Application Note
Food and animal feed

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Fast Determination of Inorganic Arsenic (iAs) in Food and Animal Feed by HPLC-ICP-MS

Method compliant with EU regulations and in accordance with two CEN standards on the analysis of iAs

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Introduction

It is well known that the two inorganic forms of arsenic (iAs), arsenite, As(III), and arsenate, As(V), are more toxic than the organo-arsenic species (*1*). The difference in the toxicity of As species is why regulations in many countries specify the need to determine the iAs content of food and feedstuffs. For example, European (EU) regulation 1881/2006 (*2*) includes maximum levels of iAs in rice and rice products—between 0.1 and 0.3 mg/kg. EU directive 2002/32/EC (*3*) states that the iAs content must be lower than 2 mg/kg in certain feedstuffs, such as feeds that contain seaweed. The European Commission is expected to establish maximum levels for iAs in other food commodities, especially foods that are widely consumed such as drinking water, milk, and dairy products. These foods have the potential to contribute more to the total dietary uptake of iAs.

HPLC-ICP-MS is widely used for As speciation studies. However, the separation of As species typically requires a long column, resulting in measurement times

greater than 10 minutes. To shorten the measurement time significantly, a fast HPLC-ICP-MS method was developed by Jackson (*4*) and further modified by Gray *et al* (*5*). These methods have been used successfully for the analysis of iAs in rice (*6*) and wine samples (*7*).

To optimize a routine method that is suitable for official control of iAs in food and feedstuffs, we based this work on those previous studies. The method also needed to be compliant with current European regulations and meet the analytical requirements of two CEN standards on the determination of iAs in food and feed. The two CEN standards are EN16802:2016 (food) (*8*) and prEN17374:2019 (animal feed) (*9*). The sample preparation guidelines stated in the two CEN standards specify oxidation of As(III) to As(V) during the extraction procedure, which simplifies the determination of iAs in foods and animal feeds.

In this study, the chromatographic conditions were optimized to separate iAs (as As(V)) from dimethylarsinic acid (DMA), and monomethylarsonic acid (MA) in under two minutes. Method optimization and evaluation were performed using an Agilent 1260 HPLC coupled to an Agilent 8900 Triple Quadrupole ICP-MS (ICP-QQQ). The method can also be run using a single quadrupole ICP-MS, as the ICP-QQQ was operated in single quadrupole mode with helium (He) as a collision gas. The analytical performance of the method was tested, including limits of detection (LOD) and quantification (LOQ), linearity, spike recoveries, precision, and accuracy. The method was validated with suitable beverage, food, and animal feed reference materials (RMs).

Experimental

Reagents and standards

The As(V) standard solution for ICP-MS was bought from SCP Science (Canada). The As(III) solution and DMA salt were bought from Sigma Aldrich (USA), and MA was bought from Santa Cruz Biotechnology (USA). The arsenobetaine (AB) was a certified reference material (ERM-AC626) that was bought from the Institute for Reference Materials and Measurements (IRMM, Belgium). For the mobile phase, ammonium carbonate ($(NH_4)_2CO_3$, trace metal grade 99.999%, Alfa Aesar, USA), and methanol (HPLC grade from VWR Chemicals, USA) were used.

As(V) calibration standards (representing iAs) were prepared in duplicate between 0.05 and 50 µg/L using the mobile phase as the diluent. MA and DMA were also added to the calibration standards to check the separation of the iAs peak from the organic compounds. The separation of the peak of the AB standard from the iAs peak was also tested. The As(III) standard was used to demonstrate the quantitative oxidation to As(V) during the sample preparation step (data not shown).

Reference materials and samples

A list of samples and RMs used in this study is given in Table 1. Some of the iAs concentrations in the RMs were certified, reference values, or values established by collaborative trials and proficiency tests.

Table 1. Overview of samples and reference materials used in the study.

Sample/RM (Full name)	Sample/RM (Short name[a])	iAs Conc (mg/kg)	Comments
Water from Plastic Bottle (Commercial Sample)	Bottled water	-	-
Apple Juice (Commercial Sample)	Apple juice	-	-
Rice (ERM-BC211)	Rice	0.124 ± 0.011[b]	Certified value
White Rice Flour (NMIJ CRM 7503-b)	White rice flour	0.153 ± 0.010[b]	Certified value
Brown Rice Flour (NMIJ CRM 7533-a)	Brown rice flour	0.530 ± 0.016[b]	Certified value
Leek (DTU Food)	Leek	0.086 ± 0.012[c]	Collaborative trial
Chili Powder (FAPAS T07288QC)	Chili powder	0.775 ± 0.024[d]	Proficiency test
Bovine Liver (ERM-BB185)	Bovine liver	-	-
Fish Protein (NRCC-DORM-4)	Fish protein	0.27 ± 0.038[c]	Collaborative trial
Blue Mussels (DTU Food)	Blue mussels	0.33 ± 0.049[c]	Collaborative trial
Complete Marine Based Feed (DTU Food)	Marine based feed	0.802 ± 0.122[c]	Collaborative trial
Mixed Corn Poultry Feed (FAPAS)	Mixed corn poultry feed	0.299 ± 0.010[d]	Proficiency test
Kelp Powder (NIST SRM 3232)	Kelp powder	0.247 ± 0.019[e]	Reference value
Hijiki (NMIJ 7405a)	Hijiki	10.1 ± 0.5[b]	Certified value

[a] *... Short names of samples and RMs are used in the document*
[b] *... Expanded uncertainty with the coverage factor k = 2 (level of confidence of approx. 95%)*
[c] *... Standard deviation of all the results from collaborative trial (duplicates from different laboratories)*
[d] *... Uncertainty determined in proficiency test based on standard deviation of all the results and number of participants*
[e] *... Expanded uncertainty with the coverage factor k = 2.36*

Sample preparation

The sample preparation guidelines stated in the two CEN standards were followed (*8, 9*). For all solid samples, 0.1 M HNO_3 in 3% (v/v) H_2O_2 was used as the extraction solution. Approx. 0.2 g of solid sample was mixed with 10 mL of the extraction solution. To obtain the same acid concentration after mixing with the sample at a ratio of 1:1, a more concentrated extraction solution was prepared for liquid samples. 5 mL of liquid sample was mixed with 5 mL

of the more concentrated extraction solution (0.2 M HNO_3 in 6% (v/v) H_2O_2). Samples were heated in a water bath at 90 °C for 60 min. The extracts were then centrifuged for 10 min at 4000 rpm. The supernatants were transferred into filter vials (0.45 µm pore size). The vials containing the extracts were refrigerated at 4 °C before analysis. Because the Hijiki sample had a high iAs content (10.1 ± 0.5 mg/kg), the extract was further diluted 20-fold with the extraction solution and filtered, before analysis.

Instrumentation

An Agilent 1260 HPLC with a binary pump was coupled to an Agilent 8900 ICP-QQQ. The HPLC was fitted with a PRP-X100 (5 µm, 50 x 2.1 mm) column and matching PRP-X100 analytical guard column (Hamilton Company, USA). The 8900 ICP-QQQ was equipped with a standard sample introduction system comprising a glass concentric nebulizer, quartz spray chamber, quartz torch with 2.5 mm i.d. injector, and nickel-tipped interface cones. The ICP-QQQ was operated in single quadrupole mode. Helium was used as a collision gas in the ORS^4 collision/reaction cell (CRC) to remove the potential interference from $^{40}Ar\ ^{35}Cl$ on ^{75}As. Although the 8900 offers higher sensitivity and lower detection limits than single quadrupole ICP-MS, the method could be run on the Agilent 7800 or 7900 ICP-MS, which are also fitted with an ORS^4. The instrument operating conditions are summarized in Table 2.

Table 2. HPLC-ICP-MS operating conditions.

ICP-MS	
RF Power (W)	1550
Nebulizer Gas (L/min)	1.06
Sampling Depth (mm)	8
Spray Chamber Temperature (°C)	2
Peristaltic Pump Speed (rps)	0.5[a]
Scan Mode	Single Quad
He Cell Gas Flow (mL/min)	3.5
Monitored *m/z*	^{75}As, ^{35}Cl
HPLC	
Column Temperature	Ambient
Injection Volume (µL)	5
Mobile Phase	40 mM ammonium carbonate in 3% methanol, pH 9
Elution	Isocratic
Mobile Phase Flow (mL/min)	0.65
Run Time (min)	5[b]

[a] … *Used for drain only*
[b] … *Run time was set to 5 min to make sure that no other compounds eluted after the iAs peak. Figures 1 and 2 do not show the entire chromatogram*

Results and discussion

Optimization of a fast method

The method was based on the approach used in two previous studies (*4, 5*). The relatively short anion exchange column was packed with small sized particles, allowing the use of a higher mobile phase flow rate and low volume injections.

Different mobile phase flow rates of 0.45, 0.5, 0.55, and 0.65 mL/min were tested. The fastest elution of arsenic compounds was achieved at 0.65 mL/min, without compromising the separation of the peaks. Higher flow rates were not tested since leaks were observed, due to the high backpressure. Since As(III) was oxidized to As(V), fewer As species were determined, allowing a stronger mobile phase to be used.

The chromatographic method achieved baseline separation of iAs from other arsenic species in less than two minutes, without compromising resolution, as shown in Figures 1 and 2. Figure 1 shows overlaid chromatograms of the individual arsenic species standards and Figure 2 shows overlaid chromatograms for a representative calibration set of standards from 0.05 to 50 µg/L. As shown, iAs (as As(V)) was well separated from the other As species.

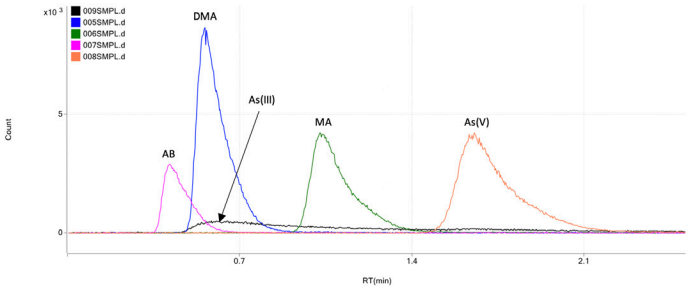

Figure 1. Overlaid chromatograms of individual As species standards.

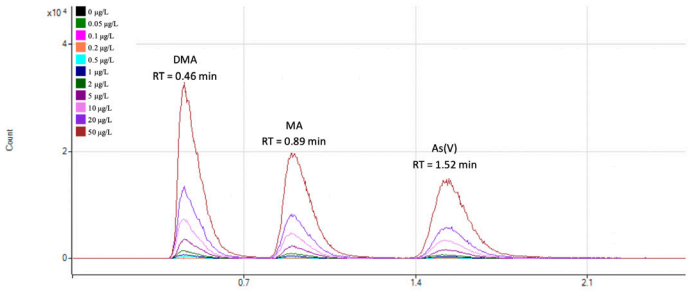

Figure 2. Overlaid chromatograms of calibration standards between 0.05 and 50 µg/L prepared in the mobile phase.

Helium was used as a collision gas in the ICP-MS to remove the potential interference from $^{40}Ar^{35}Cl^+$ on $^{75}As^+$. To check the retention times (RT) of Cl^+ and As^+, both ions were monitored at *m/z* 35 and *m/z* 75, respectively. Figure 3 shows that the RT of the chloride peak (black chromatogram) is distinct from the As(V) peak (green chromatogram), which complies with one of the requirements listed in EN16802:2016 and prEN17374:2019. The absence of a peak at the RT of Cl in the green chromatogram (although MA partly overlaps the Cl peak) suggests that $^{35}Cl^{40}Ar^+$ was removed in He mode.

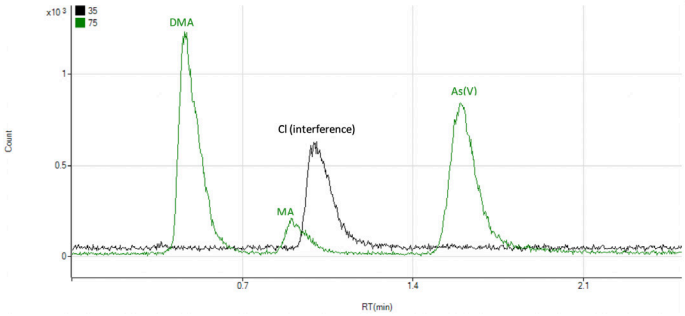

Figure 3. Chromatogram of Rice ERM-BC211 showing the RT of As species (green chromatogram) and the chloride peak (black chromatogram).

Linear calibration

The calibration curve for iAs (as As (V)) showed good linearity, as shown in Figure 4.

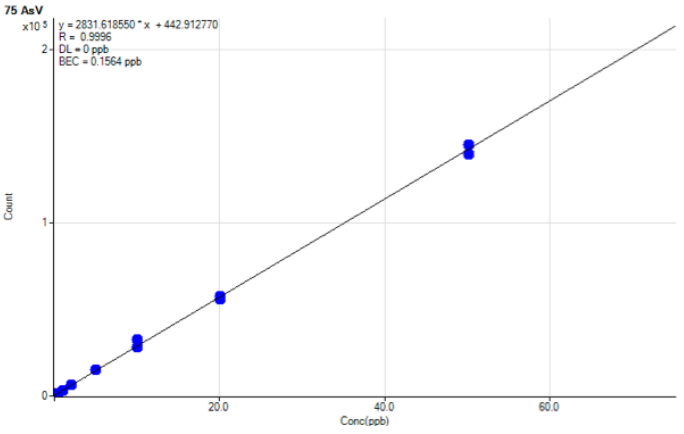

Figure 4. Calibration curve for iAs (represented by As(V)).

Limits of detection and quantification

To evaluate the LOD and LOQ, 10 blank samples of the extraction solution were analyzed. The iAs peak of the As contaminant in the blank samples was integrated and the LOD and LOQ in solution were calculated using a 3-fold and 10-fold standard deviation, respectively. The LODs and LOQs in the samples were also calculated. The values for the solid samples are based on a test portion size of 0.2 g extracted with 10 mL of the extraction solution. The values for the liquid samples are based on 5 mL of sample diluted to 10 mL with the extraction solution. All LODs and LOQs are given in Table 3.

Table 3. LODs and LOQs for iAs calculated in solution, and in the solid and liquid samples.

	iAs in Solution (µg/L)	iAs in Solid Sample (µg/kg)	iAs in Liquid Sample (µg/L)
LOD	0.040	1.99	0.08
LOQ	0.133	6.64	0.27

The LOQ value for solid samples of 6.64 µg/kg (0.0066 mg/kg) satisfies the European legislation for iAs determination in food (rice) of < 0.04 mg/kg (*10*). The LOQ is also well below the maximum iAs value of 2 mg/kg specified in European regulations for animal feed (*3*). The sensitivity of the method is sufficient for official control of iAs in food and feedstuffs.

Spike recoveries and evaluation of matrix effects

Several samples were spiked with iAs at two different concentration levels to evaluate potential matrix effects. Bottled water, apple juice, and bovine liver were selected as "unknown" samples, since no prior information on the iAs content was available for these samples. The iAs concentration of the two other spiked samples (leek and blue mussels) was obtained from collaborative trials.

Table 4 shows the spike recoveries were within 100 ± 10% range for all samples apart from apple juice. The recoveries for apple juice were outside the 100 ± 10% range for both the low and high concentration spike levels, indicating matrix effects.

Table 4. Spike recoveries of iAs in selected samples.

Sample	Low Spike Concentration (µg/L)	Spike Recovery (%)	High Spike Concentration (µg/L)	Spike Recovery (%)
Bottled Water	0.5	109 ± 9	1	90.0 ± 0.6
Apple Juice	0.5	119 ± 1	1	111 ± 1
Bovine Liver	0.5	110 ± 3	1	104 ± 5
Leek	2	102 ± 10	5	106.7 ± 0.7
Blue Mussels	5	95 ± 16	10	96 ± 4

The slope of the external calibration curve (solid line) was compared with the slopes of the standard addition curves (dotted lines; Figure 5). The trend lines for the standard addition curves are shown to help the comparison. For most of the samples, the difference in the slope between the external calibration curve and the standard addition curve was lower than 10%. The small difference suggests only minor matrix effects for these matrices, so external calibration can be used for the quantification of iAs in these samples. However, the difference in the two slopes for apple juice was higher than 20%, so standard addition is required for the quantification of iAs in apple juice.

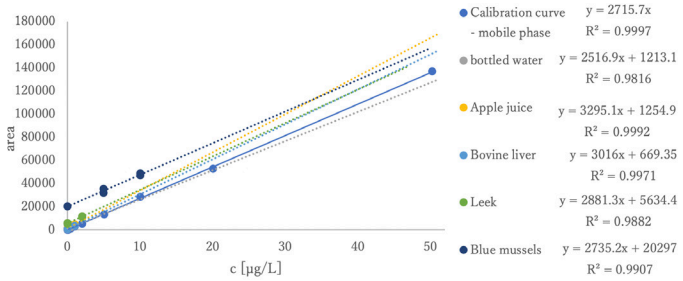

Figure 5. Comparison of the slope of the external calibration curve with the slope of the standard addition curve.

Precision and accuracy

All samples and RMs were prepared in triplicate and analyzed on the same day. The results of the three replicates were used to evaluate the precision of the method, which is reported as relative standard deviation (RSD). To evaluate the accuracy of the method, the average of the three replicates was compared to an RM target value. The results are reported as recovery.

The measured results for iAs in different samples are shown in Table 5. Recoveries were calculated using external calibration curves prepared in the mobile phase, as well as with the standard addition curves generated for selected samples. The original calibration for iAs was updated periodically through the sequence by running a QC check standard.

Precision of the measurements ranged from 0.3 to 9.4% RSD and iAs recoveries ranged from 81.8 to 110.7% of their certified, reference, or informative values. The results show that the method is suitable for the analysis of the different drinks, food, and feed samples analyzed in this study.

Table 5. Quantitative results for iAs content in samples and RMs with %RSD and recoveries.

		External Calibration Curve			Standard Addition	
Sample/RM	Target Value (µg/kg)	Measured Conc (µg/kg)	RSD (%)	Recovery (%)	Measured Conc (µg/kg)	Recovery (%)
Bottled Water[a]	-	0.81 ± 0.08[b]	9.4	-	0.96[b]	-
Apple Juice[a]	-	0.85 ± 0.06[b]	6.8	-	0.76[b]	-
Rice	124 ± 11	119 ± 3	2.1	96 ± 2	-	-
White Rice Flour	153 ± 10	151 ± 2	1.0	99 ± 1	-	-
Brown Rice Flour	530 ± 16	496 ± 9	1.8	94 ± 2	-	-
Leek	86 ± 12	95 ± 2	1.8	111 ± 2	87	101.6
Chilli Powder[a]	775 ± 24	860 ± 30	3.6	111 ± 4	-	-
Bovine Liver	-	12.5 ± 0.8	6.5	-	12	-
Fish Protein	270 ± 38	272.4 ± 0.8	0.3	100.9 ± 0.3	-	-
Blue Mussels	330 ± 49	337 ± 4	1.3	102 ± 1	350	106.1
Marine Based Feed[a]	802 ± 122	650 ± 30	4.1	82 ± 3	-	-
Mixed Corn Poultry Feed[a]	299 ± 10	293 ± 5	1.7	98 ± 2	-	-
Kelp Powder	247 ± 19	230 ± 20	7.5	94 ± 7	-	-
Hijiki	10100 ± 500	8800 ± 600	7.0	88 ± 6	-	-

[a] *... no dry matter correction*
[b] *... in µg/L*

Figure 6 shows chromatograms of several of the sample matrices. Each chromatogram meets the requirements listed in the EN16802:2016 and prEN17374:2019 standard methods, which state that the chromatograms must show:

1. Selective separation of arsenate (As(V)) from the other arsenic compounds.
2. As(V) is separated from the nearest peak by a full peak width at 10% peak height.
3. As(V) is separated from the chloride peak (as shown in Figure 3).

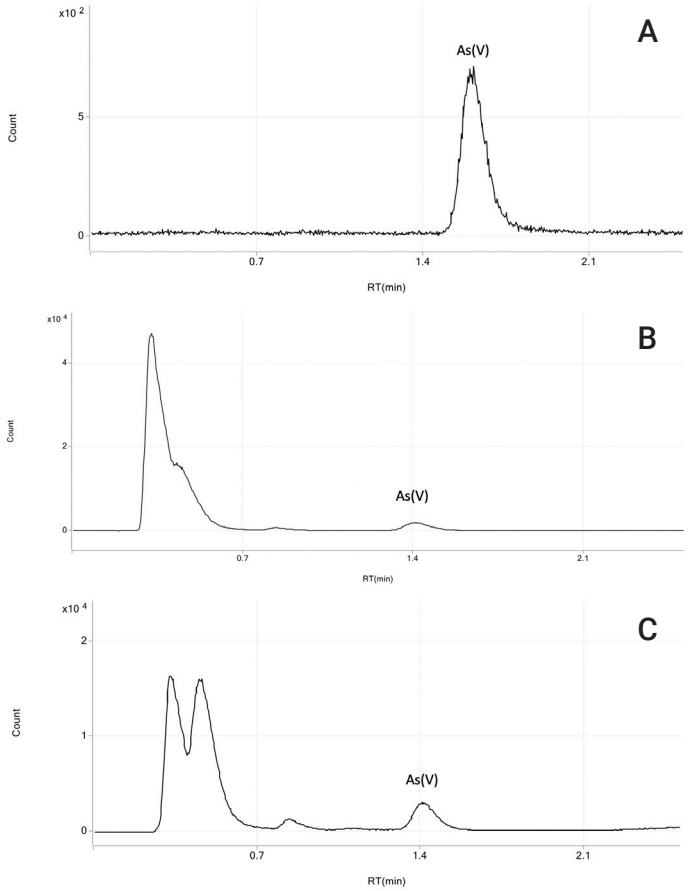

Figure 6. Chromatograms of several sample matrices. A: Chromatogram of Leek (DTU Food). B: Chromatogram of Fish Protein (NRCC-DORM-4). C: Chromatogram of Blue Mussels (DTU Food).

The data presented in this note was run over several days, and minor differences in the mobile phase composition and column conditioning led to some variation in the RTs between sequences. However, RT stability within each sample sequence was excellent, as shown in Table 6.

Table 6. Retention time stability for iAs peak in all standards and samples for three separate sample sequences.

	Average RT (min)	RSD (%)
Sequence 1 (n = 45)	1.61	1.24
Sequence 2 (n = 61)	1.41	1.28
Sequence 3 (n = 82)	1.85	1.73

Conclusion

A fast HPLC-ICP-MS method was optimized for the analysis of iAs in beverages, food, and animal feed matrices. Using a small injection volume, short ion-exchange column, high strength mobile phase, and oxidizing As(III) to As(V) with H_2O_2 during sample preparation, iAs could be determined in less than two minutes. The fast analysis time significantly increased sample throughput compared to methods that use a conventional ion-exchange column.

Following clear separation of As(V) from the organic As species and detection using an Agilent 8900 ICP-QQQ, LODs of 1.99 µg/kg (solid samples) and 0.08 µg/L (liquid samples) were obtained for iAs. The LOQs were lower than the LOQs established by European legislation for the analysis of iAs in food and feed samples. Several reference materials of different matrices with certified, reference, or informative values of iAs were analyzed with good accuracy (81.8 to 110.7%) and precision (0.3 to 9.4% RSD).

The HPLC-ICP-MS method is suitable for official control of iAs in food and feedstuffs as it complies with European regulations and meets the CEN standards' analytical requirements (*8, 9*). Given the toxicity of iAs, this fast, routine method provides valuable information on the safety of products that are widely consumed. It also enables food and feed producers to meet regulatory requirements for the analysis of iAs in their products.

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