The truth about arsenic in rice samples

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ABSTRACT

This technical article highlights Agilent's collaboration with scientists and researchers of agricultural universities and research institutes in India for the detection and quantification of arsenic in rice using LC-ICP-MS instrumentation and applied methods. Arsenic is a naturally occurring element found in many compounds in combination with either inorganic or organic substances. Even if present in ultra-trace levels in food, arsenic contaminations are toxic and harmful to humans. Since inorganic arsenic is more toxic in nature than organic arsenic, such studies are essential to ensure that the inorganic arsenic levels in rice (including Basmati rice) are no higher than the maximum acceptable concentration of 0.10 mg/kg, as per EU guidelines. Such studies not only help stakeholders, traders, and exporters in the food industries in India, but also regulators who make collective decisions for social benefits. This will lead to gains in food safety and quality assurances, while global consumers can trust that their rice is safe to eat.

The facts of rice

Rice is one of the most essential commodities, as more than half of the world's entire population consume it at 20% of their daily calorie intake(1). Scientifically, rice is known as *Oryza*; *Oryza sativa* is one of the most common species(2). With more than 90,000 samples of cultivated rice and wild species stored at the International Rice Gene Bank(3), Asia currently produces and consumes over 90% of the world's rice(4). Nutritionally, rice is a good source of fiber, vitamins B1 (thiamine) and B6, magnesium, phosphorus, selenium, and manganese. For these reasons, rice is seen as a staple food for many.

When we consider the Indian subcontinent, rice is even more important. Not only is India the second-largest producer of rice in the world, with 20% of production, but it is also the largest consumer, with approximately 1.2 billion people depending upon rice for their livelihood(5).

Concerns about rice safety

Arsenic is an element with the chemical symbol As. It is also a known metalloid and classified as a Group-A carcinogen. According to the World Health Organization (WHO), in addition to skin cancer, long-term exposure to arsenic may also cause cancers of the bladder and lungs(6). The International Agency for Research on Cancer (IARC) has classified arsenic and arsenic compounds as carcinogenic to humans, also when present in drinking water(6).

Arsenic contamination in groundwater is an issue that affects millions of people around the world. Naturally occurring high concentrations of arsenic in groundwater are particularly problematic in regions such as the Ganges Delta, causing serious arsenic poisoning to large populations. Not only limited to drinking-water supplies, the accumulation of arsenic favors the flooded cultivation practice of rice fields, thus causing absorption in rice grains farmed for human consumption.

While rice is estimated to contain 10-20 times more arsenic than other cereal crops(7), food safety assessments in India – currently the world's largest exporter of rice(8,9) – must be routinely performed to ensure that rice products are non-toxic for human consumption.

The science behind arsenic contamination in groundwater and rice

Rice acquires essential micronutrients from soil and groundwater though transporters. However, during this process, other non-essential elements like arsenic are absorbed by the plant with the help of several phosphate transporter genes and proteins(10).The poten-

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tial for arsenic accumulation in rice is increased in comparison to other plants because it requires more water to grow. Once arsenic is entrapped in the rice plant, it exists mostly in its reduced form (i.e., arsenic (III)), forming organo-arsenic complexes that may, in turn, be transported to the vacuoles of the rice plant, where they accumulate. Arsenic species known to be present in rice and rice products are mainly arsenite (As (III)), arsenate (As (V)), methylarsonic acid (MMA), and dimethylarsinic acid (DMA), which can be classified into two forms, namely: inorganic arsenic (arsenite and arsenate) and organic arsenic (MMA and DMA).

The form and concentration of arsenic depends on several factors, including:

- Whether the water is oxygenated (for example, arsenites predominate under reducing conditions such as those found in deep well waters);
- The degree of biological activity (which is associated with the conversion of inorganic arsenic to methylated arsenic acids);
- The type of water source (for example, open-ocean seawater, surface freshwater, or groundwater); and
- The proximity of the water source to arsenic-rich geological formations and other anthropogenic sources.

Inorganic species are known to be more toxic compared to organic arsenic species(10). Although both forms of inorganic arsenic are potentially harmful to human health – even if present in minute concentrations – As (III) is considered more harmful than As (V) due to its lower lethal dose (LD_{50}) value of 14 mg/kg and 55 mg/kg, respectively, at oral intake(11).

Over the past several years, many scientific reports and media articles have been published regarding serious health issues due to the presence of arsenic in Indian rice. Various national and international regulatory agencies such as the WHO, Codex, the EU Council Directive, and the Food Safety and Standards Authority of India (FSSAI) – have set up stringent norms to ensure the safety and quality of rice samples that may be subject to arsenic contamination before batches are distributed worldwide.

According to the EU Commission Regulation 2015/10006, the maximum concentrations of inorganic arsenic (the sum of As (III) + As (V)) in rice samples that are destined for the production of food for infants and young children is 0.10 mg/kg(12). By contrast, for non-parboiled milled rice (polished or white rice) and parboiled rice, and husked rice, these values are 0.20 and 0.25 mg/kg, respectively(12). Similarly, according to the Codex Alimentarius Commission (including the Food and Agricultural Organizations of the United Nations (FAO) and the WHO), the maximum residual levels (MRLs) for polished rice and husked rice are 0.2 and 0.4 mg/kg, respectively(13). The FSSAI has not directly specified limits of inorganic arsenic in rice. However, it has set the maximum limit of total arsenic concentrations in infant food to 0.05 mg/kg(14).

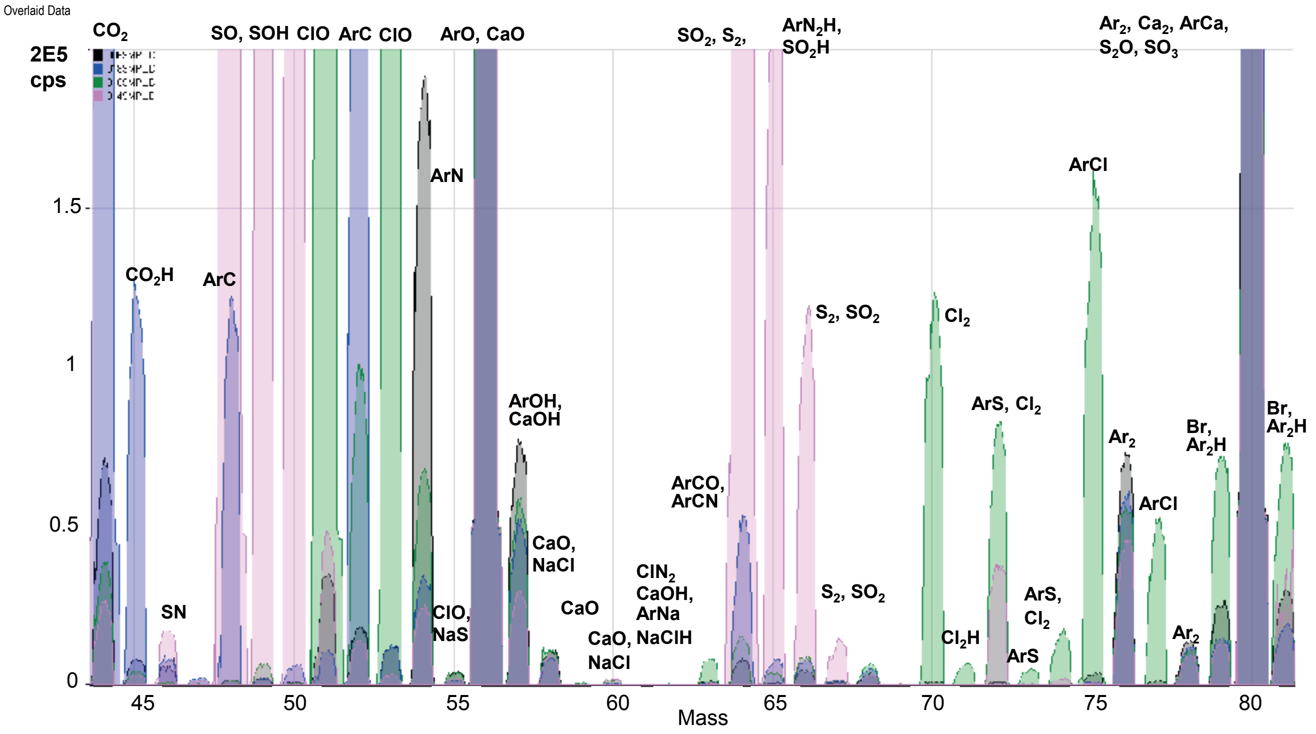
To meet these stringent limitations of arsenic concentrations in rice, there has always been a need for advanced analytical techniques to ensure the reliability of contamination results. Therefore, the quality of analytical data is vital, since it will form the basis of decisions regarding arsenic contamination in rice.

Innovations in combinatory liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS) techniques for arsenic contamination in rice

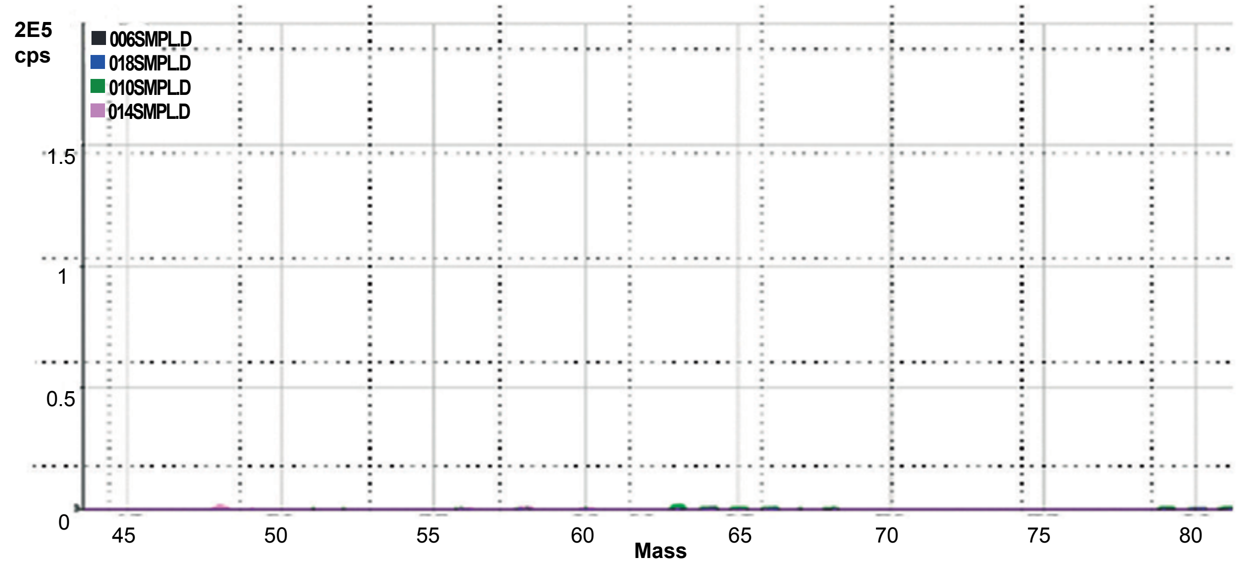
At Agilent Technologies in India, the team is working closely with various agricultural universities, research institutes and commercial testing labs (CTLs) (such as Bihar Agriculture University (BAU) and Basmati Export Development Foundation (BEDF)), for the estimation of different species of arsenic in rice and various food products at low trace levels. Experts from Agilent are working together with scientists and researchers from BAU and BEDF to provide precise, accurate, sensitive, and selective analytical methods for the detection and quantification of arsenic species in rice samples using high-performance liquid chromatography (HPLC) with inductively coupled plasma-mass spectrometry (ICP-MS). Specifically, the Agilent 7800 ICP-MS featuring fourth-generation ORS (octopole collision/reaction cell) is applied.

Dr. Anupam Dixit and his team at BEDF in India specialize in analytical quality evaluations of Basmati rice. In particular, LC-ICP-MS techniques are utilized for the detection and quantification of nutritional elements and heavy metals, including arsenic speciation with low levels of As (III) and As (V) in rice/Basmati rice to meet MRLs prescribed in various national and international regulatory requirements. Additionally, his team has developed and validated methods and has provided training for other elemental impurities and best practices on LC-ICP-MS and GC-ICP-MS with reference to Basmati rice.

Dr. Anupam Dixit explains that, in his lab, "Analytical studies carried out on LC-ICP-MS for the finding and study of As (III) and As (V), MMA, and DMA in rice (Basmati rice) are being done routinely with Agilent instruments. This will help exporters to ascertain the quality of Basmati rice. All the methods developed and validated are precise, accurate, and achieve low detection limit." The ORS feature of the 7800 ICP-MS in the helium mode is a



(a) No Gas Mode: All peaks are due to polyatomic ion interferences



(b) Helium Mode: Removed polyatomic ion interference.

Fig. 1: Effectiveness of ORS at removing polyatomic ion interference in a blank matrix containing 5% HNO_3 + 5% HCl + 1% IPA + 1% H_2SO_4

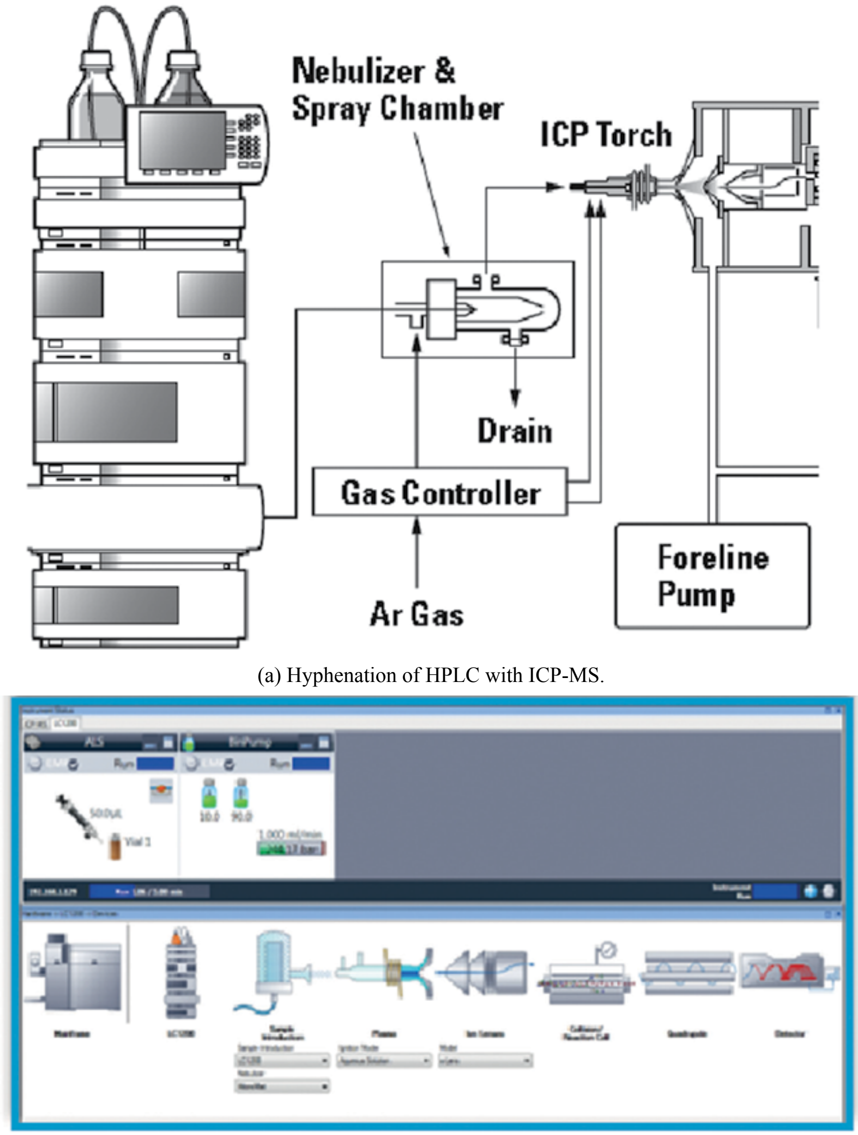
more effective application for interference removal in complex matrices. The helium mode has several advantages over reaction gas:

- It is inert, and therefore, no new reaction product ion interferences are formed;
- It does not react with analyte ions, so loss of sensitivity by reaction does not occur; and
- It is effective against all polyatomic overlaps, so method development is

simple for the end-user, and accurate multi-element analysis is possible in complex matrices.

Figure 1 shows the effectiveness of Agilent ORS in removing polyatomic interferences, since argon (Ar) and calcium (Ca) may form $Ar^{40}Cl^{35}$ and $Ca^{40}Cl^{35}$ on the same isotope of arsenic at *m/z* 75.

The 7800 ICP-MS has been used for the estimation of total arsenic in different samples of rice (including Basmati rice). The Agilent ZORBAX SB-Aq (4.6 mm id × 250 mm, 5 µm) reversed-phase column is used at room temperature for the LC-ICP-MS analysis of As (III), As (V), MMA, and DMA species of arsenic in rice. In various studies, SRM 1568b is used to verify accuracy and precision of results. All the rice samples, including SRM 1568b, are crushed and sieved to make rice flour, followed by the extraction of arsenic species into solution. Mobile phase (pH 4.3) consists of 20 mM citric acid and 5 mM sodium hexane sulfonate, with an injection volume of 5 µL and a mobile-phase flow rate of 1.2 mL/min. The exit of the column is connected with the nebulizer of the ICP-MS as shown in Figure 2. The purpose of the HPLC column is to separate different arsenic species under optimized conditions, and the ICP-MS is used as a detector for the separation of arsenic species based on *m/z*. During the study, all polyatomic interferences at *m/z* 75 are removed using helium gas with a flow of 4.3 mL/ min and KED 3.0 V. Data is interpreted using Agilent MassHunter integrated with chromatographic software, as shown in Figure 2. Peaks and other analytical results for different species of arsenic are shown in Figure 3. Results for the SRM are presented in Table 1.



(a) Hyphenation of HPLC with ICP-MS.

(b) Agilent MassHunter integrated with chromatographic software.

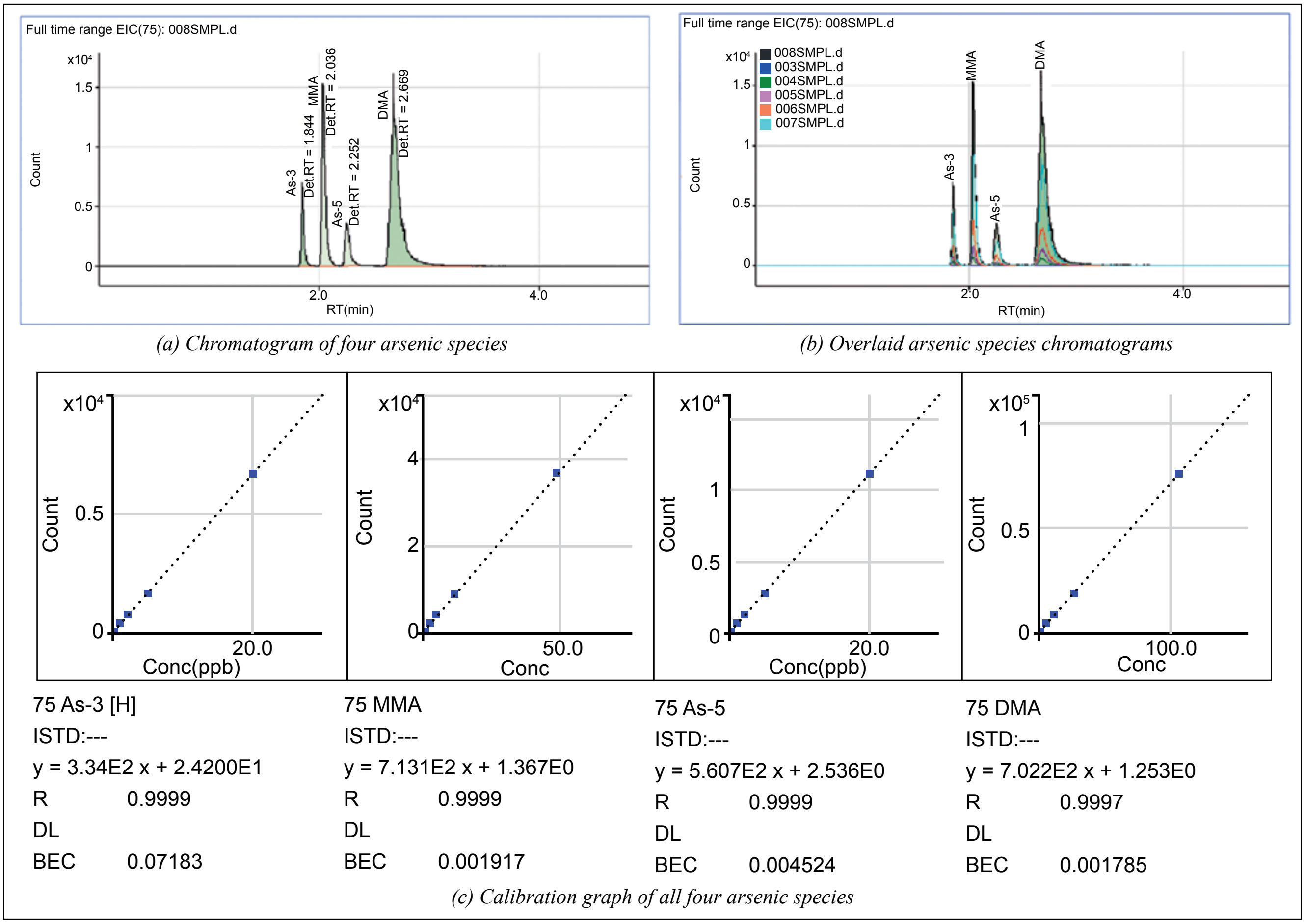
Fig. 2: Hyphenation of HPLC with the Agilent 7800 ICP-MS for arsenic speciation.

CONCLUSION

Agilent is providing innovative solutions to agricultural scientists and researchers to ensure the success of food safety efforts by providing high-through-

Table 1: A recovery study in SRM 1568

Element	Replicates			Average, mg/kg (n=3)	% RSD	Certified Value, mg/kg	% Recovery
	I	II	III				
iAs	0.0906	0.0928	0.0925	0.0920	1.30	0.092 ± 0.010	99.96
DMA	0.188	0.184	0.187	0.186	1.11	0.180 ± 0.012	103.3
MMA	0.0114	0.0117	0.0115	0.0115	1.32	0.0116 ± 0.0035	99.43



(a) Chromatogram of four arsenic species

(b) Overlaid arsenic species chromatograms

(c) Calibration graph of all four arsenic species

Fig. 3: Chromatograms and calibration graph of arsenic species.

put tools to efficiently and reliably monitor the quality of rice (particularly, Basmati rice). With optimized solutions for simultaneous estimations of organic and inorganic arsenic species, using the most sensitive detector and patented technology for minimizing matrix interference, Agilent helps customers achieve the most suitable solution to detect low levels of inorganic arsenic contamination in rice products. With this approach, food testing laboratories can confidently measure contaminants that pose a threat to global rice stakeholders.

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