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Determination of vitamin B_{12} in pharmaceutical preparations by electrothermal atomic absorption spectrometry

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A method for the determination of cyanocobalamine (vitamin B_{12}) in different pharmaceutical preparations by electrothermal atomic absorption spectrometry, is proposed. The samples to be analyzed were dissolved either in distilled water in ultrasonic bath or with acidification by hydrochloric acid. From the obtained results of cobalt concentration, the amount of vitamin B_{12} was calculated. The procedure was verified by a method of standard additions and by comparing with a HPLC technique. The precision of the method, as a relative standard deviation, was in the range from 3 to 5.5%. The detection limit for cobalt in the pharmaceutical preparation, calculated as 3σ of the blank, was 0.001 μg g^{-1} (*i.e.* 0.025 μg g^{-1} of vitamin B_{12}).

Keywords: vitamin B_{12}, determination, pharmaceutical preparations, electrothermal atomic absorption spectrometry

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Vitamin B_{12}, cyanocobalamine ($C_{63}H_{88}CoN_{14}O_{14}P$), plays an important role in human metabolism. Different types of pharmaceuticals are of interest as preparations suitable for the prevention and treatment of vitamin B_{12} deficiency.

The cyanocobalamine has been assayed by different physico-chemical (1–19) and biological (20–22) methods. It has been usually determined by spectrometric (1–11), chromatographic (12–19) or microbiological (20–22) procedures. The microbiological method is very sensitive. However, it requires highly diluted solution which considerably reduces the accuracy.

Several methods have been reported for the determination of vitamin B_{12} by flame atomic absorption spectrometry (4–9) or electrothermal atomic absorption spectrometry (10–11). The application of the atomic absorption spectrometry is based on the indirect measurement of the vitamin through determination of cobalt. Flame AAS has been applied in the samples in which the content of vitamin B_{12} is relatively high. Diaz (6) used pre-mix air acetylene flame atomic absorption spectrometry in the determination of B_{12}. Moudrou and Bres (5) carried out the analysis of vitamin B_{12} by previous mineralization of the pharmaceutical tablets with sulfuric acid. They found that the presence of NaCl,

* Correspondence

Na_2SO_4, $NaCH_3COO$ or calcium gluconate decreases the absorbance of cobalt. Therefore, they carried out the analysis in the presence of *iso*butylic acid for increasing the sensitivity. For the same reason, Suzuki *et al.* (8) added 75% of saturated oxine solution for the vitamin B_{12} assay in some cobalt containing proteins.

The electrothermal atomic absorption spectrometry has been used for the determination of low concentrations of vitamin B_{12} in pharmaceutical and vitamin preparations (10, 11). The method of Peck *et al.* (10) involves the use of flameless atomic absorption spectrometry. He also optimized the parameters which may affect the precision and accuracy for cobalt. With this technique, a calibration graph was obtained using aqueous standards of cobalt salt. Recently, a new method was reported by Akatsuka and Atsuya (11) for analysis of vitamin B_{12} as cobalt in pharmaceutical samples with solid sampling technique by electrothermal atomic absorption spectrometry.

In this work we suggest a modification of the Peck's method (10) for the determination of vitamin B_{12} as cobalt by electrothermal atomic absorption spectrometry. This modification includes a simple sample preparation, application of vitamin B_{12} standards for calibration and improved instrumental parameters and technique. The method is verified by standard additions method and by comparing with a HPLC technique (18, 19, 25). By this method, very low concentrations of vitamin B_{12} in different pharmaceutical and vitamin preparations can be determined.

EXPERIMENTAL

Instrumentation

A Perkin-Elmer Model 703 and Model 1100B atomic absorption spectrophotometers equipped with a deuterium background corrector, HGA-400 and HGA-700 graphite furnaces. A cobalt hollow cathode lamp was used as a source and background correction was applied through the course of analysis. Pyrolytically coated graphite tubes with platforms were used. Operation conditions for cobalt determination were established by extensive testing and they are given in Table I.

A Perkin-Elmer high performance liquid chromatograph Model 250 with binary pump, LC-235 diode array detector and Vydac pharmaceutical column (C18) were used. The samples were introduced in the column by sample loop injector with an effective volume of 20 μL. The Omega software package V2.50 was used for data handling and storage. The operation conditions and the program for the HPLC determination of vitamin B_{12} are given elsewhere (18, 19, 25).

Reagents and samples

All reagents were of analytical grade. Stock solution of vitamin B_{12} was prepared by dissolving the appropriate amount of crystalline vitamin B_{12}, p.a. (E. Merck, Darmstadt, Germany). The concentration of vitamin B_{12} was 0.1 g L^{-1}, corresponding to a cobalt concentration of 0.004 g L^{-1}, and from this solution other diluted solutions were prepared.

Table I. Instrumental parameters for cobalt determination by electrothermal atomic absorption spectrometry

Wavelength (nm)	240.7
Spectral band pass (nm)	0.2
Lamp current (mA)	20
Calibration mode	absorbance, peak height
Background correction	deuterium arc lamp
Graphite furnace	
Dry	
Temperature (°C)	120
Time (s)	20
Ramp time (s)	10
Char	
Temperature (°C)	1300
Time (s)	20
Ramp time (s)	15
Atomize	
Temperature (°C)	2600
Time (s)	5
Ramp time (s)	0
Cleaning	
Temperature (°C)	2650
Time (s)	5
Ramp time (s)	1
Gas	argon

The investigated samples are from different pharmaceutical companies: Krka (Novo Mesto, Slovenia), Galenika (Belgrade, Yugoslavia), Alkaloid (Skopje, Macedonia) and Vitaminka (Prilep, Macedonia).

Procedures

Depending on the vitamin B_{12} concentration in the corresponding pharmaceutical samples (tablets, injections, powder), different amounts were taken for analysis. In the analysis of tablets, two tablets of each type were dissolved in 2 mL od distilled water in an ultrasonic bath and the solutions were centrifuged. The samples can be dissolved in 2 mL HCl solution (1 mol L^{-1}) as well. The solutions were then filtered and used for analysis. The amount of powder samples for analysis was 5 to 10 g and they were treated in a similar way like the tablets using 5 mL of the solvent. The stock solution of B_{12} (0.1 g L^{-1}) was prepared by dissolving crystalline vitamin B_{12} (Merck) in distilled water and the standard solutions were then prepared from it. These solutions (20 μL) were applied in the graphite tube (AAS) and in the sample loop (HPLC).

RESULTS AND DISCUSSION

Vitamin B_{12} is present in different pharmaceutical and vitamin preparations, solid (tablets, powder) or liquid (injections). Its concentration can be very low: below 1 μg per tablet or below 1 μg in a 10 g of multivitamin powder sample. It has been found that vitamin B_{12} in the analyzed pharmaceutical preparations can be completely dissolved in distilled water using ultrasonic bath. Namely, the undissolved solid was analyzed and no traces of cobalt were found.

The optimal charring temperature was found by extensive testing; loss of cobalt was not observed in this step (Fig. 1). The charring temperature applied by Peck (10) was much lower than the one we used and those given in the literature (20).

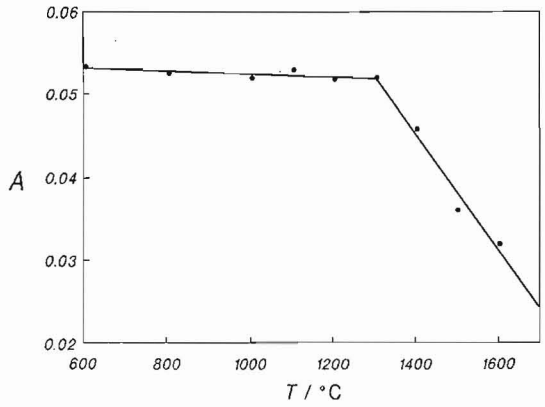

Fig. 1. Determination of optimal charring temperature.

Usually, in pharmaceutical or vitamin preparations with vitamin B_{12}, calcium pantotenate is added both as vitamin and as a stabilizing component for vitamin B_{12}. Because of that, the possible influence of calcium pantotenate on the cobalt determination was investigated. Series of solutions with same concentration of cobalt and different concentrations of calcium pantotenate were prepared. It was found that, even when calcium pantotenate is present in very high concentrations (up to 50 mg per tablet) its interference effect is practically negligible (no changes in cobalt absorbance were noticed).

To verify this procedure, vitamin B_{12} was determined in several pharmaceutical (tablets) and vitamin preparations. Some of the samples were prepared with standard additions of vitamin B_{12} and treated in the same manner. The results of the determination of vitamin B_{12} (as cobalt) are given in Tables II and III. It can be seen that the results obtained by the standard addition method are satisfactory (recovery from 95.49 to 103.3 %).

The determination of vitamin B_{12} in some pharmaceutical preparations was also performed using the HPLC technique (18, 19, 25). The results obtained with both the AAS and HPLC technique are in good agreement and they are given in Table IV together with the relative deviation of the HPLC results to the AAS results.

Table II. Content of B_{12} in pharmaceutical samples (tablets) obtained by standard addition method determined by ETAAS

Preparation sample No.	Content of B_{12} (μg) per tablet Added	calculated	found	Recovery (%)
B-complex tablets – Krka				
1	–	–	2.18	–
2	0.5	2.62	2.66	99.2
3	1.0	3.18	3.19	100.3
4	2.0	4.18	4.24	101.4
B-complex tablets – BEVIPLEX – Galenika				
1	–	–	1.02	–
2	2.97	3.99	3.81	95.5
3	5.95	6.97	6.92	99.3

Table III. Concentration of B_{12} in pharmaceutical samples obtained by standard addition method determined by ETAAS

Preparation sample No.	Concentration of B_{12} (μg g^{-1}) Added	calculated	found	Recovery (%)
Multivitamin preparation – DUOVIT – Krka				
1	–	–	0.125	–
2	0.1	0.225	0.224	99.5
3	0.2	0.325	0.324	99.7
Multivitamin preparation – MAKROVIT – Krka				
1	–	–	0.095	–
2	0.10	0.195	0.188	96.4
3	0.20	0.295	0.290	98.3
4	0.30	0.395	0.408	103.3
Multivitamin preparation – CEVITANA Orange – Vitaminka, Prilep				
1	–	–	0.062	–
2	0.149	0.155	0.157	101.3
3	0.297	0.359	0.352	98.1
4	0.595	0.657	0.669	101.8
Multivitamin preparation – CEVITANA Lemon – Vitaminka, Prilep				
1	–	–	0.10	–
2	0.149	0.259	0.255	98.7
3	0.295	0.395	0.386	97.7
4	0.595	0.695	0.680	97.8

A calibration curve was constructed in the range of 0–20 ng cobalt or 0–500 ng vitamin B_{12} and was found to be linear over this range. It is important to note that the calibration curve should be made using the absorbance value from the determination of cobalt from the solutions of vitamin B_{12} not from the cobalt salts solution. Namely, it was found that the absorbance of cobalt is higher if the solution is prepared from vita-

Table IV. AAS and HPLC results of determination of vitamin B_{12}

Pharmaceutical preparation	A (AAS) μg per tablet	B (HPLC) μg per tablet	$\frac{B-A}{A} \cdot 100$
MAKROVIT	1.091	1.104	1.19
DUOVIT	1.038	1.051	1.25

min B_{12}. This can be explained by the effect of the presence of compounds with high carbon content on absorbance (23, 24).

The standard deviation (σ) for 1 ng of cobalt is 0.05. A relative standard deviation for this method is from 3.5 to 5.0% ($n = 9$). The detection limit for cobalt in the pharmaceutical preparations, calculated as 2σ of the blank, is 0.001 μg g^{-1}. This corresponds to 0.025 μg g^{-1} of vitamin B_{12}.

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S A Ž E T A K

Određivanje vitamina B_{12} u farmaceutskim pripravcima elektrotermičkom atomsko-apsorpcijskom spektrometrijom

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Predložena je metoda određivanja cijanokobalamina (vitamina B_{12}) u različitim farmaceutskim pripravcima elektrotermičkom atomsko-apsorpcijskom spektrometrijom. Uzorci se otope u destiliranoj vodi ili klorovodičnoj kiselini i sadržaj vitamina B_{12} se izračuna iz izmjerenih koncentracija kobalta. Relativna standardna devijacija određivanja jest 3 do 5.5%, a granica detekcije kobalta jest 0.001 μg g^{-1} (tj. 0.025 μg g^{-1} vitamina B_{12}).

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