HPLC DAD Determination of Some Vitamins B Group Concentrations In Suaeda Aegyptiaca Following Solid Phase Extraction

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INTRODUCTION

Halophytes are plants that can live in saline and alkaline soils conditions containing approximately 200 mM NaCl. They store inorganic ions and produce a high osmotic potential. Some halophytes are also able to live in regions immersed with saline water. Some halophytes are also able to live in regions immersed with saline water (e.g. coastal salt swamps) [1-4].

S. aegyptiaca is a member of the Chenopodia. There are 26 families of halophytes species in Iran and more than 70% of them has belonged to the Chenopodiaceae family[4]. They grow during both high and low tides from April to October. The growth rates of S. aegyptiaca decreased after July and then increased after August. The leaf of S. aegyptiaca has been used as a medicine for hepatitis and antiviral activity. The young leaf of it often mixed with other vegetable to reduce their saltiness[5-6].

The vitamins are an essential group of organic compounds that are minor, but essential required for normal growth human and animal bodies [5]. The vitamins are classified in to fat-soluble vitamins (Vitamins A, D, E and K) and water-soluble vitamins(Vitamins B,C) .The B vitamins is water-soluble that support metabolism, skin, muscle tone, immune and bone health, nervous system. Vitamins B₉ essential in synthesis of DNA and RNA, B₂ in protein metabolism and B₁ is important in bone health.nervous system and cell growth [7-9].There are several methods available, which including capillary electrophoresis, electrochemical methods, spectrophotometry ,spectrofluorimetry, tin layer chromatography (TLC) and high performance liquid chromatography (HPLC) several chemometrics methods, such as Kalman filtering, principal component regression and partial least squares, have been used for determination of vitamins[10-14].

Solid Phase Extraction (SPE) has commonly been used as a technique for preconcentration and separation of various inorganic and organic species. SPE is used to enhance the selectivity and sensitivity of the method as it allows for discriminatory binding of analyte to a solid support where it will be accumulated and subsequently eluted with a small volume of solvent. This technique has advantages of higher enrichment factor, absence of emulsion, safety with respect to hazardous samples, minimal costs due to low consumption of reagent, environment friendly, flexibility and easier incorporation into automated analytical techniques. In addition, some pre-concentration step such as solid-phase extraction (SPE) is necessary before HPLC to remove interfering components [15-18].

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The main objectives of the present study to determine of amount B group vitamin in leaves and stems of *S.aegyptiaca* by HPLC-DAD. The proposed method was successfully applied to determine some B groups vitamin in halophyte samples. Finally to the best of our knowledge there are no published research studies about determination of vitamins B group concentrations in *S. aegyptiaca*.

MATERIAL AND METHODS

1.1. Chemicals and reagents:

All reagents were of analytical grade and were purchased from Merck (purity >99%). Water used in all the experiments was ultrapure water. High purity ultrapure water was obtained from Millipore, Milli-Q (Bedford, MA, USA). HPLC-grade methanol (MeOH) were from Merck (Germany). Solvents, calibration and real samples were used to perform the HPLC application were filtered through 0.45 µm nylon filter membranes filter paper (Varian, USA). All stock solutions and working standards were stored at 4°C and brought to room temperature (25°C) before use.

1.2. Instrumentation:

High performance liquid chromatography and UV–visible Diode-Array (DAD 2100 Knauer). Software was used to calculate peak areas, Ez Chrom Elite. Chromatographic separation was conducted on C_{18} (5µm particle size) and coupled to DAD detector. DAD detector was performed between 200 and 400 nm with the spectral resolution of 1.0 nm and integration period of 0.4 s per spectrum. pH value measurements were performed on a pH meter system (Metrohm, model 827, Switzerland) with a combined glass electrode was used for pH measurements. Before using, the pH meter was calibrated against standard Merck buffers (pH=4 and pH=7). 320R Hettich centrifuge (Germany) and a Sonorex Digital 10P ultrasonic bath (Germany) were used. The vitamin standards were of analytical-reagent grade from Sigma (Sigma–Aldrich, Deisenhofen-Germany). All stock solutions and working standards were stored at (4°C) and brought to room temperature (25°C) before use.

1.3. Study Area:

The present study was carried out the common halophytes *S. aegyptiaca* in three different region of Dashti in Bushehr province, Iran, in early summer, 2013 (Fig.1). The area under study includes a part of the coastal areas of the north of Persian Gulf south of the country that regarding political divisions this area is a part of Bushehr province. This plant is native to the south, southern-east and Persian Gulf coastal and is recognized as Kakol Bushehr province.

RESULTS AND DISCUSSIONS

1.4. Sample Preparation and Chromatographic conditions:

After drying at (50°C) for 1 h to a constant weight, the samples were separated and weighed individually. The dried samples were homogenized and grounded using a mortar. Finally, concentration of B groups vitamin contain B1, B2 and B9 were determined using HPLC apparatus. The SPE method of Cho et al.[19-20] was used for the extraction of water-soluble vitamins. To obtain the best chromatographic conditions and short separation time influences of the analytical parameters including effect pH ratios of the solvents, temperature and flow rate were investigated. The aim of this work was to develop a simple, accurate and sensitive HPLC method for the simultaneous determination of group B vitamins determination in *S. aegyptiaca* samples(Fig.2).

1.5. Optimization of pH:

In this work, various phosphate buffer (pH 3.0–6.0) were investigated to improve the resolution and peak symmetry. In this work variation of pH plays an important role in the separation process and it was found that at higher (pH 6.0) and lower (pH 3.0) values the tailing of peak was more and also resolution was poor. Finally pH =3.5 was chosen as the optimum value for resolution better peak shape and short run time.

1.6. Optimization effect pH ratios of the solvents:

Various ratios of the solvents were tested. It was found that a mobile phase included a certain ratio of methanol and water gave symmetric peak shapes. A mobile phase containing Water/methanol mixture phosphate buffer solution pH=3.5, flow rate 1.0 mL min⁻¹ was used as the mobile phase and an elution gradient allowing the complete analysis of all the in less than 10 min, whose characteristics are reported in (Table 1) was chosen. With this mobile phase composition, the retention times of simultaneous determination of vitamins B group, B₂,B₉ and B₁ were 2.185, 4.692 and 6.218 min, respectively.
1.7. Optimization Effect temperature:
The mobile phase was pumped at various temperatures to column in the range of 20–45 °C. Temperatures (25 °C) was selected to be the optimum temperature for the separation of vitamins B group peak shapes and heights were improved and retention times were decreased with (25 °C) temperature without affecting peak areas and resolution. By using above optimum conditions improve the resolution and peak symmetry.

1.8. Method evaluation:
The limit of detection (LOD) determined as three times the standard deviation of 10 blank measurements divided by the slope of the calibration curve was less than 8 ng kg\(^{-1}\). The precision estimated from the relative standard deviation was less than 6%. The calibration curves for the analytes over the desired concentration ranges exhibited good linearity. The correlation coefficients for all calibration curves were beyond 0.999. The accuracy of the proposed method was estimated by determining the recoveries of the analytes by spiking experiments. The recoveries in samples at concentration levels of 10 mg kg\(^{-1}\) were found to be 89% and 100%. The accuracy of the element concentration data for spiked samples is shown in (Tables 2).

1.9. Application of the proposed method to real samples:
The results showed a vitamins B\(_1\), B\(_2\) and B\(_9\) in leaves of S.aegyptiaca lower than stem. The concentration of vitamins in leaves and stems of S.aegyptiaca have been shown in (Table 3). Separation of vitamins B group, B\(_1\), B\(_2\) and B\(_9\) in leaves and stems of S.aegyptiaca are showed in (Fig.3). 3D figure of Separation of vitamins B group in retention time are showen in (Fig.4).

Conclusions:
In this method, the variables affecting parameters such as chromatographic conditions and optimization of conditions were studied. Phosphate buffer solution pH=3.5, flow rate 1.0 mL min\(^{-1}\), temperatures 25 °C was chosen as the optimum value for resolution better peak shape and short run time. Advantages of this method were low limit of detection (LOD), 8 ng kg\(^{-1}\), the relative standard deviations (RSD) below 6%, recoveries of all the vitamins were in the range of 89% - 100%, and the linear correlation coefficients (R) 0.999.

Evaluation of the vitamin B group composition of various S.aegyptiaca can be used to achieve the levels and importance of ions in different weather conditions and also obtain the organs of plant that can be used to prepare an extract of important and useful ions for humans. Leaves of the plant have been traditionally used as a medicine for hepatitis and it has been reported antibacterial, antioxidant activities, etc.

ACKNOWLEDGMENTS
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Fig. 2: Wavelength vitamins B1(B215 nm), B3(238) and B4(254).

Fig. 3: Separation of vitamins B group, B1, B2 and B4 in leaves and stems of *S. aegyptiaca*.

Fig. 4: 3D figure of retention time of vitamins B group 2.185(B2), 4.692(B4) and 6.218(B1).

**Table 1**: Scheme of gradient elution programme used in HPLC analysis for determination of vitamins B1,B2,B6.

<table>
<thead>
<tr>
<th>Time(Min)</th>
<th>%H2O</th>
<th>%MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Method evaluation for the determination of vitamin B1, B2, B3 by HPLC DAD.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>R</th>
<th>LOD1 (mg kg⁻¹)</th>
<th>RSD(%)</th>
<th>Recovery1 (%)</th>
<th>Recovery2 (%)</th>
<th>Recovery3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>0.9997</td>
<td>6.6</td>
<td>4.1</td>
<td>89.5</td>
<td>95.8</td>
<td></td>
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<tr>
<td>B2</td>
<td>0.9999</td>
<td>7.2</td>
<td>1.2</td>
<td>96.4</td>
<td>98.2</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>0.9994</td>
<td>5.8</td>
<td>5.7</td>
<td>100</td>
<td>98.9</td>
<td></td>
</tr>
</tbody>
</table>

1- LOD – Limit of detection, 2-Stems, 3-leaves

Table 3: Concentration analysis of vitamins B by HPLC DAD (mg kg⁻¹).

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Leaves (mg kg⁻¹)</th>
<th>Stems (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>9±2.1</td>
<td>12±1.6</td>
</tr>
<tr>
<td>B2</td>
<td>12±1.7</td>
<td>14±2.3</td>
</tr>
<tr>
<td>B3</td>
<td>23±1.5</td>
<td>26±1.3</td>
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REFERENCES
