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Application News

Sensitive and Rapid Analysis of Inorganic Arsenic in Food and Animal Feed Samples Using LC-ICP-MS

Kana Matsuno , Kosuke Naka

User Benefits

- Inorganic arsenic can be separated from organic arsenic and chlorine interference peaks in a short 6.5-minute runtime and analyzed with high sensitivity.
- Even in samples that contain large amounts of organic arsenic such as seafood, inorganic arsenic can be separated and measured.
- Inorganic arsenic in a wide variety of food and animal feed samples can be accurately quantified.

■ Introduction

Arsenic (As) exists in multiple chemical forms, and among them inorganic arsenic (iAs) is generally more toxic than organic arsenic. For this reason, many countries require the monitoring of inorganic arsenic in food and animal feed.

In the EU, regulatory limits for inorganic arsenic in food and feed are specified in Commission Regulation (EU) 2023/915[1] (food) and Directive 2002/32/EC[2] (feed). In addition, (EU) 2025/1891[3] established new maximum permitted concentrations for inorganic arsenic in seafood, effective September 17, 2025. In the United States, the Baby Food Safety Act 2021[4] also includes the maximum levels of inorganic arsenic in baby food.

Food matrices can contain large amounts of organic arsenic or chlorine, which may interfere with analytical results. Therefore, it is necessary to remove the effects of these interferences.

In this Application News, inorganic arsenic in a variety of foods and animal feeds was quantified using LC-ICP-MS (Fig. 1) based on EN 16802:2016 (food) [5]and EN 17374:2020 (animal feed) [6] .

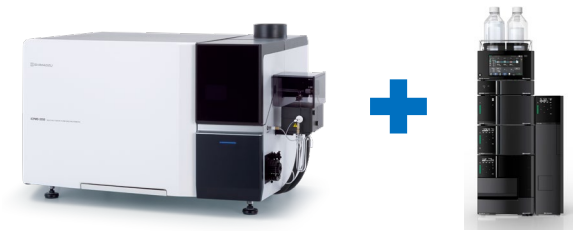

Fig. 1 LC-ICP-MS System

■ EN 16802: 2016 and EN 17374: 2020

EN 16802 is a method for quantifying inorganic arsenic in food samples by LC-ICP-MS, and EN 17374 is the corresponding method for feed.

Both methods require oxidation of arsenite (As(Ⅲ)) in the sample preparation process so that total iAs (As(Ⅲ) + As(Ⅴ)) is analyzed as arsenate (As(Ⅴ)).

Therefore, it is necessary to confirm that As(Ⅲ) is completely oxidized and is detectable as As(Ⅴ), and that the inorganic As (As(Ⅴ)) peak is separated from organic As peaks and from ^{35}Cl peak, which can form $^{40}Ar^{35}Cl$ and interfere with ^{75}As.

■ Samples

The following 14 samples were prepared for analysis:

- Rice cereal (baby food)
- Apple jelly (baby food)
- Strained corn (baby food)
- Infant formula (powdered milk, baby food)
- Sea bream (muscle (edible portion))
- Tuna (muscle (edible portion))
- Squid (mantle (edible portion))
- Ham
- Orange juice
- Japanese sake
- Animal feed (corn-derived)
- Brown rice certified reference material (CRM) NMIJ 7532-a
- Brown rice CRM NMIJ 7533-a
- Hijiki (brown algae) CRM NMIJ 7405-b

■ Extraction Solutions and Standards

- Extraction solution 1 (for solid samples) and diluent

Extraction solution 1 and diluent were prepared so that nitric acid is 0.1 M and hydrogen peroxide is 3 v/v%.

- Extraction solution 2 (for liquid samples)

Extraction solution 2 was prepared so that nitric acid is 0.2 M and hydrogen peroxide is 6 v/v%.

- Arsenic standard reagents

The following standard reagents were used:

arsenite (As(Ⅲ)) (Fujifilm Wako Pure Chemical), arsenate (As(Ⅴ)) (National Institute of Advanced Industrial Science and Technology, AIST), monomethylarsonic acid (MMA) (AccuStandard), dimethylarsinic acid (DMA) (AIST), arsenobetaine (AsB) (AIST), and arsenocholine (AsC) (Fujifilm Wako Chemical).

- Calibration standards

Arsenic standards excluding As(Ⅲ) were mixed and diluted with diluent to prepare the calibration standards. The concentration of each arsenic species in the calibration standards was 0.1–20 µg/L.

- Internal standard solution

A commercial Ga standard solution was diluted with 1 v/v% nitric acid to a concentration of 0.5 mg/L.

- Cl interference check solution

NaCl was dissolved in diluent to prepare a chloride interference check solution with 100 mg/L of Cl.

■ Sample Preparation

The sample preparation was performed with reference to EN 16802 and EN 17374 method.

- Samples

Approximately 0.5 g of each solid sample wes weighed (animal feed was approximately 0.2 g). 10 mL of extraction solution 1 were added to each weighed solid sample. To match the matrix of the solid samples, 5 mL of each liquid sample was mixed with 5 mL of extraction solution 2. Samples were extracted by heating them in a water bath at about 90 ˚C for 60 minutes, then cooled to room temperature. The extracted solutions were centrifuged (4,000 rpm, 10 min, 4 ˚C), and the supernatants were filtered through 0.45 µm PTFE filters to obtain the analytical solutions. To verify the validity of the analysis, samples spiked with arsenic standard solutions prior to extraction were prepared in the same way.

Brown rice CRM NMIJ 7533-a and hijiki CRM NMIJ 7405-b, which contain high levels of iAs, were further diluted 2-fold and 200-fold, respectively, with diluent. Orange juice and animal feed were further diluted 5-fold and 2-fold, respectively, with diluent to reduce matrix effects. For all other samples, the filtrates after the above procedure were used directly for measurement without any additional dilution.

■ Instrument Configuration and Analytical Conditions

Samples were analyzed with the LC-ICP-MS system, which consisted of the ICPMS-2040/2050 connected to a Nexera™ XS inert (Fig. 1). LabSolutions™ ICPMS TRM software was used to control the ICPMS-2040/2050 system and Shimadzu LC units. This enables everything from sample injection to chromatogram analysis to be performed via a single software program.

Table 1 shows the instruments configuration and analytical conditions for HPLC, and Table 2 shows the instruments configuration and analytical conditions for ICP-MS. The mini torch used in the analysis can reduce argon gas consumption to about two-thirds of that of a conventional torch. Therefore, mini torch system is quite effective for saving running costs especially in LC-ICP-MS analyses, which tend to have relatively long runtimes.

Table 1 Analysis Conditions of HPLC

System	: Nexera XS inert
Column	: HAMILTON PRP-X100 (10.0 µm, 250 mm × 4.1 mm)
Eluent	: 100 mM ammonium carbonate + 3% v/v methanol + 400 mg/L sodium sulfate[7), 8)] (pH 9.3)
Flowrate	: 1.2 mL/min
Temp. of Column Oven	: 30 °C
Injection Volume	: 50 µL
Measurement Time	: 6.5 min/sample
Vial[*1]	: Shimadzu Vial, LC, 1.5 mL, Polypropylene

*1: P/N : GLC-IVS-100

Table 2 Analysis Conditions of ICP-MS

Instrument	: ICPMS-2040/2050
Nebulizer	: Nebulizer DC04
Torch	: Mini-torch
Chamber	: Cyclone Chamber (electronically cooled)
Sampling Cone	: Nickel
Skimmer Cone	: Nickel
Internal Standard Spike	: Online Internal Standard Addition Kit
RF Power	: 1.20 kW
Sampling Depth	: 6.0 mm
Flowrate of Plasma Gas	: 9.0 L/min
Flowrate of Auxiliary Gas	: 1.10 L/min
Flowrate of Carrier Gas	: 0.77 L/min
Flowrate of Dilution Gas	: 0 L/min
Peristaltic Pump for Internal Standard	: 20 r.p.m. Tubing with 0.25 mm I.D.
Collision Gas	: He
Flowrate of Cell Gas	: 3.0 mL/min
Cell Voltage	: -23 V
Energy Filter	: 7 V

I.D.: internal diameter

■ Analysis of Standard Solutions

Fig. 2 shows the chromatograms of calibration standards (0.1–20 µg/L) for AsC, AsB, DMA, MMA, and iAs (As(Ⅴ)). The iAs (As(Ⅴ)) peak was successfully separated from the organic As peaks and detected. Since this Application News focuses on the sample preparation method and analytical method for iAs, separation of AsC and AsB was not performed. Although DMA and MMA can be separated, the sample preparation method used here is optimized for iAs analysis, so the validity of the pretreatment for DMA/MMA must be confirmed. In samples that contain large amounts of organic As (e.g., AsB in seafood), tailing of AsB may affect quantification of DMA and MMA. If separation of all species (AsC, AsB, DMA, MMA, As(Ⅴ), and As(Ⅲ)) is required, please refer to Related Application 1.

Fig. 3 shows the calibration curve for iAs with excellent linearity. To determine the limit of detection (LOD) in solution, the 0.1 µg/L standard was measured 10 times and the LOD in solution was calculated as the concentration corresponding to three times the standard deviation of those measurements. The LOD in solution was converted to LODs in the samples by applying the dilution factor for each sample. Table 3 lists the regulatory limits and the LODs. Even for the strictest iAs limit (0.01 mg/kg) specified by the Baby Food Safety Act 2021, sufficient sensitivity was achieved.

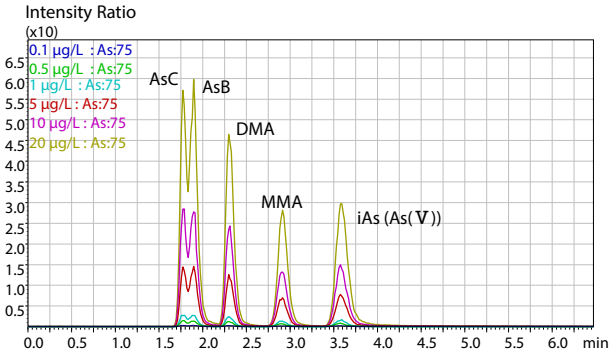

Fig. 2 Chromatograms of As Standard

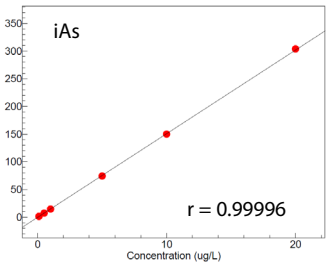

Fig. 3 Calibration Curve for iAs
(Vertical Axis: Intensity Ratio, Horizontal Axis: Concentrations)

Table 3 Regulatory Values and Detection Limits

Samples	Regulatory Values (mg/kg)				LODs in samples (mg/kg)	LOD in analytical solution (µg/L)
	Commission Regulation (EU) 2023/915	Commission Regulation (EU) 2025/1891	The Baby Food Safety Act of 2021	DIRECTIVE 2002/32/EC		
Brown Rice	0.25	-	-	-	0.0004[*1]	0.02
Hijiki	-	-	-	-	0.04	
Rice Cereal	0.02	-	0.015	-	0.0004	
Apple Jelly	0.02	-	0.01	-	0.0004	
Strained corn	0.02	-	0.01	-	0.0004	
Infant formula	0.02	-	0.01	-	0.0004	
Sea Bream	-	0.1	-	-	0.0004	
Tuna	-	0.1	-	-	0.0004	
Squid	-	0.05	-	-	0.0004	
Ham	-	-	-	-	0.0004	
Orange Juice	0.02	-	-	-	0.0002[*2]	
Japanese sake	-	-	-	-	0.00004[*2]	
Animal Feed	-	-	-	2	0.002	

Detection limits in analytical solution (µg/L): 3 × σ (standard deviation of 10 replicate measurements of the 0.1 µg/L standard) × slope of the calibration curve
Detection limits in each sample: detection limits in analytical solution (µg/L) × dilution factor
*1: The dilution factors for NMIJ 7532-a and 7533-a differ; values are converted on the basis of a 20× dilution.
*2: Units are mg/L

■ Oxidation from As(Ⅲ) to As(Ⅴ)

The following two As(Ⅲ) standard solutions were prepared to verify the oxidation of As(Ⅲ) to As(Ⅴ).

- As(Ⅲ) 5 µg/L without pretreatment

To avoid oxidation, the As(Ⅲ) standard was diluted in ultrapure water to a concentration of 5 µg/L.

- As(Ⅲ) 5 µg/L with pretreatment

The As(Ⅲ) standard was diluted with extraction solution 1 (diluent) to 5 µg/L and then processed in the same way as the samples: heated in a bath at about 90 ˚C for 60 minutes, then filtered through a 0.45 µm PTFE filter.

Fig. 4 shows chromatograms for the As(Ⅲ) 5 µg/L without pretreatment (red line), the As(Ⅲ) 5 µg/L with pretreatment (green line), and a calibration standard 5 µg/L (blue line). Without pretreatment, As(Ⅲ) appeared as a broad peak, but after pretreatment a peak was obtained at the same retention time as As(Ⅴ). When the As(Ⅲ) 5 µg/L with pretreatment was quantified using the iAs calibration curve prepared from As(Ⅴ) standards, the measured concentration was 4.83 µg/L. These results confirm that As(Ⅲ) is oxidized to As(Ⅴ) by the pretreatment.

Because As(Ⅲ) is oxidized to As(Ⅴ) during the pretreatment, total iAs (As(Ⅲ) + As(Ⅴ)) can be analyzed as As(Ⅴ).

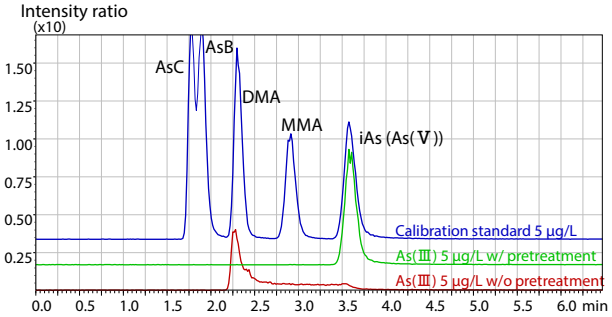

Fig. 4 Chromatograms for As(Ⅲ) Standard Solutions
(described with baseline shift)

■ Removal of Cl Interference

Since chlorine can form $^{40}Ar^{35}Cl$ in the plasma, which may interfere with ^{75}As, EN 16802 and EN 17374 require that the iAs peak be separated from the ^{35}Cl peak.

Fig. 5-a shows the chromatogram of chlorine obtained by analyzing the Cl interference check solution. The chromatograms of Cl and calibration standards are presented in Fig. 5-b. The Cl and iAs peaks are resolved, confirming that Cl does not affect the iAs analysis.

In addition, to get rid of the $^{40}Ar^{35}Cl$ interference, helium collision mode was used for analysis. No $^{40}Ar^{35}Cl$ interference at *m/z* 75 was observed at the retention time of chlorine, confirming that the $^{40}Ar^{35}Cl$ interference was sufficiently removed (Fig. 5-c).

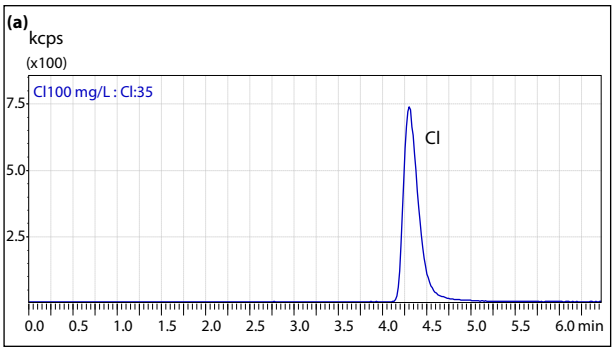

Fig. 5 Removal of Interference derived from Cl
(a)Chromatogram of ^{35}Cl

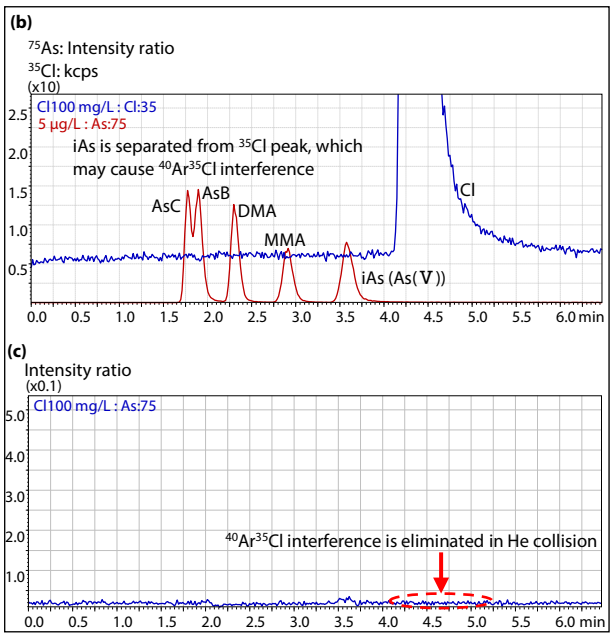

Fig. 5 (Continued) Removal of Interference derived from Cl
(b) Separation of iAs from Cl
(c) Removal of $^{40}Ar^{35}Cl$ Interference in He Collision

■ Separation of iAs from Organic As in Samples

Fig. 6 compares the chromatograms of spiked orange juice sample with and without dilution after filtration. When orange juice was analyzed after filtration without further dilution, a retention time shift, which is likely due to matrix effects, was observed (Fig. 6-a). In contrast, the sample that was filtered and then diluted 5× (total 10× dilution) showed suppression of the retention time shift (Fig. 6-b). Thus, when matrix effects cause retention time shifts, dilution can reduce those effects. For comparison, Japanese sake (which is a liquid sample with a smaller dilution factor like orange juice) did not show a considerable shift of retention time even without post-filtration dilution (see Fig. 6-a).

Separation of the iAs peak in seafood samples that contain large amounts of organic As was also confirmed. Fig. 7 shows chromatograms of 5 µg/L spiked samples of sea bream, tuna, and squid. Although tuna and squid contained large amounts of organic arsenic (particularly AsB), chromatograms demonstrated that the analysis of iAs in the 5 µg/L spiked seafood samples was not affected by huge peaks of organic As.

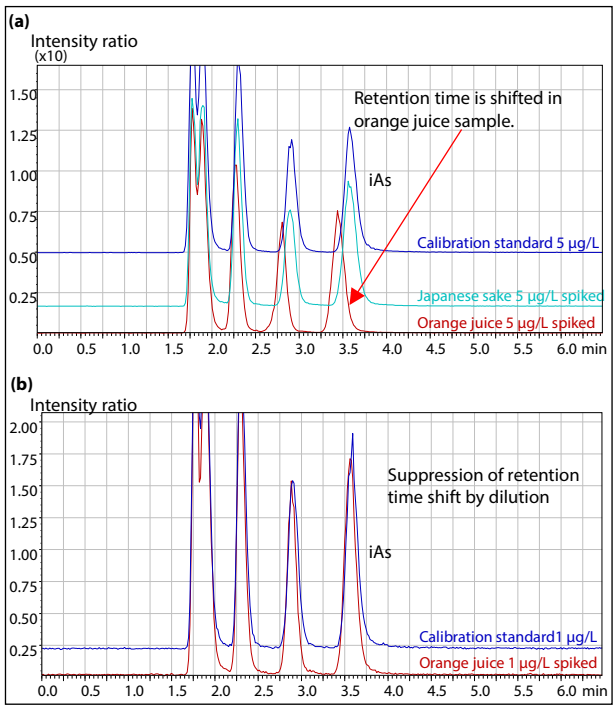

Fig. 6 Chromatograms of Orange Juice (described with baseline shift)
(a) Chromatograms of Orange Juice Analyzed w/o Post-filtration Dilution
(b) Chromatograms of Orange Juice Analyzed w/ 5× Post-filtration Dilution

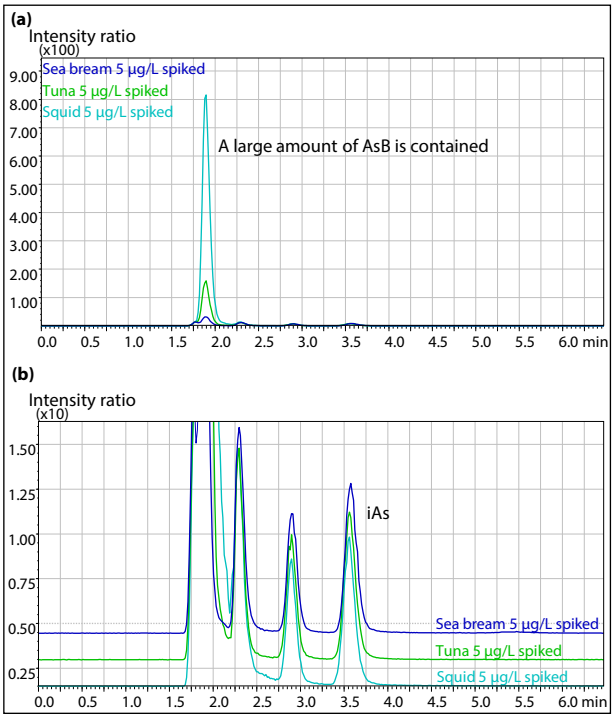

Fig. 7 Chromatograms of Seafood Spiked Sample
(a) Chromatograms of Seafood Spiked Sample, (b) Enlarged Chromatograms of Seafood Spiked Sample (described with baseline shift)

■ Analysis of the Certified Reference Materials

To verify the validity of the sample preparation process and LC-ICP-MS analysis, iAs in the brown rice CRMs NMIJ 7532-a and NMIJ 7533-a, and the hijiki CRM NMIJ 7405-b were quantified. The chromatograms are presented in Fig. 8. In the chromatogram of hijiki, a peak for an arsenic compound different from the four organic arsenic species in this study was observed next to the DMA peak, but no effect on the iAs peak was observed.

Table 4 shows the results. Quantified values for all three CRMs fell within their certified ranges, confirming the validity of the pretreatment and LC-ICP-MS method. Note that the certified values for the CRMs are given on a dry-weight basis, so the reported quantitative results were also converted to dry-weight equivalents.

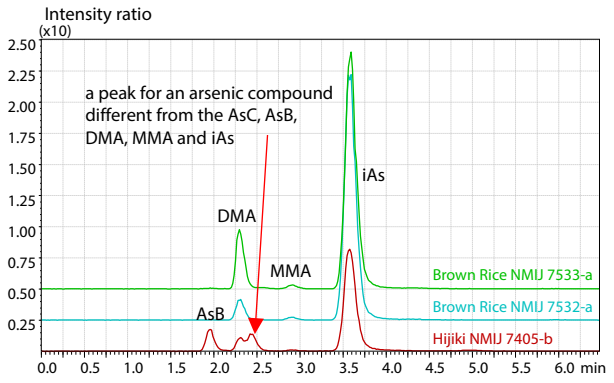

Fig. 8 Chromatograms of CRMs
(described with baseline shift)

Table 4 Results of CRM

Samples	LODs in samples (mg/kg)	Certificated Values (mg/kg)	Quantitative Results (mg/kg)
Brown rice NMIJ 7532-a	0.0004	0.298 ±0.008	0.291
Brown rice NMIJ 7533-a	0.0008	0.530 ±0.016	0.527
Hijiki NMIJ 7405-b	0.04	24.4±0.7*1	24.7

*1: Cerificated value as As(Ⅴ)

■ Spike Recovery Test

Spike-recovery tests were performed on 11 samples excluding the CRMs. The results are shown in Table 5. Good recoveries within 96–113% of iAs were obtained across a range of food and animal feed sample matrices, demonstrating that this method is accurate, robust, and reliable for the analysis of iAs in various food and feed samples.

Table 5 Spike Recovery Test

Samples	Spiked Conc. (µg/L)	Unspiked Samples (µg/L)	Spiked Samples (µg/L)	Recoveries (%)
Rice Cereal	5	4.39	9.78	108
Apple jelly	5	0.12	5.23	102
Corn	5	N.D.	5.21	104
Infant formula	5	N.D.	5.63	113
Sea bream	5	0.09	5.43	107
Tuna	5	N.D.	5.27	105
Squid	5	N.D.	5.24	105
Ham	5	N.D.	5.42	108
Orange Juice	1	N.D.	1.09	109
Japanese sake	5	0.56	5.38	96
Animal Feed	5	0.14	5.57	109

N.D.: below the detection limit
Spike recoveries (%) = (Spiked samples - Unspiked samples) / Spiked Conc. × 100

■ Analytical Results

Table 6 shows the quantified iAs results expressed as concentrations in the samples.

For many samples, the quantified iAs values were below the detection limits. In contrast, rice cereal and the animal feed contained relatively higher amounts of iAs. This is likely due to their grain-derived origin, which is known to be associated with higher iAs levels.

Table 6 Quantitative Results of iAs in Samples

sample	LODs in samples (mg/kg)	Quantitative results in samples (mg/kg)
Rice Cereal	0.0004	0.0864
Apple jelly	0.0004	0.0024
Corn	0.0004	N.D.
Infant formula	0.0004	N.D.
Sea bream	0.0004	0.002
Tuna	0.0004	N.D.
Squid	0.0004	N.D.
Ham	0.0004	N.D.
Orange Juice	0.0002*1	N.D. *1
Japanese sake	0.00004*1	0.0011*1
Animal Feed	0.002	0.013

Quantitative results in the samples: results in analytical solutions (µg/L) × dilution factor
N.D.: below the detection limits
*1: unit is mg/L

■ Conclusion

In this Application News, quantitative analysis of inorganic arsenic using the Shimadzu LC-ICP-MS system was conducted on 14 different food and feed matrices. Some foods and feeds contain large amounts of organic arsenic or chlorine, but the peak of inorganic arsenic was able to be separated from those interfering peaks with a short analysis time of 6.5 minutes per sample.

Analyses of certified reference materials and spike recovery tests yielded reliable results, confirming that a single method can quantitatively determine inorganic arsenic across a wide variety of food and animal feed samples. Furthermore, sufficiently low detection limits were achieved even for samples with stringent control concentrations, such as baby food.

These results demonstrate the capability of the Shimadzu LC-ICP-MS system for accurate, sensitive, and rapid analysis of inorganic arsenic in food and animal feed samples with diverse matrices.

<References>

1) COMMISSION REGULATION (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006
2) DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002
3) Commission Regulation (EU) 2025/1891 of 17 September 2025 amending Regulation (EU) 2023/915 as regards maximum levels of inorganic arsenic in fish and other seafood
4) US House of Representatives, The Baby Food Safety Act of 2021, accessed Jan 2026
5) EN 16802:2016: Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS
6) EN 17374:2020: Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICP-MS
7) Tadashi Taniguchi, Hiroaki Tao, Mamoru Tominaga and Akira Miyazaki, "Sensitive determination of three arsenic species in water by ion exclusion chromatography-hydride generation-inductively coupled plasma mass spectrometry", J. Anal. At. Spectrom., 1999,14, 651-655
8) Tetsuya Nakazato, Tadashi Taniguchi, Hiroaki Tao, Mamoru Tominaga and Akira Miyazaki, "Ion-exclusion chromatography combined with ICP-MS and hydride generation-ICP-MS for the determination of arsenic species in biological matrices" , J. Anal. At. Spectrom.,2000,15,1546-1552

<Related Applications>

1. Determination of Arsenic Species in Apple Juice by LC-ICP-MS Analysis, Application News No. 01-00802
2. Analysis of Heavy Metals in Baby Food Using ICP-MS, Application News No. 01-01039A
3. Speciation Analysis of Mercury in Seafood by LC-ICP-MS and Introduction of Autosampler Automatic Dilution Function, Application News No. 01-00877B
4. Speciation Analysis of Chromium by LC-ICP-MS Based on ISO 24384, Application News No. 01-00809

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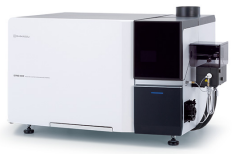

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