Comparison of High-Performance Ion Chromatography and absorptiometric methods for the determination of phytic acid in food samples

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Abstract. The objective of this paper consists in defining the interest of a new high-performance ion chromatography method (HPIC) with chemically suppressed conductivity detector for phytic acid determination in food samples. Firstly, accuracy and precision of the HPIC method have been measured. Secondly, the HPIC method and a classical absorptiometric method were compared. The HPIC method was more sensitive and selective than the absorptiometric method which leaded to an 27% overestimation of the phytic acid content in legume seeds. Because of it is rapid and easy to perform, the HPIC method appears to be particularly suitable for routine analysis of food samples.

Key words. Phytic acid – liquid chromatography – absorptiometry – food analysis.

Introduction

Phytic acid, myo-inositolhexaphosphate (Fig. 1), is a common constituent of many plant foods [1]. This molecule is highly charged with six phosphate groups extending from the central inositol ring structure [2] and therefore is an excellent chelator of mineral ions [3]. Since phytate cannot be absorbed and humans have limited capacity for

Fig. 1. Structure of Inositol 1,2,3,4,5,6-hexaphosphate (or Phytic acid).
Materials and methods

Analytical instruments

Beckman (Fullerton, USA) DU 70 spectrophotometer was used for colorimetric determination.

High Performance Ion Chromatography (HPIC) analyses were performed with a 4500i Dionex (Sunnyvale, USA) liquid chromatograph. The separation was carried out on an Ómnipac Pax-100 anion-exchange column (25 cm x 4 mm I.D., Dionex, Sunnyvale, C.A., USA) equipped with an Omnipac Pax-100 anion suppressor. The separation was performed by gradient elution using three solutions: solvent A = deionized water, solvent B = deionized water:isopropanol 50:50 (VIV). Solution C was deionized water. The mobile phase was a mixture of three solutions A, B and C with a final volume of 1 L. Solution B was deionized water:isopropanol 50:50 (VIV). Solution C was deionized water. All the mobile phases were passed throughout.

Samples preparation. Sample solutions were diluted with a Gilson (Middleton, USA) semi-automatic dilutor 401 prior to injection. A 200 pL constant volume injection loop was used for injection.

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phytic acid solution

tions of the sample with increasing standard additions of a pea (flour) were repeatedly analysed daily for four days.

0.0001 pmol L⁻¹.

Precision for replicate injection of reproducibility).

than 10: the limit was 0.57%. To determine the method reproducibility, six different samples from the same batch of ungerminated cow

of phytic acid was attained by 54

range and correlation coefficients obtained are shown in table II. In order to determine whether b (y = ax + b) was significantly different from 0 or not, the hypothesis b = 0 was tested. As the test was accepted, the calibration curve passing through zero can be used O,

reduction of conductivity of eluent and enhances the conductivity of the analytes. The retention time of phytate was proportional to the phytic acid concentration over the entire period (Fig. 2). The area under the conductivity peak is proportional to the phytic acid concentration over the entire range and correlation coefficients obtained are shown in table II. In order to determine whether b (y = ax + b) was significantly different from 0 or not, the hypothesis b = 0 was tested. As the test was accepted, the calibration curve passing through zero can be used O,


The analysis of the variance (One way ANOVA) was carried out using the Sigmastat Software (Jandel) according to the conventional procedures.

Results and discussion

Firstly, we tested the linearity, accuracy, precision and reproducibility of the HPIC method. Secondly, we compared the results obtained on the samples by the absorbometric and HPIC methods.

Phytic acid with concentrations ranging from 0.01 mmol L⁻¹ to 0.16 mmol L⁻¹ was analysed on an anion exchange column with chemically suppressed conductivity detector using three solvents mixture for gradient elution. The more phosphate group were retained on the column. The elution of phytic acid was attained by 54 – 55% 200 mmol L⁻¹ NaOH solution. An anion micromembrane suppressor (AMMS) was applied with conductivity detection. This suppressor was continually regenerated with 50 mol L⁻¹ sulfuric acid in order to neutralize. The use of suppressor reduces the conductivity of eluent and enhances the conductivity of the analytes. The retention time of phytate was 6.0 ± 0.2 min with no day-to-day variation over a 5-month period (Fig. 2). The area under the conductivity peak is proportional to the phytic acid concentration over the entire range and correlation coefficients obtained are shown in table II. In order to determine whether b (y = ax + b) was significantly different from 0 or not, the hypothesis b = 0 was tested. As the test was accepted, the calibration curve passing through zero can be used O,
with the classical absorptiometric method for use in our laboratory.

We studied the two methods to determine their precision and accuracy for the determination of phytic acid and to select the most appropriate experimental protocol. Each sample of phytate standard solutions was prepared and analyzed in five replicate with HPIC and absorptiometric methods. The data of mean, standard deviation and precision by each method are shown in table III. The HPIC method was clearly more accurate than the absorptiometric method and could be directly applied to samples without prepurification.

Several kinds of cereals were extracted and phytate was quantified by the HPIC method and by the conventional absorptiometric method. Data obtained by the absorptiometric method, regardless of the cereal flour used, were su-

Table III. Accuracy and precision of methods (absorptiometric and HPIC).

<table>
<thead>
<tr>
<th>Phytate (mmol L⁻¹)</th>
<th>Colorimetric Method</th>
<th>HPIC Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>465 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D.Ö</td>
<td>Precision⁵</td>
</tr>
<tr>
<td>0</td>
<td>1.570 ± 0.118</td>
<td>9.33%</td>
</tr>
<tr>
<td>0.0286</td>
<td>1.405 ± 0.049</td>
<td>4.34%</td>
</tr>
<tr>
<td>0.057</td>
<td>1.179 ± 0.064</td>
<td>6.77%</td>
</tr>
<tr>
<td>0.0857</td>
<td>0.966 ± 0.069</td>
<td>9.29%</td>
</tr>
<tr>
<td>0.114</td>
<td>0.779 ± 0.077</td>
<td>13.0%</td>
</tr>
</tbody>
</table>

⁵ Mean and Standard Deviation of five standards.

Table IV. Comparison of two methods for determining phytic acid in food samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorptiometric Method (A)</th>
<th>HPIC Method (B)</th>
<th>% of overestimation⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millet souna NG</td>
<td>0.807 ± 0.06</td>
<td>0.587 ± 0.06</td>
<td>27.3</td>
</tr>
<tr>
<td>Cowpea NG</td>
<td>1.32 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>26.5</td>
</tr>
<tr>
<td>Millet souna G</td>
<td>0.49 ± 0.07</td>
<td>0.351 ± 0.02</td>
<td>28.6</td>
</tr>
</tbody>
</table>

⁶ Mean ± SD of three replicate samples.

100 – (B × 100/A).
Application of multivariate mathematical-statistical methods to compare reversed-phase thin-layer and liquid chromatographic behaviour of tetrazolium salts in Quantitative Structure-Retention Relationships (QSRR) studies

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3 Reanal Fine Chemicals, Budapest, Hungary

Abstract. The retention parameters of seven mono- and nine ditetrazolium salts were investigated by reversed-phase liquid chromatography (RP-LC) using polyethylene-coated alumina support with various concentration of eluent-mixtures and by reversed-phase thin-layer chromatography (RP-TLC) using the same support and eluent-mixtures. Principal component analysis (PCA) followed by non-linear mapping (NLM) and varimax rotation was used for the determination of molecular substructures and physicochemical parameters accounting for the separation in the different methods and circumstances. Calculations proved that the steric and electronic parameters have the highest influence on the retention of tetrazolium salts. This investigation uncovered that the thin-layer chromatography is strictly independent from liquid chromatography. It has been established that multivariate mathematical-statistical methods are the most appropriate ways for evaluation of large data matrices in various chromatographic departures.

Key words. Tetrazolium salts - thin layer chromatography - liquid chromatography - principal component analysis - cluster analysis - non-linear mapping - varimax rotation.

Introduction

The widely used reversed-phase liquid chromatographic separation technique is especially useful to the separation of bioactive molecules [1].

Although the support is generally silica with covalently bonded hydrocarbons [2] or various polymers to the polar surface [3], the alumina is more frequently used in conjunction with an polar phase supports such as polystyrene [9] and polyethylene chloride [10] have been coated to silica or alumina basis.

The polymer-coated alumina supports typically and successfully used the investigation or separation of basic solutes hardly eluted from silica-based supports such as various proteins [11], peptides [12], other bio-macromolecules [13], alkaline compounds [14] and a lot of drugs [15] and diagnostic materials [16] as well.

The tetrazolium-derivatives are wide-spreadly used in the field of biology research [17], human medicine care [18] especially in the field of endocrinology, gastroenterology, cytology-histology, angiology, microbiology and virology, in microbiological laboratories [19] and in the field of bio-inorganic chemistry.

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