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Short communication

Determination of vitamin B_{12} in multivitamin tablets by multimode high-performance liquid chromatography

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Abstract

Quantitative determination of vitamin B_{12} in B-complex tablets was performed by using multimode high-performance liquid chromatography. The multivitamin tablets (B_1, B_6 and B_{12}) were sonicated for 30 min in methanol–water (50:50, v/v) and diluted to appropriated volume with the same solvent. The resulting solution was filtered and the filtrate was analysed on a phenylpropanolamine bonded silica column (15 cm×4.6 mm I.D., 5 μm). The optimized mobile phase was 30 m*M* phosphate buffer (pH 3.00) containing 6% (v/v) acetonitrile at a flow-rate of 1 ml min^{-1} and the detection was measured at 361 nm. The calibration graph prepared using standards was linear from 0.05 to 0.25 μg. The determination limit was 25 ng, the relative standard deviation was 0.47% and recovery from tablet solution was 100%. An analysis was completed in 5 min. The new method is simple, rapid and precise.

Keywords: Pharmaceutical analysis; Vitamins

1. Introduction

The problem of determination of vitamin B_{12} is of importance in the analysis of this vitamin in multivitamin formulations. Separation of vitamin B_{12} from others in the pharmaceutical preparations by HPLC have been reported by various workers [1–7]. The most common method is reversed-phase high-performance liquid chromatography (RP-HPLC) using isocratic elution [1,2]. This method is successful when the amounts of vitamin B_{12} and others, such as vitamin B_1 and B_6, is similar. For vitamin B_1–B_6–B_{12} formulations, the ratios between B_{12} and the others are in the range 1:100 to 1:1000, and RP-HPLC in the isocratic mode could not be used because the chromatographic peaks are not well resolved. In order to solve this problem, gradient elution was applied [3–7]. However, the method is time consuming and has low reproducibility. The alternative is to change the selectivity of the stationary phases to vitamin B_{12} by using multimode chromatography. A multimodal support was designed by coupling DL-phenylpropanolamine to silica and the chromatographic behavior of various compounds was investigated [8]. The retention of all compounds on this stationary phase depend on a mixed mechanism, including ionic and hydrophobic interactions. It is of interest to apply this stationary phase to the analysis of vitamin B_{12} in multivitamin formulations.

In this report, the retention of vitamins B_1, B_6 and B_{12} on phenylpropanolamine-bonded silica was investigated by modifying the pH of the mobile phase in the isocratic mode. An analytical application to the determination of vitamin B_{12} in the vitamin B_1–B_6–B_{12} tablets is given.

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2. Experimental

2.1. Apparatus

The chromatographic apparatus consisted of HPLC system (Spectra-Physics, San Jose, CA, USA) with Spectra system gradient pumps P-4000 and a Chrom-A-Scope UV–Vis spectrophotometric detector (Barspec, Rehovot, Israel). Chromatograms were recorded and processed with Barspec data system (Barspec).

2.2. Reagents

The phenylpropanolamine-bonded silica was prepared as previously described [6]. Secondary standard of vitamin B_{12} was from Division of Drug Analysis (Department of Medical Science, Ministry of Public Health, Patumtani, Thailand). Secondary standards of vitamin B_1 (thiamine mononitrate) and vitamin B_6 (pyridoxine hydrochloride) were from the Research and Development Institute (The Government Pharmaceutical Organization, Bangkok, Thailand). HPLC-grade acetonitrile was from Merck (Darmstadt, Germany). The water was deionized and distilled. All reagents were of analytical grade.

2.3. Sample of the vitamin B_1–B_6–B_{12} tablets

The sample used was Princi-B Fort [Sanofi (Thailand), Bangkok, Thailand], Lot. No. B278078, manufacturing date 18 August 1997. Each tablet contains: vitamin B_1 (thiamine mononitrate, 250 mg), vitamin B_6 (pyridoxine hydrochloride, 250 mg), vitamin B_{12} (cyanocobalamin, 1 mg).

2.4. Chromatographic conditions

The phenylpropanolamine support was packed into a 150×4.6 nm I.D. stainless steel column by conventional high-pressure slurry-packing procedures [9]. The retention of vitamin B_1, B_2 and B_{12} was studied using 30 m*M* phosphate buffer (pH 3.0–6.5), 6% (v/v) acetonitrile, as eluent and detection was at 273 nm. A sample of the vitamin B_1–B_6–B_{12} tablets was analyzed by using 30 m*M* phosphate buffer (pH 3.0) containing 6% (v/v) acetonitrile, as eluent and detection was at 361 nm. In all cases, the flow-rate was 1 ml min^{-1}.

2.5. Chromatographic behavior of vitamins B_1, B_2 and B_{12}

Stock solutions of each vitamin were separately prepared using methanol–water (50:50, v/v) as solvent at concentrations of 0.2 mg ml^{-1}. A 5-μl volume was injected onto the column.

2.6. Analysis of vitamin B_{12} in the vitamin B_1–B_6–B_{12} tablets

Calibration: A calibration graph for vitamin B_{12} was measured in the range 2.5–12.5 μl ml^{-1} using methanol–water (50:50, v/v) as the diluting solvent.

Sample preparation: ten tablets of Princi-B Fort were placed in a 500-ml volumetric flask and 350 ml of methanol–water (50:50, v/v) added. After sonication in an ultrasonic bath for 30 min the solution was diluted to volume with the same solvent and mixed well. The solution was then filtered through What-

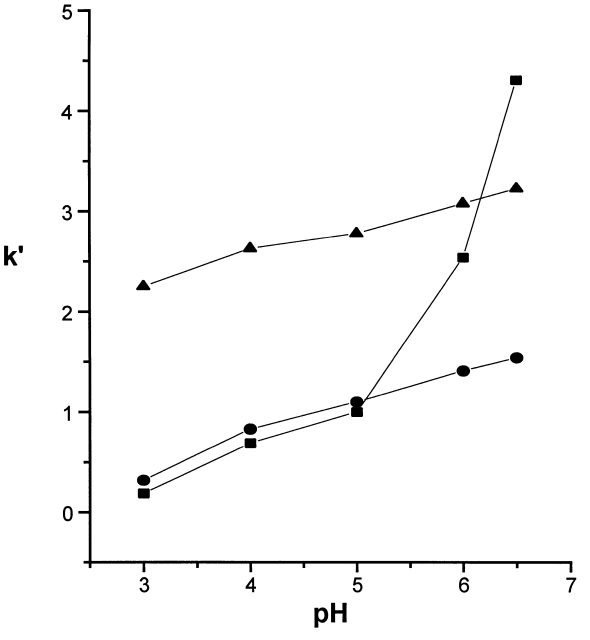

Fig. 1. Effect of pH on capacity factors (*k′*) of some basic water soluble vitamins: (■) vitamin B_1, (●) vitamin B_6, and (▲) vitamin B_{12}. Stationary phase: phenylpropanolamine bonded silica, 150×4.6 mm I.D., 5 μm; mobile phase: 30 m*M* phosphate buffer, pH 3.0–6.5 with 6% (v/v) acetonitrile; flow-rate: 1 ml min^{-1}; UV 254 nm.

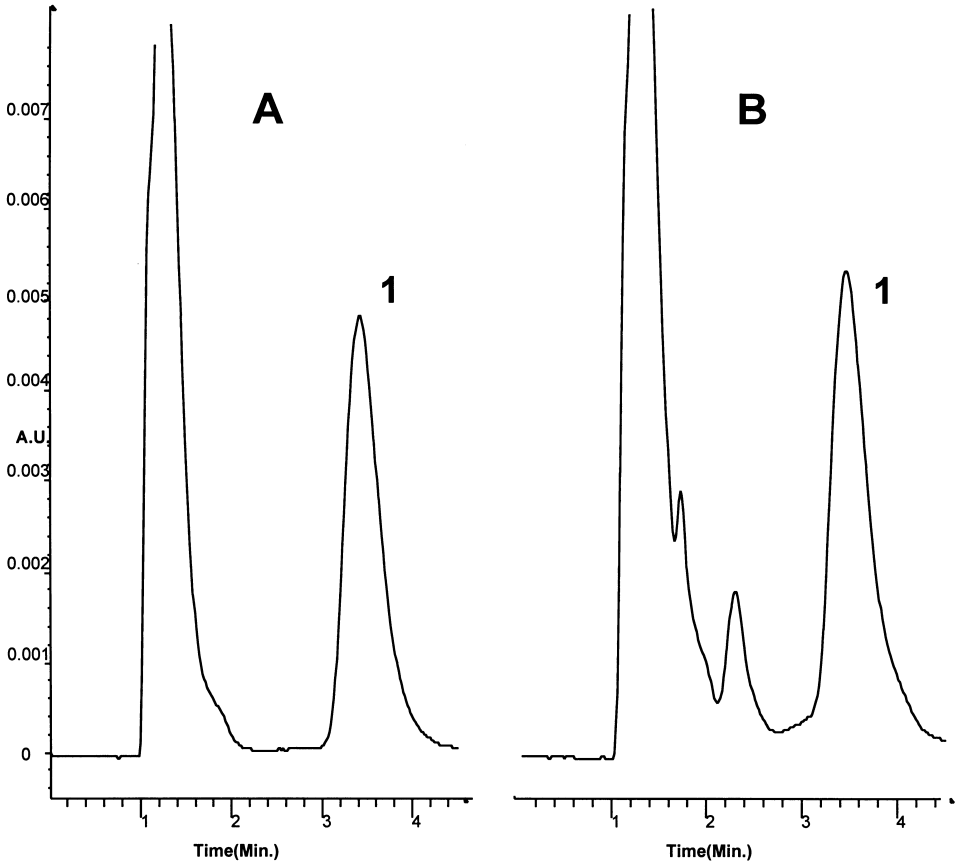

Fig. 2. Chromatograms of (A) vitamin B_{12} 0.15 μg, (B) Princi-B Fort tablets containing 1.032 mg vitamin B_{12}. Stationary phase: phenylpropanolamine bonded silica, 150×4.6 mm I.D., 5 μm; mobile phase: 30 m*M* phosphate buffer, pH 3.0 with 6% (v/v) acetonitrile; flow-rate: 1 ml min^{-1}; UV, 361 nm. Peak 1=vitamin B_{12}.

man No. 1 filter paper (Whatman, Maidstone, UK) and the first 10-ml filtrate discarded. A 25-ml volume of the filtrate was diluted to 50 ml using the same solvent.

A 20-μl portion of each of final solutions was injected onto the column after filtration through a 0.2-μm membrane filter (Whatman).

3. Results and discussion

The effect of eluent pH on the capacity factors of vitamins B_1, B_2 and B_{12} on phenylpropanolamine-bonded silica is shown in Fig. 1. The capacity factors of the vitamins decreased markedly with decreasing pH. The decrease in retention of vitamins B_1, B_6 and B_{12} was 23-, 5- and 1.4-fold, respectively, when the pH changed from 6.5 to 3.0. The results show the same behavior as organic basic compounds on this stationary phase in previous work [8]. Vitamin B_{12} is well resolved from the others between pH 3 and 5. In this study, pH 3 was selected for the analysis of vitamin B_{12} in the multivitamin tablets.

Application of the method to the analysis B_{12} in the vitamin B_1–B_6–B_{12} tablets (Princi-B Fort) were performed by using methanol–aqueous solution as extracting solvent in order to increase the recovery of vitamin B_{12} from the sample preparation because the solubility problem of thiamine mononitrate in water and the chromatogram is shown in Fig. 2. The calibration graph was linear in the range 2.5–12.5 μg ml^{-1} with the regression equation, $y = -286.8 + 90254.2x$ ($r^2 = 0.9995$), where x and y are the injected amount (μg) and peak area, respectively.

Table 1
Recovery study: final solution of sample solution containing vitamin B_{12} at a concentration of 1032 ng ml^{-1}

Added (ng)	Found (ng)	Recovery (%)	RSD (%)	*n*
826	826.4	100.5	0.20	6
1032	1036.1	100.4	0.27	6
1238	1244.7	100.5	0.26	6

The limit of detection and quantitation were 5 and 25 ng, respectively. The precision of injection was demonstrated by replicate injections of the standard solution (Fig. 2A) and the relative standard deviation (RSD) of peak area was 0.32% ($n=8$). There was no interfering peak in the chromatogram of the sample (Fig. 2B) and the analysis time was 5 min. The average amount of vitamin B_{12} in a tablet was 1.032 mg with an RSD of 0.47% ($n=8$) for intra-day assay. For the inter-day assay, the average amount of vitamin B_{12} in a tablet was 1.025 mg with RSD of 0.62% ($n=8$). Method validation regarding recovery was achieved by adding the standard vitamin B_{12} at 80–120% of the amount found in the sample solution and the results are illustrated in Table 1. The recovery is approximately 100% at all concentrations with the average RSD value not more than 0.27%. The values demonstrate the high accuracy of the method and was found to be acceptable for routine drug analysis. Applications of the method to determine vitamin B_{12} in other types of complex matrices, which contain water-soluble and fat-soluble vitamins, sugars, metal ions, oily particles or proteins will be further explored. The ability to simultaneously separate compounds of different properties [8] renders the column a valuable tool in pharmaceutical analysis.

4. Conclusion

Development of a method for determination of vitamin B_{12} in B_1–B_6–B_{12} tablets using a phenylpropanolamine bonded silica in the isocratic mode was carried out. The application of the method to the analysis of vitamin B_{12} in B-complex tablets is demonstrated and shown to be simple and rapid with a high degree of accuracy and precision.

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