APPLICATION UPDATE 73406

Determination of phytic acid in beans, wild rice, and almonds

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Keywords: Dionex IonPac AS11 column, RFIC, Reagent-Free IC, phytate, myo-inositol hexakisphosphate, AN295, IP6

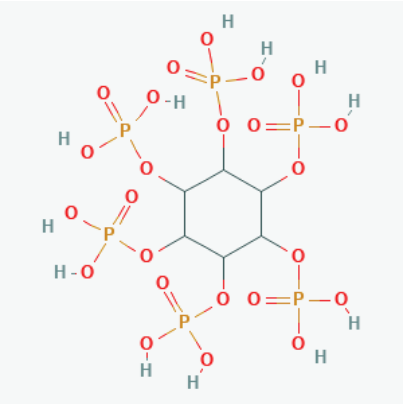


Figure 1. Phytic acid (Myo-Inositol hexakisphosphate)

Introduction

Phytic acid (myo-inositol hexaphosphoric acid or myo-inositol hexakisphosphate) (Figure 1) or phytate when in the salt form, sometimes abbreviated to IP6, is abundant in plant seeds, such as legumes, nuts, and grains. Phytic acid serves as storage for cations and phosphorus, a source of energy for germination, and a wound signal in plants.[1–4] In animals, only ruminant animals have phytase to metabolize phytic acid. Monogastric animals, including humans, do not naturally make phytase.[5,6] Other inositol phosphate compounds, such as IP2, IP3, IP4, and IP5, have important biological functions associated with calcium-dependent signaling channels and apoptosis in cancer cells.[7,8]

In addition to being nutritionally unavailable to most animals, the nutritional benefits of phytic acid are controversial. Phytic acid, a potent chelator of metals, deleteriously impacts bioavailability of important minerals necessary for metabolomic processes.[5–10] By binding the minerals, phytic acid prevents absorption and thereby accessibility to create important metal-centered biomolecules. In contrast, phytic acid also reduces free iron, thereby reducing the potential to form metabolism-damaging free Reactive Oxygen Species (ROS).[9,10] Therefore, determinations of phytic acid are needed for researchers studying botanical processes and the impact of phytic acid rich foods on human and animal nutrition.

Published phytic acid analysis methods use anion-exchange separation and suppressed conductivity detection[11] or visible absorption detection after a post column reagent addition.[12] However, acid digestion was used, which lengthens the workflow and increases complexity by requiring additional sample pretreatment prior to sample analysis. Other researchers have reported that phytic acid can be released by milling the grain and fermentation at mildly acidic pH.[5–10] More recently nutritionists reported that significant amounts of phytic acid were removed by soaking the seeds in water.[6] Determinations of phytic acid were previously reported in mung beans, soybean, and black sesame samples;[11,12] however, analysis of other high phytic acid-containing samples is needed.

In this application update, phytic acid was determined in extracts of whole and ground kidney beans, pinto beans, wild rice (aquatic grass seed), and almonds. Municipal drinking water was used as the extractant to mimic home preparation and to reduce the complexity of the sample preparation. Phytic acid is converted to the phytate anion in the presence of the strong base, and then separated using electrolytically generated 65 mM KOH and detected by suppressed conductivity (Thermo Scientific Dionex Application Note 295 (AN295)).[11] Phytic acid as phytate eluted from the column within 7 min. However, other late-eluting compounds in the samples can elute in the chromatogram of the next injection, thereby requiring longer run times. If phytic acid is the only ion of interest and shorter runs are desired, a wash step of 75 mM KOH for 10 min can be added after phytic acid elutes. The extractions of phytic acid in whole and ground kidney beans were also measured over several days following the same method.

Experimental

Equipment

Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system*

- Single Pump SP, isocratic configuration.
- Eluent Generator EG module
- Detector Chromatography DC module with one 6-port injection valve
- CD Conductivity Detector (P/N 079829)

Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control option

*Or Thermo Scientific™ Dionex™ Integrion HPIC system, RFIC model (P/N 22153-60305) with Integrion CD Conductivity Detector (P/N 22153-62034)

Table 1 lists the consumable products needed for the IC system.

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2 Software, version SR10

Table 1. Consumables list for the Dionex ICS-6000 HPIC system

Product name	Description	P/N
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kit	Dionex IC PEEK Viper fittings kit for 2 mm Dionex ICS-6000 HPIC systems with CD Detector	302965
Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator cartridge	Anion eluent generator cartridge for HPIC systems	075778
Thermo Scientific™ Dionex™ CR-ATC™ 600 Electrolytic trap column	Continuously regenerated anion trap column used with the Dionex ICS-6000 HPIC and Dionex Integrion HPIC systems	088662
Thermo Scientific™ Dionex™ HP EG Degasser Module	Degasser installed after Dionex CR-TC trap column and before the injection valve, used with eluent generation	075522
Thermo Scientific™ Dionex™ ADRS™ 600 suppressor, 2 mm	Suppressor for 2 mm anion columns	088667
Thermo Scientific™ Dionex™ IonPac™ AG11 Guard Column	Anion guard column, 2 × 50 mm	044079
Thermo Scientific™ Dionex™ IonPac™ AS11 Analytical Column	Anion analytical column, 2 × 250 mm	044077
Thermo Scientific™ Dionex™ AS-AP Autosampler vial kit options	1.5 mL polypropylene, package of 100 vials and caps	079812
	10 mL polystyrene, package of 100 caps and septa	055058
Thermo Scientific™ Dionex™ IC PEEK Viper™ Sample Loop	2.5 µL PEEK viper sample loop	302899
Syringe filters	Syringe filters suitable for IC, 0.45 µm, PES	725-2545
Thermo Scientific™ Nunc™ 50 mL conical centrifuge tubes	Sample tubes for centrifuging the extract	Fisher Scientific 12-565-270

Conditions

Columns:	Dionex IonPac AG11 guard (2 x 50 mm) and Dionex IonPac AS11 analytical column (2 × 250 mm)
Eluent:	65 mM KOH
Eluent source:	Dionex EGC 500 KOH eluent cartridge, Dionex CR-ATC 600 trap column, and high pressure degas module
Flow rate:	0.25 mL/min
Injection volume:	2.5 µL
Column temperature:	35 °C
Detection/suppressor compartment:	20 °C
Detection:	Suppressed conductivity, Dionex ADRS 600 suppressor, 2 mm, 41 mA, constant current and AutoSuppression recycle modes
Background:	<1 µS/cm
Noise:	<1 nS/cm
System backpressure:	~2400 psi (100 psi = 0.689 MPa)
Run time:	20 min*

*Samples may have late eluting compounds that require longer run times or a wash step for 75 mM for 10 min after phytic acid elutes.

Reagents

Degassed ASTM Type I deionized (DI) water[13]

Phytic acid, dodecasodium, Biosynth International Inc. (Fisher Scientific P/N 50-121-7886)

For retention time standards:
Sodium phosphate, monobasic monohydrate, ACS certified (Fisher Scientific P/N S369-500)

Sodium sulfate, anhydrous, ACS certified (Fisher Scientific P/N S421-500)

Thermo Scientific™ Dionex™ 1000 mg/L Sulfate (P/N 037160)

Phytic acid, dodecasodium, hydrate, (Aldrich P/N 27,432-1)

Phytic acid, 40% solution (Fluka P/N 80180-50mL)

Standard and sample preparation

Retention time standards

1000 mg/L individual stock standards were prepared by adding 1.479 g of sodium sulfate or 1.4529 g of sodium monobasic phosphate, monohydrate in 1 L of DI water. Individual 20 mg/L retention time standards were prepared by diluting the 1000 mg/L stock standards with DI water.

Phytic acid standards

1000 mg/L Individual stock standards were prepared by adding 100 mg of reagent, corrected for sodium content, to 100 mL of DI water. Calibration standards, 1, 2, 5, and 10 mg/L, were prepared from the Biosynth Industries 1000 mg/L stock standard and DI water. Store at 5 °C.

Samples

Whole samples

Beans (10 g kidney and 10 g of pinto), wild rice (16 g), and raw almond (15 g) samples were extracted overnight (15 h) in 50 mL of City of Sunnyvale (California) municipal drinking water. The solutions were decanted, and the supernatant was centrifuged, filtered (0.45 µm), diluted (2.5-fold for the beans, 20-fold for almonds, and 50-fold for wild rice samples) with DI water according to the flow path in Figure 2, and analyzed by IC.

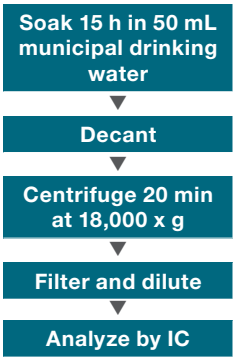


Figure 2. Sample preparation flow path for whole samples

Ground samples

For ground samples, ~5 g samples were ground into small particles using an electric coffee grinder, followed by extraction of 0.5 g in 50 mL of municipal drinking water for 16 h. An aliquot of the solution was filtered (0.45 µm), and diluted 4-fold with DI water prior to analysis.

Extended extraction samples

Similar samples were prepared for a 3-day extraction test: 0.5 g of ground sample and 15 g of whole sample were extracted in 50 mL of municipal drinking water at room temperature. Extract aliquots were sampled over the duration of the experiments, filtered (0.45 µm), and diluted with DI water (3-fold or 4-fold) prior to analysis.

Instrument setup and installation

The Dionex ICS-6000 is a modular, high pressure ion chromatograph, which can be configured as a Reagent Free™ IC (RFIC™) system, often using two pumps, two EGs, two injection valves, and two CD detectors. In this application, only a single pump, eluent generator, injection valve, and CD detector were used.

To set up the Dionex ICS-6000 system, install power and USB cables, and power-up the IC, autosampler, and computer according to Figure 3 and the instrument's operator's manual[14]. Thermo Scientific Technical Note 129[15] can also be used as a resource. Add DI water to the eluent bottles and prime the pump.

To electronically configure the IC system, start the Thermo Scientific™ Chromeleon™ Instrument Services program, then start the Instrument Controller program by selecting the *configure instruments* link. Add the Dionex ICS-6000 system SP module, EG module, DC module, and the AS-AP Autosampler module, as described in Table 2. Check and correct the configuration for any errors. Save and close the configuration program.

Plumbing the ICS-6000 HPIC system, RFIC model

Plumb the Dionex ICS-6000 IC as an RFIC system using the Dionex IC PEEK Viper fittings as indicated and as shown in Figure 3. The schematics are also illustrated on the inside doors of the Dionex ICS-6000 IC system. Direct the waste lines to the waste containers. The initial system pressure was <2000 psi, requiring additional backpressure tubing. Add yellow (0.003 in i.d., 0.0762 mm i.d.) PEEK tubing after the degas module and before the IC injection valve to bring the IC system pressure to ~2200 psi.

Conditioning electrolytic devices and columns

Important: Do not remove consumable tracking tags on the columns and consumable devices. These tags are required for consumables monitoring functionality.

Hydrate and condition the Dionex EGC 500 KOH eluent generator cartridge and Dionex CR-ATC 600 continuously regenerated anion trap column according to their product manuals, or the Consumables Conditioning instructions in the Chromeleon Console, in the Consumables drop down menu.[16,17] Condition the columns as described in the Dionex IonPac AS11 product manual using the QAR conditions (12 mM KOH, 30 °C at 0.25 mL/min for 30 min) while directing the effluent to waste.[18] Install the conditioned columns according to Figure 3.

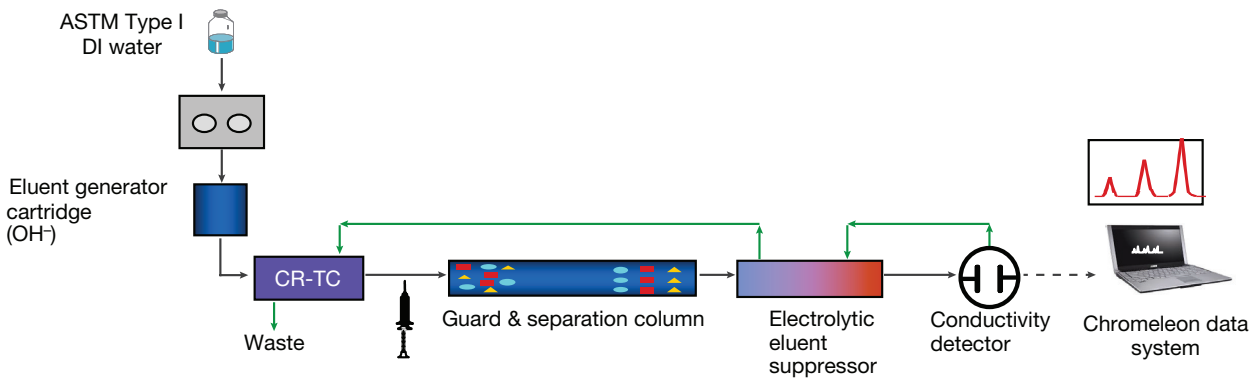


Figure 3. IC flow diagram

Table 2. Electronic configuration parameters

Module	Tab	Action
DP	General	Select Browse, Select serial number to link module to Instrument
	Device	Link pump to Instrument
EG	General	Select serial number to link module to Instrument
	Cartridges	Link to instrument. Check EGC_1 and link to Pump 1
DC	General	Select serial number to link module to Instrument, Select Instrument
	Detectors	Double click on CD, Link to Pump 1
	Thermo controls	Check Compartment_TC and Column_TC
	Suppressors	Double click Suppressor1, Link to Pump 1
	High pressure valves	Double click InjectValve1, Link to Pump 1, select control by autosampler.
AS-AP autosampler	General	Select serial number to link module to Instrument
	Sharing	This option is present, select Instrument
	Segments/pump link	Select 10 mL PolyVials or 1.5ml vials for "Red", "Blue", and "Green". Leave the pump and TTL links empty.
	Options	Select 1200 buffer loop, 250 µL syringe, temperature control, and push mode. Enter "2.5" µL in sample loop.

Hydrating the suppressor

To hydrate the Thermo Scientific™ Dionex™ ADRS™ 600 suppressor, follow the instructions in the Suppressor Installation Checklist that is included with the suppressor.[19] Install the suppressor according to Figure 3. To ensure that the suppressor is within backpressure specifications, follow the instructions in the Suppressor Installation Checklist. The backpressure increase due to the suppressor should be <50 psi, whereas the backpressure applied after the suppressor should be <100 psi. Operation of the Dionex ADRS 600 suppressor is thoroughly discussed in the suppressor manual.[20]

System startup, conditioning, and consumables device monitoring

Equilibrate the IC using the Quality Assurance Report (QAR) conditions for the Dionex IonPac AS11 column with the effluent leaving the CD detector in recycle mode, (CD Out to suppressor Regen In, suppressor Regen Out to CR-TC Regen In, and CR-TC Regen Out to waste). Equilibration is recommended until the total conductivity is <2 µS/cm. Using the Chromeleon Instrument Method Wizard, create a new IC instrument method using the column's Quality Assurance Report (QAR) conditions, and a new processing method. Approve the consumables in the Consumables Tracking panel located on the Chromeleon console (Select Chromeleon console, Consumables, Inventory, Scan and Approve, and close the window). Start the Chromeleon sequence and compare the results against QAR report.

Calculating total phytic acid in samples

Total phytic acid (measured as phytate) in samples is reported in the literature as "g of phytate" in "100 g of sample".

1. To calculate total phytic acid (mg), first correct for dilution factor and extraction volume (L).
   Amount of phytic acid in solution (mg) = measured concentration x dilution factor x extraction volume (L)
   Example: mg of phytic acid = 0.862 mg/L x 2.5 (dilution factor) x 0.050 L = 0.108 mg

2. Correct for sample weight fraction of 100 g.
   Phytic acid (mg) normalized to 100 g sample weight = Amount of phytic acid in solution (mg) x (100 (g)/ sample weight (g))
   Example: 0.108 mg x 100 (g)/(10 (g) sample = 1.08 mg/100 g of sample

3. Convert mg of phytic acid to g
   Phytic acid (g/100 g sample weight) = Phytic acid (mg/100 g sample weight)/1000 (mg/g)
   Example: Phytic acid (g/100 g sample weight) = 1.08 mg/100 g of sample/1000 = 0.00108 g/100g

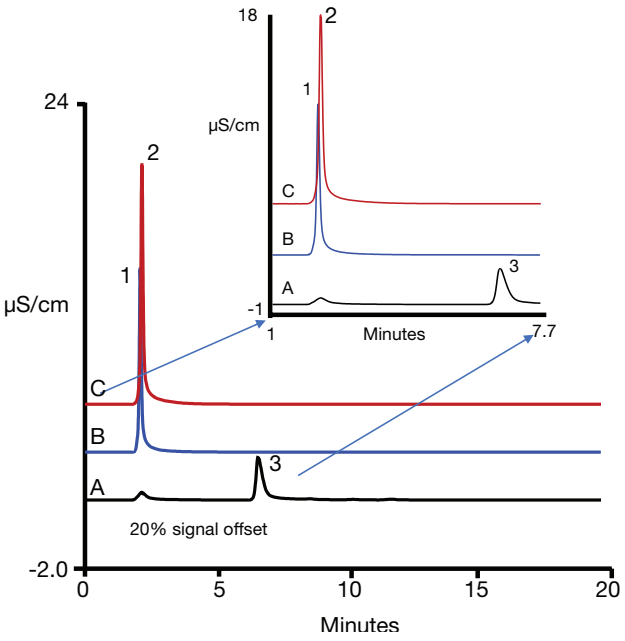
Results

The Dionex IonPac AS11 anion-exchange column is a low capacity very low hydrophobic column prepared with 13 µm diameter microporous resin beads that are functionalized with alkanol quaternary amines. The column is optimized for fast elution of organic acids and multivalent strongly retained ions, such as phytate. The suitability of

the Dionex IonPac AS11 column for phytate determinations was previously demonstrated in Application Notes 295 and 1070. The application conditions from AN295 were selected for this update.

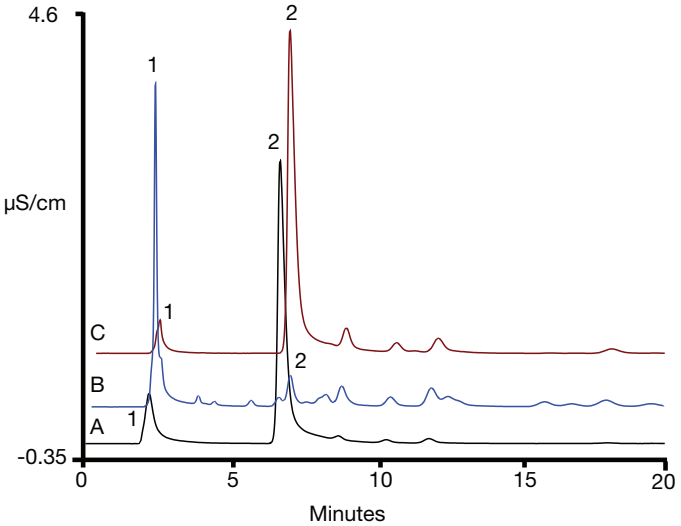
To evaluate the method, the retention times of phosphate and sulfate were compared with phytate to confirm that the ions were well-resolved from phytate. Figure 4 shows the chromatograms of single standard ions, phosphate, sulfate, and phytate using 65 mM KOH on the Dionex IonPac AS11 column. The experiments confirm that phosphate and sulfate, eluting at 1.9–2.2 min, are well resolved from phytate, eluting at 5.9 min.

To evaluate phytate in the presence of other inositol phosphate compounds and the method stability, phytate standards were prepared from different sources: Fluka 40% solution, Aldrich reagent, and Biosyn International reagent (Figure 5). A 20% offset was used for data presentation. Standards A and C (Aldrich reagent, and Biosyn) were predominantly phytate, whereas in Standard B (Fluka 40% solution) phosphate predominates over phytate, as well as other smaller peaks believed to be inositol phosphate compounds. The method stability was determined by measuring phytate retention time and peak area reproducibilities (n = 3) in 20 mg/L standards.



Columns:	Dionex IonPac AG11, IonPac AS11, 2 mm i.d.
Eluent:	65 mM KOH
Eluent source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column
Flow rate:	0.25 mL/min
Inj. Volume:	2.5 µL
Temperatures:	Column: 35 °C, Detector: 20 °C
Detection:	Suppressed conductivity, Dionex ADRS 600, 2 mm, constant current and recycle modes, 41 mA
Standard:	A: 21 mg/L Phytic acid; B: 10 mg/L sulfate, C: 10 mg/L phosphate
Peaks:	1. Sulfate 2. Phosphate 3. Phytic acid as phytate

Figure 4. Phosphate, sulfate, and phytic acid standards



Columns:	Dionex IonPac AG11, IonPac AS11, 2 mm i.d.
Eluent:	65 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column
Flow Rate:	0.25 mL/min
Inj. Volume:	2.5 µL
Temperatures:	Column: 35 °C, Detector: 20 °C
Detection:	Suppressed conductivity, Dionex ADRS 600, 2 mm, constant current and recycle modes, 41 mA
Standards:	10% signal, 1% time offset. Phytic acid. A: 21 mg/L (Biosyn International) B: 19 mg/L from 40% solution (Fluka), C: 20 mg/L (Aldrich)
Peaks:	1. Phosphate 2. Phytic acid as phytate

Figure 5. 20 mg/L phytic acid standards

In Table 3, phytic acid as phytate prepared from reagent phytic acid (Standards A and C) shows good stability as indicated by the good retention time reproducibilities with 0.1-0.11 RSDs and good peak area reproducibilities with 0.43-1.2 RSDs. In contrast, Standard B prepared from a 40% solution had lower retention time and peak area reproducibilities, with RSDs of 1.0 and 10.3, respectively. Phosphate was the dominant peak in Standard B, indicating that a portion of phytate or other inositol phosphate compounds have degraded to phosphate.

To determine the calibration range and response to concentration, the peak area was measured in triplicate from 1 to 10 mg/L (1, 2, 5, and 10 mg/L standards) prepared from Biosyn International reagent. The responses were linear with coefficient of determination (r^2) of 0.9987. The reproducibility and calibration results confirm the results reported in AN295.

Samples

Extraction of whole samples

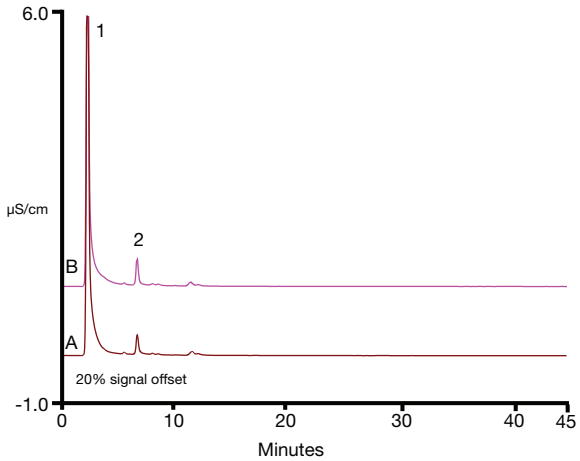
The method was applied initially to diluted, filtered, and centrifuged extracts of whole kidney and pinto beans, almonds, and wild rice samples. The purpose of these experiments was to determine whether phytic acid would be removed after an overnight extraction (soak) typically done to dried beans before food preparation. To minimize the carryover of small unknown compounds, the chromatography run time was extended to 45 min. To evaluate the accuracy, recoveries of added standards in the extracts of whole beans were determined. The results summarized in Table 4 show 1 to 8 mg/L of phytate extracted with good recoveries, 88 to 90%, except the pinto beans, which had 40% recovery. Figures 6 and 7 show chromatograms of the diluted, filtered extracts of whole almonds and wild rice with added standard. Phytate is well resolved from other anions.

Table 3. Summary of reproducibility results

Phytic acid standard		Reproducibility	
		Retention time (RSD)	Peak area (RSD)
A	20 mg/L (Biosyn International)	0.11	1.2
B	21 mg/L from 40% Solution (Fluka)	1.0	10.3
C	21 mg/L (Aldrich)	0.10	0.43

Table 4. Whole sample extractions. Summary of results and recovery of added standards

Sample extract	Extracted phytic acid	Recovery results	
	Amount found (mg/L)	Added phytic acid (mg/L)	Recovery (%)
2.5-fold diluted pinto beans	-	0.5	39.5
2.5-fold diluted kidney beans	0.86	0.5	90.4
20-fold diluted raw almonds	2.36	1.0	94.5
50-fold diluted wild rice	8.00	5.0	88.4

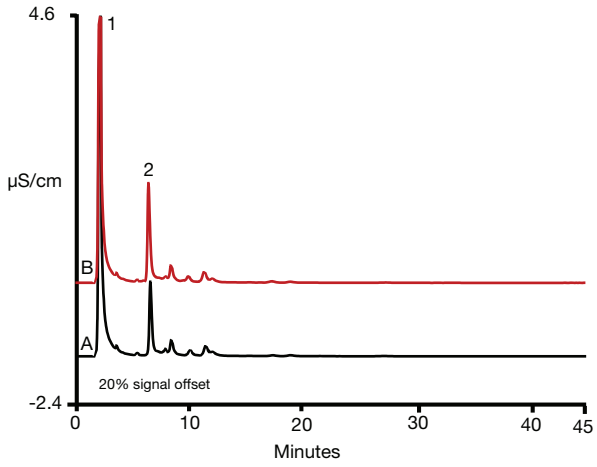


Columns: Dionex IonPac AG11, IonPac AS11, 2 mm i.d.
Eluent: 65 mM KOH
Eluent Source: Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column
Flow Rate: 0.25 mL/min
Inj. Volume: 2.5 µL
Temperatures: Column: 35 °C, Detector: 20 °C
Detection: Suppressed conductivity, Dionex ADRS 600, 2 mm, constant current and recycle modes, 41 mA
Sample Prep.: 15 g of whole almonds/50 mL Sunnyvale municipal water 15 hr, centrifuge 20 min 18000 x g, filter (0.4 µm, PES)
Samples: A: 20-fold diluted, centrifuged, filtered extract of almonds
B: Sample A + 1 mg/L phytic acid as phytate

Peaks:		A	B
	1. Phosphate	--	--
	2. Phytic acid as phytate	2.36*	3.08

Calculated total phytate: 0.0148 g/100 g almonds

Figure 6. 20-fold diluted extract of whole almonds A) without and B) with added 1 mg/L phytic acid



Columns:	Dionex IonPac AG11, IonPac AS11, 2 mm i.d.
Eluent:	65 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column
Flow Rate:	0.25 mL/min
Inj. Volume:	2.5 µL
Temperatures:	Column: 35 °C, Detector: 20 °C
Detection:	Suppressed conductivity, Dionex ADRS 600, 2 mm, constant current and recycle modes, 41 mA
Sample Prep.:	15 g of whole wild rice/50 mL Sunnyvale municipal water 15 hr, centrifuge 20 min 18000 x g, filter (0.4 µm, PES)
Samples:	A: 50-fold diluted, centrifuged, filtered extract of wild rice B: Sample A + 5 mg/L phytic acid as phytate

		A	B
Peaks:	1. Phosphate	--	--
	2. Phytic acid as phytate	8.0*	11.4

Calculated total phytic acid as phytate: 0.1333 g/100 g rice

Figure 7. 50-fold diluted extract of whole wild rice A) without and B) with added 5 mg/L phytic acid

Total extraction from whole and ground samples

To determine whether the overnight extraction of whole beans was efficient, the extraction was repeated with ground beans, rice, and almond samples, as typically done as part of an analytical method for these types of samples. The results were converted to total phytate (g per 100 g sample) for comparison (Table 5). The extraction of phytate from whole beans, almonds, and rice was negligible, ranging from non-detected to 0.133 g/100 g of sample. As expected, higher extractions were obtained from the ground samples, (0.05 to 0.312 g/100 g sample).

Table 5 also compares the recovery of the amount extracted from whole and ground samples to literature values[6]. However, the samples described in the literature had extensive sample preparation treatment, including grinding, acid digestion with a strong acid or acids, and treatment with various SPE trap columns to remove the acids, soluble fiber, and non-polar compounds.

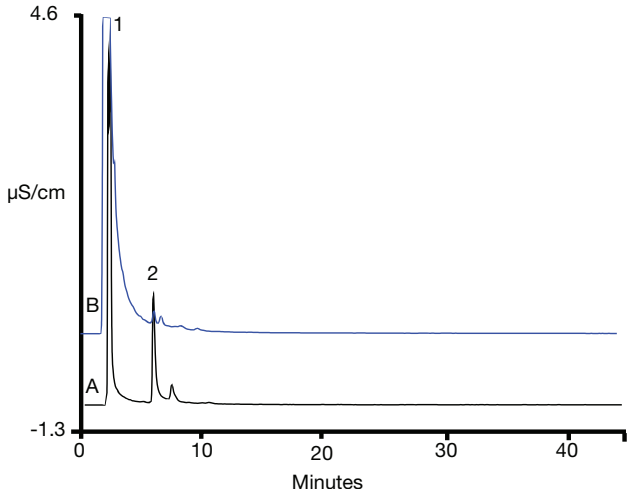
As compared to the lowest reported results, these experimental results were unacceptably low for an analytical method, 0.1–6% was recovered from non-ground samples, and 8–52% from ground samples. In addition, while the extraction was improved by grinding the samples, the extraction ranged from as low as 8% to as high as 52% suggesting an inconsistent extraction.

Table 5. Comparison of extractions of whole and ground samples

Sample	Sample extraction	Measured phytic acid as phytate (mg/L)	Total phytic acid as phytate* (g/100 g)	Recovery, based on literature values** (%)
Pinto beans	Whole, 10 g / 50 mL, 15 h, 2.5-fold diluted	—	—	—
	Ground, 0.5 g / 50 mL, 16 h, 4-fold dilution	5.56	0.223	37.1
Kidney beans	Whole, 10 g / 50 mL, 15 h, 2.5-fold diluted	0.86	0.0010	0.17
	Ground, 0.5 g / 50 mL, 16 h, 4-fold dilution	7.82	0.312	52.1
Almonds	Whole, 15 g / 50 mL, 15 h, 20-fold diluted	2.36	0.0148	4.22
	Ground, 0.5 g / 50 mL, 16 h, 4-fold dilution	1.39	0.0504	8.41
Wild rice	Whole, 16 g / 50 mL, 15 h, 50-fold diluted	8.00	0.133	6.06
	Ground, 0.5 g / 50 mL, 16 h, 4-fold dilution	5.39	0.216	35.9

*Total phytic acid as phytate g/100g = measured concentration (mg/L) corrected for extraction and dilution, and normalized to g of phytic acid as phytate and 100 g of sample

** Recovery based on lowest reported values. Reported values (g/100g): Pinto beans (0.6-2.38), kidney beans (0.6-2.38), almonds (0.35-9.42), wild rice (2.2)[6]



Columns:	Dionex IonPac AG11, IonPac AS11, 2 mm i.d.		
Eluent:	65 mM KOH		
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column		
Flow Rate:	0.25 mL/min		
Inj. Volume:	2.5 µL		
Temperatures:	Column: 35 °C, Detector: 20 °C		
Detection:	Suppressed conductivity, Dionex ADRS 600, 2 mm, constant current and recycle modes, 41 mA		
Samples:	Extract from kidney beans A: 3-Fold Diluted Extract from 0.5 g of ground beans B: 2.5-Fold Diluted Extract from 15 g of whole beans		
Sample Prep.:	Extract 15-16 h in 50 mL Municipal drinking water, centrifuge 20 min 18000 x g, filter (0.4 µm, PES)		
Data offset:	15% signal offset		
		A	B
Peaks:	1. Phosphate	--	--
	2. Phytic acid as phytate	7.81*	0.86*
	Calculated total phytic acid* A. 0.312 g per 100 g B: 0.0010 g per 100 g		

Figure 8. Comparison of extraction of phytate in A) ground and B) whole kidney beans

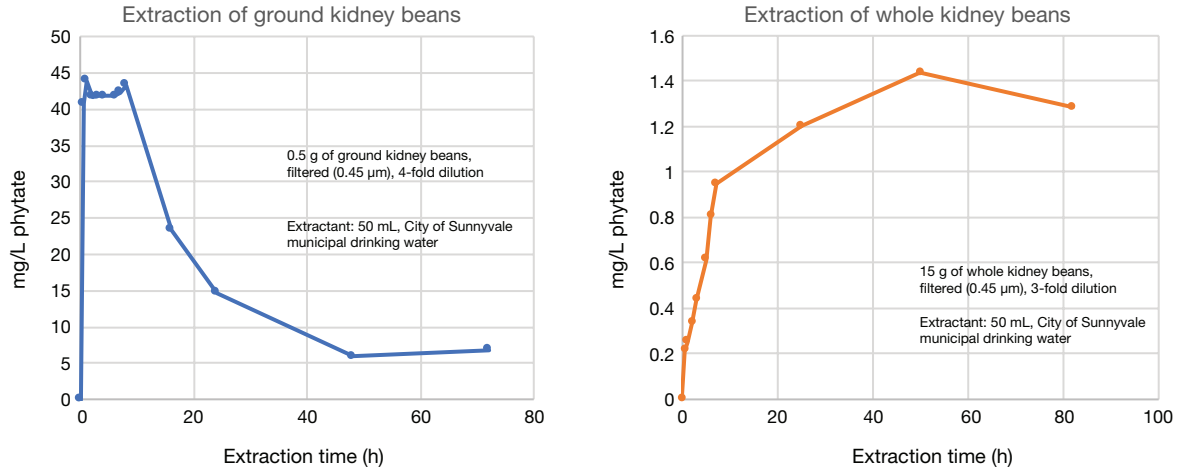


Figure 9. Extraction time course

Extended extraction

To evaluate the effect of extending the extraction time on the measured phytic acid as phytate, sample aliquots of extractions from whole and ground kidney beans were taken over 3 days. The results shown in Figure 9 indicate that the phytic acid extraction yield of whole kidney beans increased up to 50 h, whereas the concentration decreased after 8 h with ground kidney beans. The experiments show that soaking the whole beans overnight or for 3 days was inadequate to remove phytic acid prior to food preparation or as a sample preparation method for determining total phytate.

Conclusion

This application update confirms the analytical method demonstrated in AN295. The method has good reproducibility, <1% RSDs, and good recovery from added standards.

Water extraction of whole seeds (beans, rice, or almonds), which is typically done as part of food preparation, removed negligible amounts of phytic acid, 1–6% of reported values. Grinding the samples into small particles improved the extraction efficiencies to 8–52%. Although water extraction is relatively easy, acid digestions are required for total phytate as demonstrated in AN295. More information on this application update and other applications on phytic acid and inositol phosphate compounds can be found in the Thermo Fisher Scientific AppsLab digital library of applications.[21]

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