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Determination of phytate in high molecular weight, charged organic matrices by two-dimensional size exclusion-ion chromatography

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ABSTRACT

A two-dimensional chromatography method for analyzing phytate or other ionic targets in matrices containing high molecular weight, charged organic species is described. Prior to quantification by anion exchange chromatography, the sample matrix was prepared by size exclusion chromatography, which removed the majority of the matrix. Quantification of phytate on the AS11-HC was sensitive (0.25 μM, 0.17 mg/l) and reproducible (4.6% RSD) allowing this method to provide baseline separation of phytate from a manure extract within 14 min. The method is simple, requiring only sample filtering, reproducible (between-run variation 5% RSD) and linear from 0.38 to 76 μM (0.25–50 mg/l). The method is suitable for routine determination of phytate in high organic matrices such as manure extracts.

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1. Introduction

In ion chromatography (IC), interferences from sample matrices can inhibit sufficient separation and quantification of target analytes [1]. Generally, there are two approaches that the analyst can use to eliminate interfering matrices. Matrix elimination, either by selectively retaining the target and washing through the matrix, or retaining the problematic matrix components and collecting the target. Both are considered solid phase extraction (SPE) techniques, which are the hallmark of sample preparation [1]. For the removal of general organics, sample prep phases, which contain styrene-divinylbenzene resin or alkyl reversed phase material, are commonly used to remove aromatic or alkyl organics form the sample matrix. Charged organics, such as humic and fulvic acids are generally not well retained by these types of resins without neutralizing the sample pH. Without the removal of organics, IC columns quickly become fouled [2–4]. With both approaches however, SPE methods typically do not account for variation in samples or sample matrices. In this work, we examine the utility of size exclusion chromatography as an approach for matrix removal based on molecular weight.

The size exclusion chromatography approach for matrix removal described herein was developed in response to the need to isolate and measure myo-inositol hexakisphosphate, or phytate in a high dissolved organic matter extract (see Supplementary material for more information). Characterizing phosphorus in natural surface waters, wastewaters, soils and manures is important for understanding the complexities of phosphorus losses to streams, rivers, and lakes that result in eutrophication of the freshwater aquatic environment. While phytate is only one fraction of organic phosphorus, its ubiquity in nature as the primary plant phosphorus storage molecule makes it of particular interest. Additionally, phytate like most of the organic phosphorus fraction interacts with organic carbon making it challenging to isolate prior to analysis. Several extraction procedures, such as Mehlich-3 and basic-EDTA, have been developed which are intended to remove bound phosphorus from soil minerals and the organic carbon fraction [5,6]. Many of the standard methods for analyzing phytate in these extracts, however, are either quite encumbering (solid phase extraction-liquid chromatography or nuclear magnetic resonance) [7,8] or nonspecific (Inductively coupled plasma, colorimetry) [9–11]. In the case of phytate analysis, a size exclusion method provides a common procedure regardless of the sample matrix. Recently, ion chromatography (IC) has been gaining popularity as the analytical technique for speciating phosphorus [12–15]. Additionally, size exclusion chromatography has been shown as an effective method of fractionating large molecular weight humic acids [16,17]. By expanding on this work, the same principles can be applied to fractionating sample matrices based on size prior to analysis. With the matrix removed, a heartcut injection

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onto a second column, which has a comparatively clean matrix allows reliable separation and quantification of phytate by electrical conductivity [18]. Here, we demonstrate large molecular weight exclusion for the quantification of phytate in a water extract of poultry manure as well as small molecular weight exclusion for eliminating interferences from matrix components following a poultry litter extraction.

2. Materials and methods

2.1. Materials

All chromatography separations were performed with a ThermoFisher-Dionex ICS5000+ chromatography system (Sunnyvale, CA, USA). The ICS5000+ system is equipped with 2 fully capable chromatography channels, which can be integrated together. The first channel used for matrix removal included an automated sample injector, a 4-channel gradient pump, 6-port high-pressure valve, 10 μl injection loop, a (5 μm 4.6 × 300 mm) SEC-300 size exclusion column, conductivity detector, and a 4-channel variable wavelength detector. The second channel included a high-pressure isocratic analytical pump, electrolytic eluent generator, 6-port high-pressure valve, (0.75 × 80 mm) MAC-200 anion concentrator column, 2 × 250 mm AS-11HC ion exchange column, electrolytic suppressor and conductivity detector. Instrument control and data acquisition was done using Chromeleon 7.2 software (ThermoFisher-Dionex, Sunnyvale, CA, USA).

All reagents and solutions used were of analytical grade. A ROES-75 Reverse osmosis water system capable of producing 16 MΩ water (APEC Water Systems, City of Industry, CA, USA), was added to the RFIC system and polished in-line using CIRA-10 electrodialytic water purification unit (Trovion, Campbell, CA, USA) prior to entering the high-pressure pump on both channels. Additionally, the size exclusion column used one mM HCl (pH 3.0) as the mobile phase. Standards were made using ACS grade phytic acid dipotassium salt.

2.2. Methods

Two common extraction procedures were performed on poultry manure sourced from a local farm, which does not fortify their poultry feed with phytase enzyme. All manure samples were dried, grinded and sieved as discussed in [19]. Samples were then extracted with deionized water following the method of Kleinman et al. [20]. Manure samples were also extracted by shaking 2.5 g of manure with 25 ml of Mehlich-3 solution (0.2 M CH_3COOH +0.25 M NH_4NO_3+0.015 M NH_4F+0.013 M HNO_3+0.001 M EDTA) for 5 min [6].

Separation of phytate was achieved using a 2-dimensional system by matrix removal in the first dimension, followed by ion exchange chromatography in the second. A 10 μl filtered, undiluted sample was injected on to the size exclusion column (Fig. 1A), which was used to separate the matrix components based on molecular weight (Fig. 1B). At 6.1 min, the valve between the first and second dimension was actuated to capture the effluent from the SEC-300 for 15–20 s using a MAC200 anion concentrator column (Fig. 1C). After the 15–20 s interval, the valve was actuated again to inject the captured fraction onto the ion exchange column (Fig. 1D). The total runtime for the analysis was 14 min under the operating conditions shown in Table 1.

The minimum width of the heartcut was determined using several different molecular weight standards (Polyacrylic acid M. W. 15 K, 8 K, 1200), and formic acid as the marker for the total permeation time). Once the permeation time between the 1200 M. W. marker and the formic acid was determined, subsequent

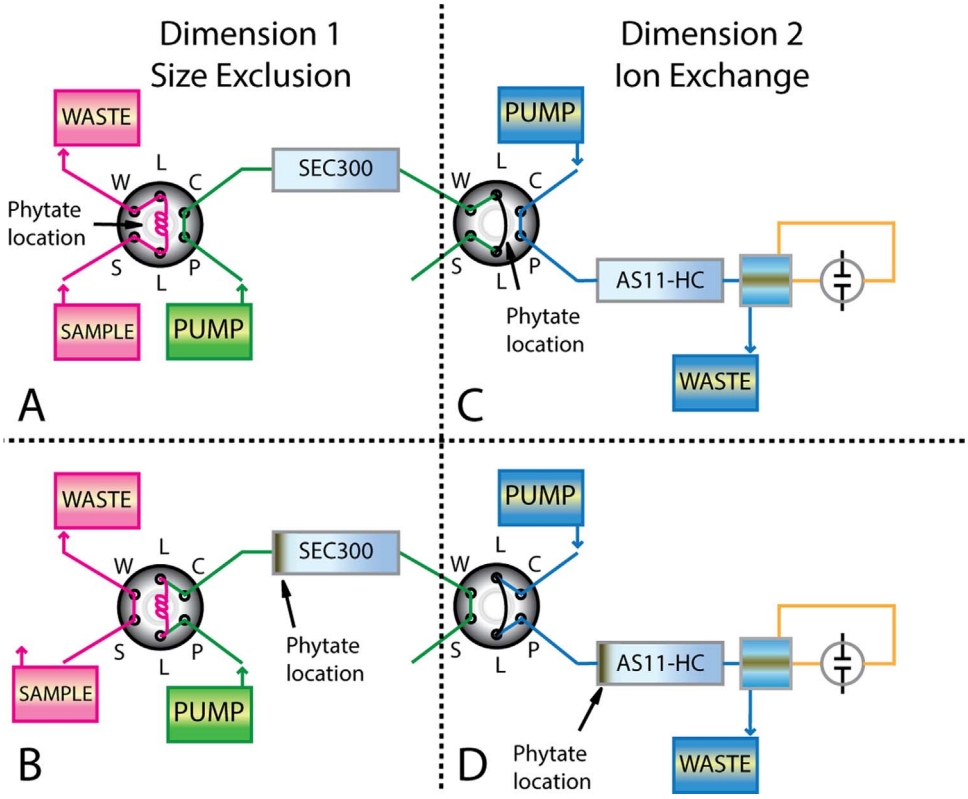

Fig. 1. Schematic of two-dimensional chromatography system. *Color online only. A: Loading manure matrix into the SEC loop (between valve "L" labels). B: Sample is injected onto the SEC column (matrix exclusion). C: Concentrator column on second valve collects the phytate fraction from the SEC outlet (between valve "L" labels). D: Phytate fraction (now on the concentrator) is injected onto the anion exchange column prior to detection.