

Vitamins are natural substances found in plants and animals. With few exceptions, the human body cannot synthesize them. Consequently, they must be supplied by the diet or by dietary supplements. Vitamins are essential for the normal growth, vitality, and for the prevention and cure of many health problems and diseases together with other essential nutrients such as proteins, fatty acids and carbohydrates.

Vitamins are required only in tiny amounts by organism (micrograms or milligrams per day). Therefore, the methods for their separation and determination should be very sensitive. One possible method is the high performance liquid chromatography equipped with the electrochemical detection.

This Diploma Thesis is focused on finding of optimal conditions for separation of folic acid, biotin and vitamin B12. These water-soluble substances belong to the B group vitamins. Their main sources are meat, eggs, brewer's yeast or pulses.

The separation was carried out on chromatography column Lichrospher 60, RP – select B (240 x 4.0 mm i.d., 5 μ m) obtained from Merck. The analysis was achieved by using the electrochemical detector Coulochem III with sensitivity of 1 μ A. The flow rate was set to 1 ml/min and the current inserted on the working electrodes was 600 mV and 900 mV.

The mobile phase number 11 containing 24% of methanol was established as the most suitable. The appropriate inorganic phase was composed of 50 mM phosphate buffer, pH was adjusted to 3.55.

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