

VITAMIN A/E

DETERMINATION OF VITAMIN A AND E IN SERUM OR PLASMA

HPLC METHOD

Products

Vitamin A/E Reagent Set

Vitamin A/E

Calibration Standard

Vitamin A/E

Deproteinization Reagent

Vitamin A/E

Mobile Phase Reagent

Vitamin A/E Control

Low Level

Vitamin A/E Control

Normal Level

Vitamin A/E Control

High Level

Analytical Column, C18 Inertsil ODS-3

100 x 3 mm (5 µm packing)

Guard Column

SS 10 x 2 mm

Chromsep Hardware Kit

for 10 cm column, incl. spacer 1 cm

Reaction Tubes 1.5 ml with cap

Reaction Tubes 1.9 ml with cap

Product no.

2795

2838

2843

2844

2840

2841

2842

28208

28141

23082

2555

2547

Quantity

50 - 70 Tests

1 x 2 ml

1 x 60 ml

1 x 500 ml

1 x 1 ml

1 x 1 ml

1 x 1 ml

1 x 1 Column

1 x 5 Columns

1 x 1 Kit

1 x 1000 Tubes

1 x 1000 Tubes

Clinical Background

Vitamin A

Retinol, retinaldehyde and retinoic acid are collectively known as vitamin A, and it was first identified in 1920 and, being the first vitamin, it was named vitamin A under the alphabetical nomenclature system. Retinol is not obtained directly from the diet, but rather is generated in the intestine from the enzymatic cleavage of beta-carotene, alpha-carotene and beta-cryptoxanthin, which are obtained from fruits and vegetables. Animal products such as egg yolk, milk, liver and fat contain retinyl esters which are hydrolyzed to retinol in the intestine. Retinol is transported in serum to target cells complexed with a specific Retinol-Binding Protein (RBP) and transthyretin, a thyroxin-binding protein. In the target cells, retinol is oxidized to retinal and retinoic acid. Retinal is utilized in the synthesis of rhodopsin in the cones, and is necessary for night vision. The first nutritional deficiency disease to be identified and studied was night blindness.

So symptoms associated with a deficiency of vitamin A are night blindness, changes in the eyes, poor bone growth, weak tooth enamel, slow growth, and dry skin.

Vitamin A has been shown to enhance gap junction communication in a dose-dependent manner, and may promote normal cell growth. Vitamin A is necessary for maintenance of healthy epithelial tissue and can prevent the inception or progress of skin cancers by stimulating normal cell differentiation. Vitamin A therapy has been useful for inhibiting or suppressing tumour growth in the mouth, breast, bladder, cervix, lung and skin. Other functions include mucous production and normal bone growth. Its high concentration in the liver is due to the fat-soluble nature of this polyene biochemical, although because of the storage mechanism, excessive doses of vitamin A can be very toxic. Hypervitaminosis A, usually resulting from overmedication leads to diverse symptoms such as headache, skeletal pain, hepatomegaly and haematological abnormalities.

Vitamin E

Vitamin E is widely distributed in foodstuffs. Vitamin E is the primary fat soluble antioxidant and it assists the preventing peroxidation of unsaturated fatty acids and the subsequent formation of cell-damaging free radicals. These free radicals increase the fragility of erythrocytes. For this reason, premature newborns whose red blood cells are more fragile than those of adults are often treated with Vitamin E to prevent haemolytic anaemia. Vitamin E therapy, in some cases, may have a favorable effect on moderate and severe cases of the retinopathy of prematurity⁶ and the retinopathy of abetalipoproteinemia.

Foodstuffs contain the Vitamin E isomers α , β , γ -tocopherol, where α -tocopherol is the most abundant isomer in normal plasma (> 90%) and is also the most biologically active.

Vitamin E deficiency can produce the following symptoms: cirrhosis of the liver, coeliac disease, cystic fibrosis, and low red blood count. Large doses of Vitamin E can be toxic and a physician should supervise large doses.

Assay Principle

After protein precipitation and extraction retinol and α -tocopherol can be determined fluorometrically on an isocratic HPLC system, with separation from interferences of the analytes by using an ODS-3 column. This approach is sensitive and simple. As these vitamins are detected at different wavelengths, a detector with programmable wavelength switching is very useful for simultaneous analysis of Vitamin A and Vitamin E in a single HPLC run. In the first run we measure the fluorescence (λ excitation 315 nm; λ emission 455 nm) which is proportional to Vitamin A concentration and after 3.5 minutes, we measure the fluorescence again (λ excitation 295 nm, λ emission 340 nm) which is proportional to Vitamin E concentration in the sample.

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