Food Chemistry 147 (2014) 215–219

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Combined use of HPLC–ICP-MS and microwave-assisted extraction for the determination of cobalt compounds in nutritive supplements

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ARTICLE INFO

Article history:
Received 31 August 2012
Received in revised form 12 April 2013
Accepted 27 September 2013
Available online 6 October 2013

Keywords:
Reversed-phase chromatography
Inductively coupled plasma mass spectrometry
Cobalt speciation analysis
Nutritive supplements
Electrospray ionisation-mass spectrometer

ABSTRACT

Speciation analysis of cobalt in nutritive supplements has been carried out using HPLC and ICP-MS equipped with a membrane desolvation sample introduction system as detector. In this study, cobalt containing compounds, namely Co(II), cyanocobalamin (CN-Cbl) and hydroxylcobalamin (OH-Cbl), were well separated by reversed phase HPLC with a C8-HPLC column as the stationary phase and 8 mmol L^{-1} ammonium acetate in 22% v/v methanol solution (pH 4) as the mobile phase using isocratic elution. Detection limit was in the range of 0.008–0.014 μg Co L^{-1} for various Co species. Over 98% of the total cobalt species was extracted in nutritive supplements using a 0.5% v/v HNO_3 solution in a microwave field; and the spike recovery was in the range of 92–108% for various species. The HPLC–ICP-MS results showed a satisfactory agreement with the total cobalt concentrations obtained by ICP-MS analysis of completely dissolved samples.

1. Introduction

Cobalamin and cyanocobalamin, complexes of cobalt with tetrapyrrole, play important role in protein synthesis and energy metabolism (Mandal, Mandal, Ghosh, & Dey, 2009). Vitamins are crucial for maintaining good health in humans and lack of sufficient amount of any of them can cause serious diseases (Castelli, Wong, Friedman, & Riley, 2011). In order to prevent cobalamin deficiency disorders, consumption of nutritive supplements and foodstuffs with cobalamin is commonly practiced. In such samples determination of cobalt species is an important pursuit. The official microbiological assay methods for the analysis of water-soluble vitamins, though very sensitive are extremely time-consuming and sometimes not completely specific (Tanner & Barnett, 1986; Tanner, Barnett, & Mountford, 1993).

Inductively coupled plasma mass spectrometry (ICP-MS) is a versatile trace element detector with superior analytical capabilities. As a detection technique, ICP-MS provides the advantages of low detection limit, multi-element detection, and element- and isotope-specific detection capabilities. Several methods of liquid chromatography (LC) and capillary electrophoresis (CE) coupled with ICP-MS have been reported for cobalt speciation analysis (Baker & Miller-Ihli, 2000; Chassaigne & Lobinski, 1998; Chen & Jiang, 2008; Makarov & Szpunar, 1999; Yanes & Miller-Ihli, 2004; Zafra-Gomez, Garballo, Morales, & Garcia-Ayuso, 2006). Coupling of HPLC to ICP-MS gained much attention due to its easiness of sample preparation, simplicity of interface to the detector and specificity of the signal intensity of the determined element. The reported HPLC–ICP-MS procedures used gradient elution. There is a need for simpler procedures with superior LODs. Identification of Co species in HPLC–ICP-MS is based on retention times of standard solutions. However, there is a possibility that another species of Co can have same retention time as that of our species of interest. The ambiguity can be avoided using ESI-MS (Chassaigne & Lobinski, 1998).

Microwave-assisted digestion has gained wide acceptance as a rapid method for sample decomposition in inorganic analysis. Recently, it has also been verified as an appropriate tool for rapid preparation of solid samples for organometallic speciation analysis (Tseng, De Diego, Martin, & Donard, 1997; Yeh & Jiang, 2005). The aims of the present work are to develop a rapid microwave-assisted extraction procedure and a rapid and accurate method for the speciation analysis of cobalt in nutritive supplements using HPLC–ICP-MS. It is based on the coupling of HPLC with on-line selective detection of cobalt by ICP-MS. The effluent was directly introduced into ICP-MS for the detection of Co at *m*/*z* 59. The optimization of the HPLC–ICP-MS technique for the determination of cobalt compounds in nutritive supplements, are also described.

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http://dx.doi.org/10.1016/j.foodchem.2013.09.141

Table 1
Equipment and operating conditions.

ICP-MS instrument	PE-SCIEX ELAN 6100 DRC II
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	1.325 L min^{-1}
Nebulizer gas flow rate	1.0 L min^{-1}
RF power	1100 W
Acquisition mode	Peak hopping
Auto lens	On
Point per peak	1
Dwell time	50 ms
No. of sweeps	10
No. of readings	450
Ion monitored	^{59}Co
HPLC system	
Pump	Hitachi model L-7100
Injector	Rheodyne 7725i
Stationary phase	Perkin Elmer C8, 3 μm diam. particles, 3.0 mm id × 33 mm length
Mobile phase	22% v/v methanol, 8 mmol L^{-1} NH_4OAc
Flow rate	1.2 mL min^{-1}
Sample loop volume	100 μL
ARIDUS system	
Sweep gas flow rate	1.2 L min^{-1}
Nitrogen gas flow rate	0 mL min^{-1}
Spray chamber temperature	110 °C
Membrane temperature	140 °C
ESI-MS instrument	Bruker amazon SL
Dry temperature	200 °C
Nebuliser gas pressure	15 psi
Dry gas flow rate	8 L min^{-1}
Capillary voltage	−4750 V
Accumulation time	100 ms
Scan range	150–1400
MRM amplitude	1.0 for OH-Cbl and 1.0 for CN-Cbl

2. Materials and methods

2.1. Apparatus and conditions

An ELAN 6100 DRC II ICP-MS (Perkin-Elmer SCIEX, Concord, ON, Canada) was used for the determination of cobalt. Samples were introduced by a membrane desolvation sample introduction system (Aridus-I, Cetac, Omaha, NB, USA). The operating conditions of ICP and nebulizer were optimised by continuous introduction of a tune solution containing 1 μg L^{-1} Co(II) in mobile phase. The operating conditions of the membrane desolvation ICP-MS used in this work are summarized in Table 1.

A HPLC pump (Hitachi, Model L-7100), injector (Rheodyne 7725i) and reversed phase HPLC column (Perkin-Elmer C8 silica based, 3 μm diam. particles, 3.0 mm i.d. × 33 mm length) comprised the HPLC system. Samples were loaded with a syringe into a 100 μL sample loop. All separations were performed at room temperature under isocratic conditions. Each separation was attempted under several different combinations of column, concentration of buffer, organic modifier concentration, etc. The conditions listed in Table 1 are those that yielded the best chromatogram of the various sets tested, based on better resolution and shorter separation time. The column outlet was connected to the pneumatic nebulizer of the ICP-MS device through a polytetrafluoroethylene (PTFE) tubing (0.17 mm i.d. × 720 mm length). Due to the difference between the flow rate of the LC mobile phase and the optimum flow rate of the Aridus-I sample introduction system, in this study a splitter was inserted in between the column and nebulizer (Bluemlein, Krupp, & Feldmann, 2009). Only 10% of the effluent was delivered to the Aridus nebulizer. The schematic diagram of the HPLC–ICP-MS system is shown in Fig. S1 (Supplementary content).

The HPLC–ESI-MS/MS system used consisted of a Hitachi Model L-2130 HPLC pump and an amaZon SL Ion Trap Mass Spectrometer (Bruker Daltonics, Bremen, Germany). The mass spectrometer was equipped with an electrospray interface as the ionisation source, which was operated under the conditions indicated in Table 1.

2.2. Reagents

Analytical-reagent grade chemicals were used without further purification. Cyanocobalamin (CN-Cbl) and hydroxocobalamin hydrochloride (OH-Cbl) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Co(II) standard solution was from Fisher (Fire Lawn, NJ, USA). HPLC-grade methanol, formic acid and ammonium acetate (NH_4OAc) were obtained from Merck (Darmstadt, Germany). To prepare the solution to be used as a mobile phase, suitable amount of ammonium acetate was dissolved in HPLC-grade methanol (Merck) and deionized water (Milli-Q water purification system, Millipore, Bedford, MA, USA) to the desired concentration. The mobile phase solution was adjusted to pH 4.0 with formic acid.

2.3. Sample preparation and extraction

The applicability of the method to real samples was demonstrated by the analysis of two B Complex Tablet samples and a Chlorella Tablet sample purchased locally. The samples were ground by a ceramic mortar and pestle and sieved by a Retsch VE1000 sieving machine (Retsch, Haan, Germany). The powders with particle size under 150 μm were collected for following experiments. A simple and rapid microwave-assisted extraction procedure was used for the extraction of cobalt species from the samples studied (Lin, Chang, & Jiang, 2008). A CEM MARS (Matthews, NC, USA) microwave digester equipped with temperature sensors was used as the extracting device. Approximately 0.2 g of powder samples were accurately weighed into 15 mL polyethylene centrifuge tubes and 5 mL of 0.5% v/v HNO_3 solution was added into each tube. The tubes were then inserted into the 500 mL beaker having 100 mL water. The microwave system was programmed to heat the water at 90 °C for 10 min and the ramp time was set as 4 min. After microwave heating, the samples were allowed to cool and directly centrifuged for 5 min at 3743*g* (MIKRO 22R, Hettich, Germany). The supernatants were diluted by another 40-fold with mobile phase (22% v/v methanol, 8 mmol L^{-1} NH_4OAc) and all the supernatants were filtered through a PVDF filter (Pall Corporation, Ann Arbor, MI, USA) of 0.2 μm porosity before HPLC separation. The concentrations of various cobalt species were determined by external calibration method based on peak area. The spike recoveries were determined by spiking 0.2 g of samples with suitable amounts of cobalamins and Co(II) standard solutions, dried and then extracted by HNO_3 solution. The standards spiked were 5 μg g^{-1} each of cobalamins and 10 μg g^{-1} of Co(II) in Chlorella Tablet and 2 μg g^{-1} each of cobalamins and Co(II) in B Complexes 1 and 2. The samples were also digested completely using pressurized microwave digestion procedure using HNO_3. The powder samples (0.2 g) were weighed into closed Teflon PFA vessels, 5 mL concentrated nitric acid was added and the vessels were heated inside a CEM MARS microwave digester to decompose the sample. After cooling, the digests were transferred into 10 mL volumetric flasks and made up to the volume with pure water. The stock solutions were diluted to the appropriate volume followed by introduction into the ICP-MS for cobalt determination. The cobalt concentration in the samples was quantified by means of external calibration with 1 μg L^{-1} of rhodium as the internal standard. The Aridus-I sample introduction system was employed to minimize the $^{43}Ca^{16}O^{+}$ interference on $^{59}Co^{+}$ determination. The extraction efficiency was computed by comparing the total Co