Journal of Chromatography, 145 (1978) 169—172
Biomedical Applications

CHROMBIO. 096

Note

Determination of plasma α-tocopherol by high-performance liquid chromatography

B. NILSSON, B. JOHANSSON, L. JANSSON and L. HOLMBERG

Department of Clinical Chemistry and Department of Pediatrics, University of Lund, Malmö General Hospital, Malmö (Sweden)

(First received June 10th, 1977; revised manuscript received August 5th, 1977)

Vitamin E, a fat-soluble vitamin, is chemically composed of four forms of tocopherol (α, β, γ, δ). α-Tocopherol is the most abundant member of the series (ca. 12—36 μmole/l plasma) in human serum and is also biologically the most active form. A large number of methods [1—3] exists for the determination of tocopherols in plasma samples but most of these are based on indirect measurement of vitamin E. In such methods carotenoids often interfere with the analysis [3].

In this paper we describe a fast accurate method to determine α-tocopherol in plasma by high-performance liquid chromatography. To our knowledge this technique has not been used for plasma samples. The method has been applied to plasma from healthy individuals as well as to plasma from pre-term newborn infants. Detection of vitamin E deficiency in the plasma of such infants may explain anaemia [4]. The results of this latter study will be reported elsewhere.

EXPERIMENTAL

Materials

n-Hexane (analytical grade, redistilled once before use), diisopropyl ether and isopropanol (spectroscopic grade) were purchased from BDH (Poole, Great Britain). α-Tocopherol was obtained from Merck (Darmstadt, G.F.R.) and the mixture of β-, γ- and δ-tocopherol was a generous gift from AB Ferrosan (Malmo, Sweden). α-Tocopheryl acetate was of purum quality from Fluka (Buchs, Switzerland).

Apparatus

A Waters Model ALC/GPC 204 liquid chromatograph equipped with a U6K loop injector and a Model 440 UV spectrophotometer was used. The outlet of the injector was connected to a stainless-steel column (60 cm × 2 mm I.D.) packed with Corasil I (purchased from Waters Assoc., Milford, Mass., U.S.A.). The column was eluted with *n*-hexane—diisopropylether (96:4) at a flow rate of 60 ml/h. The eluent was de-gassed by ultrasonication for 15 min before use. The absorbance at 280 nm was monitored at a chart speed of 0.5 cm/min. Peak areas were automatically obtained by means of a Varian CDS 111 chromatography data system.

RESULTS AND DISCUSSION

Preparation of standard solutions

Eight standard mixtures of α-tocopherol and α-tocopheryl acetate in *n*-hexane were prepared and analyzed by high-performance liquid chromatography. The solutions were stored under nitrogen in a refrigerator when not in use. The plot of peak area ratios *vs.* concentration ratios for the components in the calibration mixtures shows a good linearity (correlation coefficient, r = 0.9986) for α-tocopherol concentrations in the range 6—60 μmole/l.

Preparation of samples

Human blood collected in EDTA tubes was immediately centrifuged and the plasma fraction was pipetted off. In a typical experiment 500 μl 99.5% ethanol, containing 26.6 μmole/l α-tocopheryl acetate, were added to 500 μl plasma. Five-ml conical centrifuge tubes were used. After the addition of 500 μl *n*-hexane the tube was stoppered and the sample was carefully cyclo-mixed. After centrifugation for 10 min at 30000 *g*, 15 μl of the organic layer was directly injected into the column. The smallest volume of plasma that was extracted, was 100 μl.

Chromatogram

As shown in Fig. 1, α-tocopherol (d) and the internal reference compound (c) are well separated (retention times 5.1 min and 3.3 min, respectively) but there are other peaks in the chromatogram that are not completely resolved. To identify some of these peaks, vitamin K_1, vitamin A, β-carotene and the β-, γ- and δ-tocopherols were injected separately and as a mixture. These compounds were also added one by one to a sample. The compounds which are extractable with *n*-hexane are indicated in the chromatogram (a—e). Unfortunately, γ-tocopherol, which has been determined from plasma samples (after silylation of the phenol group) by gas chromatography [5], could not be completely separated from other compounds in our system. Perhaps the use of a fluorescence detector instead of a UV detector, as used by Van Niekerk [6] and by Abe and co-workers [7,8] in their studies on free tocopherols in plant extracts, would have improved the method. To make sure that no peaks in our system were hidden under the α-tocopherol peak, the sample was recycled several times. No separation into further peaks was observed.

Another mobile system was also tested in which isopropanol (0.15%) was

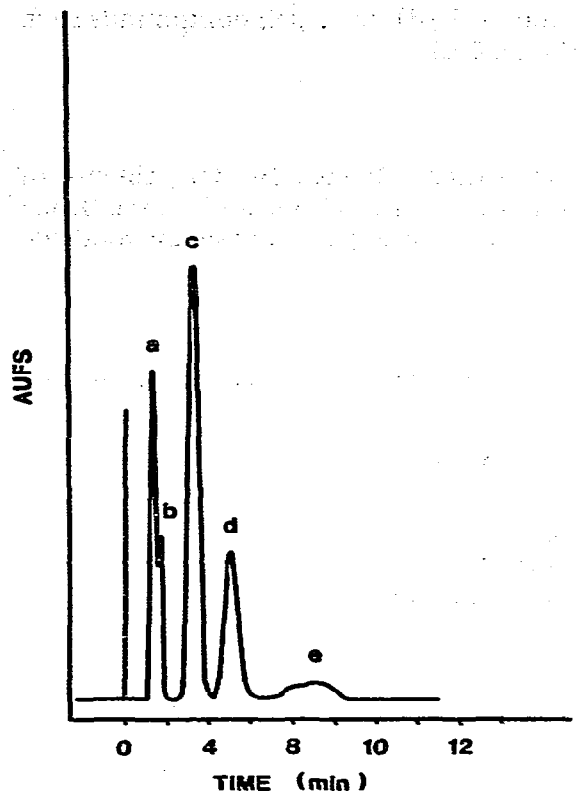

Fig.1. High-performance liquid chromatogram of α-tocopherol (d) in serum Peaks: a, β-carotene; b, vitamin K; c, α-tocopheryl acetate. The broad peak, e, was found to be due to both β- and γ-tocopherol and some other unidentified compounds. Experimental conditions are given in the text.

exchanged for diisopropyl ether (4%). The results of the α-tocopherol determination in this system compared to the one used above agreed within 2.4%. The concentration of α-tocopherol in plasma from fourteen healthy individuals varied between 17.0 and 39.9 μmole/l. This range is in good agreement with that found in the gas chromatographic study [5].

The precision and reproducibility of the method was tested by injection of the same plasma sample three times a day over a period of three days. Small variations in retention times were noticed but this did not disturb the analysis. The mean value of α-tocopherol was found to be 39.9 μmole/l, with an S.D. of 2.31 μmole/l, giving a coefficient of variation of 6.0%.

The lower limit of detection of a sample (5 μl injected) was about 6 μmole/l. The limit could be lowered if α-tocopherol was added to the sample in order to reach the linear range of the method. The recovery of added α-tocopherol to the sample was 100%. The minimum detectable amount of pure α-tocopherol was found to be 8.4 pmole, which value corresponds to twice the noise level.

The method described here is both fast and easy to apply and is suitable for small sample volumes (100 μl). It can be recommended as a complement

to the widely used spectrophotometric method [3] in which compounds such as the carotenoids often interfere with the analysis.

ACKNOWLEDGEMENTS

We wish to thank Mr. L. Malmberg at Varian (Malmo, Sweden) for use of their chromatography data system. Grants from the Greta and Johan Kock Foundation and the Albert Pahlsson Foundation are gratefully acknowledged.

REFERENCES

1 R.H. Bunnell, in R. Gyorgy and W.N. Pearson (Editors), *The Vitamins*, Vol. 6, Academic Press, New York, 2nd ed., 1967, p. 261.
2 R.H. Bunnell, *Lipids*, 6, (1971) 245.
3 J. Fabianek and J. DeFilippi, *Clin. Chem.*, 14 (1968) 456.
4 P.R. Dallman, *J. Pediatr.*, 85 (1974) 742.
5 J. Lehmann, *Lipids*, 6 (1971) 535.
6 P.J. van Niekerk, *Anal. Biochem.*, 52 (1973) 533.
7 K. Abe and G. Katsui, *Vitamins*, 49 (1975) 259.
8 K. Abe and G. Katsui, *J. Nutr. Sci. Vitaminol.*, 21 (1975) 183.