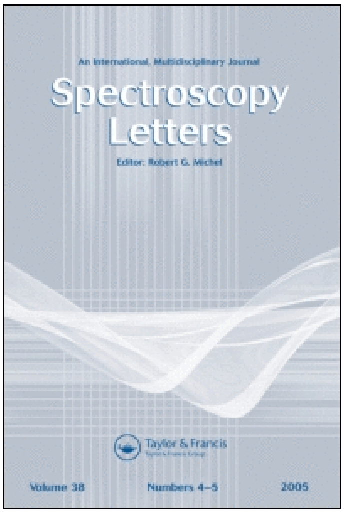

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Rapid Determination of Vitamin B_{12} by Inductively Coupled Plasma Mass Spectrometry in Multivitamin Tablets

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ABSTRACT In the current work, a rapid, simple, and sensitive inductively coupled plasma mass spectrometry (ICP-MS) method for determining vitamin B_{12} in pharmaceutical preparations is proposed. The calibration graph plotted with five concentrations of vitamin B_{12} was linear with a regression coefficient $r^2 = 0.9994$. The detection limit was 0.05 ng/mL for vitamin B_{12}. At a pump rate of 30 rpm, an analysis cycle of vitamin B_{12}, including sampling and washing, could be accomplished in 0.5 min with the relative standard deviations of 4.2%, 3.5%, and 2.6% for 0.25 ng/mL, 2.5 ng/mL, and 25 ng/mL, respectively. The method has been applied successfully in the determination of vitamin B_{12} in multivitamin tablets.

KEYWORDS cobalamin, ICP-MS, pharmaceuticals, vitamin B_{12}

INTRODUCTION

Vitamin B_{12} (cobalamin; $C_{63}H_{88}CoN_{14}O_{14}P$), which contains a cobalt ion within a tetrapyrrole ring, is a very important biochemical species in human physiology (Fig. 1). It helps maintain healthy nerve cells and red blood cells and is also necessary to make DNA, the genetic material, in all cells.[1,2] A deficiency of vitamin B_{12} may lead to fatigue, weakness, nausea, constipation, weight loss, and even disorder as severe as Addisonian pernicious anemia.[3]

Vitamin B_{12} is naturally found in animal foods including fish, milk or milk products, eggs, meat, and poultry.[4] However, it is usually insufficient to only depend on the human diet for the normal amount of the vitamin. To prevent such deficiency diseases, more and more people are advised to take adequate amount of vitamin B_{12} daily by eating vitamin tablets and fortified foods. Thus, it is of great importance to develop a rapid, simple, and sensitive analytical method for quality control of commercial vitamin B_{12} product.

The determination of vitamin B_{12} in tablets is most commonly accomplished by spectrophotometry,[5–7] atomic absorption spectrometry,[8] electrochemical analysis,[9,10] capillary electrophoresis,[11] and high performance liquid chromatography.[12–14] In addition, other analytical techniques have also been applied in the determination of vitamin B_{12} in complicated

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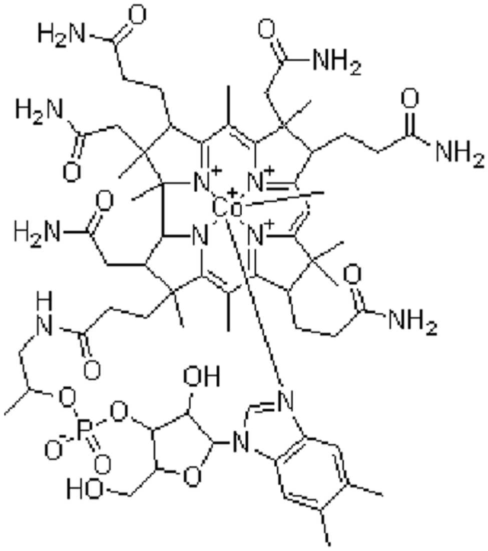

FIGURE 1 Vitamin B_{12} chemical structure.

matrices, involving chemiluminescence,[15] microbiological assay,[16] and radioassay.[17,18]

Inductively coupled plasma mass spectrometry (ICP-MS) is a rapid, sensitive, and selective analytical method, offering an attractive alternative for determination of inorganic elements. However, the method of determination of vitamin B_{12} in pharmaceutical preparations by ICP-MS has not been reported. In this work, a rapid, sensitive, and selective analytical method for the determination of vitamin B_{12} in pharmaceutical preparations by ICP-MS is proposed. Free cobalt could be removed from the sample solution, after the sample solution goes through a cation exchange resin column. Cobalt could be released completely from vitamin B_{12} under acid condition. Hence, the concentration of vitamin B_{12} could be determined by ICP-MS using ^{59}Co isotope. This method has low detection limit of 0.05 ng/mL for vitamin B_{12} and wide linear range covering five magnitude orders and has a high frequency of analyzing samples, about 10 s in one analyst cycle. This method has been applied successfully in the analysis of vitamin B_{12} tablets. It has been shown that the proposed method is very suited to monitoring the quality of commercial vitamin B_{12} products.

MATERIALS AND METHODS

Materials and Decontamination

All the material used was decontaminated with the following procedure: 5 days soaking in HCl 20% (v/v) and another 5 days in HNO_3 20% (v/v). Each soaking was cleaned with ultrapure water (Milli-Q, MA, USA). Finally, all material was dried in a clean environment (Cleaning grade is 1000).

Reagents and Standards

All the reagents were of suprapure grade. Ultrapure water (Milli-Q) was used for the preparation of solution. A stock solution of vitamin B_{12} (1.0 mg/mL) was prepared by dissolving 0.1000 g crystalline B_{12} in 100 mL distilled water. Calibration standards with concentrations of 0.1, 1, 10, 25, and 50 ng/mL were prepared in 2% hydrochloric acid by appropriate dilution of the stock solution. Gallium (Ga) was used to prepare the internal standard. The internal standard solution of Ga was prepared to 250 ng/mL by diluting the commercial stock solution (Xi'an Chemical Co., Xi'an, China) of 1 mg/mL.

Instrumental

The results were obtained on a Thermal Elemental-X Series ICP-MS with a quadrupole reaction system, concentric nebulizer, impact pelletier nebulizer chamber, and nickel cones. The operating parameters are shown in Table 1. The schematic illustration of an ICP-MS instrument for the determination of vitamin B_{12} is shown in Fig. 2.

TABLE 1 X Series ICP-MS Operating Parameters

Operating parameters	Parameter setting
Forward power	1200 W
Cool gas flow	13.0 L/min
Auxiliary gas flow	1.0 L/min
Nebulizer gas flow	0.88 L/min
Nebulizer	Std. glass concentric
Torch std.	1.5-mm injector torch
Spray chamber	Quartz impact bead
Spray chamber temperature	3°C
Sample uptake rate	0.8 mL/min
Interface	HPI
Detector mode	Simultaneous
No. of sweeps	40
Acquisition duration	10 s
Points/mass	1
No. of replicates	3 × 20 s
Channel spacing	0.02
Resolution	0.69 amu

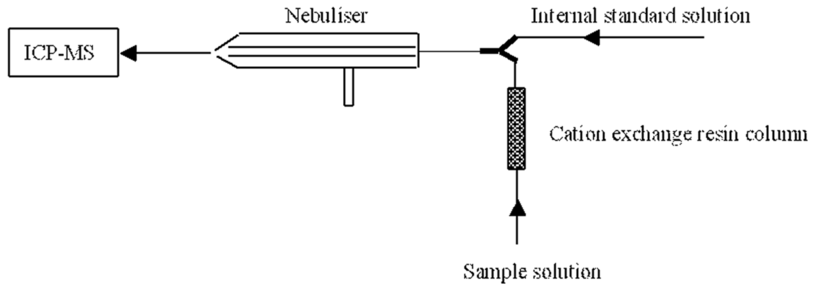

FIGURE 2 The schematic illustration of an ICP-MS instrument for the determination of vitamin B_{12}.

Sample Preparation

An amount of 0.2 mol/L hydrochloric acid was employed to acidify pharmaceuticals according to the literature.[15] About 20 vitamin B_{12} tablets were weighed and ground to fine powder. Then, a sample containing approximately 1.000 g vitamin B_{12} was weighed accurately, transferred into a 100-mL brown calibrated flask, and diluted with 0.2 mol/L hydrochloric acid.

One milliliter of the prepared sample was added to 100 µL of the internal standard and 4 mL Milli-Q water. Reagent blanks were prepared in a similar way. All the solutions were analyzed using the acquisition parameters outlined in Table 1. All data were corrected for internal standard counts. As proposed, the pharmaceutical preparation samples were analyzed directly by ICP-MS after being diluted appropriately.

RESULTS AND DISCUSSION

Spectral Interferences

Cobalt is considered to be an easy trace element to be quantified by ICP-MS due to less interference from molecular ions like $^{40}Ar^{19}F^+$ and $^{14}N^{45}Sc^+$. Only the ^{59}Co isotope was used for quantification. During the measurements of ^{59}Co in multivitamin tablets, it was verified that interferences caused by the species $^{40}Ar^{19}F^+$ and $^{14}N^{45}Sc^+$ were rare due to little concentration of F and Sc in multivitamin tablets.

Nonspectral Interferences

Nonspectral interferences were usually changes in the analytical signal (drift) and matrix effect, which could be detected and corrected if signals were monitored by an internal standardization.[19] The mass ^{69}Ga was chosen as the internal standard element to compensate the drift and to improve precision of measurement by correcting for the various plasma noises sources.[20]

Effect of Hydrochloride Concentration

To release free cobalt from vitamin B_{12}, hydrochloric acid was used for the acidification of vitamin B_{12}. Some preliminary experiments showed that 0.2 mol/L of hydrochloric acid was concentrated enough to provide the acid conditions necessary for the decomposition of vitamin B_{12}. In this case, the concentration of hydrochloric acid in the sample solutions was 0.1 mol/L, which is exactly in

TABLE 2 Comparisons with the Current Techniques

Method	Sample type	Linear range	Detection limits	Measurement cycle	Reference
Capillary electrophoresis–mass spectrometry	Nutritive supplements and chlorella foods	—	0.3 ng/mL	Need extract	[22]
UV–Vis spectrophotometry	Synthetic mixtures and pharmaceutical formulation	10–80 mg L^{-1}	—	Need extract	[23]
Liquid chromatography	Foodstuffs		3 ng g^{-1}	16 h	[24]
The proposed method	Pharmaceutical preparations	1.5×10^{-10} to 8.2×10^{-5} g/mL	50 pg/mL	0.5 min	

TABLE 3 **Results of Determination of Vitamin B_{12} in Pharmaceutical Preparations**

Sample[a]	Added (ng/mL)	Obtained (ng/mL)	RSD (%)	Recovery (%)	Content of vitamin B_{12} (ng/g) Obtained	Content of vitamin B_{12} (ng/g) Labeled
Tablet 1	0	12.29	2.66	94.6	614.5	620
	12.50	24.12	1.56			
Tablet 2	0	12.76	2.54	96.7	638.0	620
	12.50	24.85	1.12			
Tablet 3	0	12.83	2.43	98.9	641.5	620
	12.50	25.19	1.22			

[a] Batch no. 20070315, 20070405, and 20070211 (Shanghai Jinwei Pharmaceutical Co. Ltd.).
RSO, relative standard deviation.

agreement with that described by Zhou et al.[21] Hence, 0.2 mol/L hydrochloric acid was used in subsequent experiments.

Linearity and Limit of Detection

A series of standard solutions of vitamin B_{12} were detected by ICP-MS. It was found that ICPS (integral counts per second) of ^{59}Co was proportional to the concentration of acidified vitamin B_{12} in the range 1.5×10^{-10} to 8.2×10^{-5} g/mL with the detection limit of 50×10^{-12} g/mL. The regression equation is $I_{\text{cps}} = 982\ C_{\text{vitamin B12}} + 58$ and $r^2 = 0.9994$. The RSDs of five determinations were 4.2%, 3.5%, and 2.6% for 0.25 ng/mL, 2.5 ng/mL, and 25 ng/mL vitamin B_{12}, respectively. At a flow rate of 2.0 mL/min, a complete determination of analyte, including sampling and washing, could be accomplished in 0.5 min, giving a throughput of 120 per hour with a RSD of less than 5.0%. Comparisons with the current techniques are listed in Table 2. It is shown that different methods were used to analyze vitamin B_{12} for different samples. For most samples, pretreatments were needed commonly. The proposed method requires only conversion of the pharmaceutial sample into solution. Because one cycle of analysis takes only 0.5 min, the proposed method is very suited to monitoring the quality of commercial vitamin B_{12} products.

Determination of Vitamin B_{12} in Pharmaceutical Preparations

Following the procedure detailed above, the proposed method was applied to the determination of multivitamin tablets. The results are shown in Table 3. The content of vitamin B_{12} in multivitamin was in agreement with the marked value. The results show that the proposed method could be used in quality control of vitamin B_{12} products.

CONCLUSIONS

We have developed a simple and sensitive ICP-MS method for the determination of vitamin B_{12}. The presented method has prominent advantages including simple pretreatment, high sensitivity, and analytical efficiency as well. The method offers the promise for routine quality control of pharmaceuticals. This novel method can be applied in the throughput analysis of vitamin B_{12} in multivitamin tablets.

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