CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Serum Vitamin E

Purpose

Determine Vitamin E (α-tocopherol) in a small sample (25 µL) of serum.

Vitamin E deficiency has been studied mainly in laboratory animals. The manifestations of vitamin E deficiency in these animals are diverse and include infertility, degeneration of the renal tubular epithelium, depigmentation of the incisors, production of red blood cells that are unusually fragile, extensive capillary damage, encephalomalacia, and muscular dystrophy.

Vitamin E deficiency in adult human beings has been described in instances of impaired intestinal lipid absorption (vitamin E is fat soluble). A low serum tocopherol concentration was accompanied by muscular weakness, creatinuria, and red cells that were unusually fragile in the presence of dialuric acid. These symptoms disappeared after administration of α-tocopherol.

In children various neuropathologic and myopathic abnormalities as well as neuromuscular diseases have been associated with vitamin E deficiency. In premature infants and newborns it has been suggested that vitamin E plays an important role in membrane metabolism or maintenance, and in the protection against hyperbilirubinemia, retrolental fibroplasia, bronchopulmonary dysplasia, and intraventricular hemorrhage. Serum levels of α-tocopherol are generally lower in infants than in adults.

Existing Methods

Colorimetric, LCUV, and GLC methods have been published. The colorimetric method requires 1.0 mL of serum. The LCUV methods require 0.1 mL or 1.0 mL of sample and have a reported detection limit of 800 ng/mL and 100 ng/mL respectively.

Reference

Determination of Vitamin E in Microsamples of Serum by Liquid Chromatography with Electrochemical Detection, P.P. Chou, P.K. Jaynes, and J.L. Bailey, Clin. Chem., (1985), 31: 880-882.

Conditions

Detector: BAS LC-4A/17
Electrode: TL-5, glassy carbon
Potential: +1.0 V vs Ag/AgCl
Column: 5 µM C-18 reverse-phase (150 x 4.6 mm)
Mobile Phase: 95% methanol, 5% sodium acetate buffer (pH 5.0)
Detection Limit: 0.1 mg/L, injection volume was 20 µL
Linear Range: 0 to 100 mg/L

Sample Preparation

To 25 µL of sample (plus internal standard) add 100 µL of ethanol. Vortex mix for 5 seconds, add 100 µL of heptane and vortex mix for an additional 10 seconds. After phase separation, transfer 50 µL of the upper heptane layer to a small glass container and evaporate to dryness with a stream of nitrogen. Reconstitute the residue in 50 µL of methanol and inject 20 µL.

Clinical Application

Monitoring serum levels of α-tocopherol. A sample size of only 25 µL is required, allowing the method to be used for the determination of serum vitamin E in the neonatal population.

Related References

1. CV Notes on α-tocopherol and α-tocopherol quinone.

2. α-Tocopherol and its metabolite, α–tocopherol quinone, can be determined in the same sample by utilizing dual detectors in the parallel or series mode. Refer to "Current Separations" Vol. 4, No. 3, and

Vol. 5, No. 3 for details regarding the use of this technique.

3. Vitamin E: Biochemical Hematological, and Clinical Aspects, B. Lubin, and L.J. Wachlin (eds.), Annals of the New York Academy of Science, Vol. 393, 1982, New York, NY.

4. Detection of Quinone Metabolites by HPLC with Reductive Electrochemical Detection, M.T. Smith, D.S. Fluck, D.A. Eastmond, and S.M. Rappaport, Life Chemistry Reports, in press.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not been tested by BAS technical staff.

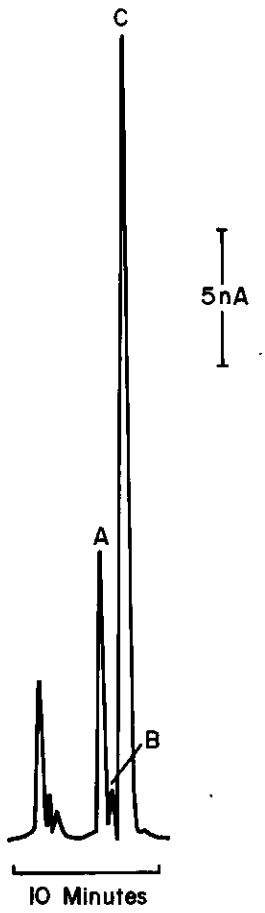

Figure 1. Chromatogram of α-tocopherol in a serum sample worked up as described in text (see Sample Preparation): A) δ-tocopherol, B) β and γ-tocopherol, and C) α-tocopherol.