Journal of **Kim Il Sung** University (Natural Science)
Vol. 3 No. 3
Juche103(2014)

On-Line Solid-Phase Extraction and HPLC Determination of Water Soluble Vitamins in Infant Formula using Acetylcellulose as Sorbent

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Abstract The acetylcellulose fiber was prepared, characterized and explored as sorbent for flow injection solid-phase extraction on-line coupled with high-performance liquid chromatography ("FI-SPE-HPLC") for determination of trace water soluble vitamins in infant formulas. Vitamin B1, B2, B6, Bc and PP were used as model analytes. The precolumn packed with the acetylcellulose fiber was shown to be promising for solid-phase extraction of vitamins in infant formula samples with subsequent HPLC separation and UV detection. With extraction of 0.25mL of sample solution, the enhancement factors for the vitamins studied ranged from 6 to 12, depending on the shape, property of the vitamins. Detection limits of SPE-HPLC method were established : vitamin B1 0.25μg/mL, vitamin B2 0.066μg/mL, vitamin B6 0.24μg/mL, vitamin Bc 0.24μg/mL, vitamin PP 0.10μg/mL. The developed method is simple, sensitive and cost-effective.

Key words solid-phase extraction, acetylcellulose fiber, high-performance liquid chromatography, water soluble vitamin

Introduction

It is well known that vitamins are an essential diverse group of compounds that are needed in relatively small amounts to sustain life and good health. UV-Vis spectrophotometry [4], fluorimetry [5], capillary electrophoresis [6], microbiology [7] and HPLC [1－3] have proposed for the determination of vitamins. Nevertheless, there is no single analytical approach to determine water soluble vitamins within a complicated matrix in a single run.

Generally, infant formula was diluted with solution consisted of trichloroacetic acid to eliminate protein and evaporated to definite volume in a rotary vacuum evaporator, because of consisting of trace vitamins [1].

In this work, we developed a on-line solid-phase extraction high performance liquid chromatographic method using acetylcellulose as sorbent to determine water soluble vitamins in infant formula. The method was successfully applied to the determination of water soluble vitamins in infant formula.

1. Experiment

1.1. Chemical materials

All reagents were of the highest available purity and at least of analytical grade. Doubly

deionized water (DDW, 18MΩ/cm) obtained from a ULTRA PURE was used throughout. Vitamin B1, B2, B6, Bc and PP of 1 000mg/L were prepared by dissolving suitable amounts in DDW directly. Working standard solution and their mixtures were prepared daily by stepwise diluting the stock solution just before use.

1.2. Apparatus

The chromatographic system consisted of Agilent HP-1100 Series G 1311 A QUAT pump and G 1311 A VWD variable wavelength detector. All separations were achieved on an analytical reversed-phase column (ZORBAX Eclips SB-C18 3.5μm 4.6mm i.d×75mm). The ChemStation software was used to acquire and process spectal and chromatographic data.

Micropump for chromatograph was used for the SPE of vitamins. Tygon pump tube was used for delivering the sample solution. Small bore (0.5mm) PTFE tubing were adapted for all connections, which were kept the shortest possible length to minimize the dead volume.

The column used for the SPE preconcentration of vitamins was a precolumn (1.5cm × 4mm i.d.) dry-packed with 100mg of acetylcellulose fiber. The two ends of the precolumn were plugged with glass wool. Before measurements, the precolumn was conditioned by flashing with mobile phase until the detector response reached stability.

Chromatographic resolution for the separation of water soluble vitamins was performed as follows. Firstly, we performed the isocratic elution with solution of 0.1% trichloroacetic acid for 3min and a linear gradient elution to 0.1% trichloroacetic acid-methanol (30 ∶ 70) for 7.5min, followed by isocratic elution for 1.5min.

The flow rate of mobile phase was 0.8mL/min and the column temperature was maintained at 40℃. Detection wavelength was 265nm.

1.3. Procedures of the SPE

The developed FI manifold for the two different valve positions is shown in Fig. 1.

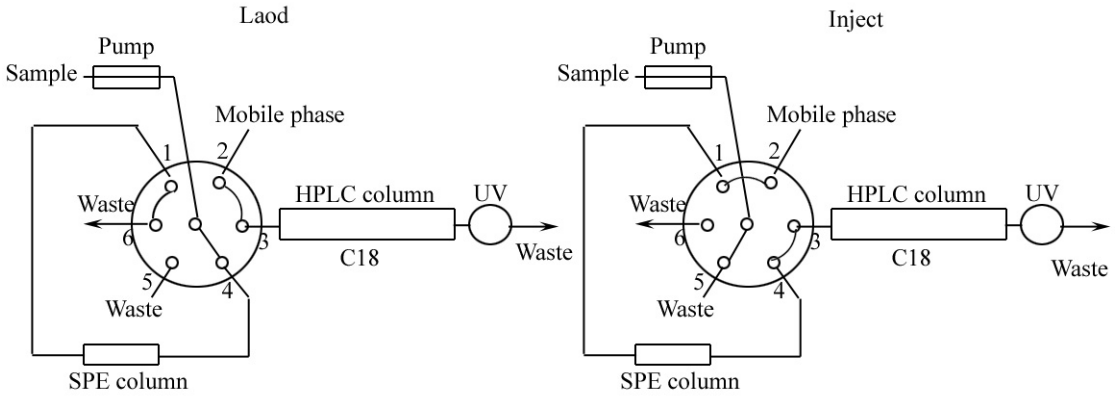

Fig. 1. Manifold for on-line SPE-HPLC determination of water soluble vitamins in infant formula

First, the sample solution was introduced onto the precolumn packed with the acetylcellulose fiber at a flow rate of 0.5mL/min for 30s while the HPLC injector valve was in the load position so that the vitamins were preconcentrated by the precolumn and the unwanted

water went to waste. Second, the analytes adsorbed on the precolumn were eluted in the back flash mode by the HPLC mobile phase at a flow rate of 0.8mL/min into the chromatographic separation column by switching HPLC valve from "Load" to "Inject" position. As such, the sample band in the precolumn was compressed into a narrow band before entering the analytical column and the band broaden effect was reduced [8]. Third, the HPLC injector valve was turned to the "Load" position for next sample preconcentration while the analytes were separated in the chromatographic separation column to improved sample throughput. In this way, a complete cycle of the SPE and HPLC separation of the vitamins lasted 15min. The peak areas were calculated at 265nm wavelength and used for data evaluation.

1.4. Sample pretreatment

Firstly, 10g of the infant formula was extracted with 80mL of DDW in an ultrasonic bath for 30min. After adding 5.00mL of 5% trichloroacetic acid, the mixture was shaken vigorously and placed in a water bath for 30min at 50℃. Secondly, the supernatant was filtered and then neutralized by KOH solution. The solution was transferred into a 100mL volumetric flask and diluted to volume with DDW, followed by filtering through a 0.45μm filterable membrane.

2. Results and Discussion

2.1. Factors affecting the flow injection SPE of water soluble vitamins

The effect of sample pH on the sorption of water soluble vitamin was tested with a mixture of these five vitamins(1μg/L for vitamin B1, B2, B6, Bc and PP, respectively) at a sample loading flow rate of 0.5mL/min for 30s preconcentration (Fig. 2).

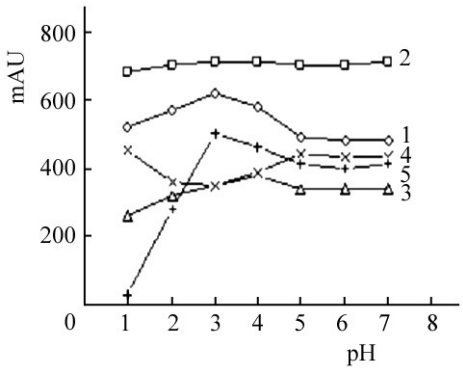

Fig. 2. Effect of pH on the preconcentration performance of water soluble vitamins
1－vitamin B1, 2－B2, 3－B6, 4－Bc, 5－PP; Concentration of each vitamins 1μg/L

The results shown in Fig. 2 illustrate that the maximum chromatographic peak areas of vitamin B1, B2, B6, Bc and PP are in the pH range of 5.0～7.0, 1.0～7.0, 3.0～7.0, 5.0～7.0, 5.0～7.0, respectively.

The results show that all of the vitamins of interest can be effectively adsorbed by acetylcellulose fibers-packed precolumn in the pH range of 5.0～7.0.

2.2. Effect of sample loading flow rate and total preconcentration time

The influence of sample loading flow rate on the sorption preconcentration of vitamins was investigated with a mixture of the water soluble vitamins (1μg/L for B1, B2, B6, Bc and PP, respectively) for 30s preconcentration (Fig. 3).

The results show that the chromatographic peak areas of B1, PP increased almost linearly up to 0.8mL/min, while B6, B2 increased linearly up to 1mL/min and B6 increased linearly up to 2mL/min. The effect of sample loading time on the adsorption of the vitamins (the concentration as above) was investigated at a sample flow rate of 0.5mL/min (Fig. 4).

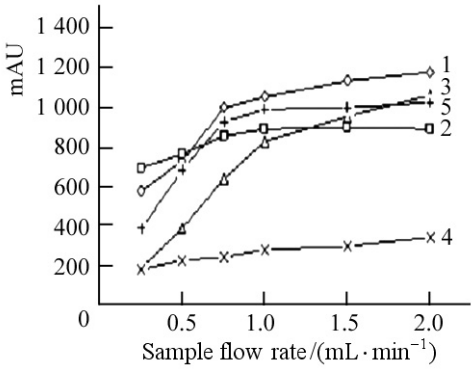

Fig. 3. Effect of sample loading flow rate on the preconcentration of water soluble vitamins
1－Vitamin B1, 2－B2, 3－B6, 4－Bc, 5－PP; sample loading time 30s

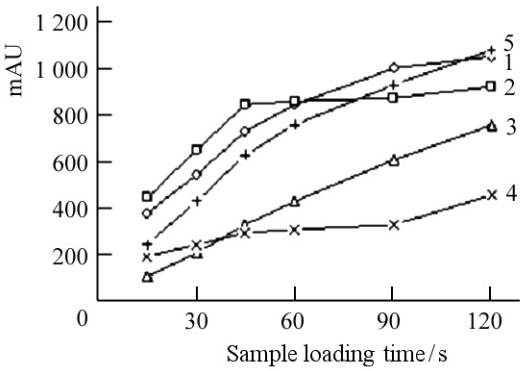

Fig. 4. Effect of sample loading time on the preconcentration of water soluble vitamins
1－Vitamin B1, 2－B2, 3－B6, 4－Bc, 5－PP; sample loading flow rate 0.5mL/min

The chromatographic peak area increased almost linearly as the sample loading time increased up to 45s for B1 and B2, and up to 60s for Bc and PP, and up to 120s for B6.

Based on the above results, we selected the following condition for the flow injection SPE preconcentration of the water soluble vitamins : the sample loading time 30s, sample loading flow rate 0.5mL/min, sample pH 5.0～7.0.

2.3. Desorption of the adsorbed vitamins from the acetylcellulose fiber-packed precolumn

For simplicity, the HPLC mobile phase(0.1% trichloroacetic acid) was used for the desorption of the adsorbed vitamins from the acetylcellulose fiber-packed precolumn. The time required for quantitative desorption of the adsorbed vitamins when the HPLC injector valve should turn to the “load” position for next SPE during the HPLC separation of the analytes this cycle. It was found that the chromatographic peak areas of the vitamins increased remarkably as the desorption from 0.5 to 1.5min, and then levelled off. Accordingly, 2.0min desorption was selected to ensure the complete stripping of the adsorbed vitamins from the acetylcellulose fiber-packed precolumn. Once the adsorbed vitamins was quantitatively stripped from the acetylcellulose fiber-packed precolumn, the HPLC injector valve turned to the “load” position for next preconcentration so that the current HPLC separation and the next preconcentration proceeded in parallel.

2.4. Analytical figures of merit

The analytical characteristic data of the developed on-line solid-phase extraction preconcentration method coupled with HPLC for the determination of water soluble vitamins were summarized in

table 1. The precision (R. S. D) for 11 replicate injections of a mixture of 1μg/L of each analyte was in the range of 8.0～10.1% and 2.1～3.1% for the peak area and retention time. With the consumption of 0.25mL sample solution, the enhancement factor defined as the ratio of the sensitivity obtained by on-line SPE-HPLC to that obtained by conventional HPLC with direct injection of 20μL solution ranged from 6.3 to 12.2. The linear concentration range were from 0.5 to 5μg/mL for each vitamins, respectively.

Table 1. Characteristics data of the developed on-line solid-phase extraction preconcentration for HPLC determination of trace levels of water soluble vitamins under the optimal conditions

Vitamin		B1	B2	B6	Bc	PP
Enhancement factors[1)]		8.1	12.2	6.3	9.6	9.6
Detection limit(S/N 3)/($\mu g \cdot mL^{-1}$)		0.25	0.066	0.24	0.24	0.10
Precision[2)](n=11)/%	t_R/min	2.1	2.2	3.1	2.3	2.7
	mAU	9.5	8.9	8.5	8.0	10.1
Linear concentration range of the calculation graph/($\mu g \cdot mL^{-1}$)		0.5～5	0.5～5	0.5～5	0.5～5	0.5～5
Sample consumption/mL				0.25		

1) compared with direct injection of 20μL sample solution, 2) using a mixture of 1μg/mL of each vitamins

To evaluate the accuracy of the method, five infant formula samples analyzed for trace levels of vitamins by the developed method. A standard addition calibration protocol was employed for quantification. The recoveries were estimated by comparing the increased peak area of the chromatographic peak of each analyte due to spiking to that of the chromatographic peak of each analyte in standard solution with the same concentration as spiking. The analytical results obtained by the developed method were given in table 2.

Table 2. Recovery of water soluble vitamins from infant formula samples a)(n=11)

Vitamin	Added/($\mu g \cdot g^{-1}$)	Recovery/%	RSD/%
B1	5.0	95.0	2.35
B2	5.0	102.3	2.38
B6	5.0	93.6	2.95
Bc	5.0	91.2	3.23
PP	5.0	98.5	2.40

The recoveries of 5μg/g of each water soluble vitamins spiked in these infant formula samples(4μg/g for B1, 7.9μg/g for B2, 4.2μg/g for B6 and 0μg/g for Bc and PP, respectively) were 91～102%, respectively.

To evaluate the usefulness of the developed method, the analytical results determinated water soluble vitamins in infant formula sample is shown in table 3. The chromatograms of water soluble vitamins are shown in Fig. 5.

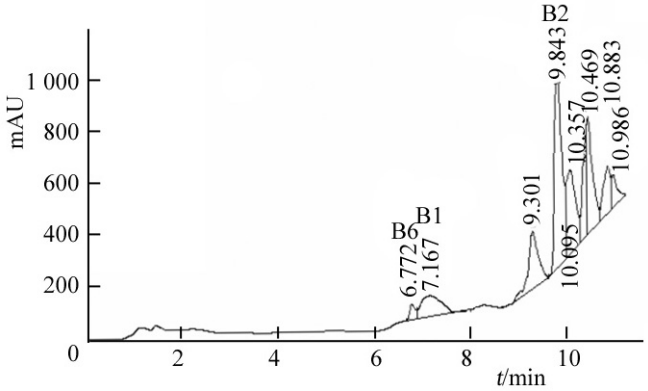

Fig. 5. Chromatogram of a infant formula sample

Table 3. Analytical results for water soluble vitamins in infant formula samples

Vitamins	Concerntration /(μg · g^{-1})	RSD/% (n=5)
B6	5.6	6.3
B1	4.0	7.5
B2	8.1	4.1
Bc	n.d.	n.d.
PP	n.d.	n.d.

n.d. not detected

Conclusion

The potentiality of acetylcellulose as sorbent for flow injection SPE of some of water soluble vitamins was explored. The results in this work demonstrated the feasibility of acetylcellulose as sorbent for flow injection SPE on-line coupled with HPLC-UV for the determination of water soluble vitamins in infant formula samples.

References

[1] Claudia Garnero et al.; Anal. Chim. Acta, 659, 159, 2010.
[2] L. A. Kozhanova et al.; Journal of Analytical Chemistry, 57, 49, 2002.
[3] Véronique Ducros et al.; Analytical Biochemistry, 401, 7, 2010.
[4] B. Morelly; J. Pharm. Sci., 84, 34, 1995.
[5] H. B. Li et al.; J. Anal. Chem., 368, 836, 2000.
[6] H. Okamoto et al.; J. Chromatogr., A 986, 153, 2003.
[7] M. A. Kall; Food Chem., 82, 315, 2003.
[8] N. Masqué et al.; J. Chromatogr., A 793, 257, 1998.