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Use of Solid Phase Extraction for Sample Clean-up and Preconcentration of Vitamin B_{12} in Multivitamin Tablet before HPLC-UV, UV and Atomic Absorption Spectrophotometry

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ABSTRACT

A solid phase extraction method for sample clean-up and preconcentration of vitamin B_{12} in multivitamin tablet and its determination by HPLC-UV, UV and atomic absorption spectrophotometry is reported. Solid phase parameters such as maximum loading capacity and breakthrough volume were 400 µg and 1750 mL of 0.001 ppm for this vitamin respectively. A proper mobile phase used for HPLC was water/acetonitrile (75:25, v/v) with pH = 3.5 (adjusted with acetate buffer) at 25°C. Limit of detection and linear dynamic range for HPLC, UV and atomic absorption were 0.1, 0.1-25.0; 0.4, 0.4 -15.0; 2.7, 2.7-300.0 (µg/mL) respectively. Recovery of vitamin B_{12} was about 96%.

Key words: cyanocobalamin (vitamin B_{12}), multivitamin tablet, solid phase extraction, HPLC, UV, atomic absorption (AA)

INTRODUCTION

Vitamin B_{12} (cyanocobalamin) is a member of a group of complex organo-cobalt compounds called corrinoids[1]. It is water- soluble and is naturally found in foods such as fish, meat, milk or milk products[2]. Vegetables are poor sources of vitamin B_{12} and vegetarians have low level of vitamin B_{12}[3-5]. Incorporation of vitamin B_{12} into the vegetables by absorption from their seeds has been done to solve this problem for vegetarians[6]. Numerous techniques have been developed to extend application of HPLC, including pre and post column labeling[7,8] and solid phase extraction (SPE) for determination of nutrients from foods, drugs and their metabolites from biological fluids[9-11]. The SPE strategy generally comprises the isolation and preconcentration of the analytes from a complex matrix by adsorption on to an appropriate sorbent, removal of interfering impurities by washing with a suitable solvent system and selective recovery of the retained analytes with a suitable solvent. HPLC is a useful tool for separation and determination of various vitamins[12-15]. Vitamin B_{12} were determined by spectrophotometery[16,17] microbiological[18,19], chemiluminescence[20], fluorescence[21], voltammetry[22] and enzymology[23] methods. Spectrophotometry method is not suitable for a complex sample matrix[16]. Microbiological method is tedious and time-consuming because it requires that the tissue be cultured and preserved. This paper studies the determination of vitamin B_{12} in multivitamin tablets by HPLC-UV, UV and atomic absorption spectrophotometry after solid phase extraction (SPE). Appropriate conditions for SPE (using a Bond-Elut C_{18} cartridge) and HPLC such as: percentage of organic modifier, pH of mobile phase and temperature examined and suitable conditions was chosen for determination.

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MATERIALS AND METHODS

I. *Chemicals*

The cyanocobalamin (vitamin B_{12}), HPLC grade acetonitrile and methanol used were from Fluka (Buchs, Switzerland). Glacial acetic acid, NaOH and HNO_3 were obtained from Merck (Darmstadt, Germany). Water used was double distilled deionized. Mobile phase was filtered by 0.45 µm filter (Millipore, Bedford, MA, USA).

II. *Apparatus and Conditions*

The chromatographic measurements were carried out with HPLC system equipped with a series 10 LC pump, UV detector model LC-95 set at 360 nm, and model 7125 manual injector. AA determination was carried out using an atomic absorption spectrophotometer model 2380, set at 240 nm, all from Perkin-Elmer (Norwalk, CT, USA). Column used was C_{18} (250 × 4.6 mm, 5 µm) from Waters (Milford, USA). UV measurements were performed with a double beam UV-Vis spectrophotometer model 2100 from Rayleigh Analytical

Instrument (Beijing, China) set at 360 nm. SPE cartridge was Bond-Elut C_{18} (3 mL, 300 mg) from Varian (Harbor City, CA, USA). Cartridge was conditioned with 5mL of methanol and then with 10 mL of deionized water. Adjustment of pH of HPLC mobile phases was done by model 3030 Jen way pH meter (Leeds, UK). The mobile phase used for determination of vitamin B_{12} by HPLC was water/acetonitrile (75/25, v/v) with pH = 3.5 (adjusted with acetate buffer) and flow rate of 1 mL/min. The column temperature was controlled by a water circulator bath at 25°C.

III. *Sample Preparation*

The average weight of ten multivitamin tablets was 3.700 g. Ten tablets were finely powdered and a portion of powder equivalent to one average tablet weight (0.3700 g) was dissolved in 100 mL of deionized water. This solution was filtered and fat soluble vitamins were extracted with 10 mL of n-hexane from filtrate. Ten milliliter of aqueous layer containing vitamin B_{12} was passed through a solid phase cartridge (Bond-Elut C_{18}). Retained vitamin B_{12} eluted with 8 mL of 90% methanol. The eluate was dried in a water bath at 50°C in vacuum and residue was dissolved in 1mL of deionized water and determined by HPLC and UV instruments. For determination by atomic absorption, sample preparation is similar to the above with the exception that residue was dissolved in 1 mL of 0.1 N of HNO_3 instead of deionized water. Stability of vitamin B_{12} solutions was investigated after 24 hours and no degradation of this vitamin was observed.

RESULT AND DISCUSSION

I. *Selection of a Suitable Solvent and Solvent Volume for Elution of Vitamin B_{12} from SPE Cartridge*

To obtain a suitable solvent for elution of vitamin B_{12} from cartridge, different percentages of methanol and acetonitrile solutions in water were examined. Optimal elution of vitamin B_{12} was achieved using 10 mL volumes of 50% acetonitrile or 90% methanol in water (Figure 1A). For determination of suitable volume of elution solvent, different volumes (2, 4, 6, 8, 10 mL) of 50% acetonitrile and 90% methanol in water were used for elution of retained vitamin B_{12} from cartridge. Methanol (90%) and acetonitrile (50%) in water needed for elution, 8 and 10 mL respectively (Figure 1B), so 90% methanol in water was chosen for elution of this vitamin.

II. *Determination of Maximum Loading Capacity of Solid Phase Cartridge*

Maximum loading capacity of solid phase cartridge for adsorption of vitamin B_{12} was determined by passing different volumes (5-120 mL) of 5 μg/mL aqueous standard solutions of this vitamin from the cartridge. Retained vitamin was eluted with 8mL of 90% methanol solution in water, dried in a water bath at 50°C in vacuum. Residue was dissolved in proper volume (The aim of proper volume is a volume which sample must be dissolved in order to be in the range of calibration curve) of deionized water and analyzed by HPLC. Maximum loading capacity of the cartridge was found to be 400 μg (Figure 2).

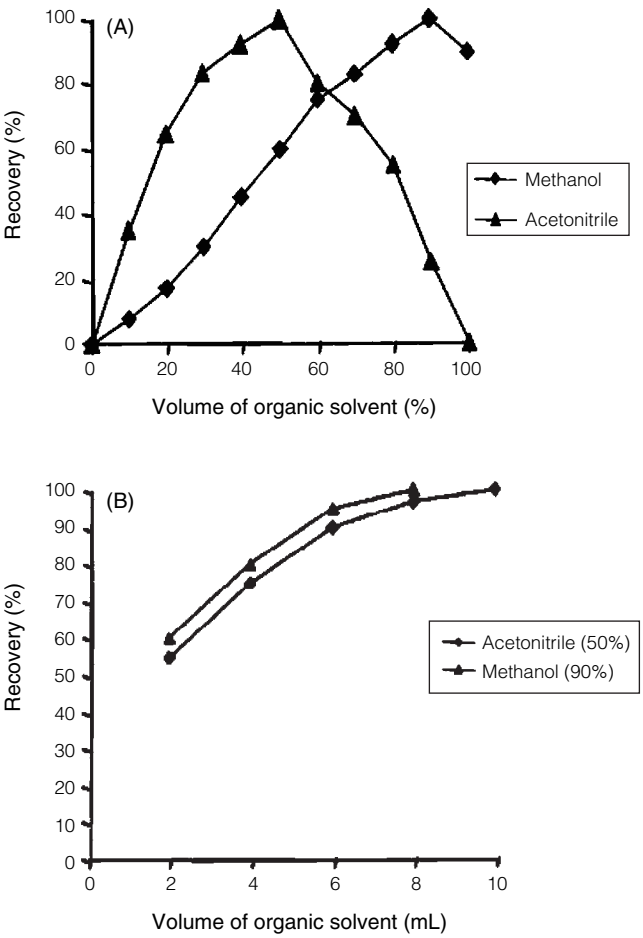

Figure 1. Selection of type and volume of elution solvent for SPE using HPLC. (A) Recovery against percentages volume; (B) Recovery against volumes of elution solvents. Conditions: cartridge, Bond-Elut C_{18}; sample loaded: 5 mL of 2 ppm of vitamin B_{12} solution ; elution volume for (A): 10 mL; HPLC mobile phase, water/acetonitrile (75/25, v/v); pH = 3.5; flow rate = 1 mL/min; column, C_{18} (250 × 4.6 mm, 5 μm); λ = 360 nm; n = 4.

III. *Determination of Breakthrough Volume for SPE*

An assay for determination of breakthrough volume was performed according to the procedure described by Hennion[24]. It consists of preconcentrating samples of increasing volumes, each containing the same amount of analyte. As the sample volume increases, the analyte concentration decreases. While breakthrough does not

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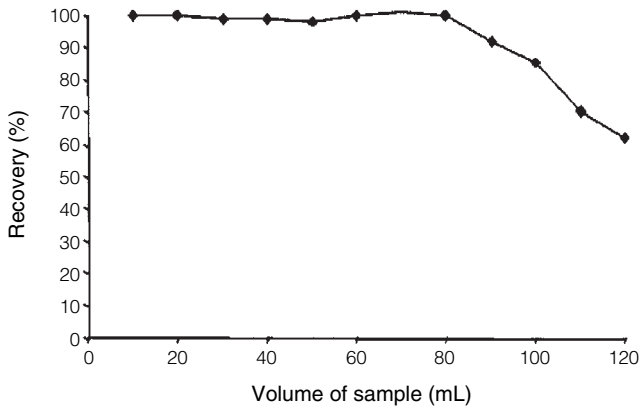

Figure 2. Maximum loading capacity of Bond Elut C_{18} cartridge for adsorption of vitamin B_{12}. Conditions: elution solvent, 8 mL of 90% methanol in water; sample loaded, 5-120 mL of 5 ppm of vitamin B_{12} solutions; other conditions as Figure 1.

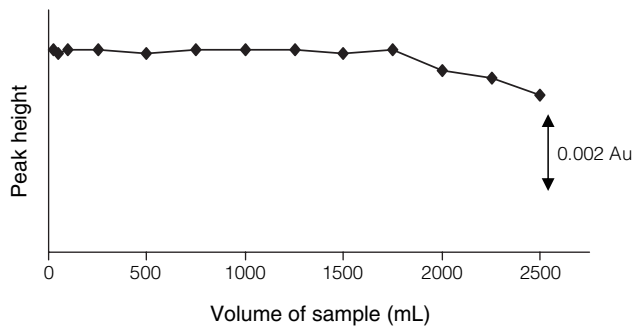

Figure 3. Breakthrough volume of cartridge. Conditions: sample loaded, 25-2500 mL of standard solutions containing 2 μg of vitamin B_{12}; other conditions as Figure 2.

occur, the amount preconcentrated is constant but when breakthrough occurs the recoverd amount is reduced. For determination of break-through volume, 2 μg of standard of vitamin B_{12} was dissolved in 25-2500 mL of deionized water. Each solution passed through the cartridge, eluted with 8 mL of 90% methanol solution in water, dried in a water bath at 50°C in vacuum. Residue was dissolved in 1 mL of deionized water and injected into the HPLC system. Results showed that the break-through volume was 1750 mL (Figure 3).

IV. *Comparison of LOD, LDR and %RSD of HPLC, UV and AA*

Limit of detection (LOD), linear dynamic range (LDR) and relative standard deviation (%RSD) of three methods were obtained and compared. The limits of detections were calculated on the basis of 3Sb/m, where "Sb" is the standard deviation of blank and is equal to 1/5 P-P Noise when only mobile phase was passing through the column for 30 min and "m" is the slope of calibration curve. The RSD of three methods was

Table 1. Comparison of LOD, LDR and %RSD for determination of vitamin B_{12} in multivitamin tablet using SPE

Method	LOD (μg/mL)	LDR (μg/mL)	%RSD
HPLC[a]	0.1	0.1-25.0	2.5
UV[b]	0.4	0.4-15.0	3.3
AA[c] for vitamin B_{12}	2.7	2.7-300.0	4.0
AA for cobalt solution	0.1	0.1-15.0	3.0

[a]HPLC conditions: mobile phase, water/acetonitrile (75/25, v/v); pH = 3.5; flow rate = 1 mL/min; column C_{18} (250 × 4.6 mm, 5 μm); λ = 360 nm; Temp. = 25°C; injection volume = 10 μL.
[b]UV conditions: quartz cell; λ = 360 nm.
[c]AA conditions: flame; air: acetylene; λ = 240 nm.

Table 2. Amount of vitamin B_{12} in each multivitamin tablet using HPLC, UV and AA method

Method[a]	Determined amount (μg)	Stated amount (μg)
HPLC	5.8 ± 0.1	6.0
UV	5.6 ± 0.2	6.0
AA	5.7 ± 0.2	6.0

[a]Conditions as table 1.

obtained from 8 replicate measurements of vitamin B_{12}. The values of LOD, LDR and %RSD are shown in Table 1. Results in this table show that HPLC is more sensitive for determination of vitamin B_{12} than UV and AA and has a wider LDR. Atomic absorption spectroscopy is not very sensitive for determination of vitamin B_{12} because each molecule of vitamin B_{12} has only one atom of cobalt to provide the atomic absorption signal[25]. Precision of three methods was compared using statistical method and there was no difference between the precision of these three methods.

V. *Determination of Vitamin B_{12} in Multivitamin Tablets*

Determination of vitamin B_{12} in multivitamin tablets was performed by standard addition method. Determination by HPLC and UV was performed at λ = 360 nm and by atomic absorption at λ = 240 nm. Amount of vitamin B_{12} in multivitamin tablets are shown in table 2. Typical chromatograms of vitamin B_{12} in standard solution and multivitamin tablet are shown in Figure 4.

CONCLUSIONS

Application of SPE proved to be a suitable technique for sample clean-up and preconcentration of cyanocobalamin in multivitamin tablet. Effect of type of elution solvent and their volumes, maximum loading capacity and breakthrough volume for SPE were studied. Comparison of LOD, LDR and RSD for HPLC, UV and AA showed that HPLC is more sensitive for

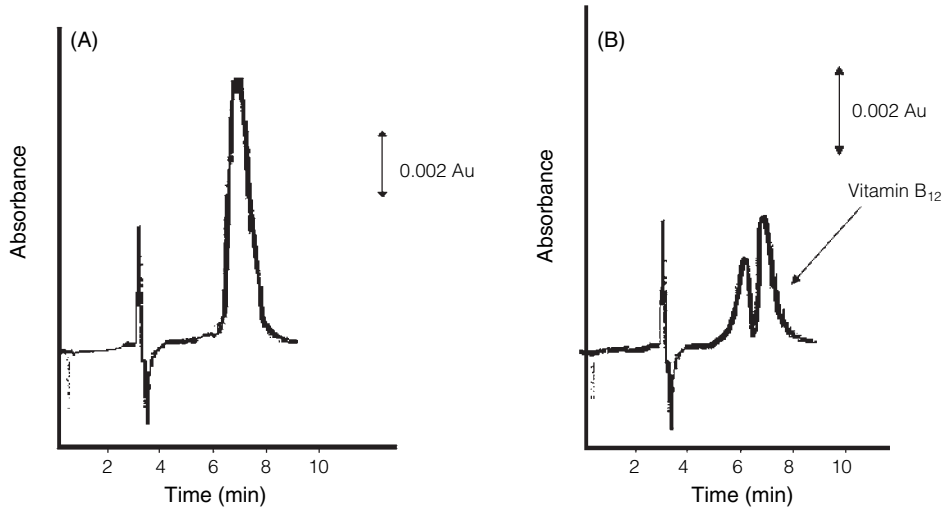

Figure 4: Typical chromatograms of vitamin B_{12} in (A) 5 ppm standard solution and (B) multivitamin tablet. Conditions: mobile phase, water/acetonitrile (75/25, v/v); pH = 3.5; flow rate = 1 mL/min; column C_{18} (250 × 4.6 mm, 5 μm); injection volume = 10 μL; λ = 360 nm; Temp. = 25°C.

determination of vitamin B_{12} than UV and AA and has a wider LDR. Comparison of precision of three mentioned methods using statistical method showed no differences between the precision of these three methods.

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