Journal of Chromatography A, 1354 (2014) 109–116

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Chromatographic separation of arsenic species with pentafluorophenyl column and application to rice

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ARTICLE INFO

Article history:
Received 16 January 2014
Received in revised form 28 May 2014
Accepted 28 May 2014
Available online 4 June 2014

Keywords:
Arsenic speciation
Rice
HPLC–ICPMS
Pentafluorophenyl column
Volatile mobile phase

ABSTRACT

Arsenic species, including arsenous acid, arsenic acid, methylarsonic acid, and dimethylarsinic acid, were determined using HPLC–ICPMS. The species were separated with a Discovery HS F5 column and a simple, volatile, and isocratic mobile phase of 0.1% (v/v) formic acid and 1% (v/v) methanol. The Discovery HS F5 column with a pentafluorophenyl (PFP) stationary phase gave sharp peaks and full separation of the arsenic species in 5 min, and other PFP columns showed lower performance. This separation method was applied to arsenic species analysis in rice. The extraction of arsenic from rice samples was performed using 0.15 M nitric acid. The methodology was validated by use of certified reference materials, NMIJ CRM 7503-a and NIST SRM 1568a, and extremely low arsenic rice samples as blank samples.

1. Introduction

Arsenic is one of the most well known toxic elements. The toxicity of arsenic depends on its chemical species. Arsenite (As(III)) is more toxic than arsenate (As(V)), and inorganic arsenic is thought to be more toxic than organic arsenic [1]. So, in arsenic speciation of foods and drinks, inorganic arsenic (As(III) + As(V)) and organic arsenic species are separately evaluated [2]. HPLC (or IC) coupled with ICPMS has been widely used for arsenic speciation analysis [3–8]. Various chromatographic separation modes are used for arsenic speciation analysis, including anion-exchange, cation-exchange, reversed phase, ion-pair reversed phase, ion exclusion, and size exclusion. Anion-exchange columns, especially the Hamilton PRPX-100, have been the most frequently used for many applications for approximately 20 years [9–11]. Phosphate buffer is frequently used as the mobile phase for anion-exchange separation; however, nonvolatile salts in the buffer can deposit on the sampler and skimmer cones, resulting in signal instability and time lost cleaning the instrument parts. Xie et al. separated As(V), As(III), methylarsonic acid (MAA), and dimethylarsinic acid (DMAA) in 12 min using a cation-exchange column and a mobile phase of 10 mM ammonium nitrate and 0.0025% (v/v) nitric acid in isocratic mode [12]. However, peaks of As(V) and MAA partially overlapped. Mobile phases of nitric acid and/or ammonium nitrate were also used under gradient elution conditions [13–15]. Ammonium carbonate and ammonium hydrogen carbonate have been used occasionally as volatile buffers for gradient elution programs for surface water, urine, fish, and muscle samples [16–19]. The mobile phases are alkaline, so chromatographic turbulence must be considered in analyses of acidic analytical solutions, such as rice extracts. Francesconi et al. have recently reported on arsenic speciation in foods containing rice using a PRP-X100 column and aqueous malonic acid at pH 5.6 under isocratic elution. Volatile mobile phases and fast (<6 min) separation are essential for routine everyday analyses using HPLC–ICPMS [20]. In this study, we investigated HPLC conditions for arsenic speciation analysis using a simple volatile mobile phase, isocratic elution, and silica-based pentafluorophenyl (PFP) columns, which are less expensive than polymer columns, such as the PRP-X100. The PFP columns are classified into a higher shape selectivity group using principal component analysis [21,22]. The PFP interacts with analytes through dipole–dipole, π–π charge–transfer, and ion–exchange interactions [23]. Since the 1990s [24], the columns have been used to separate small polar analytes in basic pharmaceuticals [25], alkaloids [26], taxanes [27], cancerostatic platinum compounds [28], betaine metabolites [29], and catecholamines [30]. However, there are no reports for arsenic species. This HPLC system was applied in the analysis of arsenic in rice, which is an important application, particularly since rice is

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http://dx.doi.org/10.1016/j.chroma.2014.05.072
0021-9673/

a critical staple in most parts of the world. Arsenic contamination of rice is a major concern, and its regulation is now discussed in CODEX [31].

2. Material and methods

2.1. Reagents and standards

Water was purified using a Milli-Q system (Nihon Millipore, Tokyo). TAMAPURE-AA-100 series ultrapure 68 wt% nitric acid (HNO_3) and 35 wt% hydrogen peroxide (H_2O_2) (Tama Chemicals, Tokyo); LC/MS grade methanol (MeOH) (Kanto Chemical, Tokyo); column chromatography grade 98 wt% formic acid (HCOOH), column chromatography grade acetic acid (CH_3COOH), and guaranteed reagent grade ammonium formate (Nacalai Tesque, Kyoto, Japan); methylarsonic acid (MAA), oxalate pH standard solution (pH 1.68), phthalate pH standard solution (pH 4.01), and phosphate pH standard solution (pH 6.86) (Wako Pure Chemical Industries, Tokyo); HPLC grade phosphoric acid (H_3PO_4) (Merck Ltd., Japan); and ICP standard Rh (1000 μg mL^{-1}) (SCP Science, QC, Canada) were used. An anion mixed standard solution IV (Kanto Chemical) was used for phosphate analyses in rice extracts. For the As(III) standard, a JCSS arsenic standard solution was purchased from Wako Pure Chemical Industries. The manufacturer prepared it from sodium arsenite and hydrochloric acid. Before use, we checked using HPLC–ICPMS that it contained only As(III). NMIJ CRM 7912-a (99.53 mg As kg^{-1}), 7913-a (25.11 mg As kg^{-1}), and 7901-a (24.40 mg As kg^{-1}) were used as the As(V), DMAA, and arsenobetaine (AsB) standards, respectively. For total arsenic analysis of rice, calibration standards of 0.1, 0.2, 0.5, 1, 2, and 5 μg As L^{-1} were prepared from the As(V) standard and 1 wt% HNO_3 by stepwise dilution. The calibration standards contained 25 μg L^{-1} of Rh as an internal standard. For arsenic speciation analysis, mixed standard solutions of As(III), As(V), MAA, and DMAA were prepared from each standard solution using stepwise dilution with 0.15 M HNO_3. To validate the analytical method, NIST SRM 1568a rice powder and NMIJ CRM 7503-a white rice powder were used.

2.2. Sample preparations

Rice samples were purchased from markets in Japan and stored at 4 °C before grinding. Rice samples with extremely low arsenic levels were cultivated in our dry field using aerobic treatments to reduce the arsenic concentration [32]. Rice grains were finely ground using a CryoMill (Retsch GmbH, Haan, Germany) with a 25 mm ball and a 50 mL vessel, both made of stainless steel. The milling time was set to 150 s × 2 for 7.5 g of rice grain. The milled powder was stored at 4 °C before analysis. To assess the water content, 1 g of milled powder was dried in an oven at 105 °C for 48 h. In this paper, the arsenic concentrations in the samples are expressed as dry weight after moisture content correction.

2.3. Digestion

For total arsenic analysis, the milled rice samples were digested using an aluminum heat block (DTU-1C, Taitec Co., Saitama, Japan). Rice powder (0.5 g) was predigested at an ambient temperature with 2 mL of HNO_3 in a 50 mL centrifuge tube and was then heated at 105 °C for 1 h. After cooling to ambient temperature, 1 mL of H_2O_2 was added to the yellow solution. The solution was heated to 105 °C for 2 h. After cooling to ambient temperature, water was added to make a total volume of ca. 25 mL. The precise net weight was calculated by subtracting the initial weight of the tube.

Table 1
Instrumental parameters for arsenic speciation in rice.

ICPMS	
RF power (W)	1450
Nebulizer gas flow (L min^{-1})	0.9
Auxiliary gas flow (L min^{-1})	1.2
Plasma gas flow (L min^{-1})	14.0
Collision gas flow (He) (L min^{-1})	6.0
Collision gas flow (H_2) (L min^{-1})	3.0
Dwell time (ms)	200
Cone voltage (V)	50
Spray chamber	cyclone type with a cold jacket (20 mL volume, 0 °C)
Ions monitored	^{75}As (and $^{40}Ar^{37}Cl$ in preliminary analyses)
HPLC	
Sample loop (μL)	200
Injection volume (μL)	10
Column	Sigma-Aldrich Discovery HS F5
Column size (mm)	250 × 4.6
Column particle size (μm)	5
Column temperature	Ambient
Mobile phase	0.1% (v/v) formic acid containing 1% (v/v) methanol
Mobile phase flow (mL min^{-1})	1.0
Elution mode	Isocratic
Integration mode	Peak area

2.4. Instruments

A Platform ICPMS (Micromass, Manchester, UK) with a hexapole collision cell was used to determine arsenic concentration. To reduce the dead volume, a MicroMist (0.1 mL min^{-1}) nebulizer (Glass Expansion, West Melbourne, Australia) was used. The detailed instrumental parameters are listed in Table 1. Because we confirmed that no chromatographic peak of $^{40}Ar^{37}Cl$ (m/z 77) was seen in the sample analyses due to the addition of collision gas, only ^{75}As (m/z 75) was monitored in the following analyses [33]. If this method is modified or unknown samples are analyzed, monitoring of m/z 77 is recommended for the mathematical correction of ^{75}As. A 10 μg As L^{-1} calibration standard of each arsenic species for speciation or a 5 μg As L^{-1} calibration standard of As(V) for total arsenic analysis was included in every set of 10 subsamples to confirm that each peak area after the internal standardization are within 90–110% of the initial value. A 3033 HTS autosampler, a 3001 inert pump (Shiseido, Tokyo), and a DG-1580-53 degasser (ERC Inc., Saitama, Japan) were used for HPLC and flow injection (FI). Phosphate ion concentration in selected rice extracts was measured using a DIONEX DX-500 IC with an ED40 electrochemical detector, an RFC-10 reagent-free controller, and an ASRS 300 anion self-regenerating suppressor (2 mm) (Thermo Fisher Scientific K.K., Yokohama). A DIONEX IonPac AS20 (250 mm × 2 mm, 7.5 μm) anion exchange column was used at 30 °C. The mobile phase was sodium hydroxide aqueous solution, 15.75–35 mM (0–6 min), 35 mM (6–12 min), and 35–15.75 mM (12–15 min). The flow rate was 0.2 mL min^{-1}. The injection volume was 10 μL. Centrifugation was done using a high-speed refrigerated centrifuge, CR 22G (Hitachi Ltd., Tokyo).

2.5. Determination of total arsenic concentrations

The digested solution was filtered through a 0.45 μm polyethersulfone membrane of a 13 mm syringe filter device (Nihon Millipore, Tokyo) before analysis. After the addition of Rh as an internal standard (25 μg L^{-1}), the total arsenic concentration was determined by FI-ICPMS with a 1 wt% HNO_3 mobile phase at a 0.4 mL min^{-1} flow rate. The injection volume was 50 μL. The FI peak was integrated and the concentration was determined by a four- or six-point calibration of arsenic standards in the range