



## Fat soluble Vitamins A, D, E, K

### Introduction

Vitamins A, D, E and K belong to the fat soluble vitamins. These vitamins are involved in several biochemical processes in the body. Briefly, **vitamin A** (retinol) plays a role in vision and bone growth. It comes from animal sources such as eggs, meat and dairy products. Beta-carotene, a precursor, comes from fruit and vegetables (paprikas, carrots).

**Vitamin D** (ergocalciferol, D2, cholecalciferol, D3) promotes the adsorption of calcium in the body, essential to development of bones and teeth. Vitamin D is made by the body when exposed to sun light, or via our diet of cheese, butter, fish, and milk.

Tocopherol, or **Vitamin E**, is an important antioxidant. Antioxidants protect cells against the effects of free radicals, which are potentially damaging by-products of the body's metabolism.

**Vitamin K** denotes a group of 2-methilo-naphthoquinone derivatives. They are needed for the posttranslational modification of certain proteins, mostly required for blood coagulation. Normally it is produced by bacteria in the intestines.

### Method

The HPLC system consists of reversed phase column and a apolar mobile phase (Table I). For EC detection an electrolyte is required therefore lithium perchlorate was added to the mobile phase. A VT03 cell with glassy carbon electrode has been used. Under these extremely apolar conditions the HyREF is the best choice for a reference electrode.

Only when Vitamin K must be analysed a second pre-reduction cell is applied.

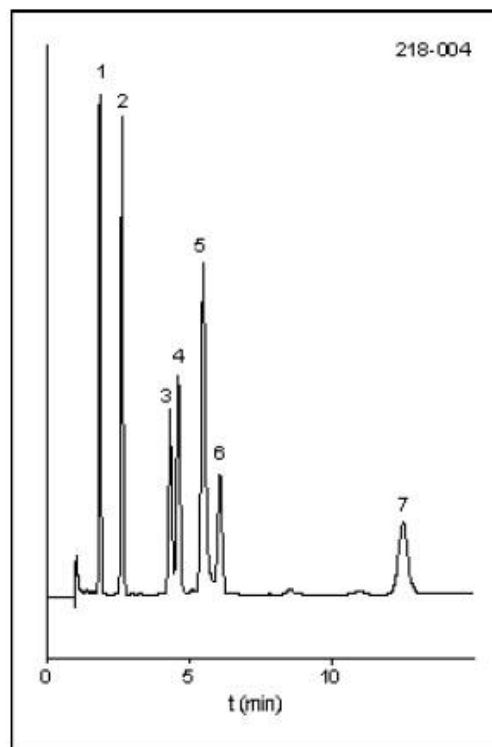


Fig. 1. Analysis of vitamin A (1), vitamin A acetate (2), vitamin D2 (3), vitamin D3 (4), vitamin E (5), vitamin K2 (6) and vitamin K1(7). Scale: 240 nA full scale.

Table 1

Set-up	
<b>Column</b>	Spherisorb ODS 100 x 4.6 mm, 3 µm
<b>Flowrate</b>	1.0 mL/min
<b>Sample</b>	Vitamins A and A acetate: 5 µM, E: 10 µM, D2, D3, K1, K2: 100 µM
<b>Mobile phase</b>	100 mmol/l lithium perchlorate, 96 % MeOH
<b>Temperature</b>	30 °C for column and flowcell
<b>E-cell</b>	1050 mV vs. Hy-REF, Only for vit K: pre-reduction: -400 mV

### Recommendation

The advised configuration for this application is the ALEXYS Analyzer using an autosampler with sample cooling option.

PART NUMBERS AND CONFIGURATION	
<b>180.0035C</b>	ALEXYS Analyzer – coded
<b>110.4305</b>	VT03 flowcell, 3 mm GC, HyREF