ANALYSIS OF PHOSPHOLIPIDS IN NATURAL SAMPLES BY NP-HPLC AND CORONA CHARGED AEROSOL DETECTION

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Overview

Purpose: To develop HPLC methods for quantitation of phospholipids in natural products, including foods and ingredients, using an HPLC system with a charged aerosol detector.

Methods: One NP HPLC method, using the Thermo Scientific™ Hyperil GOLD™ SIL column, was created for the determination of six phospholipids and was used to quantify phospholipids in foods and ingredients.

Results: Samples of foods or ingredients were analyzed for content of phospholipids using the developed method. Sensitivity for the phospholipids varied from limits of detection between 10 – 22 ng o.c.

Introduction

Phospholipids are a broad class of lipids that can be divided into glycerophospholipids (GPLs) and sphingolipids. Both groups show great structural diversity. Phospholipids are amphiphilic molecules, having a hydrophobic head group, and a lipidic fatty acid tail. Several families of GPLs exist biologically, differing in the type of polar head group present, and include: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), cardiolipin, and phosphatidylglycerol (PG). Each compound contains many species resulting from differences in their fatty acid composition. For example, PC may contain several different fatty ligands, which will result in multiple peaks by reversed phase chromatography. Normal phase liquid chromatography (NP- HPLC) uses differences of polar moieties to separate analytes, and provide more quantitative data with less effort. The Thermo Scientific™ Dionex™ Corona™ Vee™ Charged Aerosol Detector, a sensitive mass-based detector, is ideally suited for the direct measurement of phospholipids, as they are non-volatile and non-chromophoric compounds. It offers excellent sensitivity (down to low nanogram amounts on column), a dynamic range of over 4 orders of magnitude, and similar inter-analyte response independent of chemical structure. This developed method is based on an original publication by Rombaut, R., et al. (J. Dairy Sci., 2005, 88, 482), that enables the direct measurement of a number of GPL and GL species, each as near-single peaks. As shown in Figure 1, the detector utilizes nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube, leaving analyte particles, which become charged in the mixing chamber. The charge is then measured by a highly sensitive electrometer, providing reproducible, nanogram level sensitivity. This technology has greater sensitivity, dynamic range and precision than ELSD and refractive index (RI), is a gradient compatible and is simpler to operate than a mass spectrometer (MSM). Compounds do not have to possess a chromophore (unlike UV detection) or be ionized (as with MS).

This sensitivity, combined with the linearity that is possible with use of the Corona Power Function, provides a unique and complete analytical solution for sensitive, reproducible, and routine analysis of non-chromophoric analytes.

Methods

Standard and Sample Preparations

Standards were dissolved in methanol / chloroform (1:1), at a concentration of approximately 2.00 mg/mL, including the sodium salt for the PI and PS analytes. Standards were diluted sequentially by half to provide a calibration range of 78 – 10,000 ng o.c. of each analyte.

Egg Yolk Sample:

In a 2 mL glass vial, 90.3 mg yolk was placed. To this, 150 μL of methanol / chloroform (1:1) was added. The mixture was vortex-mixed. To precipitate proteins, 500 μL of acetone-water was added and the mixture was vortex-mixed. Another 1,350 μL of methanol / chloroform (1:1) was added, vortex-mixed, and allowed to settle. The supernatant was centrifuged at 10,000 g for 3 minutes, and the supernatant was analyzed directly.

Lecithin:

To an HPLC vial, 1.1 mg of Lecithin (Laboratory Grade, Fisher Scientific) was added. The sample was dissolved in 1100 μL of methanol / chloroform (1:1) and centrifuge filtered through a 0.2 micron filter, 10,000 g for 3 minutes. Supernatant was analyzed directly.

Koil Oil:

An HPLC vial, 2.50 mg of oil was added. To this, 1000 μL of methanol / chloroform (1:1) was added. The solution was vortex-mixed and analyzed directly.

Liquid Chromatography

HPLC System: Thermo Scientific™ Dionex™ Ultimate™ LC System with LPG340H-DP pump (normal phase), WPS3000 RS autosampler, and TCD3000 RS column oven

HPLC Column: Thermo Scientific™ Hyperil™ SIL 3 μm, 3.0 x 150 mm

Column Temperature: 50 °C

Mobile Phase A: 0.5% diethylamine-formate, pH 3.0

Mobile Phase B: 150 mM NaCl, 0.5% diethylamine-formate, pH 3.0

Mobile Phase C: Isooctane

Flow Rate: 0.3 – 0.8 mL/min

Injection Volume: 2–5 μL

Detector: Corona Vee SD Charged Aerosol Detector

Data Analysis

All HPLC chromatograms were obtained and compiled using Thermo Scientific™ Dionex™ Chromleon™ Chromatography Data System software, 6.8 SR 13.

Results

Calibrations

Five phospholipids were used to calibrate the method for sample analysis: PE, PI, PS, PC, and LPC. A chromatogram of the standard solution at approximately 5000 ng o.c. is shown in Figure 2. Each analyte solution was injected threefold, plotted, and fit to a linear regression, as shown in Figure 3. As can be seen from the plot, all plots were linear, and five of the analytes had nearly the same response factor, which is a typical characteristic of charged aerosol detectors with non-volatile analytes. Other phospholipids, PA, PG, and SPH were not included in these analyses. PI and PS eluted at the retention time of 4.8 minutes, or the small peak seen just after PE, in Figure 2.

The correlation coefficients, R², for all analytes was greater than 0.995 across the entire dynamic range, from 78 to over 10,000 ng o.c. Along with these values, the sensitivity results, based on signal to noise calculations for LOD and LOQ, are provided in Table 1.

FIGURE 1. Schematic and functioning of charged aerosol detection

Sample Analysis—Lecithin

The lecithin sample was analyzed directly, with chromatogram shown in Figure 5, finding PE, possibly PA (peak at 4.59 minutes), PI, PS, and LPC. The LPC peak retention time was observed by matrix peak. Composition and total phospholipids results are provided in Table 3.

FIGURE 2. HPLC chromatogram of phospholipids at 5000 ng o.c. in lecithin.

Sample Analysis—Koil Oil

The krill oil sample was analyzed directly, with chromatogram shown in Figure 4, finding the major phospholipids present, mainly PC, PE, and LPC and the minor PI and PS phospholipids. Relative phospholipid composition and total phospholipids results are provided in Table 4. The values obtained matched the AOCS method results within 15%.

FIGURE 4. HPLC-CAD chromatogram showing phospholipids found in koil oil.

Table 3. Results of laboratory grade lecithin analysis, including total phospholipids and mass % of each phospholipid in amount of lecithin.

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Found (% Mass)</th>
<th>LOQ (% Mass)*</th>
<th>LOD (% Mass)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>4.7</td>
<td>0.14</td>
<td>0.03</td>
</tr>
<tr>
<td>PI</td>
<td>1.7</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>PS</td>
<td>61.2</td>
<td>2.88</td>
<td>0.90</td>
</tr>
<tr>
<td>PC</td>
<td>31.3</td>
<td>1.55</td>
<td>0.52</td>
</tr>
<tr>
<td>LPC</td>
<td>15.6</td>
<td>0.33</td>
<td>0.13</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>5.06</td>
<td>1.49</td>
</tr>
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Table 4. Results of kril oil analysis compared to literature values, including total phospholipids and relative composition of total phospholipids.

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Conclusions

A method for the determination of phospholipids in various samples was developed using normal phase HPLC and the Corona Ve SD Charged Aerosol Detector. Sensitivities for the phospholipids were < 25 ng o.c. LOD. Linear calibration curves were found for all five phospholipid analytes, with correlation coefficients > 0.955 over four orders of magnitude. Phospholipid compositional results matched orthogonal results very well, and quantity amounts were similar, whereas differences were more likely due to sample preparation rather than chromatographic method conditions.

References