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Practical applications of element-specific detection by inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectrometry to ion chromatography of foods

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Abstract

Three practical examples are presented to demonstrate the utility of element-selective detection for ion chromatography (IC). The determination of As species in a liquid health food supplement by IC with inductively coupled plasma atomic emission spectroscopy (IC–ICP-AES) is shown to confirm results obtained for total As. IC–ICP-AES is also used to investigate the identity of an unknown peak in a sample of shrimp commercially treated with tripolyphosphate. Finally, results are presented for the determination of residual bromate in baked goods by IC with inductively coupled plasma mass spectrometry detection.

1. Introduction

The combination of ion chromatography (IC) with inductively coupled plasma atomic emission spectroscopy (ICP-AES) or inductively coupled plasma mass spectrometry (ICP-MS) provides a powerful analytical technique. In comparison to total element determinations by atomic spectrometric techniques, this hyphenated technique provides information regarding the chemical form of a particular element. The chemical form or nature of an element can greatly affect its function, bioavailability and toxicity. In addition, the ability to monitor a specific element(s) can eliminate chromatographic interferences associated with difficult matrices. This permits accurate, reliable analyses even when the separation of analyte from matrix is incomplete. ICP-AES and ICP-MS have high elemental sensitivity and are capable of monitoring more than one element simultaneously. The coupling of ICP-AES and ICP-MS with chromatography has been reviewed by several groups [1–4]. Arsenic, lead, mercury, selenium, tin and chromium have been the subject of the majority of studies involving element-specific detection for HPLC and IC [1–4]. In this paper, three applications of element-specific detection for food analysis will be discussed.

The Food and Drug Administration is responsible for ensuring that food products in the marketplace are safe; this includes health food supplements. The determination of toxic trace elements such as arsenic in mineral supplements is thus of interest. The use of IC–ICP-AES for the determination of As provides important

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toxicity information since various species have widely differing toxicities. The technique can also be used to confirm the results obtained for total As.

Arsenic has been the element most studied by element-selective detection using both ICP-AES and ICP-MS. These studies have been reviewed elsewhere [2,3]. The advantage of element-specific detection in the IC determination of As(III) and As(V) has been demonstrated using a direct-current plasma [5,6]; however, no applications were reported.

Polyphosphates are added to seafood products to provide protection from moisture loss during processing [7]. However, the amount of polyphosphate used must be controlled in order to prevent the uptake of excess water by treated products. The potential for economic fraud through mislabeling or excessive use necessitates the development of analytical methodology for polyphosphates and their hydrolysis products. There are reports of both ICP-AES and ICP-MS detection for phosphates separated by HPLC [8,9]. In this work, ICP-AES detection is used in the investigation of an unknown peak found by IC with UV detection in samples of shrimp processed with sodium tripolyphosphate.

There is some concern regarding the health effects of residual bromate in bakery products. Potassium bromate is used in the baking industry as a dough conditioner. The majority of bromate is reduced to bromide during the baking process; however, residual bromate has been found in some baked goods. The determination of residual bromate using IC with conductivity detection is complicated because of inadequate sensitivity and the presence of interfering matrix species such as chloride [10]. The combination of gel-permeation liquid chromatography and ICP-MS has been used in the determination of several inorganic halogen species including bromate, but was not applied to bakery products [11]. IC–ICP-MS detection of bromate at m/z 79 and/or 81 provides excellent sensitivity and freedom from matrix interferences.

2. Experimental

2.1. Apparatus

The chromatographic system used consisted of a Dionex (Sunnyvale, CA, USA) system 4500i equipped with a gradient pump Module-2, liquid chromatography Module-3, variable-wavelength detector and an ASM autosampler. Analytical columns used in this work were the IonPac AS4A, IonPac AS7 and IonPac AS10 (250 × 4 mm, Dionex). Guard columns used were the IonPac AG4A, IonPac NG1 and IonPac AG10 (50 × 4 mm, Dionex).

A Thermo Jarrell Ash Model 1140 Plasma Atomcomp Polychromator ICP-AES system was used. The instrument was operated in an intensity *vs.* time data acquisition mode. The acquisition time was 3 s/point for the As detection work and 7.5 s/point for the P detection work. The acquisition time used depends on the length of the chromatographic run because the maximum number of time slices allowed in this operating mode is 100. The operating conditions were as follows: Ar coolant gas flow, 20 l/min; Ar auxiliary gas flow, 1.5 l/min; Ar nebulizer gas flow, 0.75 l/min; forward power, 950 W; P line monitored, 214.9 nm; As line monitored, 193.9 nm.

The ICP-MS system used was a VG/Fisons PlasmaQuad Model PQ2+ Turbo. The operating parameters used were as follows: Ar coolant gas flow, 14 l/min; Ar auxiliary gas flow, 1.1 l/min; Ar nebulizer gas flow, 0.82 l/min; forward power, 1350 W; resolution 0.8 u. For chromatographic sample introduction, the instrument was operated in the single ion monitoring mode with a 1-s integration time per data point. For the determination of bromate in baked goods, the ^{79}Br isotope was monitored.

The interface between the IC system and the ICP-AES or ICP-MS detector consisted of a *ca.* 48 in. (1 in. = 2.54 cm) length of 0.010 in. I.D. polyether ether ketone (PEEK) tubing. The tubing connected either the outlet of the UV cell or the conductivity detector cell to the inlet of a concentric nebulizer which is used for sample

introduction to both the ICP-AES and ICP-MS systems.

Chromatographic data obtained for both ICP-AES and ICP-MS were converted to ASCII files and imported into a spreadsheet program for evaluation of peak heights and retention times.

2.2. Reagents and standard solutions

The water used throughout this work was distilled and deionized (DDW). Sodium hydroxide used in preparing mobile phases was a 50% (w/w) solution (Fisher Scientific, Fair Lawn, NJ, USA). Nitric and perchloric acids used were double distilled (GFS Chemicals, Columbus, OH, USA). Sulfuric acid and ferric nitrate 9-hydrate were reagent grade.

Sodium arsenite and sodium arsenate standards were prepared from reagent-grade chemicals (Fisher) dissolved in DDW. Food-grade sodium tripolyphosphate was obtained from Monsanto (St. Louis, MO, USA). Sodium pyrophosphate decahydrate was obtained from Aldrich (Milwaukee, WI, USA) and sodium phosphate monobasic was obtained from EM Science (Gibbstown, NJ, USA). Stock standards of each (10 000 μg/ml) were prepared in DDW. Potassium bromide and sodium bromate stock standards (1000 μg/ml) were prepared from reagent-grade chemicals (Aldrich) dissolved in DDW.

3. Results and discussion

3.1. Determination of inorganic arsenic species in a liquid health food supplement

Element-specific detection has been used extensively in the analysis of arsenic because of large differences in the toxicity of various arsenic-containing species [2–4]. The use of conductivity detection for As(III) is difficult because of the low ionization constant of arsenous acid [6]. In this work, IC–ICP-AES is used to determine the oxidation state of inorganic As found in a liquid health food supplement.

Samples were simply diluted 1:10 with DDW and filtered through a 0.2-μm nylon syringe filter. Fig. 1 shows the separation of arsenite and arsenate spiked into a sample of the health food supplement at the 5 μg/ml level. A single standard addition was used to quantitate arsenate found in the samples. No arsenite was detected. The solution detection limits were 0.16 and 0.14 μg/ml for arsenite and arsenate, respectively. Ten background points were measured in a blank solution at the analyte retention time; and the detection limit was calculated as three times the standard deviation of these ten points divided by the sensitivity obtained for a 4 μg/l analyte standard.

Four samples were analyzed and good agreement was obtained with total As results determined directly by ICP-AES. The average and standard deviation for the total As measurements of four samples was 9.0 ± 0.1 μg/ml. In comparison, the IC–ICP-AES result was 9.1 ± 0.5 μg/ml of arsenate.

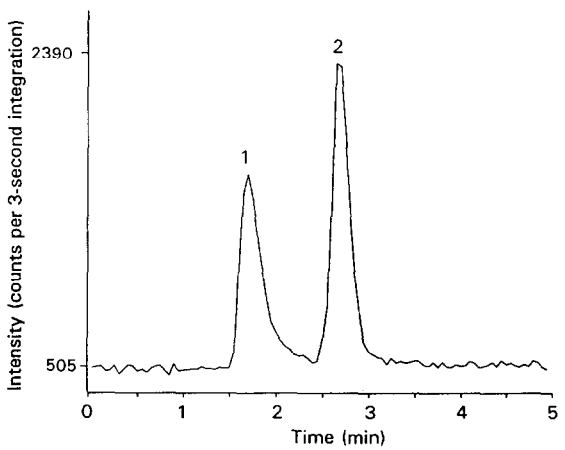

Fig. 1. As-specific detection in a liquid health food supplement. Peaks: 1 = 5 μg/ml As(III) spike; 2 = 5 μg/ml As(V) spike. Column, Dionex IonPac AS4A. Mobile phase, 40 m*M* sodium hydroxide. Flow-rate, 1.0 ml/min. Injection volume, 100 μl. Suppressor, Dionex AMMS-I. Regenerant, 12.5 m*M* sulfuric acid. Regenerant flow-rate, *ca.* 5 ml/min. Detection, ICP-AES 193.7 nm.

3.2. Investigation of polyphosphates in processed shrimp by IC–ICP-AES

A method for the determination of tripolyphosphate and its hydrolysis products in processed shrimp using IC with post-column reaction UV detection has been described elsewhere [12]. The presence of a peak of unknown identity when using UV detection was cause for some concern. The unknown peak eluted between peaks for tripolyphosphate and pyrophosphate and near the retention time for sulfate. However, a sulfate spike of the sample solution showed that the unknown was not sulfate. It was important to show that the peak was not a phosphate-containing species such as trimetaphosphate which is a known impurity in some commercially available sodium tripolyphosphates [13].

Shrimp samples were composited and prepared as described in ref. 12. Composited sample (0.5 g) was diluted 1:100 with DDW and shaken for 30 min. A portion of the extract was then filtered through a nylon syringe filter and a C_{18} sample preparation cartridge in series. Fig. 2 shows UV and ICP-AES chromatograms obtained for a sample of cooked processed shrimp which had been treated with tripolyphosphate. In this case, the outlet of the UV cell was connected directly to the ICP-AES instrument; thus the column effluent and the UV post-column reagent (0.1% ferric nitrate in 2% perchloric acid) were introduced to the plasma. The unknown peak (3) is only detected by the UV detector. Based on the strong ICP emission signal obtained for orthophosphate, pyrophosphate and tripolyphosphate peaks, it can be concluded that the unknown peak does not contain phosphorus and should not affect the analysis of tripolyphosphate and its hydrolysis products.

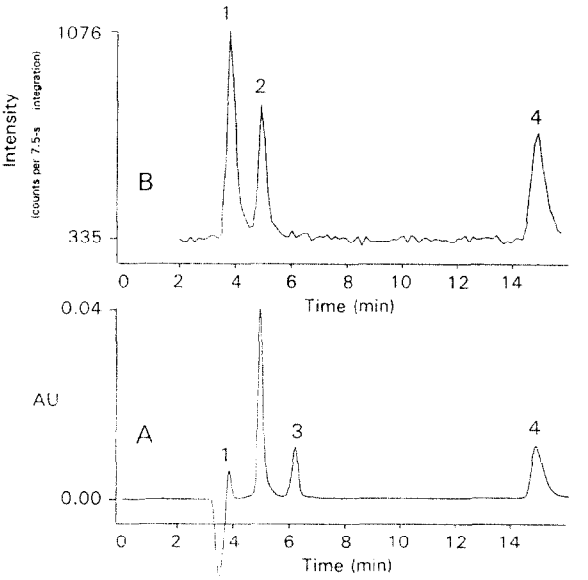

Fig. 2. Determination of polyphosphates in shrimp. (A) UV detection at 330 nm; (B) ICP-AES detection of P at 214.9 nm. Peaks: 1 = orthophosphate; 2 = pyrophosphate; 3 = unknown; 4 = tripolyphosphate. Column, Dionex IonPac AS7. Guard column, NG1. Mobile phase 70 m*M* nitric acid. Flow-rate, 0.5 ml/min. Injection volume, 100 μl. Post-column reagent for UV detection, 1 g/l ferric nitrate in 2% perchloric acid. Post-column reagent flow-rate, 0.5 ml/min.

3.3. Determination of bromate and bromide in baked goods by IC–ICP-MS

Investigations into the health risks associated with the use of potassium bromate in bread require the development of analytical methodology capable of quantitating bromate at the low ng/g level. Conductivity detection is hampered by insufficient sensitivity and interference from high chloride levels in baked goods [10]. IC–ICP-MS provides sufficient sensitivity and freedom from interferences to accurately quantitate bromate in a relatively straightforward manner.

Five slices of bread from different areas of the same loaf were cut (or torn) into small pieces approximately 1 cm^3, placed into a plastic zip-lock bag, and mixed thoroughly. A 25-g sample of the composite was accurately weighed into a clean 250-ml Nalgene HDPE bottle and 200.0–225.0 g of DDW were added. The sample was then homogenized using a Kinematica Polytron homogenizer for approximately 2 min. A portion of the sample solution was then centrifuged at 2500 *g* for 5 min (in later work, the centrifuge step was omitted). Finally, a portion of the supernatant was passed through a 0.45-μm nylon

syringe filter and an activated Alltech Maxi-clean C_{18} sample preparation cartridge. This solution served as the sample solution. The C_{18} cartridge was activated by passing through 20 ml of methanol followed by 20 ml of DDW. The first 3 ml of sample through the syringe filter and C_{18} column were discarded.

A similar procedure was followed for bun and roll samples. One or two buns (or rolls) were sampled and treated in the same manner as the five slices of bread. In more recent studies, a single bun or roll has been cut into small pieces and the entire sample is diluted 1:10 with DDW, after which the sample is prepared as described above.

Samples should be stored frozen prior to preparation and analysis. Prepared samples should be analyzed as soon as possible after preparation.

A Dionex AS10 analytical IC column and AG10 guard column with a mobile phase of 150–180 m*M* NaOH at a flow-rate of 1 ml/min has been found to provide good separation of bromate, bromide and some unidentified interferences. A micromembrane suppressor, Dionex Model AMMS-II, has been utilized to reduce the amount of Na which reaches the ICP-MS system. Large amounts of Na introduced to the ICP-MS system can cause suppression of analyte signal and response drift. Bromate and bromide elute at retention times of approximately 6 and 17 min, respectively. In bread matrix, both the bromate and bromide peaks elute at slightly shorter retention times than found with standards presumably because of matrix effects. Peak height was used for quantitation due to difficulties associated with integrating peak areas using the present method of data collection and evaluation.

Peak height response was found to be linear over the range 1 to 1000 ng/ml for bromate and 2 to 500 ng/ml for bromide. The solution detection limit for bromate was 0.6 ng/ml. This corresponds to a method detection limit of approximately 6 ng/g in baked goods. For bromide the solution detection limit was 1.0 ng/ml. Detection limit was defined as the concentration of bromate which would result in a peak height equivalent to three times the standard deviation of 30 background points for a blank solution taken at the retention time of the analyte. For 10 consecutive injections of a 10 ng/ml bromate standard, a R.S.D. of 2.1% was obtained. Figures of merit obtained using the 150 m*M* sodium hydroxide eluent are summarized in Table 1.

Initially, an eluent of 180 m*M* sodium hydroxide was used. Four replicate injections of a fully prepared sample extract from hot dog buns resulted in an average of 80 ng/g bromate and 44 μg/g bromide with R.S.D.s of 7.9 and 5.0%, respectively. In a similar experiment with "brown and serve" bread sticks, five portions of extract were filtered through five syringe filters and C_{18} cartridges and analyzed. This resulted in an average of 8.2 μg/g bromate and 37 μg/g bromide with R.S.D.s of 8.2 and 7.0%, respectively. Thus in both cases the repeatability of sample injections was less than 10% R.S.D. for both bromate and bromide.

This chromatographic system (AS10 column with 180 m*M* NaOH eluent) was used to analyze several different sample types. Table 2 shows the results obtained for seven samples of buns and rolls. Bromate was found in six of the seven

Table 1
Figures of merit

	Bromate	Bromide
Solution detection limit (ng/ml)	0.6	1.0
Short-term precision (10 ng/ml)	2.1% (n = 10)	3.2% (n = 4)
Linear range (ng/ml)	1–1000	2–500
Correlation coefficient	0.9999	0.9998
Sensitivity (slope)	909 cps/ng/ml	483 cps/ng/ml
Log–log slope	0.98	0.99

Table 2
Results for buns and rolls, 180 m*M* NaOH eluent

Description	Bromate concentration (μg/g)	Bromide concentration (μg/g)
Steak rolls	1.6, 1.1	37, 33
Twist rolls	0.7, 0.3	39, 36
Hot dog buns	1.1, 0.08	45, 44
French rolls	<0.04	16, 14
Hot dog buns	0.08, 0.09	14, 14
Hot dog buns	0.8, 0.7	23, 23
Brown and serve bread sticks	10.5	35, 36

samples at concentrations ranging from 0.8 to 10.5 μg/g. Table 3 shows the results obtained for ten samples of sliced bread. The determination of bromate using this separation system is still somewhat hampered by the incomplete resolution of bromate and an interference. Based on the amount of interference seen in these ten bread samples, a conservative estimate of the bromate detection limit (0.04 μg/g) was made. None of the sliced bread samples analyzed was found to contain bromate at a concentration above the detection limit. Spike recoveries for a 0.1 μg/g bromate spike into three of the samples are also reported in Table 3. Recoveries for the three spiked samples were 78, 86 and 134%.

The use of 150 m*M* NaOH was found to provide adequate separation of the bromate peak and unidentified interference. Fig. 3 shows a spiked and unspiked chromatogram for a sample of white bread. The spike concentration was 1 ng/ml bromate (roughly 10 ng/g of sample). The bromate peak is well resolved from any interfering peaks. The same ten sliced bread samples as listed in Table 3 were analyzed using the 150 m*M* NaOH. In each case bromate was not detected.

The chromatogram shown in Fig. 4 was obtained from a sample of "brown and serve" Italian rolls. Ten separate rolls were prepared by homogenizing the entire rolls. Bromate was present at an average concentration of 3.5 μg/g of sample. The average bromide concentration

Table 3
Results for sliced bread, 180 m*M* NaOH eluent

Description	Bromate concentration (μg/g)	Bromide concentration (μg/g)
Buttermilk	<0.04	7.0, 6.6
Whole wheat	<0.04	6.6, 6.3
Multigrain	<0.04	5.8, 5.8
Whole grain	<0.04 (134%)	5.9, 5.9
White	<0.04	7.0, 6.0
Rye	<0.04 (86%)	6.4, 6.6
Light white	<0.04 (78%)	Not determined
White	<0.04	7.0, 6.8
Light white	<0.04	32, 32
White	<0.04	5.8, 5.3

Numbers in parentheses are recovery values for a 0.1 μg/g bromate spike (10 ng/ml in solution).

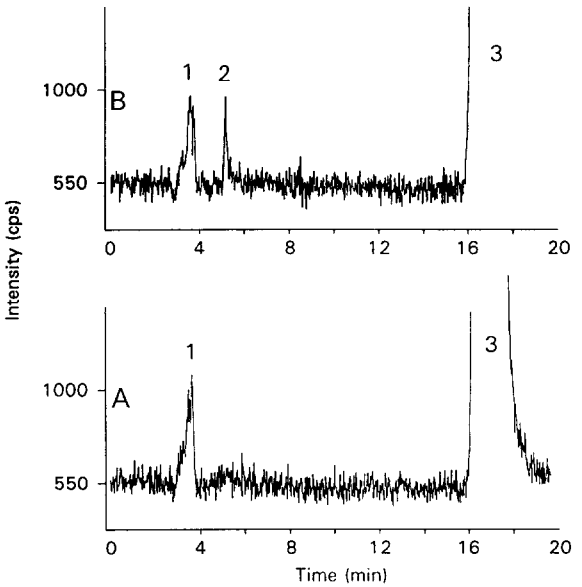

Fig. 3. Chromatograms of spiked and unspiked samples of white bread using ICP-MS detection. (A) Unspiked sample; (B) spiked sample, 1 ng/ml bromate spike. Peaks: 1 = unknown; 2 = bromate; 3 = bromide (off scale). Column, Dionex IonPac AS10. Guard column, AG10. Mobile phase, 150 m*M* sodium hydroxide. Flow-rate, 1.0 ml/min. Injection volume, 100 μl. Suppressor, Dionex AMMS-II. Regenerant, 25 m*M* sulfuric acid. Regenerant flow-rate, *ca.* 5 ml/min.

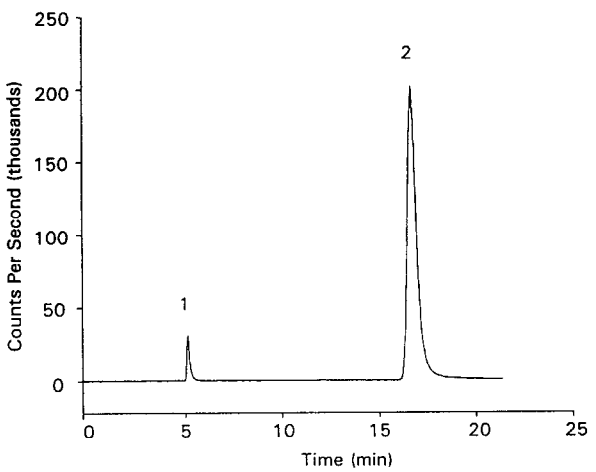

Fig. 4. Chromatogram of "brown and serve" Italian roll using ICP-MS detection. Peaks: 1 = bromate; 2 = bromide. Chromatographic conditions as for Fig. 2.

was 36 μg/g of sample. The R.S.D.s for the 10 samples were 8.1% and 8.9% for bromate and bromide concentrations, respectively.

4. Conclusions

Element specific detection for ion chromatography by ICP-AES or ICP-MS has been shown to resolve persistent separation problems in food analysis as well as provide additional confirmation of peak identity. The oxidation state of inorganic arsenic in a liquid health food supplement was determined by IC–ICP-AES. While ICP-AES is generally more tolerant of the eluents used in IC, ICP-MS provides enhanced sensitivity. The combination of IC and ICP-MS allows the requisite sensitivity and freedom from chromatographic interferences which are necessary to make an informed decision on the potential health risks associated with ingestion of bromate from baked goods.

5. Acknowledgement

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