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Determination of vitamin B12 (cobalamin) using HPLC

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Vitamin B12 belongs to water-soluble vitamins and has a complex ring system with cobalt as a central atom. This vitamin is an important cofactor for many biochemical reactions and is produced exclusively by microorganisms. The largest amounts are found in the liver and kidneys (μg/kg) and low concentrations of vitamin B12 can be found in meat, fish, egg and milk in very low concentrations. Cobalamin is the most stable form of vitamin B12. It is stable in high temperatures, but degrades in very acidic and alkaline pH values, and in the presence of oxidizing agents and light. The aim of this study was to validate a quantitative method for the determination of vitamin B12 by RP-HPLC with UV detection and to determine the vitamin B12 content in the offal of livestock. Cyanocobalamin was detected as total vitamin B12 after extraction in 50 mM sodium acetate buffer in the presence of sodium cyanide and purification on an immunoaffinity column. The extraction was preceded by the release vitamin B12 protein-bound by pepsin treatment. Both the isocratic method and the gradient method were used to validate the determination method. The gradient method was evaluated as more suitable method with the limit of detection (LOD) 7 μg/kg and the limit of quantification (LOQ) 25 μg/kg. The calibration with six concentration points of vitamin B12 was linear with a regression coefficient R2 = 1. The vitamin B12 concentrations measured by HPLC were 19.8 μg/100g in chicken liver, 47.7 μg/100g in turkey liver, 18.1 μg/100g in pork liver, 9.21 μg/100g in pork kidneys, and 46.3 μg/100g in beef liver.

Keywords: vitamin B12, HPLC-UV, liver, kidneys, meat

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Biography:

Filip Beno research focuses primarilly on the quality of meat and meat products (from farm to meat/consumer). I am also involved in the development of new recipes to reduce the salt content of meat products. We cooperate with many companies, we consult technological problems and perform analyzes of their products. I also focus on technology and processing of vegetables and fruits and their products. The study at UCT Prague includes also consultations on bachelor's and master's theses, leading, laboratory work of students and, last but not least, publishing activities.

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