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Determination of cobalt species in nutritional supplements using ICP-OES after microwave-assisted extraction and solid-phase extraction

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ABSTRACT

Cobalt content (as vitamin B_{12} and inorganic cobalt) in two nutritional supplements, namely *Spirulina platensis* and *Saccharomyces cerevisiae known as a "superfood"*, has been determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Several sample pre-treatment protocols have been applied and compared. Microwave-assisted acid digestion efficiently decomposed all cobalt-containing compounds, thus allowed obtaining total cobalt content in supplements examined. Vitamin B_{12} was extracted from the samples with acetate buffer and potassium cyanide solution exposed to mild microwave radiation for 30 min, and cyanocobalamin was separated from the extract by on-column solid phase extraction using C-18 modified silica bed. About 100% of cobalt species was extracted using the triple microwave-assisted extraction procedure. Total cobalt content was 20-fold greater in *Spirulina* tablets than the declared cobalamin content (as Co). The ICP-OES method precision was about 3% and detection limit was 1.9 and 2.7 ng Co mL^{-1} for inorganic cobalt or cyanocobalamin, respectively.

1. Introduction

Vitamin B_{12}, a tetrapyrrole complex containing cobalt ion, plays important role in metabolic processes and protein synthesis [1,2]. Its deficiency leads to pernicious anemia and neurological disorders [3]. Depending on the functional group bounded to Co, different cobalamin species are formed: cyano-, hydroxo-, methyl- and adenosylocobalamin [4]. Since vitamin B_{12} is not synthesized in the human body, nutritional supplements containing cobalamin are widely used to prevent cobalamin deficit in high-risk populations such as vegetarians [5]. Cyanocobalamin is the most chemically stable form of vitamin B_{12} and it is commonly added to human dietary supplements [6]. Some nutritional products are supplemented with exogenous vitamin B_{12}. However, algae and yeast are known to produce substantial amounts of cobalamin [7,8]. Some *Spirulina* species are grown in large scale cultivation systems and they are used for the preparation of nutritional supplements. Considering both the huge number of nutritional supplement consumers and the diversity of commercially available vitamin products, quality control of cyanocobalamin in dietary supplements is required. Since vitamin B_{12} exists in free and bound form in nutritional supplement products, it is important to quantify the total amount of the vitamin. The estimated cobalt intake from food is 5–40 μg per day, most of which is inorganic cobalt. However, many cobalt compounds are genotoxic in mammals.

Numerous analytical techniques such as microbiological test [9], radioisotope dilution [10], spectrophotometry [11], chemiluminescence [12], atomic absorption spectrometry (AAS) [13], mass spectrometry (MS) [14], capillary electrophoresis [15], and high performance liquid chromatography (HPLC) [16] have been proposed for assaying vitamin B_{12}. The recommended and official method for the quantification of vitamin B_{12} involves microbiological assay using *Lactobacillus leishmanii* as the test organism and is considered as time-consuming [9]. Isotope dilution method [10] is accurate and sensitive, but expensive due to the requirement of radio-labeled cyanocobalamin and isotopic MS analysis. Spectrophotometry [11] is hardly applicable for the samples of complex matrix and the sensitivity of this technique is too low. When quantifying various cobalt species by different spectrometric methods, it was found that the signals measured are species-dependent. Also, Grotti et al. [17] investigated the influence of the chemical species (organic or inorganic) on the ICP-MS signal for Hg, Se and Sn compounds and they observed significant differences in sensitivity for examined elements. They showed that for every examined element, species-specific calibration is required in order to achieve an accurate quantitation.

Despite many analytical methods of the vitamin B_{12} content determination available, fast, simple, cheap, specific and sensi-

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tive method for nutritional supplement quality control is still desired. The determination of cobalamin in dietary preparations is usually done either directly or indirectly by determining cobalt concentration. Various analytical methods have been proposed for indirect determination of vitamin B_{12} as cobalt. Zeng et al. [18] applied chemical vapor generation coupled with atomic fluorescence spectrometric for determination of cobalt in vitamin B_{12} samples. Bruno [19] developed a method based on formation of complex with hexamethyl-phosphoramide and thiocyanate for spectrophotometric determination of cobalamin as cobalt in pharmaceuticals preparations. However, the applicability of indirect methods for quantification of vitamin B_{12} in biological samples is limited since these methods cannot differentiate the bound and free form of cobalamin [16]. This problem could be solved by using appropriate sample preparation procedure. Kumudha and Sarada [8] studied six different extraction protocols for the extraction of vitamin B_{12} from different food materials and found that for determination of cyanocobalamin in *Spirulina platensis* the recovery of CNcob exceeded 90% when the extraction method involving the addition of acetate buffer and KCN was applied. In this case, the cobalamins are converted to cyanocobalamins using KCN. In turn, Hadjmohammadi and Sharifi [13] described an effective use of solid phase extraction (SPE) for matrix removal and preconcentration of cyanocobalamin for multivitamin tablet samples with recovery of vitamin B_{12} of about 96%. Importantly, this method can distinguish the inorganic and cyanocobalamin form of cobalt in examined samples.

The aims of the present work are to develop a simple microwave-assisted solid-liquid extraction procedure and an accurate and sensitive determination method of total cobalt, inorganic cobalt and free cobalamin content in nutritional supplements containing either naturally synthesized or exogenous cobalamin using ICP-OES. For separation of inorganic cobalt and cyanocobalamin, solid phase extraction process after microwave-assisted extraction procedure with acetate buffer and potassium cyanide was optimized.

2. Materials and methods

2.1. Reagents and samples

High grade analytical reagents were employed for the preparation of solutions. A standard stock solution of cobalt (1000 mg L^{-1}) was prepared from cobalt chloride (POCh, Poland) in 2%$_{v/v}$ HNO_3. The stock solution of vitamin B_{12} (230 mg $L^{-}1$) was prepared by dissolving 23.0014 mg of cyanocobalamin (Sigma Aldrich, Germany) with 3 mL of methanol in a 100-mL volumetric flask, and next filled up with water. Working standard solutions were prepared by serial dilution of the stock solutions of inorganic cobalt and cyanocobalamin, respectively. The cyanocobalamin standards were stored in the dark. Stability of vitamin B_{12} was investigated within 7 days and no degradation of cyanocobalamin was observed. Sodium acetate buffer solution (pH 4.6; 0.2 M) was prepared by dissolving 8.2035 g of sodium acetate (POCh, Poland) in 500 mL of water and adjusted to pH of 4.6 with acetic acid (POCh, Poland). For sample preparation, potassium cyanide (POCh, Poland) and HPLC grade acetonitrile (HPLC grade, POCh, Poland) were used. The nutritional supplements, *Spirulina platensis* algae and *Saccharomyces cerevisiae* yeast tablets, were purchased from local market.

2.2. Instrumentation

Atomic emission measurements were made with an Integra XL ICP-OES spectrometer (GBC Scientific Equipment, Australia) equipped with conical pneumatic nebulizer AR40-07(Glass Expansion Pty. Ltd., Australia). Cyanocobalamin structure degradation was examined using a Lambda 20 spectrophotometer (Perkin Elmer, USA). For both microwave-assisted digestion and extraction, a high-pressure microwave digester (double-position closed system) (Plazmatronika, Wroclaw, Poland) was used. A 6-mL SPE column was filled with a C-18 modified silica (ENVI-18, 500 mg) from Supelco (Sigma Aldrich, Austria). The column was conditioned with 2 mL of acetonitrile and then with 5 mL of water using vacuum pump (JT Baker, USA). Optimized operating conditions are given in Table 1.

Table 1
Operating conditions for determination of Co by ICP-OES after microwave-assisted acid digestion or microwave-assisted solid-liquid extraction.

ICP-OES parameters		
Sample flow rate (ml min^{-1})	1.6	
Plasma power (W)	1200	
PMT voltage (V)	575	
Nebulizer gas flow rate (L min^{-1})	0.5	
Auxiliary gas flow rate (L min^{-1})	0.5	
Cooling gas flow rate (L min^{-1})	12	
Height above coil (mm)	7.0	
Wavelength (nm)	Co II	228.61
Microwave-assisted acid digestion parameters		
Step 1		
Temperature (°C)	>100	
Time (min)	15	
Power (W)	50	
Step 2		
Temperature (°C)	>150	
Time (min)	15	
Power (W)	70	
Step 3 - cooling		
Temperature (°C)	<50	
Time (min)	15	
Power (W)	0	
Microwave-assisted solid-liquid extraction parameters		
Step 1		
Temperature (°C)	50	
Time (min)	10	
Power (W)	20	
Step 2		
Temperature (°C)	70–80	
Time (min)	10	
Power (W)	40	
Step 3 - cooling		
Temperature (°C)	<30	
Time (min)	15	
Power (W)	0	

2.3. Microwave-assisted acid digestion

Ten tablets of each nutritive supplement were ground in an agat mortar. A portion of powder equivalent to two average tablets weight (1040 mg and 765 mg of powdered samples of *Spirulina platensis* or *Saccharomyces cerevisiae* tablets, respectively) was placed in a 40 mL PTFE vessel and 2 mL of concentrated HF was added. Next, the vessel was placed on a hot plate till gentle boiling to remove silica present in tablets as a excipient. After evaporation, a sample residue was cooled and subjected to microwave-assisted pressurized digestion using 5 mL of concentrated nitric acid. After cooling, the sample solution was filtered and placed in a 10 mL volumetric flasks and filled to the mark with water. The total cobalt concentration was determined by means of external calibration based on a set of inorganic cobalt standard solutions (Fig. 1). Optimized operating conditions for microwave-assisted acid digestion are given in Table 1.