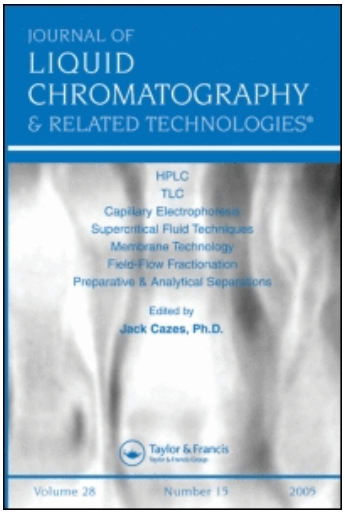

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Water-Soluble Inorganic Phosphates in Fresh Vegetables by Ion Chromatography

E. Ruiz[a]; M. I. Santillana[a]; M. T. Nieto[a]; I. Sastre[a]
[a] Sección de Componentes y Aditivos II Centro National de Alimentación Instituto, Madrid, Spain

To cite this Article Ruiz, E. , Santillana, M. I. , Nieto, M. T. and Sastre, I.(1995) 'Determination of Water-Soluble Inorganic Phosphates in Fresh Vegetables by Ion Chromatography', Journal of Liquid Chromatography & Related Technologies, 18: 5, 989 — 1000
To link to this Article: DOI: 10.1080/10826079508010407
URL: http://dx.doi.org/10.1080/10826079508010407

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(5), 989-1000 (1995)

DETERMINATION OF WATER-SOLUBLE INORGANIC PHOSPHATES IN FRESH VEGETABLES BY ION CHROMATOGRAPHY

E. RUIZ, M. I. SANTILLANA, M. T. NIETO, AND I. SASTRE
Sección de Componentes y Aditivos II
Centro Nacional de Alimentación
Instituto de Salud Carlos III
Ctra. Majadahonda-Pozuelo, Km.2,2
28220 Madrid, Spain

ABSTRACT

An alternative Ion Chromatographic method has been developed for selective separation and quantitation of water-soluble inorganic phosphate in aqueous extracts of vegetables, based on the use of an anion-exchange polymethacrylate column, borate/gluconate as eluent and conductivity detection. The method shows a good detection limit as well as a high chromatographic resolution. It is also applicable to the detection of phosphates in fresh vegetables.

INTRODUCTION

Phosphate determination is of increasing importance in environmental matters regarding eutrophization phenomena in the biomedical fields, as well as in the food and beverage industry for effluent control.

Recent studies show a considerable increase in daily phosphate intake (1,000-1,500 mg), due to changes in dietary habits (Recommended phosphate intake: 800 mg/day) (1). This increase leads to a P/Ca ratio higher than 2, with possible harmful effects on the mineral metabolism (osteoporosis, osteomalacia, decreased Mg absorption, ...) (2,3,4,5).

Generally, vegetables have high phosphorus contents due to inorganic fertilizers used in agriculture in order to obtain a higher yield and quality of these products (6) and for the purpose of antioxidant or protection from browning (7,8).

We considered the need for an analytical method which permits the study of residual inorganic phosphate in spanish vegetable produce in view of the increase in phosphate concentration levels in it, and the lack of data in the reference literature available.

Different techniques are routinely used for the determination of phosphate in foodstuffs. These include spectrophotometry (9,10), voltammetry (11) and flow injection analysis (12), but these methods suffer from various drawbacks, such as cumbersome sample preparation and long analysis time.

Ion Chromatography offers the opportunity to analyze ion species. Several applications of Ion Chromatography in analysis of phosphate in water (13), soil (14), fertilizers (15) and vegetables (16,17), have appeared in the literature.

In this work, we chose as working method an anion-exchange with conductivity detection and borate/gluconate as eluent to determine water-soluble inorganic phosphates in aqueous extracts of fresh vegetables.

MATERIAL AND METHOD

Samples

Analyzed samples were purchased from local food stores. Vegetables were kept refrigerated until assayed. Celery, chard, spinach and lettuce samples were analyzed within one day of purchase.

Reagents

All the reagents used were of analytic-reagent grade (Merck, D-6100 Damstadt, Germany). Organic solvents of high purity grade for HPLC (BDH, Poole, Dorset, UK). Ultrapure water with conductivity < 1 μS (DI water) was obtained from a Milli-Q (Millipore Corp., Bedford M.A. 01730, USA) four-bowl deionization system.

Phosphate stock standard solution was prepared at 1,000 ppm concentration by dissolving 0.1432 g of potassium dihydrogen phosphate ($K H_2 PO_4$) per 100 ml in DI water. Working standard solutions were prepared daily by appropiate dilution of the stock solution with DI water.

Sodium Borate/gluconate concentrate solution was prepared with 16 g sodium gluconate, 18 g boric acid, 25 g sodium tetraborate decahydrate and 250 ml glycerin per 1,000 ml in DI water (concentrate may be stored for up to six months).

Sodium Borate/gluconate Eluent (conductivity 270 μS, pH 8.5) was prepared with 20 ml borate gluconate concentrate, 20 ml n-butanol and 120 ml acetonitrile to 1,000 ml. It was filtered through a 0.22 μm Durapore membrane (GVWP-Millipore), and degassed by ultrasonication before use.

Equipment

Chromatographic analysis was performed on an Ion Chromatography System ILC-1 (Waters Chromatography Division; Milford, MA, USA): Manual Injector with a 100 μl loop, Conductivity Detector (430), Programmable Solvent Delivery Module (590), Data Module Integrator (745). Precolumn Guard-Pak with IC-Pak anion inserts and 4.6 cm x 75 mm IC-Pak anion HR column (also Waters).

Method

250 g of each of the representative vegetable samples was cut into pieces and chopped in a domestic mincer. The marrow and onion samples had had their

skins peeled off previously. A subsample of 10.00 ± 0.1 g (5.00 ± 0.1 g in the case of samples with phosphate concentration higher than 1,000 μg/g, so that they were within the lineal range of this method) was homogenized with 100 ml of destilled water pre-heated to 70°C in a household mixer for 2 minutes. The mixture was heated on a boiling water bath for 15 minutes with repeated shaking, to denature and precipate the proteins.

The resulting extract solution was cooled, made up to 200 ml with water in a volumetric flask and filtered through Whatman n° 44 paper. Aliquots of 5 ml of the extracts were filtered through a 0.45 μm membrane filter (Millex HV - Millipore) to clarify them.

The purification was carried out applying this solution to a classic short body cartridge for solid phase extraction Sep-Pak C18 (Millipore - Waters), which was pretreated with 5 ml of methanol and 5 ml of water. The first 2 ml eluated were discarded and aliquots of 100 μl were injected into the chromatograph.

High Performance Ion Chromatography was carried out under the following conditions: conductivity detection; eluent, Sodium Borate/Gluconate pH 8.5 (conductivity 270 μS); flow rate, 0.9 ml/min.; chart speed, 0.5 cm/min; attenuation, 512; gain, 0.01.

RESULTS AND DISCUSSION

Ion Chromatography with conductivity detection is suitable for the determination of water-soluble inorganic phosphate (18, 19 y 20). Figure 1 shows the chromatogram of a standard solution containing 20 μg/ml of dihydrogen phosphate obtained under the chromatographic conditions previously described in the method.

Linearity and sensitivity of the method were calculated from a series of standard solutions from 1 to 50 μg/ml. Relationship between peak area and

FIGURE 1: Chromatogram of a standard solution of dihydrogen phosphate (20 μg/ml).
Conditions: Water IC-Pak HR anion column with Sodium Borate/Gluconate pH = 8.5 eluent; conductivity detection; flow rate: 0.9 ml/min; injection volume: 100 μl.

dihydrogen phosphate concentration was found to be linear over the 1-50 μg/ml concentration range. Equation of the least squares regression line was $y = 0.23 - 0.26\ x$ with a correlation coefficient of 0.999.

The limit of quantitation was 0.6 μg/ml allowing a signal noise ratio of 10. The limit of detection was estimated at 0.2 μg/ml to a signal noise ratio of 3.

The mean recoveries of standards in the 1-50 μg/ml range were 100.5 ($\sigma = 4.6$; $n = 10$).

Although Busman et al (14) suggest that the use of Ion Chromatography with conductivity detection in plant anion analysis was restricted by the presence of the organic compounds or by the high levels of salts, in the Ion Chromatography method developed in our laboratory and applied to water-soluble inorganic phosphate determination in aqueous extracts of several fresh vegetables, no interferences have been found from salts (chloride, nitrite, nitrate, sulphate) and organic compounds. Figure 2 shows the chromatogram of a standard solution of Cl^-, NO_2^-, NO_3^-, $H_2PO_4^-$, SO_4^{2-} anions, obtained under the chromatographic conditions described.

Figure 3 shows a typical chromatograms obtained from several samples of fresh vegetables (tomato, lettuce and marrow), where no chromatographic interferences can be observed.

Only in samples containing high levels of nitrate, the water-soluble inorganic phosphate couldn't be correctly quantified because resulting nitrate peak masks the phosphate peak. Figure 4 shows a chromatogram of a vegetable sample (chard) with a high nitrate concentration (4,500 μg/g), where it can appreciate that, though the nitrate concentration is very high, the phosphate peak is well resolved.

Furthermore Grunau et al (17) found that phosphate quantitation in vegetables aqueous extracts is less precise than other anions, due to the potential interference from proteins in plant extracts. To minimize the problem we have

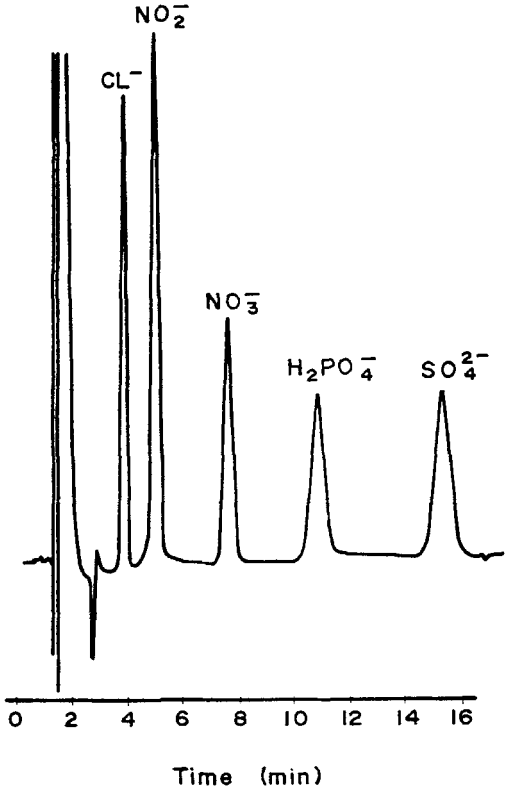

FIGURE 2: Chromatogram of a mixture of chloride, nitrite, nitrate, dihydrogen phosphate and sulphate standards (2, 4, 4, 6 and 4 respectly).
Conditions: Water IC-Pak HR anion column with Sodium Borate/Gluconate pH = 8.5 eluent; conductivity detection; flow rate: 0.9 ml/min; injection volume: 100 μl.

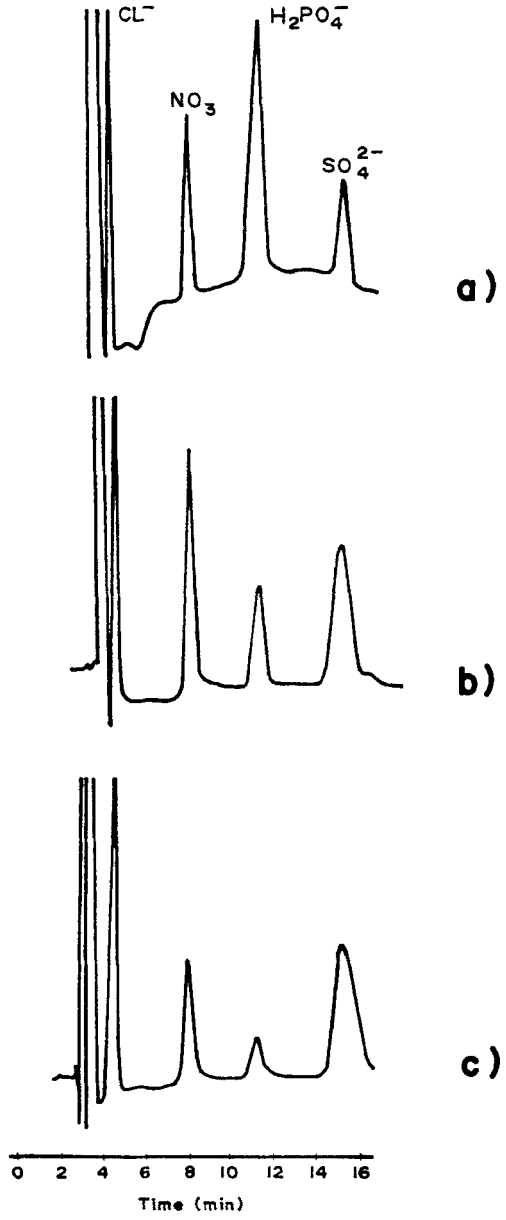

FIGURE 3: Typical chromatograms obtained from: tomato (a), lettuce (b) and marrow (c); using the proposed chromatographic method.

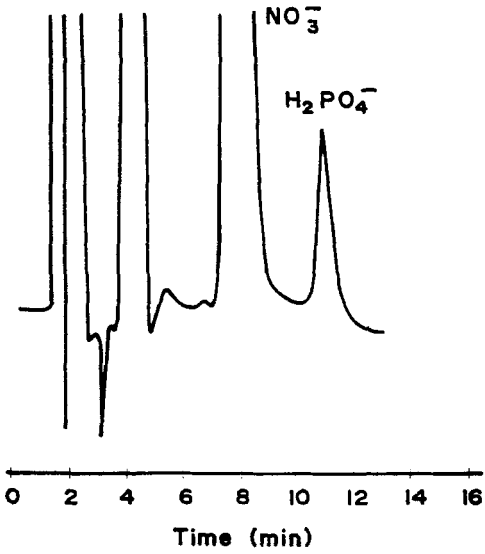

FIGURE 4: Chromatogram of a chard sample with high nitrate concentration (4,500 μg/g), obtained under the chromatographic conditions described.

chosen to desproteinize vegetable extracts routinely by boiling, primarily to avoid possible interferences and to prevent the column from clogging.

Recovery studies were performed on several fresh vegetable samples by adding known quantities of dihydrogen phosphate to the sample solution prior to the initial homogenization step. The results shown on Table 1, indicate that satisfactory recoveries were achieved for the samples tested.

A total of 76 different commercial fresh vegetable samples were analyzed: mushroom (8), celery (9), cauliflower (5), tomato (6), marrow (7), chard (8), onion (11), lettuce (10) and carrot (12). Water-soluble inorganic phosphate contents are shown on Table 2, where we can appreciate that all analyzed samples present high concentrations, the maximum amount reached being in the mushroom samples (mean value: 1,681.3 $\pm$ 393.5). In the other vegetable

TABLE 1

Percentage Recovery of Water-soluble Inorganic Phosphate from Fresh Vegetables after Extraction and IC Analysis

Food	Amount Added (μg/g)	Amount Recovered (μg/g) Mean $\pm$ SD	Recovery (%)	CV (%)
Chard	20	19.20 $\pm$ 1.69	97.20	8.80
(n=10)	40	41.18 $\pm$ 4.12	99.80	10.00
Lettuce	20	19.02 $\pm$ 1.58	97.70	8.30
(n=3)	40	40.93 $\pm$ 0.59	101.80	1.44
Spinach	20	18.01 $\pm$ 1.78	90.2	9.80
(n=3)	40	35.58 $\pm$ 1.22	89.2	3.40
Carrot	20	19.61 $\pm$ 0.49	98.2	2.49
(n=6)	40	39.99 $\pm$ 1.11	97.5	2.84
Marrow	20	20.02 $\pm$ 0.38	100.1	1.89
(n=4)	40	39.66 $\pm$ 0.58	99.1	1.46

samples analyzed, the mean values found ranged from 623.2 $\pm$ 297.4 (celery) to 357.5 $\pm$ 164.6 (carrot). Similar phosphate concentrations have been found in lettuce, spinach and tomato by Hertz et al (16).

To sum it up, several characteristics of the proposed method is a useful analytical technique for the determination of water-soluble inorganic phosphates: little sample preparation is required; it is not subject to organic, salts or protein interferences, and the precision of the technique is adequate for routine analysis, specially when the speed and cost-effectiveness of the method are considered.

TABLE 2

Water-soluble Inorganic Phosphate Contents in Commercial Samples

Sample (type)	n[a]	Mean (μg/g)	SD (μg/g)	Range (μg/g)
Mushroom	8	1681.3	393.5	1000-2174
Celery	9	623.2	297.4	222-1200
Cauliflower	5	612.2	246.6	228-925
Tomato	6	574.0	289.9	196-1060
Marrow	7	506.1	201.4	258-825
Chard	8	463.5	214.1	142-800
Onion	11	430.3	116.7	230-630
Lettuce	10	388.7	135.3	230-726
Carrot	12	357.5	164.6	156-608

[a] Duplicate determinations

REFERENCES

1. M. C. Linder, Nutrición y Metabolismo de los Elementos Mayoritarios. Nutrición: Aspectos Bioquímicos, Metabólicos y Clínicos, Ed. Eunsa, Pamplona, 1988.

2. H. F. Draper, Boll. RR. Adv. Nutr. Research, 2:90, 1980.

3. L. V. Avioli, Ann. Rev. Nutrition, 4:471-491, 1984.

4. J. Ritskes Hoinga, J. N. Mathot, J. Nutr., 122:1682-1692, 1992.

5. E. J. Brink, American Institute of Nutrition, 122 (3): 580-586, 1992.

6. P. G. Marais, J. Deist, R. B. A. Harry, C. F. G. Heyns, Agrochemophysica, 2: 7-12, 1970.

7. Y. Hayasi, K. Tajima, I. Hirono, Eisei Kagaku, 35 (3): 206-211, 1989.

8. A. Ibe, Y. Tamura, H. Kamimura, Ann. Rep. Tokyo Metr. Res. Lab. P. H., 38: 216-221, 1987.

9. C. Matsubara, I. M. Fuji, K. Takamura, Eisei Kagaku, 34 (2): 123-127, 1988.

10. G. Graffmann, W. Schneider, L. Dinkioh, Anal. Chem., 301: 364, 1980.

11. L. Campanella, M. Cordatore, Food Chemistry, 44: 291-297, 1992.

12. K. B. Male, J. H. T. Loung, Biosens. Bioelectron., 6 (7): 581-587, 1991.

13. M. A. Tabatabai, W. A. Dick, J. Environ. Qual., 12: 209-213, 1983.

14. L. N. Busman, R. P. Dick, M. A. Tabatabai, Soil Sci. Soc. Ann. J., 47: 1167-1170, 1983.

15. L. Brenman, G. Schmuckler, LC-GC INTL, 5 (10): 36-38, 1992.

16. J. Hertz, U. Baltensperger, A. Fresenius, Anal. Chem., 318: 121-123, 1984.

17. J. A. Grunau, J. M. Swiader, Commun. in Soil Sci. Plant Anal., 17 (3): 321-335, 1986.

18. Waters Ion Chromatography Cookbook, Method A-103, 1989.

19. D. S. Ryder, J. Chromatogr., 354: 438, 1986.

20. R. H. Smillie, B. Grant, J. Chromatogr., 455: 253-261, 1988.

Received: July 25, 1994
Accepted: October 31, 1994