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Cite this: *J. Anal. At. Spectrom.*, 2013, **28**, 901

A simple and sensitive LC-ICP-MS method for the accurate determination of vitamin B_{12} in fortified breakfast cereals and multivitamin tablets†

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A sensitive liquid chromatographic (LC) method coupled with inductively coupled plasma mass spectrometry (ICP-MS) has been developed for the determination of vitamin B_{12}. The method was based on efficient isocratic separation with a mobile phase consisting of 20 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) in 25/75 methanol–water mixture (volume fractions) operating at a flow rate of 0.2 mL min^{-1}. After LC separation, ionic cobalt (Co), cyanocobalamin, methylcobalamin, and hydroxocobalamin were measured as ^{59}Co by ICP-MS. For Co as cyanocobalamin, the analyte of interest of this work, the method has shown good repeatability with relative standard deviation (RSD) of 3% for ten measurements and excellent linearity between 0.1 ng g^{-1} and 100 ng g^{-1} (linear regression, $r^2 > 0.999$). The limit of detection (LOD) for cyanocobalamin was found to be less than 1 ng g^{-1}, which permits the method to be employed for the determination of ultra-trace concentrations of vitamin B_{12} in various types of dietary supplements and fortified food products. Cyanocobalamin in aqueous solution was found to decompose under the ambient light of the laboratory; therefore, dark room conditions are required for the determination of vitamin B_{12} in the form of cyanocobalamin to minimize the photon-induced decomposition. To determine total Co in a commercial high-purity cyanocobalamin using direct ICP-MS measurement as part of an effort to characterize the chemical for use as a calibrant, it was observed that quantitative measurement of Co was achieved only through a complete acid digestion. The method was applied to the determination of vitamin B_{12} in Standard Reference Material (SRM) 3233 Fortified Breakfast Cereal. SRM 3280 Multivitamin/Multielement Tablet was used for quality assurance of the cereal sample measurements. The vitamin B_{12} value of (0.187 ± 0.016) mg kg^{-1} found in SRM 3233 was comparable to (0.219 ± 0.066) mg kg^{-1} obtained by Grocery Manufacturers Association's Food Industry Analytical Chemists Committee (FIACC) using microbiological assay. The (4.38 ± 0.05) mg kg^{-1} of vitamin B_{12} found in quality assurance samples of SRM 3280 was in good agreement with the certified values of (4.8 ± 1.0) mg kg^{-1}.

Received 26th December 2012
Accepted 10th April 2013

DOI: 10.1039/c3ja30383g

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Introduction

Vitamin B_{12} is one of eight B vitamins with a key role in the normal functioning of the brain and nervous system, red blood cell formation, cell metabolism, fatty acid synthesis, and methyl donation in DNA synthesis. Deficiency of vitamin B_{12} is linked to a variety of health problems including haematological disorders and neurological disorders.[1,2] Vitamin B_{12} consists of a cobalt (Co) ion bound to a corrin ring.[3] Different forms of vitamin B_{12} are similar in the Co central ion, the four parts of the corrin ring and a dimethylbenzimidazole group, but differ in the sixth site which may contain a cyano group (–CN), hydroxyl group (–OH), methyl group (–CH_3) and/or a 5′deoxyadenosyl group (–Ado).[2] The structure of vitamin B_{12} is given in Fig. 1.

The human body cannot synthesize vitamin B_{12}, and therefore, diet is the essential source for maintaining adequate health. Naturally occurring cobalamins are only found in animal products such as meat, milk and other dairy products. A synthesized form, cyanocobalamin is the main constituent in dietary supplements and fortified foods.[4] The recent daily reference intake (DRI) report from the National Academy of Sciences recommended 2.4 μg day^{-1} for adults.[5] Apart from meat and dairy products, vitamin B_{12} is rarely found in other foods and hence a range of foods are fortified with the vitamins to reduce the incidence of deficiency-related diseases.

The dietary supplement industry in the United States is rapidly expanding, with 66% of adult Americans now considered as supplement users.[6] Consumption of dietary supplements, including vitamin and mineral supplements, represents

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3ja30383g

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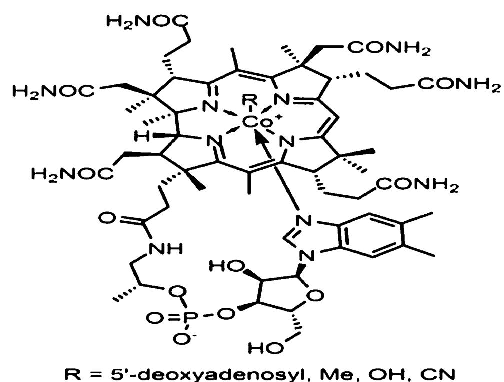

Fig. 1 Structure of vitamin B12.

an annual U.S. expenditure of more than $25 billion.[7] As the importance of vitamin B_{12} increases and a wide range of fortified foods are introduced into the market, the need for accurate measurement of vitamin B_{12} is gaining importance. Currently, the determination of vitamin B_{12} in food and dietary supplement products is routinely carried out by means of a microbiological assay (MBA),[8,9] which is time consuming and labour intensive. Also MBA lacks a high degree of specificity as food commodities can contain inactive cobalamins that can interfere with the growth of the microorganism. Other methods include radioisotope dilution assay,[10] atomic absorption spectrometry (AAS),[11,12] inductively coupled plasma mass spectrometry (ICP-MS),[13] capillary electrophoresis (CE),[14] chemiluminescence techniques,[15] biomolecular interaction analysis (BIA)[16] and a combination of fluorescence resonance energy transfer (FRET) and flow-injection analysis (FIA).[17] Liquid chromatography has recently emerged as an efficient technique for determination of vitamin B_{12}.[18–21] However, traditional LC methods lack sensitivity and most procedures need clean up and pre-concentration steps to increase the ability to measure low concentrations of vitamin B_{12}.[22–24] ICP-MS is one of the most sensitive detection methods for the analysis of trace level metal concentrations. Hence, the combination of LC-ICP-MS provides extremely selective detection, and matrix interference was minimized as the determination of vitamin B_{12} is accomplished through the measurement of the Co content. Determination of different cobalamin forms like hydroxo-, cyano-, adenosyl- and methylcobalamins is possible with reversed phase LC coupled with ICP-MS.[25,26]

Analyte stability, traceability of calibrant, and accuracy of calibration transfer are critical elements of an accurate measurement procedure. To our best knowledge, a comprehensive LC-ICP-MS based measurement procedure containing these elements has not been reported for cyanocobalamin. We show a measurement procedure that stabilized the analyte with dark room conditions, ensured traceability of calibration to International System of Units (SI), and transferred the calibration to cyanocobalamin in breakfast cereal using an efficient and sensitive LC-ICP-MS method. We validate the procedure with the determination of cyanocobalamin in Standard Reference Material (SRM) 3280 Multivitamin/Multielement Tablets.

Experimental

Instrumentation

A Series 200 LC system coupled to a DRCII single quadrupole ICP-MS (PerkinElmer, Shelton, CT, USA) was used for the determination of ionic Co, hydroxocobalamin, and cyanocobalamin. The LC system consisted of a Peltier-cooled autosampler and a quaternary pump. Separation of cyanocobalamin from ionic Co was accomplished by using an Atlantis T3 C_{18} column (2.1 mm × 150 mm) with an Atlantis T3 guard column (2.1 mm × 10 mm) (Waters, Milford, MA, USA).

An Agilent 7500 cs single quadrupole ICP-MS (Santa Clara, CA, USA) equipped with a Peltier-cooled, inert sample introduction system was used for the characterization of the cyanocobalamin calibrant.

Reagents

Optima grade (Fisher Scientific, Pittsburgh, PA) nitric acid (HNO_3) was used for sample digestion and in the preparations of samples and standards for total Co measurements using ICP-MS. HPLC grade methanol and ACS grade ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) were used for the mobile phase preparation. Cyanocobalamin, methylcobalamin and hydroxocobalamin (analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). NIST SRM 3113 Cobalt Standard Solution was used to prepare calibration standards, and NIST SRM 3167a Yttrium Standard Solution was used to prepare internal standard solutions.

Procedure

Sample preparation was conducted in a dark room illuminated with red incandescent lamps. Duplicate 2 g samples were accurately weighed from each of the six bottles of SRM 3233. Each sample was placed into a 50 mL autosampler tube along with 18 g high-purity H_2O.

A bottle of SRM 3280 contains 30 tablets. Fifteen tablets from the bottle were ground for 15 min using an automated mortar grinder with agate mortar and pestle. Two 2 g samples of SRM 3280 and four procedure blanks were prepared along with the SRM 3233 samples. The samples were shaken for 15 min, sonicated for 15 min, and centrifuged for 15 min at relative centrifugal force (RCF) of $0.2g_n$. After the centrifugation, approximately 5 g of the supernate of each sample was filtered through a 0.45 μm nylon filter and collected in a 15 mL autosampler tube. The centrifugation step was repeated for a second time with supernate liquid collected for cereal samples. An additional 2 g filtered supernate from two SRM 3233 samples and an SRM 3280 sample were collected in 15 mL autosampler tubes and set aside for density measurements. An aliquot of 0.13 g of a solution containing 0.2 μg g^{-1} yttrium (Y) was weighed into each filtrate. After the resulting solution was homogenized by gentle shaking, a 1 g subsample was transferred into a 15 mL autosampler tube. A 1 g aliquot of a solution containing 5 ng g^{-1} Co as cyanocobalamin was added to the tube to constitute a spiked sample for the purpose of quantification by the method of standard additions. Approximately

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1 mL of the spiked and 1 mL of the unspiked filtrate for each sample was transferred into two 1.7 mL centrifuge tubes, respectively. The samples were ultra-centrifuged at $10^4 g_n$ for 10 min. The supernatant from each tube was transferred into a 2 mL amber vial for LC-ICP-MS measurement.

To characterize commercial cyanocobalamin, a solution containing 40 μg g^{-1} Co as cyanocobalamin in water was prepared from the commercial chemical. Six aliquots of 0.5 g of the solution were individually transferred to six poly-fluoroalkoxy (PFA) beakers. Four procedure blanks were prepared along with the samples. Five millilitres of HNO_3 was added to each beaker, and the contents were digested on a hot plate at a surface temperature of approximately 185 °C. The contents were evaporated to near dryness. After another 5 mL HNO_3 was added, and the digestion process was repeated for a second time. A 0.11 g aliquot of 196 ng g^{-1}, Y solution was added as the internal standard. The contents were transferred to a 30 mL low-density polyethylene (LDPE) bottle and diluted to approximately 30 g with 1.5% HNO_3. Undigested samples of cyanocobalamin were prepared in triplicate each by transferring 0.5 g of the aforementioned cyanocobalamin solution and 0.11 g of the 196 ng g^{-1} Y solution into a 30 mL LDPE bottle and diluting the contents to 30 g with 1.5% volume fraction HNO_3 in water. A subsample of 0.23 g from each digested and undigested sample was diluted to 30 g in a 30 mL LDPE bottle. A 5 g aliquot of the diluted solution was transferred to a 15 mL autosampler tube, and a 5 g aliquot of a spike solution containing 6 ng g^{-1} Co was added for the purpose of quantification by the method of standard additions.

Sample analysis

For the determination of vitamin B_{12}, samples were measured by LC-ICP-MS using the separation and spectrometric parameters listed in Table 1. The sample compartment of the autosampler was covered with cardboard to keep the samples in darkness during the measurements. The Co and internal standard Y were measured in normal mode at 59 u and 89 u, respectively. All samples were measured in 2 days to reduce the possibility for degradation. For the characterization of commercial cyanocobalamin, samples were measured with the quantitative analysis mode of the ICP-MS using instrumental parameters shown in ESI, Table S1.†

Table 1 Instrument parameters for the determination of cyanocobalamin

LC separation	
Column	Atlantis T3 150 mm × 2.1 mm i.d., C_{18} reverse phase column, 3 μm particle size
Column temperature	23 °C ± 0.2 °C (room temperature)
Mobile Phase	20 mmol L^{-1} EDTA in 25% methanol balance water, volume fraction
Flow rate	200 μL min^{-1}, isocratic
Injection volume	25 μL
Mass spectrometric determination	
RF power	1300 W
Plasma gas flow	15 L min^{-1}
Nebuliser gas flow	0.9 L min^{-1}
Auxiliary gas flow	1.3 L min^{-1}
Sample introduction	IsoMist at 2 °C, equipped with a standard Meinhard concentric nebulizer

Results and discussion

Method development

Quantification of vitamin B_{12} can be achieved by the determination of Co in a sample because each molecule contains one atom of Co. However, trace amounts of free Co can be present as an impurity, resulting in a positive bias if the concentration of total Co is used to estimate the concentration of vitamin B_{12}. For accurate determinations of vitamin B_{12} in a sample, the contribution from free Co must be eliminated. Liquid chromatography is capable of separating free Co from the Co in cyanocobalamin form of vitamin B_{12}. The method was developed with the objective of an efficient separation of free ionic Co and cyanocobalamin. The flow rate for this work was optimized to 0.2 mL min^{-1} to minimize backpressure and to prevent clogging by organic carbon deposits on the torch and on the cones of the ICP-MS system.

Although Atlantis T3 column allows the use of various organic solvents for separations, the ICP-MS hyphenation constrains the usage of solvents containing large mass fraction of carbon. For solvents like acetonitrile (ACN), oxygen gas has to be used as an additive to remove the carbon, which in turn decreases the sensitivity. Hence, methanol was chosen as an eluent in this work. The chromatographic profiles of cyanocobalamin as a function of methanol contents in the mobile phase are given in Fig. 2.

The instrument was stable with no decrease in sensitivity or problems with carbon deposits up to a mobile phase composition of 30% mass fraction methanol in water. Baseline

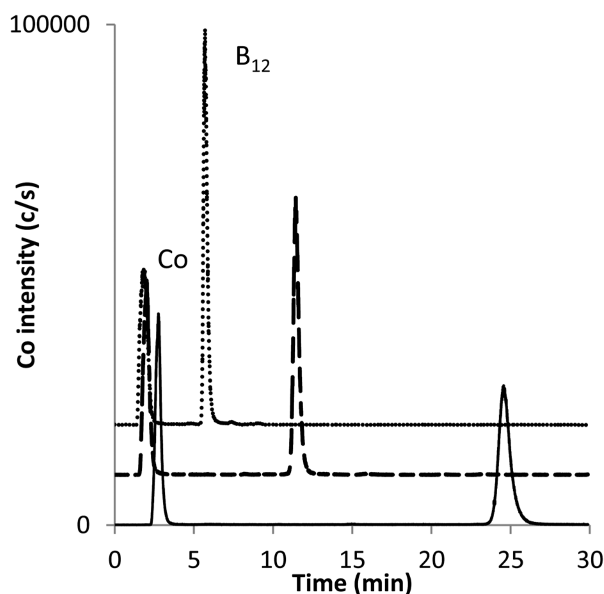

Fig. 2 Separation of ionic Co and cyanocobalamin (B_{12}) as a function of methanol in water at constant 20 mmol L^{-1} EDTA concentration. Solid, dashed and dotted lines represents 20% methanol, 25% methanol and 30% methanol.

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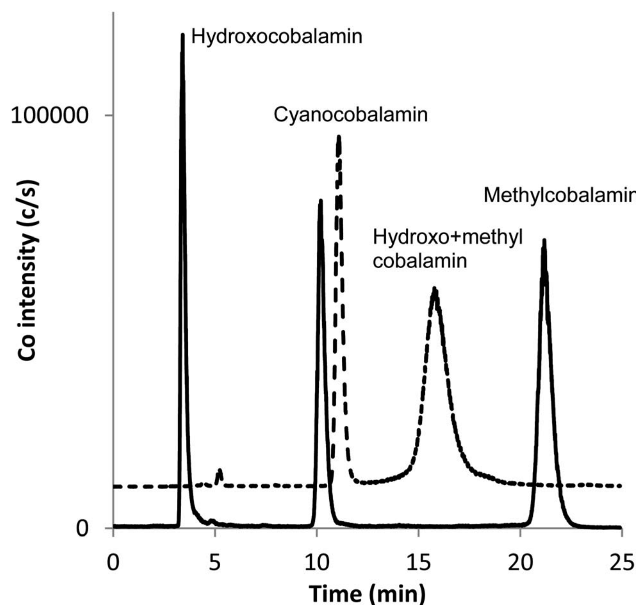

Fig. 3 Effect of EDTA addition on the separation of different forms of cobalamins. Solid line and dashed line represents with and without EDTA.

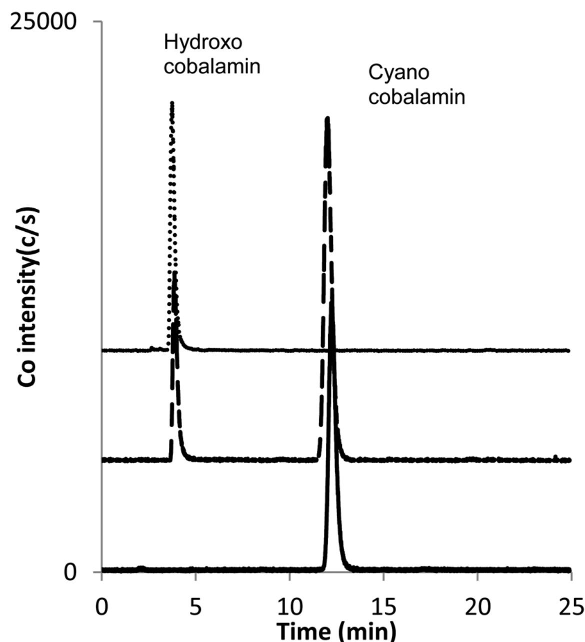

Fig. 4 Stability of cyanocobalamin (vitamin B_{12}) in presence of light. The solid line, the dashed, and the dotted lines represent chromatograms obtained with solutions of freshly prepared cyanocobalamin, 4 days old cyanocobalamin and freshly prepared hydroxocobalamin respectively.

separation was achieved in 6 min under the isocratic separation condition and ionic Co was eluted in the void volume. At methanol concentrations <20% in water, there was no significant change in ionic Co retention, but cyanocobalamin was retained strongly by the column. Increasing methanol concentration to >30% methanol in water did not appreciably improve the shape of the cyanocobalamin peak while the separation between the ionic Co and the cyanocobalamin worsened as two began to co-elute at the void volume. Also, carbon deposits were observed accumulating on the ICP-MS torch and cones at concentration greater than 30% methanol in water. Therefore, a mobile phase containing 25% methanol in water was determined to be optimum for this work.

The effect of EDTA is shown in Fig. 3. Hydroxo- and methylcobalamins were eluted together when only 25% methanol in water was used as the mobile phase. Addition of EDTA improved the separation between hydroxo- and methyl-cobalamins, whereas cyanocobalamin remained resolved from other species. Another important function of EDTA was to maintain a constant pH of 4.2. This pH was reported to be optimum for stability of hydroxocobalamin[27] and cyanocobalin.[2] Addition of 20 mmol L^{-1} EDTA was found to be the optimum concentration for separation of cobalamins. Hence the mobile phase composition of 20 mmol L^{-1} EDTA and 25% volume fraction methanol in water was used for the work as shown in Table 1.

Stability of vitamin B_{12} in ambient light

Vitamin B_{12} in the cyanocobalamin form was found to be unstable under ambient laboratory lighting conditions, and it was observed that over a period of time, the cyanocobalamin peak decreased with a commensurate increase in a new peak at 4 min. Fig. 4 shows chromatograms of a freshly prepared vitamin B_{12} solution, a 4 days old solution, and a hydroxocobalamin solution. This peak was suspected to be hydroxocobalamin, as cyanocobalamin is susceptible to photolysis.[28] The retention time of a hydroxocobalamin standard matched that of the new peak.

Accurate determination of cyanocobalamin requires that the analyte be stable during the sample preparation and measurement process. To ensure stability of cyanocobalamin, experiments were performed to study the stability of cyanocobalamin with respect to lighting conditions. Two solutions each containing 20 ng g^{-1} Co as cyanocobalamin were prepared with one stored in the dark room and the other in the ambient laboratory environment with light. A sample from each solution was measured on day 0, 1, and 4 after the preparation using the instrument parameters described in Table 1. The fraction of the decomposed cyanocobalamin *vs.* time is shown in Fig. 5. This study indicates that an aqueous solution of cyanocobalamin is stable for at least 4 days when the solution is stored in the dark. The latest results have shown that the samples kept in a dark room in amber bottles were stable for at least one month. Hence, all samples and standards for the determination of cyanocobalamin were prepared in dark room conditions with lighting only from red incandescent lamps, and all samples were measured within 2 days after preparation.

Purity of the vitamin B_{12} standard

The commercial cyanocobalamin was characterized for use as a calibrant in the determination of vitamin B_{12} in candidate SRM 3233. Duplicate measurements were made of two solutions prepared from the commercial chemical containing nominally 100 ng g^{-1} and 1000 ng g^{-1} Co as cyanocobalamin.

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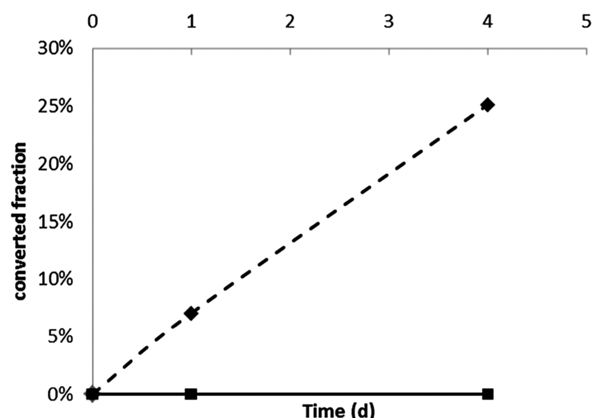

Fig. 5 Stability of 20 ng g^{-1} Co as cyanocobalamin aqueous solution under ambient (light) and dark conditions. The dashed line and the solid lines mark hydroxocobalamin (converted from cyanocobalamin) found in solutions in ambient and dark storage, respectively.

The cobalt-containing species in these solutions were measured. ESI, Fig. S1† shows the chromatogram of the nominal 1000 ng g^{-1} Co solution as cyanocobalamin. There are no significant peaks other than the dominant cyanocobalamin peak confirming that the chemical is of high purity. Consequently, Co was used as a proxy for the determination of cyanocobalamin in the chemical.

Total Co in the chemical was determined with ICP-MS. Since cyanocobalamin is soluble in water, an attempt was made to determine the Co in the chemical after a simple dilution in water. The amount of Co found was approximated 89% of the nominal value, contrary to the purity of ≥98.5% specified by the vendor. Suspecting the sequestration of Co in the corrin ring might have reduced the efficiency of atomization and ionization of Co, an experiment was performed to compare the values of digested and undigested cyanocobalamin. Six samples containing approximately equal amounts of the commercial cyanocobalamin were subjected to acid digestion in comparison to triplicates of the same chemical prepared by simple dilution (Table 2). The means of the undigested sample are approximately 89% of the digested samples, suggesting that the complexed Co in cyanocobalamin is approximately 89% as sensitive as the free Co under the experimental conditions.

Table 2 Co in the commercial cyanocobalamin[a]

	Digested	Undigested
Sample 1	42.84	38.57
Sample 2	43.26	37.88
Sample 3	42.93	38.30
Sample 4	42.19	
Sample 5	42.77	
Sample 6	42.61	
Mean	42.77	38.25
s	0.36	0.35
Recovery	97.3%	89%

[a] All values are in mg g^{-1} units unless otherwise noted.

Consequently, a determination of Co using an undigested cyanocobalamin sample would have underestimated the cyanocobalamin in the commercial product by approximately 11%. Probably the addition of 1.5% nitric acid to a solution of cyanocobalamin was not sufficient to break down the cyanocobalamin complex thereby decreasing the intensity of the signal. Quantitative recovery of Co is achieved by digestion with concentrated nitric acid. The value obtained with digested samples, after impurity correction, is found to be 42.32 mg g^{-1} ± 0.38 mg g^{-1} Co as cyanocobalamin, *i.e.*, 97.3% ± 0.9% in the chemical. The commercial chemical has a stated purity of ≥98.5%, which is close to the determined value. The slightly lower value of the measured value might be a result of the moisture present in the chemical.

Table 3 Cyanocobalamin in SRM 3233 Breakfast Cereal (all values are in ng g^{-1} units)

Bottle # sample	*A*	*B*	Bottle mean
1	201.1	197.5	199.3
2	193.9	205.8	199.8
3	199.9	203.9	201.9
4	164.9	171.1	168.0
5	167.2	161.9	164.6
6	185.6	195.3	190.5
Mean			**187.3**
s			16.8

		Degrees of freedom (DF)
Sample replication (U_{reps})	6.9	5
Calibrant (U_{cal})	0.71	6
Weighing (B_1)	0.31	Infinite
u_c	6.9	
Effective DF	5	
K	2.57	
U	**17.6**	

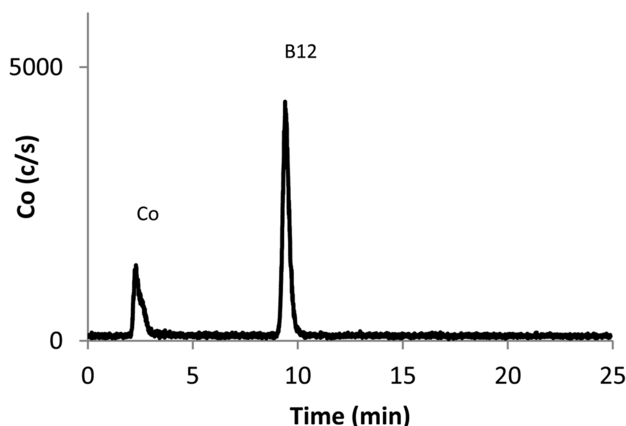

Fig. 6 A typical chromatogram of a SRM 3233 Fortified Breakfast Cereal sample.

Table 4 Comparison of the present work with other associated laboratories for determination of vitamin B_{12} in SRMs 3280 and 3233. (*Uncertainty values are based on standard deviation of measurement)*

Name of the laboratory	Reported value (mg kg^{-1})	No. of participating labs	Method used
SRM 3280 Multivitamin/Multielement Tablets			
Present work	4.38 ± 0.05	—	LC-ICP-MS
Certified value	4.8 ± 1.0	—	Microbiological assay and LC-ICP-MS
SRM 3233 Fortified Breakfast Cereals			
Present work	0.187 ± 0.016	—	LC-ICP-MS
FIACC[a]	0.219 ± 0.066	5	Microbiological assay

[a] Grocery Manufacturers Association's Food Industry Analytical Chemists Committee (FIACC).

Figures of merit

The linearity of calibration curves for Co as cyanocobalamin and ionic Co were evaluated. The area of the chromatographic peak was used for the quantification. The calibration curves for the ionic cobalt and Co as cyanocobalamin were linear over a concentration range of 0.1 ng g^{-1} to 100 ng g^{-1} with eight calibration points. The correlation coefficient for ionic Co and cyanocobalamin was found to be 0.9997 and 0.9996 respectively.

The limits of detection (LOD) were determined by values calculated based on the formula LOD = $3(s_X/Y)C_Y$, where s_X is the standard deviation of blank, Y is the B_{12} peak height and C_Y is the B_{12} concentration. The LOD values were found to be 0.04 ng g^{-1} and 0.08 ng g^{-1} for Co as cyanocobalamin (*i.e.*, 0.9 ng g^{-1} cyanocobalamin) and ionic Co, respectively. The LOD for cyanocobalamin is approximately five times better than what is reported in the literature.[25] The relative standard deviation (RSD) of peak areas as a measure for repeatability was determined to be 3% as calculated from ten replicate measurements of 10 ng g^{-1} solutions for both Co as cyanocobalamin and ionic Co.

Applications

Determination of vitamin B_{12} in fortified breakfast cereal and multivitamin tablet samples

The developed method was applied to the determination of vitamin B_{12} in SRM 3233. Table 3 summarizes mass fraction of cyanocobalamin found in SRM 3233. A typical chromatogram of Co and cyanocobalamin in a cereal sample is shown in Fig. 6.

A one-way analysis of variance (ANOVA) was performed on the bottle-to-bottle variability of the sample material. There is significant bottle-to-bottle inhomogeneity of cyanocobalamin in the cereal as indicated by the p-value (0.001) being much less than 0.05. Therefore, the bottle means were used for subsequent data analysis. Table 3 lists the results and uncertainty of cyanocobalamin in SRM 3233. It shows the uncertainties from calibrant u_{cal} and weighing B_1 are negligible relative to the uncertainty of sample replication, which is due primarily to the inhomogeneity of the sample. The expanded uncertainty of the measurement is calculated using the following equation

$$U = k\sqrt{{u_{reps}}^2 + {u_{cal}}^2 + {B_1}^2} \quad (1)$$

where k is the coverage factor from Student's t table for a 95% confidence level with the associated degrees of freedom. The results of this LC-ICP-MS measurement of SRM 3233 measurement was compared (Table 4) with the results obtained by Grocery Manufacturers Association's Food Industry Analytical Chemists Committee (FIACC) using microbiological assay method as part of an effort for the certification of vitamin B_{12} in the material. The results were in good agreement as indicated by the values overlap each other.

The amount of cyanocobalamin found in SRM 3280 was 4.38 μg g^{-1} ± 0.05 μg g^{-1}, which is in good agreement with the certified value of 4.8 μg g^{-1} ± 1.0 μg g^{-1}. A typical chromatogram for cyanocobalamin in SRM 3280 is shown in ESI, Fig. S2.†

Conclusion

The developed LC-ICP-MS method provides excellent separation between various forms of cobalamin and ionic Co. The typical values of vitamin B_{12} in dairy and meat products range from 2 ng g^{-1} to 900 ng g^{-1} with the maximum value reported in pan-fried beef liver.[29] At detection limit of 0.9 ng g^{-1}, this method is sufficiently sensitive for the determination of B_{12} in these products. Care must be taken to ensure stability of cyanocobalamin during the measurement, as the chemical is unstable in ambient light. The procedure developed in this work provides a road map for accurate determination of cobalamins in food and dietary supplements.

Disclaimer

Certain commercial items are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment identified is necessarily the best for the purpose.

Acknowledgements

We are thankful to Laura Wood for providing us the grounded samples of SRM 3280 and SRM 3233. We also thank Katherine E. Sharpless for valuable suggestions during manuscript preparation.

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Notes and references

1 G. F. M. Ball, *Bioavailability and analysis of vitamins in foods*, Chapman & Hall, London, 1997, ch. 14, Vitamin B_{12}, pp. 497–515.
2 R. R. Eitenmiller, Y. Lin and W. O. Landen, *Vitamin analysis for health and food sciences*, CRC press, 2008, ch. 11, Vitamin B_{12}, pp. 507–535.
3 R. Bonnett, J. Cannon, A. Johnson, I. Sutherland, A. Todd and E. Smith, *Nature*, 1955, **176**, 328–330.
4 H. E. Indyk, J. S. Persson, M. C. B. Caselunghe, A. Moberg, E. L. Filonzi and D. C. Woollard, *J. AOAC Int.*, 2002, **85**, 72–81.
5 Food and Nutrition Board, *Institute of Medicine, Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline*, National Academy Press, Washington D. C., 2000, pp. 306–356.
6 E. Hlasney, *Supplement Usage, Consumer Confidence Remains Steady*, Council for Responsible Nutrition, Washington D. C., 2010.
7 J. Dennis, *Nutraceuticals World*, 2010, **13**, 40–46.
8 K. Cunniff, *AOAC Official Methods of Analysis*, Association of Official Analytical Chemist Inc., Gaithersburg, 2000, p. 47.
9 C. J. Blake, *Anal. Bioanal. Chem.*, 2007, **389**, 63–76.
10 R. S. Yalow and S. A. Berson, *J. Clin. Invest.*, 1960, **39**, 1157–1175.
11 K. Akatsuka and I. Atsuya, *Fresenius. J. Anal. Chem.*, 1989, **335**, 200.
12 P. Vinas, N. Campillo, I. Lopez-Garcia and M. Hernandez-Cordoba, *Chromatographia*, 1996, **42**, 566.
13 N. Zhang, W. Wang, K. Wang, Z. Ding and Z. Wei, *Spectrosc. Lett.*, 2008, **41**(7), 332–336.
14 D. Lambert, C. Adjalla, F. Felden, S. Benhayoun, J. P. Nicolas and J. L. Gueant, *J. Chromatogr. A*, 1992, **608**, 311.
15 W. Qin, Z. Zhang and H. Liu, *Anal. Chim. Acta*, 1997, **357**, 127.
16 Y. Gao, F. Fuo, S. Gokavi, A. Chow, Q. Sheng and M. Guo, *Food Chem.*, 2008, **110**, 769–776.
17 H. Xu, Y. Li, C. Liu, Q. Wu, Y. Zhao, L. Lu and H. Tang, *Talanta*, 2008, **77**, 176–181.
18 M. Stefova, T. Stafilov, K. Stojanoski and B. CepreganovaKrstic, *Anal. Lett.*, 1997, **30**, 2723.
19 P. Vinas, N. Campillo, I. Lopez-Garcia and M. Hernandez-Cordoba, *Anal. Chim. Acta*, 1996, **318**, 319–325.
20 H. Iwase and I. Ono, *J. Chromatogr. A*, 1997, **771**, 127–134.
21 X. Luo, B. Chen, L. Ding, F. Tang and S. Yao, *Anal. Chim. Acta*, 2006, **562**, 185.
22 C. C. Jansen and J. P. Dekleijn, *J. Chromatogr. Sci.*, 1990, **28**, 42.
23 H. Iwase, *J. Chromatogr. A*, 1992, **590**, 359.
24 O. Heudi, T. Kilinç, P. Fontannaz and E. Marley, *J. Chromatogr. A*, 2006, **1101**, 63.
25 A. Makarov and J. Szpunar, *J. Anal. At. Spectrom.*, 1999, **14**, 1323.
26 E. G. Yanes and N. J. Miller-Ihli, *Spectrochim. Acta, Part B*, 2004, **59**, 891.
27 A. D. Marcus and J. C. Stanley, *J. Pharm. Sci.*, 1964, **53**, 91–94.
28 N. Baxter, J. Horsford, F. Wokes, F. W. Norris and S. J. G. Fernandes, *J. Pharm. Pharmacol.*, 1953, **5**, 723–736.
29 *Vitamin B-12 content of selected foods*, USDA National Nutrient Database for Standard Reference, Release 23.