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DETERMINATION OF VITAMIN B_{12} IN MULTIVITAMIN TABLETS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Key words: vitamin B_{12}, cyanocobalamine, determination, tablets, multivitamin, B-complex, High Performance Liquid Chromatography, HPLC

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ABSTRACT

Two procedures for separation and determination of vitamin B_{12} in multivitamin tablets by reversed phase high performance liquid chromatography are proposed. Sample preparation is very simple: tablets are dissolved in distilled water, centrifuged and filtered. The sample solution is directly applied in the sample loop injector and chromatograms are obtained with gradient elution using water-methanol and water-acetonitrile as solvents. The peak of vitamin B_{12} from samples of B-complex tablets is well separated with the two procedures. For multivitamin tablets, however, only the procedure

with water and methanol as solvents was good for separation and quantification of vitamin B_{12}. Both procedures were verified by the standard addition method and also compared to a previously developed method using electrothermal atomic absorption spectrometry for vitamin B_{12} determination.

INTRODUCTION

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

Cyanocobalamine has been assayed by different physico-chemical[1-10] and biological[11-13] methods. It has usually been determined by spectroscopic[1-5], chromatographic[6-10] or microbiological[11-13] procedures. The microbiological method is very sensitive. However, it requires a highly diluted solution which considerably reduces the accuracy.

Analyses of many vitamins have been investigated by HPLC[14-16]. Separation of cyanocobalamine and its analogs has been reported[6-10]. However, HPLC has not been used for routine determination of cyanocobalamine because experimental conditions require sample preparation with preconcentration of the ultramicro amounts of cyanocobalamine and removal of interferences by solid-phase extraction.

This paper describes separation and quantitative determination of vitamin B_{12} in multivitamin tablets by reversed phase HPLC without preconcentration steps. Sample solutions prepared from multivitamin tablets are directly subjected to reversed-phase high performance liquid chromatography (HPLC) with binary gradient elution and detection at 362 nm. Two methods were developed using water-acetonitrile and water-methanol as solvents. Both

procedures were verified by standard additions method and also compared to a previously developed method using electrothermal atomic absorption spectrometry for vitamin B_{12} determination[4]. The comparison between methods shows satisfactory results.

EXPERIMENTAL

Chromatographic conditions

A Varian high-performance liquid chromatograph with ternary pump Model 9012 and UV diode-array detector Model 9064 were used. The column was C18 15 x 0.46 cm ID, 5 μm particle size (Varian, USA). The samples were applied with a Rheodyne Model 7125 sample loop injector with an effective volume of 100 μL.

The mobile phase flow rate was 1.0 mL/min and the gradient elution programs for the two proposed procedures (water-methanol and water-acetonitrile) are given in Table 1.

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} (E. Merck) in redistilled water. Its concentration was 100 μg/mL, and by diluting this solution the calibrating solutions with concentrations 1.0; 2.0; 3.0; 4.0; 5.0 and 10.0 μg/mL were prepared.

The solvents, methanol and acetonitrile, were of HPLC grade and redistilled water was used.

The analyzed samples were tablets of two types:

- B-complex tablets- containing only B-vitamins (B_1, B_2, B_6, B_{12}, niacinamide, folic acid, calcium pantotenate and biotin).
  Vitamin B_{12} content: 150 μg/tablet.

TABLE 1

Gradient elution programmes, a) water-methanol; b) water-acetonitrile

a) H_2O-CH_3OH			b) H_2O-CH_3CN		
t/min	$\omega(H_2O)$/%	$\omega(CH_3OH)$/%	*t*/min	$\omega(H_2O)$/%	ω(50% CH_3CN)/%
0	100	0	0	100	0
15	85	15	15	80	20
25	75	25	25	75	25
35	20	80	30	0	100
40	20	80	40	0	100

- Multivitamin tablets-containing the following vitamins: β-carotene, D, E, C, B_1, B_2, B_6, B_{12}, niacinamide, folic acid, calcium pantotenate and biotin.

  Vitamin B_{12} content: 50 μg/tablet.

Sample preparation

One tablet was placed in a 50 mL tube and 20 mL of distilled water were added. The mixture was sonicated for 20 min, centrifuged for 15 min (5000 s^{-1}), filtered through sintered glass filter (pore diameter 5 μm), transferred to a 25 mL volumetric flask and completed to volume with distilled water. Depending on the vitamin B_{12} content more than one tablet can be taken for analysis.

Aliquots of the sample solutions were applied in the sample loop with volume of 0.1 mL and the peak area was determined. The amount of vitamin

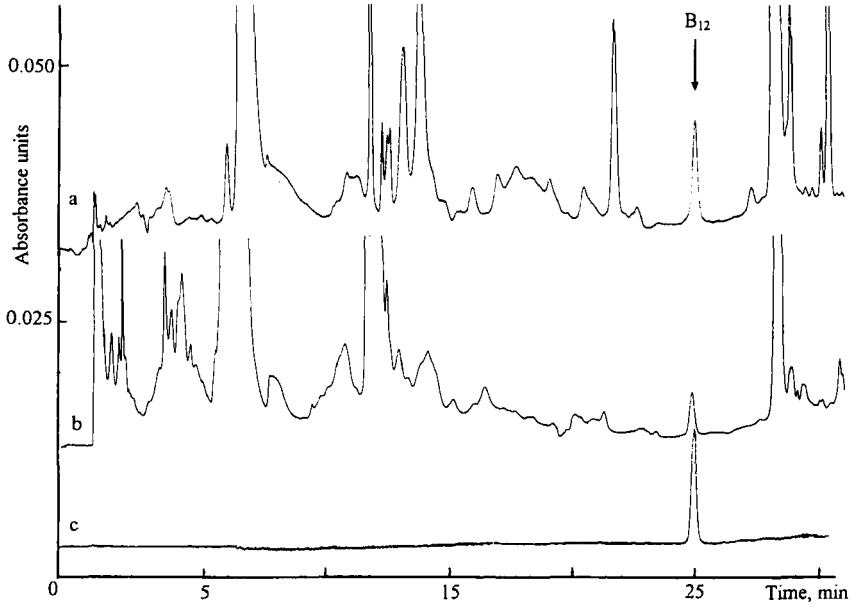

FIG. 1. Chromatograms of: a) B-complex tablets sample solution; b) Multivitamin tablets sample solution; c) Vitamin B_{12} standard solution, obtained with water-methanol gradient elution

B_{12} was determined by comparing the peak area obtained for the sample with those for the standard solutions.

RESULTS AND DISCUSSION

The HPLC chromatogram of B-complex (a), multivitamin tablets (b), and B_{12} standard solution (c) using water-methanol gradient elution (Table 1b) is shown in Fig. 1. The B_{12} peak is well separated at 25.5-26.0 min. No interference from other components was observed.

Fig. 2 shows the chromatogram of B-complex (a), multivitamin tablets (b), and B_{12} standard solution (c) using water-acetonitrile gradient elution (Table

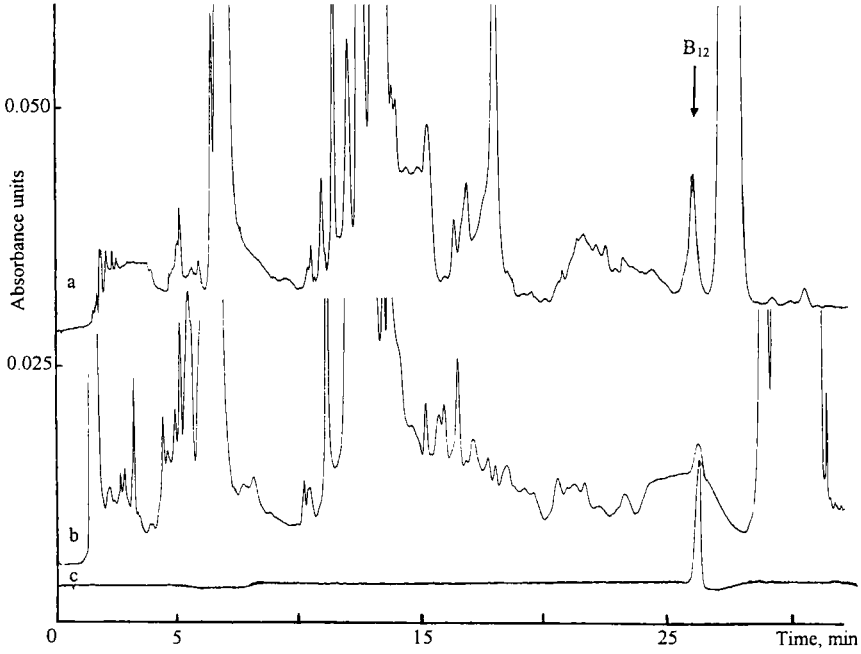

FIG. 2. Chromatograms of: a) B-complex tablets sample solution; b) Multivitamin tablets sample solution; c) Vitamin B_{12} standard solution, obtained with water-acetonitrile gradient elution

1b). Vitamin B_{12} elutes at ~26 min. The chromatogram of the B-complex sample solution shows good separation of the peak of vitamin B_{12}. In the chromatogram of the multivitamin sample the peak of B_{12} is placed on a broad peak from an interfering substance which is eluted together with B_{12} and this procedure can not be used for B_{12} analysis in this kind of sample.

A regression analysis of the peak areas relative to the used concentrations of standard solutions gives the following equation:

$$y = 8.500 \cdot x - 0.848$$

TABLE 2

Results of the vitamin B_{12} determination and standard additions method in two samples: B-complex and multivitamin tablets; a) water-methanol gradient elution; b) water-acetonitrile elution

	Sample	B_{12}, μg/mL	Standard additions Added, μg/mL	Calculated, μg/mL	found, μg/mL	%R
		3.99	2.00	3.00	2.97	99.11
	B-complex		4.00	4.00	3.93	98.23
a	tablets		6.00	5.00	4.89	97.82
		1.23	1.00	1.11	1.13	101.82
	multivitamin		3.00	2.11	2.09	98.97
	tablets		5.00	3.11	3.09	99.09
		3.90	2.00	2.95	2.89	98.12
b	B-complex		4.00	3.95	3.93	99.44
	tablets		6.00	4.95	4.80	97.10

where: y-concentration, x-peak area. The coefficient of correlation is 0.9998 and the standard deviation 0.55.

The accuracy of both procedures was verified by the standard addition method. Solutions were prepared by mixing equal volumes of sample solution and standard solution of B_{12}. Solutions of B-complex were mixed with equal volumes of standard solutions with concentrations of B_{12}: 2.0; 4.0 and 6.0 μg/mL, and multivitamin sample solutions were mixed with equal volumes of standard solutions with concentration of B_{12}: 1.0; 3.0 and 5.0 μg/mL. Satisfactory values were obtained for the recovery (97.10-101.82%). Results of the two described procedures and also those from the standard additions method are given in Table 2.

TABLE 3

Results from B_{12} analysis obtained with two HPLC procedures and ETAAS

Sample	HPLC		ETAAS
	water-methanol	water-acetonitrile	
B-complex tablet	3.99	3.90	3.92
Multivitamin tablet	1.23	-	1.26

Analysis of vitamin B_{12} was also performed by electrothermal atomic absorption spectrometry (ETAAS) using the method we developed and published[4]. Comparison of results obtained by HPLC and ETAAS is given in Table 3. It can be seen that HPLC results agree with those from the AAS analysis.

CONCLUSION

Two procedures for separation and quantitative determination of vitamin B_{12} in multivitamin tablets by HPLC without preconcentration steps are proposed. Comparison of results of these methods with results from B_{12} analysis with atomic absorption spectrometry is satisfactory. Results from the standard addition method are satisfactory, too. So, both proposed procedures can be used for vitamin B_{12} determination in solutions of B-vitamins and one for multivitamin samples.

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