A rapid monitoring method for inorganic arsenic in rice flour using reversed phase-high performance liquid chromatography-inductively coupled plasma mass spectrometry

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Abstract

A new rapid monitoring method by means of high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) following the heat-assisted extraction was developed for measurement of total inorganic arsenic species in rice flour. As(III) and As(V) eluted at the same retention time and completely separated from organoarsenic species by an isocratic elution program on a reversed phase column. Therefore, neither ambiguous oxidation of arsenite to arsenate nor the integration of two peaks were necessary to determine directly the target analyte inorganic arsenic. Rapid injection allowed measuring 3 replicates within 6 min and this combined with a quantitative extraction of all arsenic species from rice flour by a 15 minute HNO_3-H_2O_2 extraction makes this the fastest laboratory based method for inorganic arsenic in rice flour.

Keywords: *Arsenic speciation; HPLC-ICP-MS; Inorganic arsenic determination; Rice flour; Risk assessment; Extraction.*

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Highlights

- A new HPLC-ICP-MS method was developed for the rapid monitoring of inorganic As
- Inorganic As can be analyzed directly since As(III) and As(V) co-eluted
- Inorganic As can be separated from all organoarsenic compounds in rice
- Three replicates can be analyzed within 6 minutes
- Complete extraction of As species from rice flour was achieved within 15 minutes

1. Introduction

Knowledge of chemical species of elements present in biological and environmental samples is important for an understanding of the toxicity, metabolism and transport properties of the elements.[1-2] Therefore, determination of specific chemical forms of elements (speciation analysis) has become an increasingly active-research field in recent years.[3] Arsenic (As) is found in various chemical species in environmental and food samples. In general, naturally occurring organoarsenic compounds are considered to be of non-toxicity, but inorganic arsenic in the form of arsenite (As(III)) or arsenate (As(V)) is a class I carcinogen.

The Codex Alimentarius Commission, which provides the guideline for allowable concentrations of toxic elements and compounds in foodstuffs, approved a tolerance level of inorganic arsenic as 0.2 mg kg^{-1} in polished rice in 2014. A tolerance level for inorganic arsenic in unpolished rice is still under discussion but rice for baby and toddler food has an even lower maximum limit for inorganic arsenic (0.1 mg kg^{-1}).[4-6] Therefore, it is now apparent that a rapid determination of total inorganic arsenic species present in rice flour is necessary for monitoring test and/or surveillance.[6]

Prior to measurements, the arsenic species in rice flour samples are generally extracted by the techniques employing heated HNO_3 solutions.[7-10] Although most analytical procedures for arsenic in any foodstuffs are composed with similar operating steps, there are some specific conditions for the determination of inorganic arsenic in rice flour samples. The Codex Alimentarius Commission recommends the analytical methods of the arsenic species in rice flour, involving extraction with diluted acids (*e.g.* 0.15 M HNO_3 or 1 to 2 (v/v)% HNO_3, Extraction: 100 ºC for 2 h) and subsequent determination of As by HPLC-ICP-MS using a reversed phase (ODS) column.[11] On the other hand, the US Food and Drug Administration (FDA) recommends 0.28 M HNO_3 for arsenic extraction followed by IC-ICP-MS using an anion-exchange column (IC) for the determination.[12]

An ODS column is well able to separate many arsenic species; it can separate the two inorganic arsenic species, six methylated arsenic species and four arsenosugars within 10 to 15 minutes, however the peaks of As(III) and MMA tend to be very close each other. In a similar way, the peaks of DMA and arsenobetaine (AsB) and those of trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TeMA) almost overlap one another.[13-15] When a large number of measurements are carried out by using one column, the column packing is likely to deteriorate and these pairs of peaks would not be separated eventually. In addition, a complex component of eluent containing ion-pair reagents and organic contents can cause a problem at the ICP-MS interface with long-term analysis.

Each of these techniques has their own advantages and disadvantages. Anionic arsenic species can be separated by an IC column with a simple component eluent and the separation

property depends upon the component and pH of the eluent.[16-22] An IC column is useful to separate arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and some arsenosugars respectively, but it cannot separate some methylated arsenic compounds under a single set of operating conditions.

The main arsenic species in agricultural samples, in particular rice flour, are As(III), As(V), MMA and DMA.[23-24] TeMA however was found in Chinese rice as well.[25] Hence, only arsenic in the forms of As(III), As(V), MMA, DMA and TeMA are of interest when rice grains are going to be analyzed.

The Codex Alimentarius Commission regulation refers to the inorganic arsenic, i.e. the sum of As(III) and As(V). Nevertheless, most monitoring test methods for inorganic arsenic in rice flour separate As(III) and As(V) and determine them individually which makes the integration of two peaks necessary for the quantification of the target analyte inorganic arsenic. Another approach is to oxidize all As(III) to As(V) and subsequently measure all inorganic arsenic as As(V) using an anion column. A peak appearing in the void where As(III) would elute means that either the cationic species TeMA is in the rice grain extract or the oxidation of As(III) was not complete. Since As(III) and TeMA elute at the same time[25], TeMA should be separated from As(III) and As(V) to avoid ambiguity and so far no method has shown to be capable for showing both species separately.

One of the most important purposes of food analyses is to ensure the food safety based on the appropriate regulations with rapidity and efficiency. Therefore, a rapid screening method with high sample throughput is required. In recent year, a screening and a rapid measurement methods for inorganic arsenic in rice flour were reported; the former was a field test kit method based on the Gutzeit methodology and the latter a fast separation-detection method using IC-ICP-MS/MS.[26-27]

In this study a rapid monitoring method of inorganic arsenic in rice flour with HPLC-ICP-MS using a reversed phase column with a simple component eluent was developed. Chromatographic conditions, in particular eluent component and pH were optimized to determine As(III) and As(V) at the same time, and completely separate them from other organoarsenic compounds such as DMA, MMA and TeMA.

2. Experimental

2.1 Instrumentations

An ICP-MS (7500c, Agilent, Tokyo, Japan) equipped with a Micromist nebulizer (100 μL type) and a Scott spray chamber (2 °C) was used. Typical operating parameters for the ICP-MS were as follows: incident rf power was 1600 W, outer Ar gas flow rate 15 L min^{-1}, intermediate Ar gas flow rate 0.9 L min^{-1}, carrier Ar gas flow rate 0.8 L min^{-1} and make-up Ar

gas flow rate 0.4 mL min^{-1}. The ICP-MS was usually operated using He as the collision cell gas (3 mL min^{-1}) to reduce some polyatomic molecular interferences. An HPLC was used for separation of arsenic species. The exit of the HPLC column was directly connected to the nebulizer of the ICP-MS with PEEK tubing (HPLC-ICP-MS). The typical operation conditions and its performances are shown in Table S1.

Fifteen types of columns were investigated in the experiment (Tables S2 and S3). Five types of columns categorized in group I provided satisfactory performances for rapid monitoring of inorganic arsenic compounds, but ten other types of columns categorized in group II were unsuitable for the purpose. The columns of group I are as follows; A Shim-pack VP-C8 C_8 column (hereafter referred to as the C_8: particle size of the filler 5 μm, ID 4.6 mm x 250 mm, end-capped type, Shimadzu Co., Kyoto, Japan), a CAPCELL PAK C_{18} MG column (hereafter referred to as the C_{18}: particle size of the filler 5 μm, ID 4.6 mm x 250 mm, polymer-coated type, Shiseido Co., Ltd., Tokyo, Japan), a CAPCELL PAK C_{18} ACR (hereafter referred to as the C_{18} ACR: particle size of the filler 5 μm, ID 4.6 mm x 250 mm, polymer-coated type, Shiseido), a Sunrise C_{28} column (hereafter referred to as the C_{28}: particle size of the filler 5 μm, ID 4.6 mm x 250 mm, TMS end-capped type, ChromaNik Technologies Inc., Osaka, Japan), and a DEVELOSIL C_{30}-UG-5 C_{30} column (hereafter referred to as the C_{30}: particle size of the filler 5 μm, ID 4.6 mm x 250 mm, end-capped type, Nomura Chemical Co. Ltd., Aichi, Japan) were used.

The columns of group II were also tested under multiple elution conditions of eight separation modes. Details are given in the Tables S2 and S3.

A heating block system (Digi PREP, SCP Science Inc., Quebec, Canada) was used for heat assisted extraction.

2.2 Calibration standards and reagents

The Japan Calibration Service System (JCSS) arsenic standard solution (*ca*. 1000 mg L^{-1}, Kanto Chemical Co., Inc., Tokyo, Japan) was used as the source of the calibration standard solution. It is produced by dissolving As_2O_3 powder into HNO_3 solution and contains only As(III). It did not actually contain detectable levels of any other arsenic species including As(V) and was thus used as the As(III) source standard solution.

The certified reference materials of As(V) (NMIJ CRM 7912-a), the dimethylarsinic acid (DMA) (NMIJ CRM 7913-a) and the arsenobetaine (AsB) (NMIJ CRM 7901-a) supplied by the National Metrology Institute of Japan / National Institute of Advanced Industrial Science and Technology (NMIJ/AIST, Tsukuba, Japan) were used as source standard solutions.

Source standard solutions of the other organoarsenic species such as monomethylarsonic acid (MMA), trimethylarsine oxide (TMAO), tetramethylarsonium chloride (TeMA), and

arsenocholine bromide (AsC) were prepared from commercially available reagents (Tri-Chemical Laboratories Inc., Yamanashi, Japan), after their purity was evaluated (moisture, elemental analysis of C, H, O, Br and Cl, and arsenic impurities). Each compound was dissolved in water to prepare an in-house standard solution containing *ca*. 1000 mg As kg^{-1}.

The acids and ammonia solutions used were of ultrapur® grade (Kanto), and the organic solvents used were of HPLC grade (Kanto). Ammonium dihydrogen phosphate and diammonium hydrogen phosphate were of ultrapure grade (Kanto). Sodium 1-butanesulfonate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), malonic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and tetramethylammonium hydroxide (TMAH, Tama Chemicals Co., Ltd., Kanagawa, Japan) were obtained as indicated. Ultra-pure water was generated with a Milli Q-Labo filter (Nippon Millipore Ltd, Tokyo, Japan) and was used throughout.

2.3 Extraction procedure for arsenic species

Arsenic species in the rice flour samples were extracted by a heat-assisted technique using acid solvent. A portion of rice flour sample (*ca*. 0.5 g) was accurately weighed into a 10 mL glass tube and 2 g of extracting solvent were added. Four kinds of extracting solvents were used here respectively; 0.15 mol L^{-1} HNO_3, 0.28 mol L^{-1} HNO_3, 0.30 mol L^{-1} H_2O_2 and 0.20 mol L^{-1} H_2O_2+0.10 mol L^{-1} HNO_3. The tube was capped and placed in a dry heating block system at 100 °C for a period from 15 min to 2 h. After cooling to room temperature, 8 g of water was added (total liquid phase: 10 g). The tube was centrifuged at 4000 rpm for 5 min, and then the liquid phase was then passed through a 0.45 μm syringe-type polyvinylidene difluoride (PVDF) membrane filter. The filtrate was analyzed by HPLC-ICP-MS.

2.4 Certified Reference Materials (CRMs)

NMIJ brown rice flour CRMs (NMIJ CRM 7532-a and NMIJ CRM 7533-a) and NIST rice flour SRM (SRM 1568b) were analyzed to valid the newly developed rapid monitoring method.

3. Results and Discussion

3.1 Conditions for simultaneous determination of inorganic arsenic species

Besides the columns categorized in group I, the columns in group II were also examined with various types of eluent and at various pHs shown in supplementary material 1. However, unsatisfactory results were obtained for simultaneous determination of inorganic arsenic species. Therefore, the following discussion is limited to the experiments carried out using the columns in group I.

For chromatographic analysis of water-soluble arsenic species, pH of the eluent is one of

182 the most effective factors to control the separation of the compounds. The separation of
183 As(III), As(V), MMA, DMA and TeMA using the columns of group I were investigated using
184 the eluents containing 0.001 to 0.01 mol L^{-1} HNO_3 / 0.05 % methanol. With increasing HNO_3
185 concentration (that is, lower pH), As(III), MMA, DMA and TeMA were eluted faster. The
186 retention times of each species with the eluent of 0.01 mol L^{-1} HNO_3 / 0.05 % methanol are
187 shown in **Figure 1**, where the chromatography was carried out with four different types of
188 columns. Increasing carbon chain length of the chromatographic support, the retention times
189 of As(III) and As(V) closed in each other, and they completely matched when C_{28} and C_{30} ODS
190 columns were used. However, when HNO_3 eluent was applied, the peaks tended to broaden
191 and peaks of inorganic arsenic overlap to that of organoarsenic species using C_{28} and C_{30}
192 columns. On the other hand, As(III) and As(V) always eluted faster than the organoarsenic
193 species regardless of the carbon chain length of the columns applied. This is not surprising
194 since the interaction of these polar inorganic arsenic species would show less interaction with
195 the non-polar reverse phase. Tetramethylarsonium, the only cationic species under these
196 conditions eluted last among five compounds with the C_8 column, but faster than DMA with the
197 C_{18}, C_{28} and C_{30} columns. This indicates that the C_8 column may have some ion exchange
198 properties. This suggestion is also supported by the fact that the anionic As(V) elutes before
199 the neutral As(III). In addition, the specific surface area of filler of C_8 is the biggest; therefore
200 the retention time of the first eluting species was slightly longer. In contrast, only C_{18} was the
201 polymer–coated type column, and its specific surface area of filler was the smallest in the
202 columns; hence, the retention times of As species were faster than the other columns. However,
203 MMA and DMA were always separated even when using any columns in the group I.
204 Buffers and chelating agents are effective for suppressing peak tailings.[28] A series of
205 eluents containing 1 to 5 mmol L^{-1} of diammonium hydrogen phosphate / 0.05 % methanol was
206 tested; the pH of the eluents was adjusted to 2.0 with HNO_3. The eluents containing
207 diammonium hydrogen phosphate reduced the tailing of the peaks, and improved the separation
208 capacity. However, it also made As(III) and As(V) peaks split slightly from each other using
209 C_{28} and C_{30} columns. On the other hand, it was found that the eluent with lower pH was
210 effective to elute As(III) and As(V) coincidently. The ODS columns employed here are
211 usually used in pH range from 2 to 10, because the Si-C bond of the functional group will be
212 deteriorated at pH of lower than 2. Therefore, a column with high resistance to acidic
213 conditions was likely to prove valuable for simultaneous elution of As(III) and As(V).
214 A polymer-coated C_{18} ACR column, which is stable at pH range of 1 to 10, was
215 investigated using the diammonium hydrogen phosphate buffer containing HNO_3 (pH 1.5～3.0)
216 / 0.05 % methanol. Although the length of the carbon chains of chromatographic support was
217 shorter than C_{28} and C_{30} columns, the sharp peaks were observed and the retention times of

As(III) and As(V) coincided completely (Table 1), whereas the MMA, DMA and TeMA were observed at the different retention times. TeMA eluted before all other possible arsenic species (As(III) and As(V) and DMA, MMA) in rice grains using C_{18} ARC column with the diammonium hydrogen phosphate buffer containing HNO_3 (pH 1.5～2.0) / 0.05 % methanol. As(III) and As(V) were determined simultaneously as total inorganic arsenic, and the organoarsenic species were separated from the inorganic arsenic. This means that there is only one integration necessary which shortens the SOP and may prevent errors in the routine lab. Although previous methods have detected all inorganic arsenic in one peak, but a complete oxidation of As(III) to As(V) was necessary prior to the analysis. The completeness of the oxidation could often not been checked since As(III) was co-eluting with TeMA [25]. Our new method prevents this ambiguity. In addition, the concentration of diammonium hydrogen phosphate was not critical for the chromatographic separation under the operating conditions tested here.

As an optimum experimental setups, the C_{18} ACR column was selected and the eluent containing 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol was flowed at 0.75 mL min^{-1}. Under these conditions, the inorganic arsenic was separated from MMA, DMA and TeMA and determined within approximately 3.5 min.

3.2 Monitoring time

With the object of developing a rapid monitoring method, the flow rate of the eluent was investigated to achieve quick and effective separation of the inorganic arsenic from organic arsenic species, using a C_{18} ACR column (particle size 5 μm, ID 4.6 mm × 250 mm). The inorganic arsenic were well separated from the organoarsenic species at the flow rate range from 0.75 to 1.0 mL min^{-1}, although the separation capacity slightly deteriorated at the flow rate more than 1.1 mL min^{-1}, because the separation factor decrease with increasing the flow rate. Therefore, taking into consideration of chromatographic resolution and measurement rapidity for monitoring, the flow rate of the eluent was set at 1.0 mL min^{-1}, and then the measurement time of HPLC-ICP-MS was 3 min. Each chromatogram of As(III), As(V), MMA, DMA and TeMA at a flow rate of 1.0 mL min^{-1} are shown in Figure S2. Since the actual separation of inorganic arsenic from the other organoarsenicals takes only 1 minute with 2 minutes of void, samples could be injected every minute. This was demonstrated by injecting 3 replicates of one extract which was determined within 6 minutes (see Figure S3). This is a considerable improvement in analysis time.

3.3 Organoarsenic compounds

Agricultural samples occasionally contain very little amounts of TMAO, TeMA, AsB, and

AsC, although MMA and DMA were major organoarsenic compounds in rice flour samples. Therefore, the chromatographic characteristics of TMAO, AsB, and AsC under the operating conditions established above were investigated. When C_{18} ACR column was used, AsB was eluted at the same retention time as MMA, DMA, TMAO and AsC were eluted at the same retention time as TeMA. They were clearly separated from the inorganic arsenic. As the results, the organic arsenic compounds, such as MMA, DMA, TMAO, TeMA, AsB and AsC were well separated from inorganic arsenic.

3.4 Extraction of arsenic species from rice flour samples

A heat assisted technique was applied to the extraction of arsenic species from rice flour samples. The extraction efficiency was investigated using the following four extraction solutions; 0.15 mol L^{-1} HNO_3, 0.28 mol L^{-1} HNO_3, 0.30 mol L^{-1} H_2O_2, and 0.20 mol L^{-1} H_2O_2+0.10 mol L^{-1} HNO_3.[29-30] The certified reference material of NMIJ CRM 7532-a was analyzed. The extracted solutions were measured by HPLC-ICP-MS with the C_{18} ODS column using the eluent containing an ion pair reagent.[28-34] As(III), As(V) and DMA were detected after extraction using 0.15 mol L^{-1} HNO_3 and 0.28 mol L^{-1} HNO_3 (Figure 2). On the other hand, As(V) and DMA, but not As(III), were detected in the extraction solution of 0.30 mol L^{-1} H_2O_2 and 0.20 mol L^{-1} H_2O_2+0.10 mol L^{-1} HNO_3 (Figure 3), because almost all As(III) was oxidized to As(V) during the extraction process.[30] All the extraction efficiencies were approximately 100 %, since the inorganic and total arsenic concentrations were in good agreement with the certified values (Figure 4). The authors reported in the previous report that almost 100 % of the arsenic species were extracted by the heat assisted technique with acidic solvents.[30]

By the proposed method, the total inorganic arsenic is determined HPLC-ICP-MS regardless of As(III) and As(V), since they are eluted at the same retention time using C_{18} ACR column in HPLC. The conversion of As(III) into As(V) is not strictly necessary in the method. However, there are some reports that the detection performances of As(III) and As(V) by ICP-OES and ICP-MS are slightly different each other under some operation conditions. [35-37] The single inorganic species [As(V)] measurements makes the measurement errors smaller than the two species [As(III) and As(V)] measurements and probably increases a precision of the total analysis. The presence of the oxidant H_2O_2 in the extraction solution is required for the oxidization of As(III) during extraction process. H_2O_2 solution by itself was able to extract the arsenic species, but the extracted solution was highly viscous, because the proteins in rice flour were not hydrolyzed. As a result, bothering and lengthy filtration was required before chromatographic analysis. The presence of a small amount of HNO_3 in extraction solution makes the extracted solution smooth and the filtration easy. Therefore,

0.20 mol L^{-1} H_2O_2+0.10 mol L^{-1} HNO_3 was selected as the extraction solvent.

Extraction time is obviously important for a rapid monitoring test. The extraction efficiency of the heat assisted method was investigated at 100 ^{O}C using the following extraction solvents; 0.15 mol L^{-1} HNO_3 and 0.20 mol L^{-1} H_2O_2+0.10 mol L^{-1} HNO_3. Results are shown in Figure 5. The extraction time 0 is non-heating extraction process, and it just stood at room temperature for 2 h. The extraction efficiency of non-heating process for inorganic arsenic was approximately 94 %. The complete extraction of inorganic arsenic was achieved in 15 min at 100 ºC, when 0.20 mol L^{-1} H_2O_2+0.10 mol L^{-1} HNO_3 was used.

The working efficiency of monitoring tests for inorganic arsenic analysis is estimated based on the Codex Alimentarius survey method. The working efficiencies are summarized in the Table 2. The proposed technique will reduce approximately 40% of total measurement time, 50% of Ar consumption of ICP-MS, and 30% of eluent consumption of HPLC, comparing with CODEX Alimentarius survey method.

3.5 Application

The proposed method using C_{18} ACR was applied to the analysis of the CRMs (Figure 6). Analytical results of the inorganic arsenic were in good agreement with the certified values (Table 3).

The certified values of NMIJ CRM 7532-a are as follows; inorganic arsenic 0.298 ± 0.008 mg kg^{-1} and DMA 0.0186 ± 0.0008 mg kg^{-1} as As (the figure following ± indicates is the expanded uncertainty with k=2; k indicates the coverage factor), and those of NMIJ CRM 7533-a are; inorganic arsenic 0.530 ± 0.016 mg kg^{-1} and DMA 0.092 ± 0.004 mg kg^{-1} as As (k=2). Those of NIST SRM are as follows; inorganic arsenic is 0.092 ± 0.010 mg kg^{-1}, DMA 0.180 ± 0.012 mg kg^{-1}, and of MMA 0.0116 ± 0.0035 mg kg^{-1} as As (k=2).

The measurement precision for inorganic arsenic was around 3 %, even when the rice flour containing inorganic arsenic was less than 0.1 mg kg^{-1} (5 ng g^{-1} As in a measurement solution: 0.1 mg kg^{-1} × 0.5g / 10g).

4. Conclusions

A rapid monitoring method for inorganic arsenic in rice flour was achieved with HPLC-ICP-MS using C_{18} ACR ODS column. By the proposed method, As(III) and As(V) were completely separated from organic arsenic compounds and eluted at the same chromatographic retention times using simple component eluent, and thus they were detected as the inorganic arsenic at a time. Therefore, it was possible to measure inorganic arsenic selectively, easily and quickly. Moreover, the simple and dilute one component of the chromatographic eluent substantially reduces the deterioration of analytical performances of

columns and a detector of HCPLC-ICP-MS. Prior to HPLC-ICP-MS determination, the heat assisted extraction was applied to the extraction of arsenic compounds from rice flour. The H_2O_2+HNO_3 extraction solvent can extract arsenic compounds efficiently and oxidize As(III) to As(V) during the extraction process. It facilitates a reliable separation and determination of arsenic species for HPCL-ICP-MS, since it hydrolyzed proteins in extraction solution.

The proposed method requires a single standard solution, As(V), for inorganic arsenic determination, since the As(III) and As(V) are measured as a single peak of the total inorganic arsenic and Almost all As(III) is oxidized to As(V) during the extraction process. Low concentration levels of As(III) and As(V) in standard solutions sometimes interchange by oxidation-reduction reactions and their detection sensitivities are sometimes different in ICP-MS measurement. Those events can lead us to measurement errors when As(III) and As(V) are determined. No error caused by As(III) - As(V) interchange can occur in the proposed method.

The presented method was developed specifically for the rapid monitoring of inorganic arsenic in rice flour. It is also useful for monitoring inorganic arsenic in other environmental and food samples. The accurate evaluation methods might be necessary when the concentration of inorganic arsenic in a sample is very close to the tolerance values.

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Table 1 Effect of carbon length of ODS column on retention times of arsenic species.

Carbon length	C_8	C_{18}	C_{28}	C_{30}	C_{18} ACR
	Retention Time (min)				
As(III)	4.30	3.26	3.78	3.76	2.79
(peak start)	(4.11)	(3.06)	(3.62)	(3.62)	(2.70)
As(V)	4.20	3.22	3.73	3.74	2.79
(peak start)	(4.04)	(3.04)	(3.62)	(3.60)	(2.70)
MMA	4.34	3.52	4.20	4.15	2.98
DMA	4.53	3.73	4.38	4.33	3.03
TeMA	4.79	3.68	4.22	4.18	2.69

Flow rate: 0.75 mL min^{-1}.

Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate buffer / 0.05 % methanol (pH 2.0 by HNO_3)

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480 Table 2 Working efficiency for monitoring test of inorganic arsenic in rice flour samples.

Content	Step	Codex*	US FDA	Proposed method
ANALYTICAL TIME				
Sample pretreatment				
	Sample taken (e.g. *n*=20)**	20 min	20 min	20 min
	Addition of extracting solvent**	10 min	10 min	10 min
	Heating time (Extraction)	120 min	90 min	15 min
	Cooling time**	30 min	30 min	30 min
	Centrifugation time**	10 min	10 min	10 min
	Filtration time (*n*=20)**	20 min	20 min	20 min
	Dilution (*n*=20)	100 min	100 min	60 min
Calibration standards				
	Preparation of standard solution** (e.g. four points, three step dilution)	60 min	60 min	60 min
Measurement				
	Runtime (*n*=24) (samples + standards)	6 min × 24 = 144 min	15 min × 24 = 360 min	3 min × 24 =72 min
	Total analytical time (min)	514 min	700 min	297 min
	Efficiency factor (based on the Codex)	1.00	1.36	0.58
Equipment				
	Ar gas consumption of ICP-MS	20 L/min × 144 min = 2880 L	20 L/min × 360min = 7200 L	20 L/min × 72 min = 1440 L
	Efficiency factor (based on the Codex)	1.00	2.50	0.50
	Eluent consumption of LC system	0.75 mL/min * 144 min = 108 mL	1.0 mL/min * 360 min = 360 mL	1.0 mL/min * 72 min = 72 mL
	Efficiency factor (based on the Codex)	1.00	3.33	0.67

* Codex Alimentarius survey method, ** Common factor

Except a dry mass correction factor experiment

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Table 3 Inorganic arsenic (i-As) in rice flour samples obtained by the proposed method with C_{18} ACR column (mg kg^{-1}).

	Extraction 1	Extraction 2
NMIJ CRM 7532-a		
Certified value of i-As	0.298 ± 0.008 (*k*=2)	
Result*	0.295 ± 0.002	0.296 ± 0.002
NMIJ CRM 7533-a		
Certified value of i-As	0.530 ± 0.016 (*k*=2)	
Result*	0.522 ± 0.007	0.528 ± 0.007
NIST SRM 1568b		
Certified value of i-As	0.092 ± 0.010 (*k*=2)	
Result*	0.093 ± 0.003	0.094 ± 0.003

Extraction time, 1: 15 min, 2: 30 min

Extraction conditions: Temperature 100 ºC, Extracting solvent: 0.2 M H_2O_2 + 0.1 M HNO_3

*Mean ± SD (*n*=3)

Figure Captions

Figure 1 Retention times of arsenic species according to carbon length of ODS column.
Eluent: 0.01 M HNO_3 / 0.05 % methanol, Flow rate: 0.75 mL min^{-1}.

Figure 2 Chromatogram of the arsenic species in brown rice flour following extraction with solvent of 0.15 mol L^{-1} HNO_3.
Column: C_{18} ODS (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 10 mmol L^{-1} sodium 1-butanesulfonate / 4 mmol L^{-1} malonic acid / 4 mmol L^{-1} tetramethylammonium hydroxide / 0.05 % methanol (pH 3.0), Flow rate: 0.75 mL min^{-1}, Sample; NMIJ CRM 7532-a, Peaks from the left: 1st peak As(V), 2nd peak As(III), 3rd peak DMA.

Figure 3 Chromatogram of the arsenic species in brown rice flour following extraction with solvent of 0.30 mol L^{-1} H_2O_2.
Column: C_{18} ODS (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 10 mmol L^{-1} sodium 1-butanesulfonate / 4 mmol L^{-1} malonic acid / 4 mmol L^{-1} tetramethylammonium hydroxide / 0.05 % methanol (pH 3.0), Flow rate of 0.75 mL min^{-1}, Sample; NMIJ CRM 7532-a, Peaks from the left: 1st peak As(V), 2nd peak DMA.

Figure 4 Influence of solvents on extraction efficiencies.
◆ Inorganic arsenic (i-As), □ Total arsenic(total As), Certified: Certified values of inorganic arsenic and total arsenic (k=1), i-As 1 and total As 1: 0.15 mol L^{-1} HNO_3 extraction, i-As 2 and total As 2: 0.28 mol L^{-1} HNO_3 extraction, i-As 3 and total As 3: 0.30 mol L^{-1} H_2O_2 extraction, i-As 4 and total As 4: 0.20 mol L^{-1} H_2O_2 + 0.10 mol L^{-1} HNO_3 extraction, Sample: NMIJ CRM 7532-a brown rice, Extraction; temperature 100 ^{o}C, time 2 h, Column: C_{18} ODS ACR (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol, Flow rate; 0.75 mL min^{-1}.

Figure 5 Effect of extraction time on extraction efficiency.
◆ 0.15 mol L^{-1} HNO_3 extraction, ■ 0.20 mol L^{-1} H_2O_2 + 0.10 mol L^{-1} HNO_3 extraction, Sample: NMIJ CRM 7532-a brown rice,
Extraction temperature: 100 ^{o}C, 0*: Non-heating extraction at room temperature for 2 h.

Figure 6 Chromatogram of the arsenic species in NMIJ CRM 7533-a and NIST SRM 1568b rice flour.

Column: C_{18} ODS ACR (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol at a flow rate of 0.75 mL min^{-1} and 1.0 mL min^{-1} (proposed optimized condition), Extracting solvent: 0.15 mol L^{-1} HNO_3, Peaks from the left: 1st peak i-As, 2nd peak Organoarsenic species.

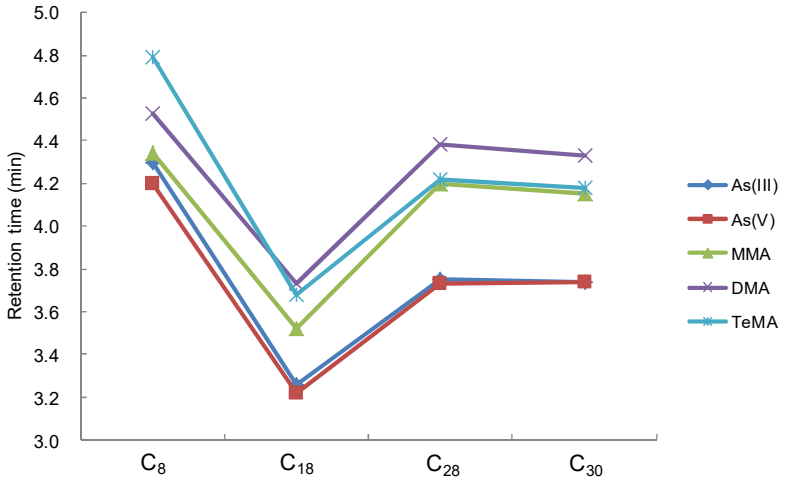


Figure 1 Retention times of arsenic species according to carbon length of ODS column. Eluent: 0.01 M HNO_3 / 0.05 % methanol, Flow rate: 0.75 mL min^{-1}.

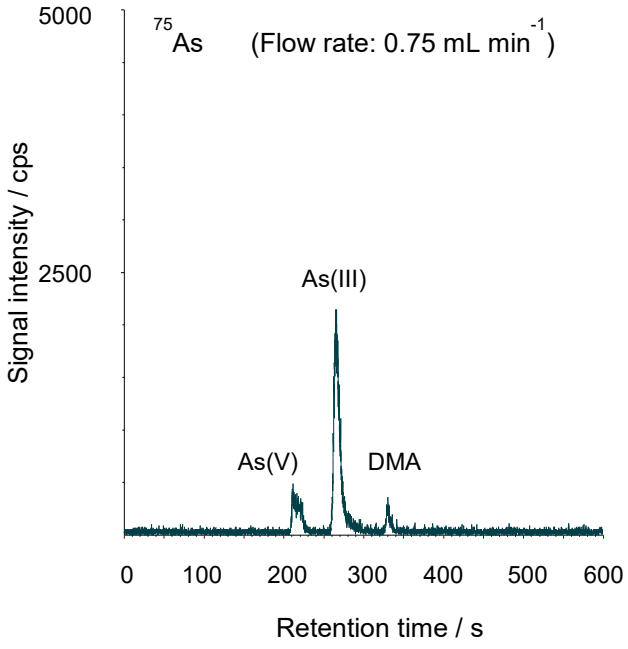
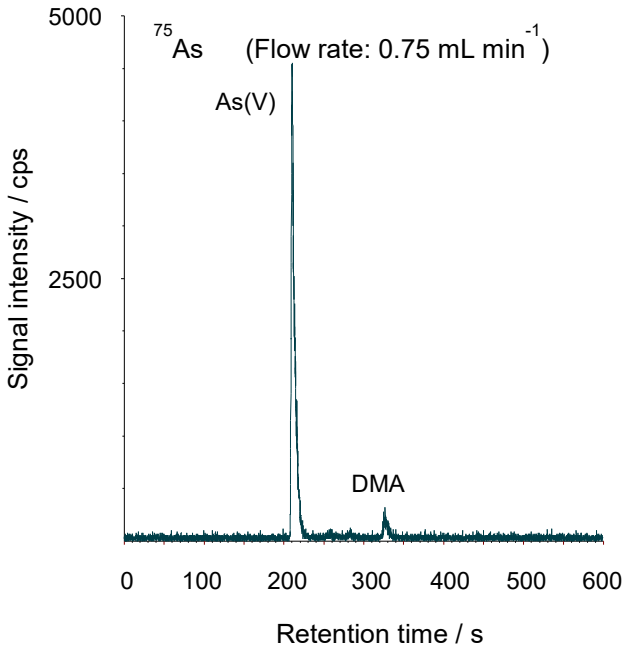


Figure 2 Chromatogram of the arsenic species in brown rice flour following extraction with solvent of 0.15 mol L^{-1} HNO_3.

Column: C_{18} ODS (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 10 mmol L^{-1} sodium 1-butanesulfonate / 4 mmol L^{-1} malonic acid / 4 mmol L^{-1} tetramethylammonium hydroxide / 0.05 % methanol (pH 3.0), Flow rate: 0.75 mL min^{-1}, Sample; NMIJ CRM 7532-a, Peaks from the left: 1st peak As(V), 2nd peak As(III), 3rd peak DMA.



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Column: C_{18} ODS (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 10 mmol L^{-1} sodium 1-butanesulfonate / 4 mmol L^{-1} malonic acid / 4 mmol L^{-1} tetramethylammonium hydroxide / 0.05 % methanol (pH 3.0), Flow rate of 0.75 mL min^{-1}, Sample; NMIJ CRM 7532-a, Peaks from the left: 1st peak As(V), 2nd peak DMA.

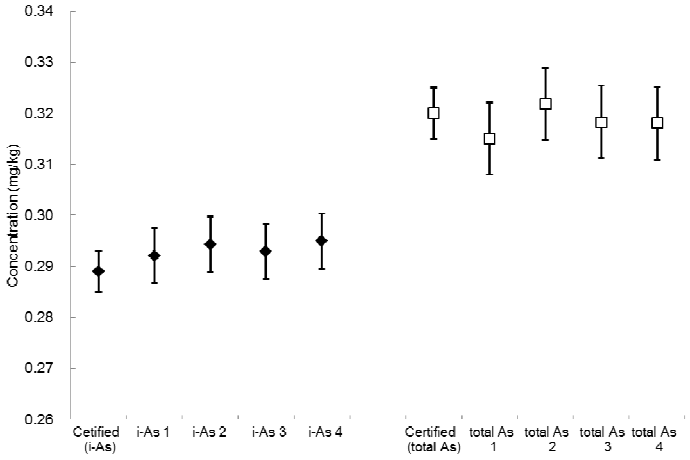

Figure 4 Influence of solvents on extraction efficiencies.

◆ Inorganic arsenic (i-As), □ Total arsenic(total As), Certified: Certified values of inorganic arsenic and total arsenic (k=1), i-As 1 and total As 1: 0.15 mol L^{-1} HNO_3 extraction, i-As 2 and total As 2: 0.28 mol L^{-1} HNO_3 extraction, i-As 3 and total As 3: 0.30 mol L^{-1} H_2O_2 extraction, i-As 4 and total As 4: 0.20 mol L^{-1} H_2O_2 + 0.10 mol L^{-1} HNO_3 extraction, Sample: NMIJ CRM 7532-a brown rice, Extraction; temperature 100 ^{o}C, time 2 h, Column: C_{18} ODS ACR (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol, Flow rate; 0.75 mL min^{-1}.

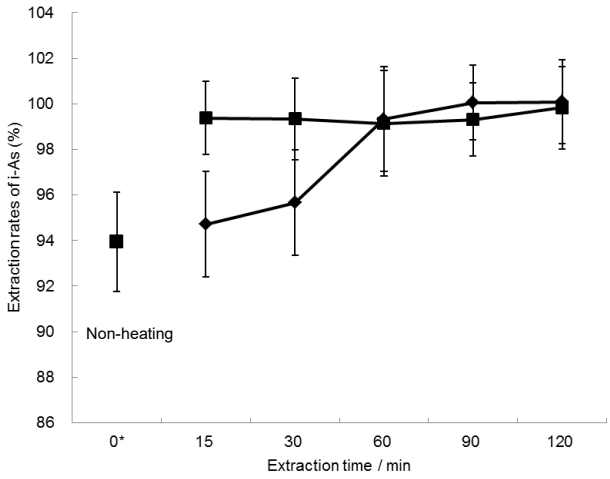

Figure 5 Effect of extraction time on extraction efficiency.

◆ 0.15 mol L^{-1} HNO_3 extraction, ■ 0.20 mol L^{-1} H_2O_2 + 0.10 mol L^{-1} HNO_3 extraction, Sample: NMIJ CRM 7532-a brown rice, Extraction temperature: 100 ^{O}C, 0*: Non-heating extraction at room temperature for 2 h.

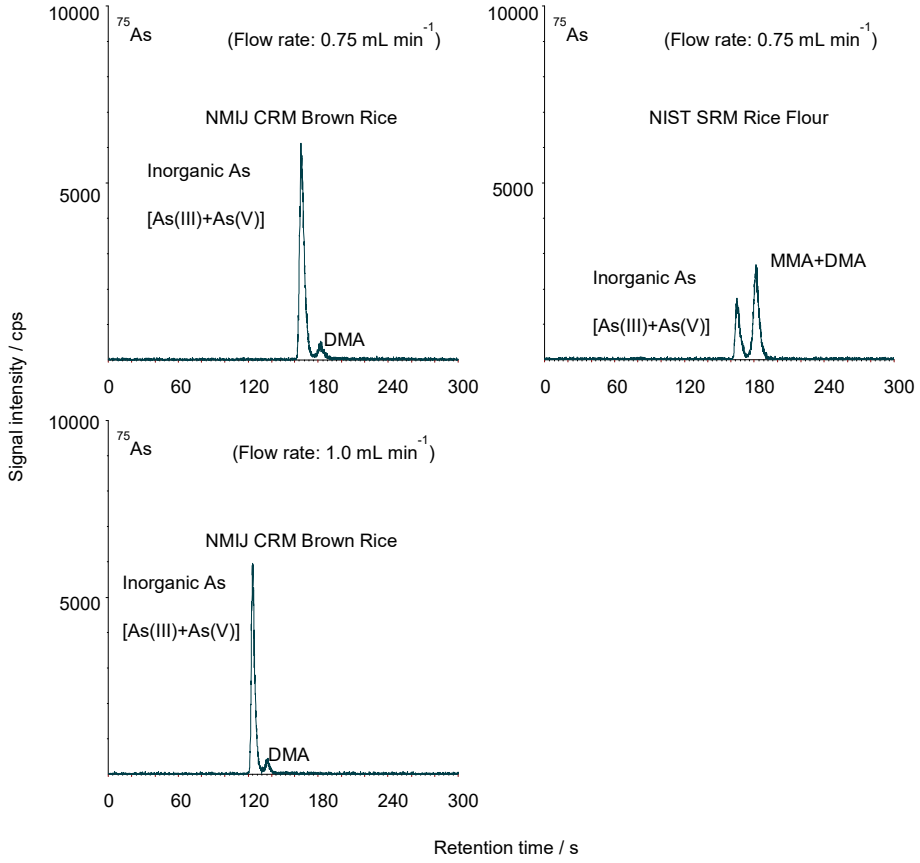

Figure 6 Chromatogram of the arsenic species in NMIJ CRM 7533-a and NIST SRM 1568b rice flour. Column: C_{18} ODS ACR (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol at a flow rate of 0.75 mL min^{-1} and 1.0 mL min^{-1} (proposed optimized condition), Extracting solvent: 0.15 mol L^{-1} HNO_3, Peaks from the left: 1st peak i-As, 2nd peak Organoarsenic species.

Supplementary material

Table S1 Measurement parameters and its analytical performances.

ICP-MS Agilent 7500c		
Plasma conditions:		
	Incident Rf power	1600 W
	Reflected power	< 2 W
	Outer gas flow rate	Ar 15 L/min
	Intermediate gas flow rate	Ar 0.9 L/min
	Carrier gas flow rate	Ar 0.8 L/min
	Make-up gas flow rate	Ar 0.4 L/min
Sampling conditions:		
	Nebulizer	Glass 100 μL (natural aspirate)
	Spray chamber	Scott type (2 ºC)
	Sample depth	7 mm from work coil
Collision / reaction mode:		
	Calibration method	He 3.0 mL/min
Data acquisition:	Dwell time	20 ms / point
Measured isotopes (*m*/*z*):		^{75}As
HPLC conditions:		
	Column	ODS columns (particle size of the filler 5 μm, ID 4.6 mm x 250 mm)
	Eluent	1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol
	Flow rate	0.75 mL min^{-1}
	Injection volume	20 μL
Analytical performances:		
	Limit of detection (3 σ)	0.01 ng g^{-1} (as As)
	limit of quantitation (10 σ)	0.03 ng g^{-1} (as As)
	Analytical precision	3 % for 5 ng g^{-1} As, 10 % for 1 ng g^{-1}

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772 Table S2: Tested columns (Column group II)

Column	Production	Typical separation mode	Particle size of filler	Column size (mm) Inside diameter × Length
PRP-X100	Hamilton	Anion-exchange	10 μm	4.1 x 150
IonPac CS12A	Dionex	Cation-exchange		3.0 x 150
PRP-X300	Hamilton	Ion-exclusion	10 μm	4.1 x 150
RSpak NN-414	Showa Denko K.K.	Reversed phase		4.6 x 150
RSpak NN-614	Showa Denko K.K.	Reversed phase		6.0 x 150
CAPCELL CORE	Shiseido Co., Ltd.	Reversed phase	2.7 μm	4.6 x 100
CAPCELL PAK ADAM S3	Shiseido Co., Ltd.	Reversed phase	3 μm	4.6 x 50
CAPCELL PAK C27 AQ	Shiseido Co., Ltd.	Reversed phase	2.7 μm	4.6 x 100
Shim-pack VP-ODS C18	Shimadzu Co.	Reversed phase	5 μm	4.6 x 250
Sunrise C18 SAC	ChromaNik Technologies Inc.	Reversed phase	5 μm	4.6 x 250

*For comparison study, typical column conditions were selected the particle size of filler 5 μm and the column size ID 4.6 mm × 250 mm. But, some columns are not on the market: therefore, the different size columns were controlled by its column pressure.

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774 Table S3: Components of the tested eluents for column group II

Component of eluent	Concentration range (mmol L^{-1})	Organic solvent (Methanol %)	pH range
HNO_3	1 to 10	0 to 0.5	-----
H_2SO_4	1 to 10	0 to 0.5	-----
Malonic acid	1 to 5	0 to 0.5	2 to 4
Malonic acid / TMAH	1 to 5	0 to 0.5	2 to 4
$NH_4H_2PO_4$	1 to 20	0 to 0.5	2 to 8
$(NH_4)_2HPO_4$	1 to 20	0 to 0.5	2 to 8
Prigine	5 to 20	0 to 0.5	2 to 4
NH_4NO_3	5 to 20	0 to 0.5	2 to 4

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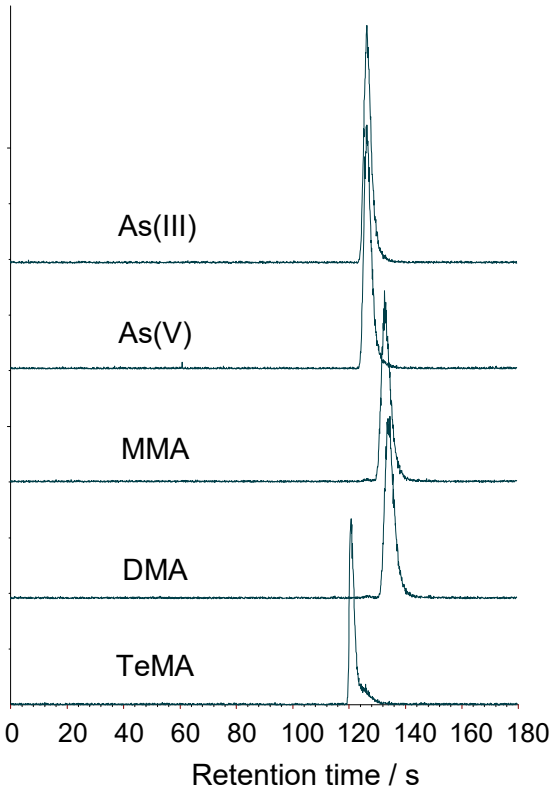

Figure S1: Chromatogram of each arsenic species under the proposed condition.
Column: C_{18} ODS ACR (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol at a flow rate of 1.0 mL min^{-1}.

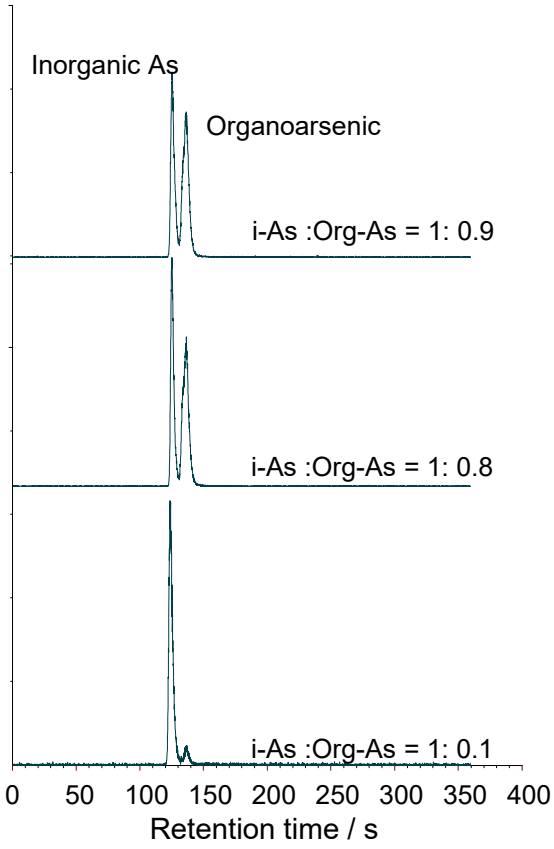

Figure S2: Chromatogram of the arsenic species with different concentrations.
Column: C_{18} ODS ACR (particle size 5 µm, 250 mm x ID 4.6 mm), Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol at a flow rate of 1.0 mL min^{-1} (proposed optimized condition), i-As: As(III)+As(V), Organoarsenic: MMA+DMA.
The peak resolutions in Figure S2 are 1.35 to 1.40. In general, it is said that complete separation is more than 1.5: therefore, approximately 0.2 % may be overlapping each other.

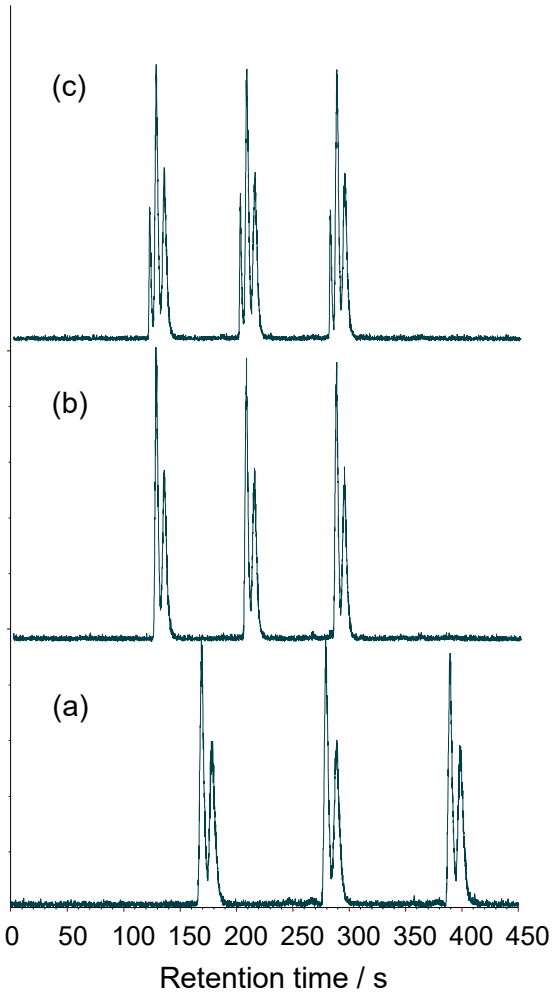

Figure S3: Demonstration results of the rapid monitoring by the proposed method.
Injection interval time: 60 s, Replication: 3, Monitoring time: 450 s, Column: C_{18} ODS ACR (particle size 5 μm, 250 mm x ID 4.6 mm), Eluent: 1 mmol L^{-1} diammonium hydrogen phosphate (pH 2.0) / 0.05 % methanol, (a) 1st peak As(III)+As(V), 2nd peak MMA+DMA at a flow rate of 0.75 mL min^{-1}, (b) 1st peak As(III)+As(V), 2nd peak MMA+DMA at a flow rate of 1.0 mL min^{-1} (proposed condition), (c) 1st peak TeMA, 2nd peak As(III)+As(V), 3rd peak MMA+DMA at a flow rate of 1.0 mL min^{-1} (proposed condition).