Pharmaceutica Analytica Acta

Shintani, Pharmaceut Anal Acta 2013, 4:1
DOI: 10.4172/2153-2435.1000207

Short Communication Open Access

HPLC Analysis of Vitamin E with Electrochemical Detection

Hideharu Shintani*

School of Engineering, Chuo University, Tokyo, Japan

Introduction

Extraction of vitamin E (V.E) from tissue usually involves saponification with ethanolic potassium hydroxide. Direct extraction without saponification, however, is used for plasma. HPLC equipped with a spectro fluorimeter (λex 298 nm, λem 325 nm) or with electrochemical detector (ECD) have enabled measurement of small amounts of tocopherols in biological samples [1-4].

Experimental

Plasma or cell suspensions

Protocol

- 0.5 ml of sample was placed in a tube.
- Phosphate buffer (pH 7.4, 150 mM, 0.5 ml) containing EDTA (0.27 mM) was added.
- Pyrogallol was added in ethanol (6% w/v, 1 ml).
- A solution of tocol (2-methyl 2-phytyl 6-chromanol; 2 μg) was added in ethanol (1 ml) as internal standard.
- It was shaked.
- It was preincubated for 2 min at 70°C.
- KOH was added (60%, 0.2 ml).
- It was incubated for 30 min at 70°C.
- n-hexane and distilled water (2.5 ml) was added. Both reagents were from Wako, chromatography grade.
- It was shaked.
- It was centrifuged for 5 min at 3000 rpm.
- 4 ml of n-hexane layer was taken and evaporated under N_2 gas.
- Ethanol was added (100 ml).
- It was analysed by HPLC.

HPLC was performed with an Irica Instruments chromatograph equipped with 250 mm×4 mm i.d. RP-18 column (Shiseido Co., Sepak C-18). The mobile phase was 100:2:7 (v/v/w) methanol:H_2O:$NaClO_2$ at 1 ml/min. The injection volume was 10 μL. Typical chromatograms detected by ECD 900 mV are illustrated in figure 1.

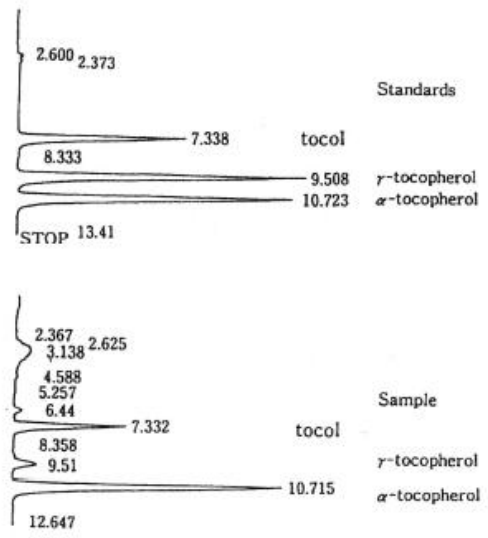

Figure 1: Typical chromatograms obtained from standards and a sample (plasma) detected with electrochemical detection (900 mV).

Results and Discussion

In figure 1 the admixture in plasma were not interfered, indicating pretreatment was attained satisfactory.

Result of HPLC with ECD detection at 900 mV application was presented. ECD detection was selective to analysis for the compounds with tautomerism such as keto ⇄ enol or amin ⇄ imine [5] with relatively lower oxidation-reduction voltage. So in case of V.E analysis, ECD detection can be applicable because phenolic OH ⇄ carbonyl tautomerism exist when voltage applied.

In addition, ECD detection was around more than 10 times sensitive than UV detection [5]. Sensitivity and selectivity of ECD detection was almost identical to fluorescence detection.

References

1. Nishio K, Ishida N, Saito Y, Ogawa-Akazawa Y, Shichiri M, et al. (2011) α-Tocopheryl phosphate: uptake, hydrolysis, and antioxidant action in cultured cells and mouse. Free Radic Bio Med 50: 1794-1800.
2. Yoshikawa S, Morinobu T, Hamamura K, Hirahara F, Iwamoto T, et al. (2005) The effect of gamma-tocopherol administration on alpha-tocopherol levels and metabolism in humans. Eur J Clin Nutr 59: 900-905.
3. Tarng DC, Huang TP, Liu TY, Chen HW, Sung YJ, et al. (2000) Effect of vitamin E-bonded membrane on the 8-hydroxy 2'-deoxyguanosine level in leukocyte DNA of hemodialysis patients. Kidney Int 58: 790-799.
4. Tamai H (1993) Measurement of vitamine E from biological samples. Nihon Rinsho 51: 939-942.
5. Shintani H, Nakamura A (1989) Analysis of a carcinogen, 4,4'-methylenedianiline, from thermosetting polyurethane during sterilization. J Anal Toxicol 13: 354-357.

***Corresponding author:** Hideharu Shintani, 1-10-60, Minamidaira, Hino, Tokyo 191-0041, Japan, Tel: +81425922336; E-mail: shintani@mail.hinocatv.ne.jp

Received January 03, 2013; **Accepted** January 22, 2013; **Published** January 25, 2013

Citation: Shintani H (2013) HPLC Analysis of Vitamin E with Electrochemical Detection. Pharmaceut Anal Acta 4: 207. doi:10.4172/2153-2435.1000207