



Determination of Vitamin B₆, B₉ and B₁₂ in Halophytes by Solid Phase Extraction Followed by HPLC-UV

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NC and MV designed the study. Authors SAH and MJL performed the statistical analysis. Author NC wrote the protocol and authors NC and AM wrote the first draft of the manuscript. Authors TMI and NC managed the analyses of the study. Authors AGG and SMAM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2017/34730

Editor(s):

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Complete Peer review History: <http://www.sciencedomain.org/review-history/22630>

Original Research Article

Received 8th June 2017
Accepted 6th December 2017
Published 8th January 2018

ABSTRACT

In this work, solid phase extraction (SPE) was proposed for simultaneous determination of trace levels of vitamin B₆, B₉ and B₁₂. High performance liquid chromatography with UV detection (HPLC-UV) was used. The experimental conditions including pH of sample and type and volume of

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extraction solvent were studied by one-factor-at-a time method. The limit of detection in the optimal conditions was 14.91, 10.03, and 7.25 ngkg⁻¹ for vitamins B₆, B₉ and B₁₂ respectively and relative standard deviations (RSD) of the analysis less than 8% (n= 3). Recoveries of vitamins B₆, B₉ and B₁₂ were in the ranges of 80–93%. The proposed method was successfully applied for the simultaneous determination of vitamin B₆, B₉ and B₁₂ in halophyte samples such as, *Salicornia*, *Suaeda monoica*, *Suaeda aegyptiaca*, *Sueada vera* and *Sueada martima*.

Keywords: Vitamins B; halophyte; HPLC-UV.

1. INTRODUCTION

Vegetable substances were constituted generally of essential organic nutrients and inorganic nutrients compounds in diets such as vitamins, minerals which are usually in very small quantity [1-2]. *Suaeda* is types of the plant also known as halophyte. *Suaeda aegyptiaca*, *Sueada vera* and *Sueada martima* are halophytes. They habitat is in the salt marshes of the northern hemisphere [2]. *Suaeda monoica* is native to much of western North America, central Canada through the western united states, the Mojave Desert in California, and in to northern Mexico[3]. *Salicornia* and *Suaeda monoica* are halophyte plants in kind of plant in the family Amaranthaceae that grow in salt marshes area, on beaches, and among mangroves. *Salicornia* species grow world wide in North America, Europe, South Asia, and South Africa. The leaves are small and scale-like. Many types are green, but their leaves turns red in autumn. The hermaphrodite flowers are wind pollinated, and the fruit is small and succulent and contains a single seed [3-4]. B vitamin complex found in a variety of foods and they play an important role in metabolism, skin, muscle tone and bone health, protein metabolism, and neurological function [2]. There are methods for determining B-complex vitamins such as spectrofluorimetry, capillary electrophoresis (CE), electrochemical methods, tin layer chromatography (TLC), and HPLC [2,4-7] have been applied. Solid phase extraction (SPE) technique was used successfully sample preparation, since a higher enrichment factor, simple and safe for use with harmful samples this technique has flexible, environment-friendly, simple too easily incorporate into automated analytical methods [1-2]. Solid-Phase Extraction as the preconcentration step Coupled with HPLC-UV for determination of vitamins B₆, B₉ and B₁₂ in *Salicornia* and *Suaeda monoica*, *Suaeda aegyptiaca*, *Sueada vera* and *Sueada martima* samples. Experimental variables affecting the recoveries and chromatographic signals such as pH, volume of extraction solvent,

and temperature of HPLC column were examined.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Vitamins B₆, B₉ and B₁₂, Methanol and acetonitrile were purchased from Sigma Aldrich (Steinheim, Germany). The stock solutions (100 mg L⁻¹) of each vitamin were prepared by dissolving them in water. All the solvents and real samples were filtered through 0.45 µm nylon filter membranes. The working solutions were freshly prepared by mixing the suitable volumes of the stock solutions and diluting with deionized water Millipore, Bedford, MA, USA).

2.2 Apparatus and Software

The chromatographic measurements for the separation and determination of vitamins B were carried out with an KNAUER HPLC system equipped with UV-VIS detector.

LPG system, micro vacuum degasser and a C₁₈ – ODS column (250 × 4.6 mm, 5 µm) column. The pH was measured using a pH meter (Metrohm 827, Switzerland) combined with a glass electrode.

3. RESULTS AND DISCUSSION

3.1 Sample Preparation

Samples were dried after separating then rinsed with distilled water. Finally, concentration of vitamin B₆, B₉ and B₁₂ were determined using HPLC. The tests were repeated three times for sample *Salicornia*, *Suaeda monoica*, *Suaeda monoica*, *Suaeda aegyptiaca*, *Sueada vera* and *Sueada martima* samples include of many component that cause chromatographic interferences with vitamins. Then solid phase extraction (SPE) method² was applied for determination of the vitamin B₆, B₉, and B₁₂. After conditions of SPE cartridge (Sep-Pak

WAT020515) with methanol and double distilled deionized water, 1.0 mL of sample was eluted by 2.5 mL methanol and 1.2 mL phosphate with 1.0 mL min⁻¹. Then the provided samples were passed through the 0.45 µm nylon filter membranes and 50.0 µL of the final extract was injected into the HPLC for subsequent analysis. To obtain best HPLC conditions and short retention time influences of the analytical parameters including, pH, ratio of mobile phases, and column oven temperature were investigated. Finally this method was successfully applied for the simultaneous determination of vitamins B₆, B₉ and B₁₂ concentration in *Salicornia*, *Suaeda monoica*, *Suaeda monoica*, *Suaeda aegyptiaca*, *Sueada vera* and *Sueada martima* samples.

3.2 Chromatographic Conditions

In this work, pH of sample plays an important role in the separation process and it was found that at higher (pH 6.0) and lower (pH 2.0) values the tailing of peak was more and also resolution was poor. pH 3.0 was found to be the best. In the consequence step, 2-mercaptoethanol, acetate, and phosphate were used to adjust the pH. Among them, phosphate was chosen as the best. Various ratios of the solvents were tested. Ratio of solvents was changed to reach high resolution between chromatograms. The best symmetry of the peak shapes was found in the mobile phase containing methanol (MeOH) and acetonitrile (ACN). Moreover, effect of column oven temperature was studied in the range of 20-35 °C while the flow rate of mobile phase was kept at 1.0 mL min⁻¹. According to the results, the temperature of 25 °C was found to be optimal

and used in the subsequent analysis. It should be mentioned that changing the flow rate of mobile phase did not affect the chromatographic peaks. Elution program for determination vitamins B are shown in Table 1.

Table 1. Scheme of gradient elution programme used in HPLC analysis for determination of vitamins B

Time(min)	% ACN	%MeOH
0	33	67
2	35	65
4	37	63
6	40	60
15	33	67

3.3 Optimization Solid Phase Extraction variables Using One at a Time Method

In order to optimize the experimental variables on the extraction recoveries, one at-a-time method were applied.

3.4 pH of Sample Solution

The pH of sample solution is an important factor and has great impacts on the recoveries [2]. pH was studied at the range of 2.0-4.0. The result was depicted in Fig. 1. According to this figure, pH 3.0 was found to be the best. In the consequence step, different chemicals including 1,4-dithioerythritol (DTE), 2-mercaptoethanol, phosphate, and acetate were used to adjust the pH. Among them, phosphate gave the best results.

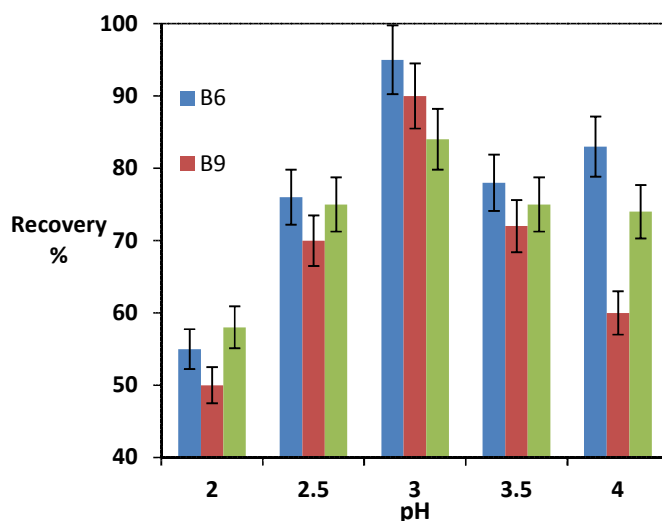


Fig. 1. pH of sample solution

3.5 Type and Volume of Extraction Solvent

Selection of suitable extraction solvent is the most important analytical parameter in SPE technique [2]. Different extraction solvents including n-hexane, dichloromethane, acetonitrile, methanol, methanol/ water, and acetone were investigated. Methanol/water was selected as the best extraction solvent due to its higher recoveries compared to the others. Effect of volume of the solvents was also studied in the range of 0.5-4.0 mL and the results are shown in Fig. 2.

3.6 Method Evaluation of the Proposed Method

3.6.1 Analytical figures of merit

In the present study, figures of merit such as the limit of detection (LOD), dynamic range, sensitivity (SEN), select (SEL) and relative standard deviations (RSD) were calculated under

the optimal conditions. Results detected that recoveries vitamin B6, B9 and B12 were in the ranges of 80–93%. The results research compared with reported literature the determination of vitamin B6, B9 and B12 demonstrate in Table 2. The analytical features are matched or better than the results reported for vitamins B₆, B₉ and B₁₂ determination.

3.7 Application of the Proposed Method to Real Samples

To evaluate applicability of the proposed method, determination of vitamins B₆, B₉ and B₁₂ in *Salicornia*, *Suaeda monoica*, *Suaeda aegyptiaca*, *Sueada vera* and *Sueada martima* samples were carried out under the optimized conditions. Recoveries of vitamins in samples were in the ranges of 80–93%. These results revealed the ability of the HPLC method for determination of vitamins in real samples. Chromatograms of the extracted vitamins in real samples are shown in Fig. 3.

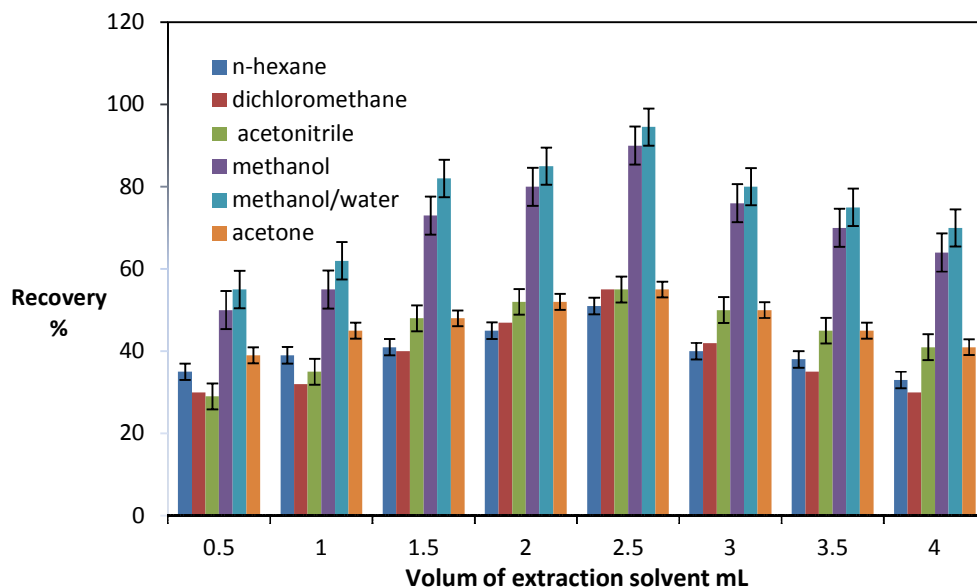


Fig. 2. Volume of extraction solvent

Table 2. Analytical figures of merit for determination of analytes in samples by applying SPE method

Vitamin	LOD ng kg ⁻¹	Dynamic range ng kg ⁻¹	SEN ng kg ⁻¹	SEL	RSD %
B6	14.91	1-200	0.71	0.84	5.1
B9	10.03	5-1000	0.28	0.45	6.4
B12	95.4	10-250	0.07	0.29	7.2

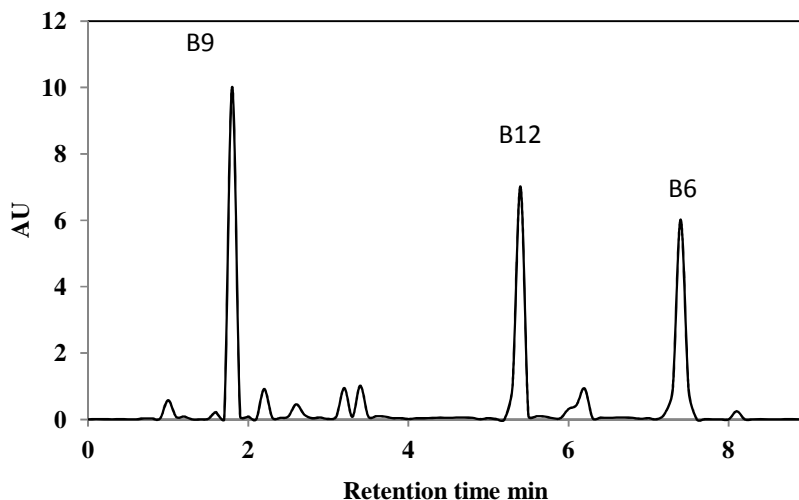


Fig. 3. Two-dimensional chromatograms of vitamins

4. CONCLUSIONS

In this research, SPE-HPLC-UV method was proposed for determination of vitamins B6, B9 and B12 in halophytes. The obtained data the ability of this method for the determination B-group vitamins in halophyte showed. The estimation of vitamins B group composition of *Salicornia*, *Suaeda monoica*, *Suaeda aegyptiaca*, *Sueada vera* and *Sueada martima* sample can be used to achieve understanding and Plays important role in human.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors would like to acknowledgements the support of Abadan School of Medical Sciences for financial support of this work (Project ID: 93u-062 and code of ethics:IR.AbADANUMS.RES.1394.38).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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