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# Use of packed-fiber solid-phase extraction for sample clean-up and preconcentration of vitamin $B_{12}$ before determination

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**Abstract**

A rapid and simple preconcentration step applying packed-fiber solid-phase extraction columns has been investigated to vitamin $B_{12}$. The extraction performance of the new method was investigated preliminarily on vitamin functional drink. The analysis used a reversed-phase $C_{18}$ column, with a photo-diode array detector at 220 nm. The samples were preconcentrated with packed-fiber solid-phase extraction columns. Good linearity was observed in vitamin functional drink. The repeatability of extraction performance, expressed as relative standard deviations, was from 3.5% to 4.3%. The limit of detection (LOD) is 5 ng $mL^{-1}$ (S/N = 3). Finally, the method had been applied for the determination of vitamin $B_{12}$ in vitamin functional drink.


*Keywords:* Vitamin $B_{12}$; Nanofibers; Packed-fiber solid-phase extraction; Preconcentration

Vitamin $B_{12}$ belongs to the group of water-soluble compounds. It is an essential nutrient linked to human growth and cell development. In the literature, a number of methods for the analysis of vitamin $B_{12}$ have been reported including microbiological method [1], chemiluminescence [2], voltammetry [3], enzymology [4], and column liquid chromatography with specific detection such as AAS [5], fluorescence [6], UV visible detection [7], and ICP-MS [8]. Recent methods for quantifying vitamin $B_{12}$ include capillary electrophoresis [9], and micro-HPLC [10], combined with detection by ICP-MS.

The HPLC method is considered as one of the most convenient methods for the determination of analytes. However, an extraction technique is sometimes necessary for trace analysis prior to HPLC to preconcentrate target molecules. The sorbents used in SPE are normally particles at micron scale, most of which are $C_{18}$ particles. New kinds of adsorbents development are an important aspect in the SPE research. Only few papers reported that submicron materials were used as the sorbents. Carbon nanotubes (CNTs), with nano-sized diameter and tubular microstructure, have been used to investigate the adsorption capability of vitamin $B_{12}$ [11]. Guo et al. have used coated mesoporous carbons with PMMA to extract vitamin $B_{12}$ [12], and Shen et al. have studied extraction methods for preconcentration of vitamin $B_{12}$ by using of activated carbon fibers (ACFs) [13]. These methods usually need long equilibrium time for several hours, and none of them were applied to real samples.

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Here, we present a new method for the direct determination of vitamin $B_{12}$ after preconcentration using packed-fiber solid-phase extraction (PFSPE). PFSPE has been used for extraction of target compounds from different samples [14–16] in previous papers. In this work, vitamin $B_{12}$, a polar compound, was preconcentrated using PFSPE. The optimized method was applied to the determination of vitamin $B_{12}$ in a drink.

## 1. Experimental

The standard of vitamin $B_{12}$ was purchased from the National Institute of Pharmaceutical and Biological Authentication of China (Beijing, China). Methanol was LC grade from Shangdong Yuwang Fine Chemical Company (Shangdong, China). The vitamin functional drink (Red Bull) was purchased from local market (Nanjing, China). All other chemicals used were of analytical grade.

The HPLC equipment used consisted of a Shimadzu LC-20A pump and a Shimadzu SPD-M20A detector (wavelength range from 190 to 800 nm) (Kyoto, Japan). The column used was VP-ODS Dikma $C_{18}$, 5 μm, 250 mm × 4.6 mm. All the measurement operations were performed at room temperature. The mobile phase was 18:82 (v/v) methanol/water containing 0.08% 1-heptanesulfonic acid sodium salt and 1.0% triethylammonia adjusted at pH 3.6 with phosphoric acid, at a constant flow rate of 0.9 mL $min^{-1}$. Aliquots of 20 μL were injected. Considering the sensitivity of sample analysis, 220 nm was selected for analyzing vitamin $B_{12}$.

The procedures for preparing polystyrene (PS), poly (styrene-co-methacrylic acid) (PS-COOH), poly (styrene-co-p-styrene sulfonate) (PS-$SO_2OH$) and polymethyl methacrylate (PMMA) nanofibers were described detailed in our previous paper [14,16], and the SEM images of nanofibers were also showed. The system for the extraction and the extraction procedure were described in previous paper [15,16].

## 2. Results and discussion

In this enrichment system, the performance of the extraction method is potentially affected by a large number of factors. First, four kinds of nanofibers, PS, PS-COOH, PS-$SO_2OH$ and PMMA nanofibers were tested to select the most efficient one for the extraction of the target molecules. As presented in Fig. 1, PS and PS-COOH showed better extraction efficiency. Then these two were chosen to carry out loading volume experiment with a spiked water solution (containing 1.0 μg $mL^{-1}$ of vitamin $B_{12}$) at various volumes. As shown in Fig. 2, both sorbents exerted decreasing extraction performance as the sample volume increased, but PS nanofibers seemed better than PS-COOH nanofibers. This phenomenon may contribute to ion exclusion effects between vitamin $B_{12}$ and PS-COOH or PS-$SO_2OH$ nanofibers in water medium, because the vitamin $B_{12}$ ($pK_a$ = 1.59) molecules exit in negative charge forms in water. On the other hand, vitamin $B_{12}$ is a polar analyte incapable of sorption to unpolar materials, but owning to the porphyrin ring structure in its molecule, there may be strong π–π interactions between porphyrin ring and phenyl in polymers composing nanofibers (PS, PS-COOH, PS-$SO_2OH$), so PMMA nanofibers behaved worst sorption efficiency. Therefore, PS nanofibers were selected as sorbent for the analysis of vitamin $B_{12}$.

Medium pH (in the range of 1–11) was examined in order to get the optimum pH conditions. The maximum extraction recovery of vitamin $B_{12}$ (96–101%) was obtained within a pH range 5–7; while pH values below and above that range lowered the recovery (57–90%). The optimal pH was finally chosen to be pH 7.

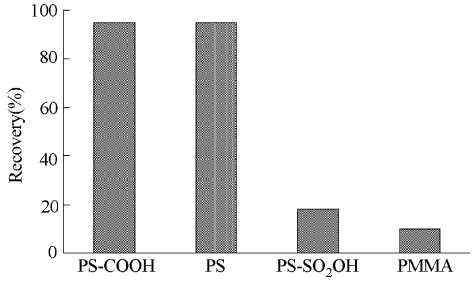


Fig. 1. Influence of different kind of nanofibers on the extraction. Extraction conditions as follows: a spiked water solution (1.0 μg $mL^{-1}$, 3.0 mL); each kind of nanofiber had an average diameter of 500 nm; pH 7; no salt addition; eluting solvent, 50 μL of 50% methanol.

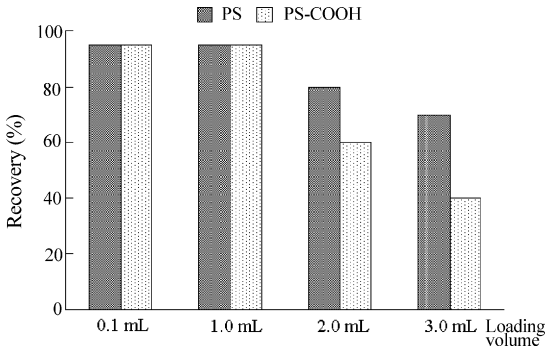


Fig. 2. Influence of loading volume on the PFSPE basing on PS and PS-COOH nanofibers. Extraction conditions as follows: a spiked water solution (1.0 μg $mL^{-1}$); each kind of nanofiber had an average diameter of 500 nm; pH 7; no salt addition; eluting solvent, 50 μL of 50% methanol.

The addition of salt often improves the extraction of analytes in conventional liquid-liquid extraction, solid-phase microextraction and liquid-phase micro-extraction through the salting-out effect. In this work, the effect of ionic strength (0–2.0 mol $L^{-1}$) on the extraction was tested with tetrabutyl ammonium hydroxide, 1-heptanesulfonic acid sodium salt, sodium chloride, sodium acetate, sodium sulfate, and sodium carbonate, which were added into the sample solution respectively. There are the same shape outlines (U type) of extraction efficiency versus salt concentration for all salts. Take sodium chloride for example, the peak area is decreased with increasing salt concentration till a lowest point, after that, the peak area is increased with increasing salt concentration. However, the high concentration buffer was not favorable because of increasing pressure on the column, as a result of the high viscosity of the sample solution. Therefore, in the present work, no electrolyte was employed.

In order to determine the optimal solvent for desorption of the extracted compounds, methanol, ethanol, and acetonitrile were examined. Compared with other solvents, methanol had the highest efficiency as the eluting solvent for vitamin $B_{12}$. Next, proportion of methanol (water as solvent; in the range 0–100%), were examined. And the results suggested that 50% methanol could meet requirement. To get the optimum enrichment conditions eluting volumes in the range 20–200 μL were tested. The peak area reached the optimal point for 50 μL eluting solvent, and after that no increase of peak area was observed. So 50 μL of 50% methanol was sufficient.

A series of experiments were employed for achieving the parameters of analytical performance. These procedures were carried out at the optimum conditions. The linearity of the calibration plot was tested on three consecutive days, seven different concentrations of vitamin $B_{12}$ in vitamin functional drink (10, 40, 80, 200, 500, 1000, and 2000 ng $mL^{-1}$) were analyzed in triplicate. Good linearity was observed in vitamin functional drink over the concentration range of 10–2000 ng $mL^{-1}$, with a correlation coefficient of 0.999 and the linear regression equation $y = 77205x + 728.67$ ($y$: peak area; $x$: concentration). The limit of detection (LOD) was defined as the signal-to-noise ratio 3:1. The LOD was 5 ng $mL^{-1}$. The precision of the assay was determined by replicate analyses of three concentration levels of vitamin $B_{12}$: 25, 50, and 100 ng $mL^{-1}$ for spiked vitamin functional drink. Relative standard deviation (RSD) was calculated as an estimation of precision. The precision of the method was evaluated as RSD intra-day: 3.5–3.9% ($n = 6$), and RSD inter-day: 3.6–4.5% ($n = 6$). The efficiency of the extraction was evaluated by means of recovery studies using the standard addition technique for samples of vitamin functional drink. The relative recoveries of vitamin functional drink was 92.4–99.2%.

The optimized method was applied to the vitamin functional drink. Identification was performed by UV spectral comparison using a photo-diode array detector. A typical liquid chromatogram of extracted vitamin $B_{12}$ from vitamin functional drink is shown in Fig. 3. The analyte could be detected after preconcentration using PFSPE method. No interference was observed in the sample, and the drink had been partly cleaned-up as shown in the figures. Applying this method, vitamin $B_{12}$ detected in three samples was 10.2, 10.7, and 10.5 ng $mL^{-1}$ separately, and the results obtained were in reasonable agreement with the amount labeled (12 ng $mL^{-1}$).

We have developed a novel method for the determination of vitamin $B_{12}$ in a drink. This new protocol involves packed-fiber solid-phase extraction technique was firstly applied to a polar analyte. This method is simple, rapid and accurate, and it costs little methanol during extraction compared with $C_{18}$ SPE cartridge. The technique was successfully applied to the determination of vitamin $B_{12}$ at trace amount level.

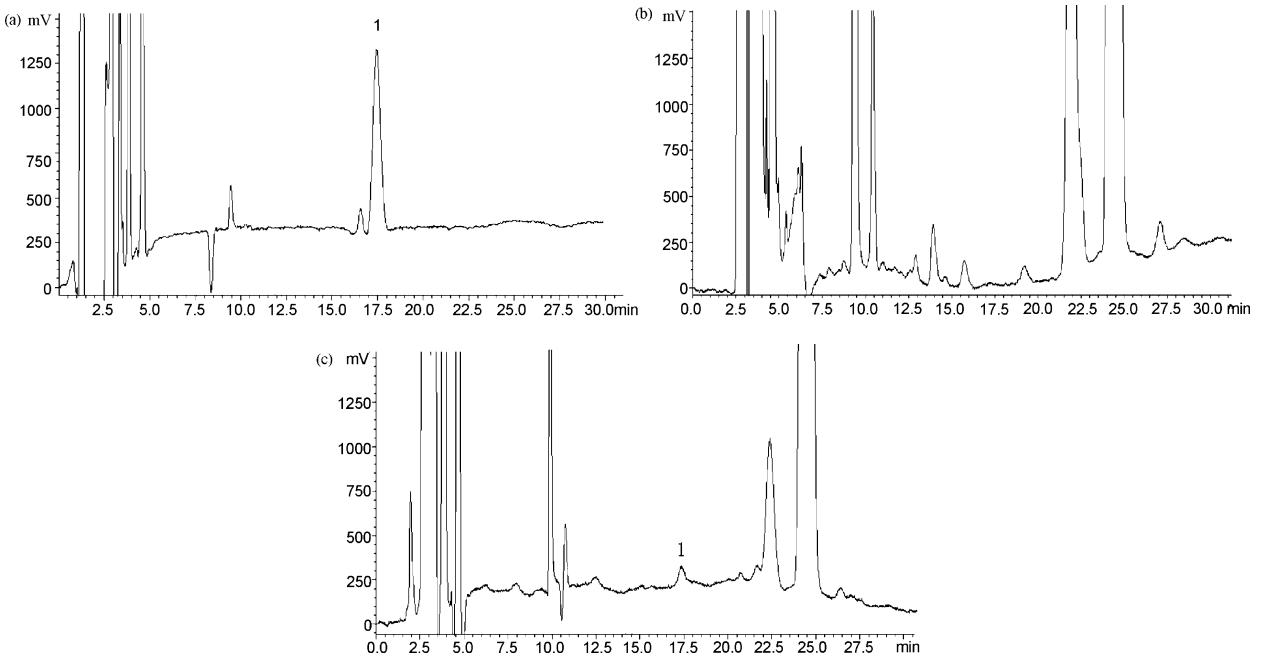


Fig. 3. Chromatograms of: (a) vitamin $B_{12}$ (1.0 μg $mL^{-1}$, water as solvent) directly inject into HPLC; (b) vitamin functional drink before extraction; (c) vitamin functional drink after extraction. Vitamin $B_{12}$: (1).

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