DETERMINATION OF VITAMINS B GROUP CONCENTRATIONS IN DOREMA Aucheri BY HPLC-UV

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Abstract

Solid phase extraction (SPE) was proposed for simultaneous extraction of trace levels of vitamins B1, B2, B3, B5, B6, B7, B9 and B12. SPE method was proposed for determination of vitamins B in Dorema aucheri. High performance liquid chromatography with UV detector (HPLC-UV) was employed to analysis the vitamins. The affecting parameters in chromatographic conditions such as, pH, ratios of the solvents and temperature were studied. Under the optimal conditions, relative standard deviations (RSD) of the analysis less than 11% (n= 3) and detection limit were detected less than 1 ng kg\(^{-1}\) for vitamins. The proposed method was successfully applied for the determination of vitamins B in Dorema aucheri.

Keywords: Vitamins B, Dorema aucheri, HPLC-UV.

Introduction

Vegetables constitute vital organic and inorganic components of the diet such as vitamins, minerals, and other nutrients which are usually in very small quantity [1-4].

Dorema aucheri Asteraceae family that grow in Iran[]. It has been used an herbal medicine to decrease the blood triglycerides, antispasmodic, bronchodilator, expectorant, kidney stone repellent, and analgesic for visceral pain[5].

B vitamins exist in an extended range of foods. The B vitamins are water-soluble an essential group of organic compounds for normal growth human and animal bodies [3-4]. They are support metabolism, skin, muscle tone, immune and bone health, synthesis of protein metabolis, and neurological function [4].
There are several methods available, which including, spectrophotometry, spectrofluorimetry, capillary electrophoresis (CE), electrochemical methods, tin layer chromatography (TLC), and HPLC have been used for determination of B vitamins concentration [6-12]. Solid phase extraction (SPE) is a most popular and increasingly useful sample preparation method. SPE method enhances the selectivity and sensitivity and reproducibility of a method by allowing separated binding of an analyte to a solid support where it collected and eluted with a small volume of solvent. This method has a higher enrichment factor, no emulsion, simple and safe for use with harmful samples, low cost, environment friendly, flexible and easier to incorporate into automated analytical methods [2-3]. The goal of this research is determination of vitamins B₁, B₂, B₃, B₅, B₆, B₇, B₉ and B₁₂ in Dorema aucheri by HPLC-UV after pre concentration by SPE.

Material and Methods

Chemicals and reagents

Vitamins B₁, B₂, B₃, B₅, B₆, B₇, B₉ and B₁₂, methanol and acetonitrile were purchased from Sigma Aldrich and Merck. The stock solutions (1 mg L⁻¹) of each vitamin were prepared by dissolving each of them in water. All standard solutions working standards were stored at 4-5 °C and brought to ambient temperature just prior to use. In throughout the experimental runs all the solvents, calibration and real samples were filtered through 0.45 µm nylon filter membranes (Varian, USA). Double distilled deionized water was used throughout which was produced by a Milli-Q system (Millipore, Bedford, MA, USA).

Apparatus and software

The chromatographic measurements were carried out with an KNAUER HPLC system equipped with micro vacuum degasser, LPG system (SCL-10Avp), UV Detector (2100: was set at 200 to 400 nm with a spectral resolution of 1.0 nm and integration period of 0.4 s per spectrum) and a C₁₈ (250 mm×4.6mm, 5µm) column. The pH was measured using a pH meter (Metrohm 827, Switzerland) combined with a glass electrode. A 320R Hettich centrifuge (Germany) and a digital 10P ultrasonic bath (Sonorex; Germany) were used.

Results and Discussion

Sample Preparation

Samples were dried after separating then rinsed with distilled water. Finally, concentration of vitamins B₁, B₂, B₃, B₅, B₆, B₇, B₉ and B₁₂ were determined using HPLC-UV. The tests were repeated three times for sample Dorema aucheri include
of many component that cause chromatographic interferences with vitamins. Then solid phase extraction (SPE) method [6] was used for determination the vitamins B. SPE is an extremely efficient method designed for simple, selective and sensitive and reproducibility sample preparation and purification prior to HPLC analysis. 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 2.0 mL 1,4-dithioerythritol(DTE) 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.

**Chromatographic conditions**

In this research, pH of sample plays an important role in the separation process and it was found that at higher (pH 4.0) and lower (pH 2.0) values the tailing of peak was more and also resolution was poor. pH was studied at the range of 2.0-9.0. In the consequence step, different chemicals including 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 and acetonitrile. 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, temperature of 25 °C was found to be optimal and used in the subsequent analysis. It should be showed that changing the flow rate of mobile phase did not affect on the chromatographic peaks. Elution program for determination vitamin B are as shown in Table 1.

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

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>% ACN</th>
<th>% MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

**Optimization Solid phase extraction variables using one-at-a time method**

In order to optimize the experimental variables on the extraction recovery, two methods were applied. In the first stage, traditional optimization method one at-a-time was used for screening the several factors to detect significant factors.
After screening out the factors with insignificant effect, the remaining factors were optimized using the hybrid Central–Composite design–genetic algorithm.

**pH sample solution**

The pH of sample solution is an important factor and can be affected the recoveries. pH was studied at the range of 2.0-9.0. pH 2.5 was found to be the best. In the consequence step, different chemicals including 1,4-dithioerythritol, 2-mercaptoethanol, phosphate, and acetate were used to adjust the pH. Among them, DTE was chosen as the best.

**Selection of extraction solvent**

Selection of suitable extraction solvent is the most important analytical parameter in SPE technique [3]. Different extraction solvents including n-hexane, 1-octanol, dichloromethane, n-hexane, acetonitrile, methanol, methanol/water, ethanol/water and acetone were investigated. Among these solvents, methanol was selected as the best extraction solvents, respectively, because that higher recoveries were obtain using these solvents in comparison with the others. Effect of volume of the solvents was also studies in the range of 0.5 - 4.0 mL and the results are shown in Figure 1. Optimal volume of methanol was found to be 2.5 mL.

**Fig 1. Separation of vitamins B group in Dorema aucheri.**

![Separation of vitamins B group in Dorema aucheri](image)

**Method evaluation and Application of the proposed method to real samples**

Relative standard deviations (RSD) of the analysis less than 11% (n= 5) and detection limit were determined less than 1 ng kg\(^{-1}\) for B vitamins. The concentration of vitamins in Dorema aucheri are shown in Table 2. Separation of vitamins B group, in leaves and stems of Dorema aucheri are shown in Figure 1.
Table-2: Concentration of vitamins B in Dorema aucheri by HPLC-UV (n=5).

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Stem*</th>
<th>Leaf*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_1</td>
<td>9.3±0.01</td>
<td>10.07±0.06</td>
</tr>
<tr>
<td>B_2</td>
<td>11.1±0.43</td>
<td>11.2±0.50</td>
</tr>
<tr>
<td>B_3</td>
<td>15±0.29</td>
<td>14±0.33</td>
</tr>
<tr>
<td>B_5</td>
<td>20.3±0.17</td>
<td>18±1.40</td>
</tr>
<tr>
<td>B_6</td>
<td>19.7±0.25</td>
<td>21.1±0.19</td>
</tr>
<tr>
<td>B_7</td>
<td>2.5±0.38</td>
<td>4.8±2.57</td>
</tr>
<tr>
<td>B_9</td>
<td>11.0±2.40</td>
<td>10.5±0.01</td>
</tr>
<tr>
<td>B_12</td>
<td>2.52±0.08</td>
<td>2.63±0.55</td>
</tr>
</tbody>
</table>

Conclusions

The evaluation of vitamins B group composition of Dorema aucheri can be used to achieve the levels and of importance of vitamins in different weather conditions and also to achieve which organs of plant can be used to prepare an extract of important and useful vitamin for use in human. The leaves of plant have been used as medicine for hepatitis traditionally.

In this work, the leaves of Dorema aucheri was evaluated for the determination of B vitamins levels under the optimized conditions. In this research, a simple and quick SPE-HPLC-UV method to simultaneously determine vitamins B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_12 in Dorema aucheri has been developed and validated in terms of sensitivity, linearity, and precision. The data obtained by this method revealed the ability of this method in the determination vitamins B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_12.

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