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Short communication

Determination of cobalt in pharmaceutical products

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Abstract

The aim of this paper is to determine the content of cobalt in pharmaceutical products (B_{12} vitamin powder, B_{12} ampoules, Centrum, Spectrum ABC and Optima Forte) by spectrometric (FAAS, GFAAS and ICP-AES) and electrometric (AdSV) analytical techniques.

The samples (∼0.5 g) were treated with a mixture of 6 mL HNO_3 and 1 mL H_2O_2 in the microwave oven. Due to the matrix effects the method of standard addition is preferred. The validity of the methods was tested by recovery studies of standard addition and results were found to be satisfactory.

Keywords: Cobalt; Spectrometric techniques; Electrometric technique; Pharmaceutical products

1. Introduction

Cobalt is an essential element for plants, animals and human body and it is present in B_{12} vitamin in 4.35% and has an important role in some biochemical metalloenzyme reactions. The presence of cobalt in food improves the synthesis of hemoglobin; the most important sources of this are kidney, liver and also green vegetables like cabbage and spinach. Bacteria, fungi and algae can synthesize B_{12} vitamin, yeasts, higher plants and animals cannot. B_{12} vitamin is a coenzyme in a lot of biochemical processes; the most important is the erythrocytes formation [1,2]. For its determination various methods were used [3,4].

The concentration of cobalt necessary per day for human body is 8 μg [5]. A cobalt deficiency can produce loss of appetite, chronic swell and pernicious anemia. High concentration of cobalt causes irritation of gastrointestinal tract, nausea, diarrhea, lung and heart diseases and inhibits some enzyme activities.

Also, B_{12} vitamin deficiency is followed by a high accumulation of iron and nickel in liver. The determination of heavy metals in pharmaceutical and food samples has a great importance for human health. Some aliments are rich in cobalt (B_{12} vitamin), like kidney, liver and brain, 0.02–2 μg/g, and others, cheese, milk, eggs and meat contain low concentration of cobalt, 2×10^{-4} to 2×10^{-3} μg/g.

The determination of cobalt in various samples (food, pharmaceutical preparations, biological and environmental samples) by different methods (FAAS, GFAAS, ICP-AES, ICP-MS, stripping voltammetry, NAA, GC–MS, etc.) represents the aim of a lot of papers [6–13].

The low cobalt content in some pharmaceutical products makes FAAS less useful and another methods should be utilized. Graphite furnace atomic absorption spectrometry is a common method applied for cobalt determination from pharmaceutical samples. Also, inductively coupled plasma atomic emission spectrometry has some advantages, offers multielement determination and is relatively free from interferences. Voltammetric methods, especially differential pulse adsorptive stripping voltammetry with dimethylglyoxime, as chelating agent is one of the most used techniques for cobalt determination [14].

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The novelty of this paper consists in a rapid and reliable technique utilized for sampling of cobalt from different pharmaceutical preparations using microwave digestion and the comparison of results obtained by spectrometric and electrometric analytical techniques. Adsorptive stripping voltammetry was adapted for cobalt determination at hanging mercury drop electrode in NH_4OH–NH_4Cl buffer.

For cobalt determination from pharmaceutical samples it was chosen two sensitive and selective analytical methods, graphite furnace atomic absorption spectrometry and adsorptive stripping voltammetry, because the samples contain also another microelements and some organic compounds, especially vitamins.

2. Experimental

2.1. Apparatus

ICP-AES determinations were carried out by means of an atomic emission spectrometer with inductively coupled plasma, SPECTROFLAME-P (SPECTRO-Analytical Instruments, Germany). The instrument has 30 fixed spectral channels that can simultaneously be monitored by the three polychromators and allows one to carry out the background correction, application of internal standard method and other facilities. The argon utilized was of spectral purity (99.998%), the cooling flow rate 12 L min^{-1}, the auxiliary flow rate 0.8 L min^{-1} and nebulizer flow rate 1 L min^{-1} all of them are functions of plasma power and some of them were automatically controlled. The consumption rate of the liquid sample was about 2 mL min^{-1}. The observation height is adjustable; it is usually 12 mm.

For FAAS cobalt determination a Carl Zeiss-AAS-30, double beam, with background correction was utilized. The flame was stoechiometric ratio air–acetylene, flow rate 1–1.2 L min^{-1}, wavelength was 240.7 nm.

For GFAAS determination a VARIAN, AA 880 spectrometer with a graphite furnace GTA 100, autosampler, and hollow cathode lamp for cobalt (VARIAN) was utilized. The sample volume was 10 μL, the split width 0.5 nm, wavelength was 240.7 nm.

For voltammetric determinations an electrochemical system, polarographic and voltammetric ensemble Trace Master 5 and POL 150 Polarographic Analyzer (Radiometer, Copenhagen) was used. The electrochemical cell contained hanging mercury drop electrode (HMDE) as working electrode, a reference Ag/AgCl electrode and platinum wire as auxiliary electrode.

The solutions were deaerated with analytical grade argon (99.998%) at the start of each experiment and a flow of argon was maintained over the solution during the experiment to prevent oxygen interference. All experiments were performed at a constant temperature of 25 °C.

2.2. Reagents

Standard stock solution of cobalt (1000 μg/mL) were prepared from metallic cobalt of spectral purity (Johnson Mathey Standards) dissolved in HNO_3 (Merck, Germany) and diluted with Milli-Q water in a 100 mL volumetric flask. The diluted solutions were prepared daily.

The reagents used were analytical grade (Merck, Germany) and all the solutions were prepared in Milli-Q (18 MΩ cm) water. HNO_3 (65%) and H_2O_2 (30%) were analytical grade (Merck, Germany).

The dimethylglyoxime (Merck, Germany) solution was made in alcoholic medium and was prepared daily.

2.3. Analytical procedure

Cobalt was determined from B_{12} vitamin powder, Merck, Germany and B_{12} ampoules, SICOMED S.A., Romania by different techniques, electrometric (stripping voltammetry) as well as by spectrometric techniques, flame atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry.

Also, cobalt was determined from three pharmaceutical products, Centrum, WHITEHALL®, USA; Spectrum ABC, WALMARK®, Czech Republic; Optima Forte, Stanley Pharmaceuticals Ltd., Canada by graphite furnace atomic absorption spectrometry and adsorptive stripping voltammetry.

The sampling procedures are very important and influence the analysis and data processing. The digestion of the samples was performed taking into account the matrix and the analytical technique used for metal ion determination.

For cobalt determination in pharmaceutical preparations firstly it was necessary digestion of the samples as following: 10 tablets were taken and homogenized. The appropriate aliquots were digested.

A Milestone model MLS-1200 Mega (Milestone Laboratory Systems, Italy) microwave (MW) oven (1000 W maximum power) equipped with six high-pressure (up to 100 bar) Teflon® containers was used for digestion.

The samples (around 0.5 g) were treated with a mixture of 6 mL HNO_3 and 1 mL H_2O_2 in the microwave oven with the following program: 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, 5 min at 650 W and finally ventilation 5 min. Five replicate assays were made.

2.4. Calibration procedure

Calibration curve of cobalt was conducted using the standard solution described previously. Triplicate registrations were made of each standard solution and each calibration curve was fitted by linear regression.

3. Results and discussion

Samples digestion in a microwave oven has some advantages over the classic digestion techniques such as the

Table 1
Comparative results obtained for cobalt determination by three analytical techniques

Sample	Amount of sample taken (mg)	FAAS			ICP-AES			AdSV		
		Confidence limit[a] (mg)	R.S.D. (%)	Recovery (%)	Confidence limit[a] (mg)	R.S.D. (%)	Recovery (%)	Confidence limit[a] (mg)	R.S.D. (%)	Recovery (%)
B_{12} powder	4.35	4.05 ± 0.07	0.43	93.10	4.34 ± 0.03	0.35	99.77	4.35 ± 0.01	0.16	100.00
B_{12} ampoules	4.35	4.48 ± 0.03	0.78	102.99	4.39 ± 0.01	0.17	100.92	4.34 ± 0.03	0.14	99.77

[a] $n = 5$; $P = 0.95$.

Table 2
The optimum parameters established for adsorptive stripping voltammetric determination of cobalt

Number of drops	3
Stirring speed	400 rpm
Purge time	300 s
Electrolysis time	45 s
Waiting time	10 s
Deposition time	0.7 s
Initial potential	−700 mV
Final potential	−1200 mV
Step amplitude	−2 mV
Pulse duration	40 ms
Pulse amplitude	−50 mV
Initial current	10 nA
Final current	10 μA

reduction of the digestion time, rapidity and the reproducibility reduced contamination of the samples.

The results obtained for cobalt determination by FAAS, ICP-AES and stripping voltammetry in B_{12} powder and B_{12} ampoules are presented in Table 1.

To check the validity of the proposed methods the standard addition method was applied by adding cobalt solution to the previously analyzed samples. The precision of the proposed method was evaluated by a replicate analysis of samples containing cobalt in different concentrations.

From these results on observed a good correlation between the values obtained by FAAS, ICP-AES and stripping voltammetry and the best results were obtained for AdSV determination.

The recoveries (93.10–102.99%) agree well enough with the nominal contents. In order to fully characterise the methods, the statistical evaluation was made.

Also, cobalt was determined in three real samples with complex matrices, Centrum, Spectrum ABC and Optima Forte. The samples were analyzed by two analytical techniques based on different principle, graphite furnace atomic absorption spectrometry (GFAAS) and adsorbtive stripping voltammetry (AdSV). The pharmaceutical products analyzed contain together with B_{12} vitamin microelements such zinc, molybdenum, chromium, nickel, vanadium, tin, manganese, copper, selenium, iron and another vitamins (A, E, C, B_1, B_2, B_6, D, K), and to determine cobalt in these samples it was necessary to utilize sensitive and selective analytical methods.

For voltammetric determination, firstly the optimum parameters (deposition time, step duration, step amplitude and pulse amplitude) were established (Table 2) and than the calibration curve was constructed by plotting current intensity against concentration.

The calibration curve for cobalt determination by AdSV was linear in the range 2–40 μg/L and linear regression equation was $y = 0.0822x + 0.0507$, the correlation coefficient of 0.9986 indicating good linearity.

Limits of detection LOD (S/N = 3:1) and limit of quantification LOQ (S/N = 10:1) for cobalt were 0.10 and 0.50 μg/L, respectively ($n = 5$).

Dimethylglyoxime was utilized for adsorptive stripping voltammetric determination of cobalt, to increase the sensitivity and selectivity of the method.

No interferences were observed in the determination of cobalt by AdSV in the presence of the common excipients and additives of the tablets, such as talc, magnesium stearate, starch, lactose, glucose and dextrose. The organic support was destroyed by dissolution of the samples. Zinc interference can be eliminated by masking with EDTA.

To check the validity of the proposed method, the results obtained by GFAAS were compared with the results obtained by AdSV (Table 3) and also standard addition method was applied. The standard addition recoveries were carried out by adding a known amount of standard to the blank excipient at three different levels. Each level was repeated five times ($n = 5$) and the results are determined using the calculation algorithms in TraceMaster 5 Software. The method of standard addition was preferred due to the complexity of the real samples.

Table 3
Determination of cobalt in different pharmaceutical products

Sample	GFAAS				AdSV			
	Co content (μg/g)	Co found[a] (μg/g)	R.S.D. (%)	Recovery (%)	Co content (μg/g)	Co found[a] (μg/g)	R.S.D. (%)	Recovery (%)
Centrum	1.88	1.89 ± 0.11	5.80	100.53	1.88	1.83 ± 0.15	4.60	97.34
Spectrum ABC	1.80	1.81 ± 0.01	0.70	100.55	1.80	1.78 ± 0.03	1.68	98.88
Optima Forte	2.60	2.50 ± 0.07	2.70	96.15	2.60	2.55 ± 0.07	2.74	98.07

[a] Results are average of five determinations.

The recoveries (96.15–100.55%) agree well enough with the nominal contents. In order to fully characterise the methods, the statistical evaluation was made.

The samples were stable in time for more than one month and the results of the cobalt determination were reproducible according the results presented in Tables 1 and 3. For the determination of the repeatability of the measurements cobalt was analyzed five times in the same sample.

4. Conclusions

Having in mind the great importance of cobalt for human metabolism, this element was determined in some complex multivitamin preparations. The determination of cobalt was made by different techniques, electrometric (AdSV) as well as by spectrometric techniques, FAAS, GFAAS, ICP-AES. Due to the fact that these techniques, AdSV and GFAAS are very sensitive and selective, they can be utilized for cobalt determination in various pharmaceutical products. GFAAS method is used for most pharmaceutical samples due to its rapidity, very low quantity of samples, possibility of automation and high sensitivity.

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