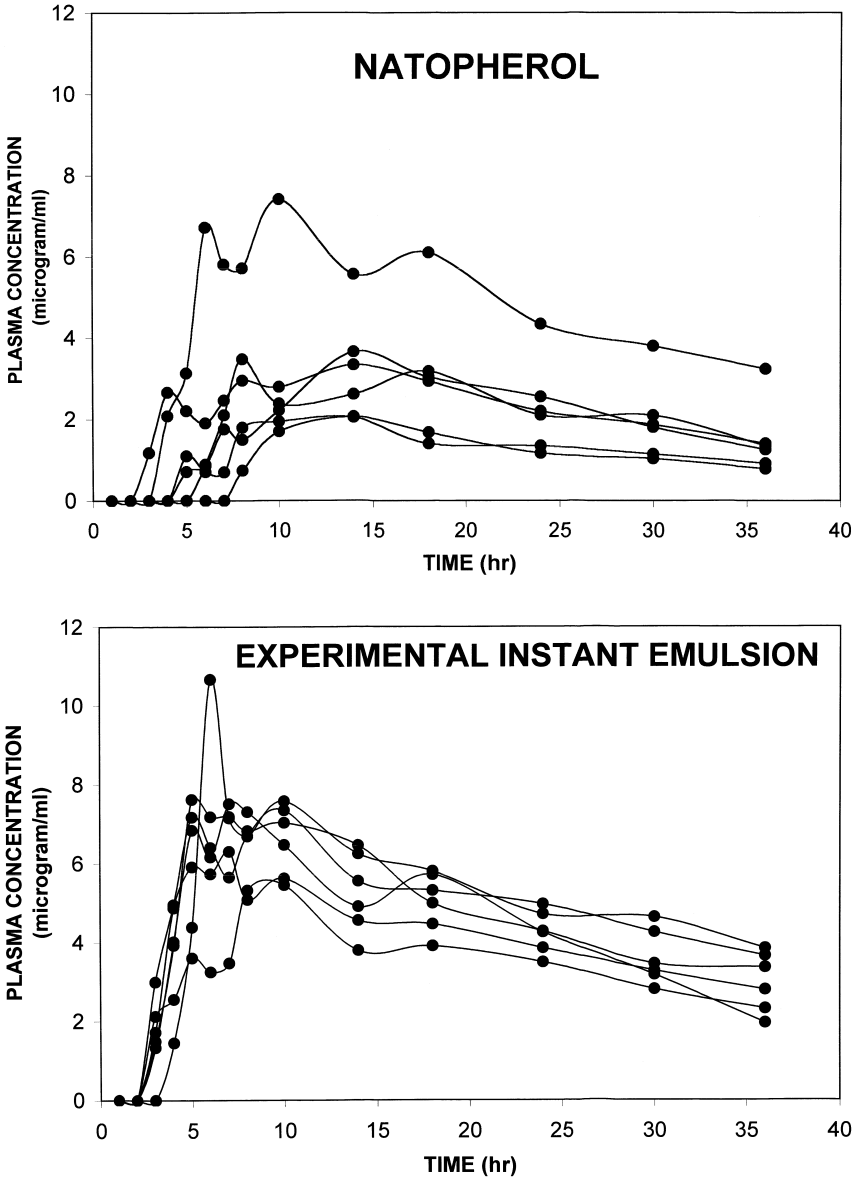

Fig. 2. Plasma concentration versus time profiles from six volunteers following the oral administration of 400 IU Natopherol® and experimental instant emulsion of α-tocopherol (after subtracting from endogenous α-tocopherol).

min and was well resolved from other interfering peaks.

The standard calibration curve after subtracting the basal concentration of endogenous α-tocopherol passed through the origin and was found to be linear with a correlation coefficient value of 0.9998 over the concentration range used.

The absolute recovery, within-day and between-day accuracy and precision values are presented in Table 1. The coefficient of variation (C.V.) values of both the within-day and between-day precision were all less than 7%, while the accuracy denoted by percentage error values were also less than 7%. These values indicate that the assay procedure has satisfactory accuracy and precision. In addition, the method has a limit of quantification of approximately 0.42 μg/ml being comparable to those reported in the literature [9–12].

The method employed in our study involved direct injection of the plasma samples after a simple one step deproteinization procedure. Recovery of α-tocopherol at various concentration values are shown in Table 1. Except for the concentration of 0.42 μg/ml, all the others showed a recovery value of greater than 90%. However, all the recovery values were found to be less than 40% when acetonitrile was used alone as the deproteinizing agent. Addition of tetrahydrofuran was found to enhance recovery, and a ratio of 3 parts of acetonitrile to 2 parts of tetrahydrofuran was found optimal. Further increases in tetrahydrofuran tended to reduce the deproteinizing activity of the mixture. Also the stability of the column was not affected by direct injection of the plasma samples after deproteinization with the present procedure. The resolution as well as retention time of the drug peak remained consistent even after analysis of over 600 samples.

The present method was applied to analyze plasma samples of 6 healthy adult male volunteers from a comparative bioavailability study of two different α-tocopherol preparations, namely Natopherol®, as 400 IU α-tocopherol in soft gelatin capsule and an experimental instant emulsion preparation containing the same dose. Fig. 2 shows the individual plasma concentration profiles of the volunteers obtained with the two preparations.

In summary, the present HPLC method is simple, specific, sensitive and suitable to be used for determination of plasma α-tocopherol in routine measurement as well as in pharmacokinetic/bioavailability studies.

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