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The use of HPLC-NAA and HPLC-ICP-MS for the speciation of As in infant food

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ABSTRACT

Arsenic and its inorganic species: As (III), As (V), dimethylarsenic acid (DMA) and monomethylarsenic acid (MMA) were determined in hypoallergenic formulas and grain porridges commercially available on Polish market, dedicated for infant 0–8 months. After quantitative extraction with 0.5% HNO_3, separation of individual species was performed by high performance liquid chromatography (HPLC), and their determination by neutron activation analysis (NAA) and inductively coupled plasma mass spectrometry (ICP-MS). Due to relatively low content of As in the analysed samples, it was only possible to determine DMA using the HPLC-ICP-MS mode. HPLC separation coupled with off-line determination by NAA enabled the determination of more extracted As species (especially inorganic) with good accuracy. Certified reference material (CRM) Rice Flour SRM 1568b (NIST) was used for the validation of both procedures.

1. Introduction

Arsenic has been classified by the International Agency for Research into Cancer (IARC) as a human carcinogen on the basis of increased cases of cancers in people exposed to arsenic at work, in the environment or through their diet (IARC, 2012). Arsenic intake from food for infants under 12 months of life was estimated at 1.3 μg/day (EFSA, 2014). Several investigations shown that rice-based infant food products contain relatively high content of inorganic As (inAs) (Meharg et al., 2008; Carbonell-Barrachina et al., 2012; Jackson, Taylor, Punshon, & Cottingham, 2012; Juskelis, Li, Nelson, & Capozzo, 2013; Signes-Pastor, Carey, & Meharg, 2016). Arsenic speciation in infant food products is needed to define risk assessment of inAs for infants, and to introduce appropriate standards to protect their health. Currently, as a rule, hyphenated techniques are used in arsenic speciation analysis (Chen & Chen, 2014; Welna, Szymczycha-Madeja, & Pohl, 2015; Jung, Kang, Jung, & Ma, 2018; Son, Lee, Kim, Lee, & Nam, 2019; Guillod-Magnin, Brüschweiler, Aubert, & Haldimann, 2018; Costa, Coelho, & Coelho, 2015). The most common detection system is ICP-MS, due to its precision, low detection limit (LOD), wide linear dynamic range etc. (Thomas, 2013). However, some elements like As could be classified as the difficult for ICP-MS, mainly due to presence of spectral interferences. Arsenic has only one isotope (m/z 75), its measurement is often impossible in chloride-containing samples because of presence $^{40}Ar^{35}Cl^+$ or $^{40}Ca^{35}Cl^+$ in plazma gas (May & Wiedmeyer, 1998). Applying the dynamic reaction cell (DRC) mode could eliminate part of polyatomic interferences that have caused problems in the direct analysis of infant formulas (D'Ilio, Violante, Majorani, & Petrucci, 2011). However, the most popular reaction gas – ammonia – is not sufficient to reduce all spectral interferences. Although $ArCl^+$ reacts with NH_3, $CaCl^+$ is unreactive due to the high Ca–Cl bond strength. So, $CaCl^+$ cannot be eliminated in this manner. As a result, in that case measurements of arsenic at trace-level were impossible. The difficulties associated with the study of chemical forms of elements in baby food became a factor stimulating the development of new methods and the modification of existing analytical procedures. Application of NAA method in the determination of separated chemical forms of elements ensures better quality of obtained analytical results (Chajduk & Polkowska –Motrenko, 2017). Moreover, the use of NAA with LC-MS enables identification of species in the case when appropriate CRMs are not available. Certified Reference Materials (CRMs) play a key role in quality assurance of analytical results. They not only allow the validation of reliable methods but also permit rigorous quality regimes and comparability of results (Dybczyński, 2002). Unfortunately relatively few CRMs can be applied for speciation measurements and are they dedicated mainly for total mercury and methylmercury contents (EVISA, 2018). Lack of CRMs for the speciation analysis somehow enforces search for alternative solutions.

The importance of the determination of inorganic As in rice based foodstuff dedicated for infants and young children was strongly

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underlined by the European Commission. The dietary exposure to inorganic arsenic for children under 3 years old, including from rice-based foods, is estimated to be about 2 to 3 fold that of adults. According to EU Regulation 1881/2006 and with later changes, dated 26 June 2015, the requirements of the maximum levels of inorganic arsenic in rice-based food are applied from 1 January 2016 (Commission & Regulation, 2015). For rice destined for the production of food for infants and young children, inorganic As content is acceptable at a level of 0.1 mg/kg of fresh samples. To meet these requirements, The European Committee for Standardization (CEN) has developed a new standard: "EN 16802:2016. Foodstuffs. Determination elements and their chemical species. Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS." (ISOIEC, 2016). Lately, HPLC-ICP-MS and HPLC-NAA was complementary used to determine arsenic species in certified reference materials by National Institute Standards and Technology (Carioni, Nomura, Yu, & Zeisler, 2014). Produced CRMs have been based on foods of marine origin and have relatively high contents of total arsenic (in the order of mg/kg). The authors demonstrated advantages of HPLC-NAA using in certification of new CRMs for arsenic speciation (Yu, Stanoyevitch, & Zeisler, 2017).

Regulations introduced in the European countries require the determination of arsenic at significantly lower concentration levels than it is present in marine food. The objective of this work was to show the benefits of the complementary application of HPLC-ICP-MS and HPLC-NAA in the speciation analysis of food samples with low arsenic total content (below 100 ng/g). Two analytical techniques with different physicochemical foundations ensures that accurate results are obtained when used in a complementary way. It is especially important, when determining some problematic element for ICP-MS like arsenic; also in the case of lack certified reference materials for arsenic speciation in food samples.

2. Materials and methods

2.1. Samples

All food samples (four cow-based milk powders, one goat-based milk powder, one soy-based milk, hypoallergic cereal not containing milk, lactose, soy protein and gluten (manufactured from high quality carob bean and rice), three flavored rice porridges (banana, forest fruit, apple), corn porridge, oatmeal porridge, buckwheat porridge, millet porridge, spelt porridge, corn-tapioca porridge were bought at the food market in Poland (Chajduk & Polkowska –Motrenko, 2018). During the selection of the samples for this research, two criteria were followed: availability on the market and their popularity. Chosen materials could be divided into two groups: the most popular brands on the market, easily available in every place in Poland: corn and rice based porridges, hypoallergenic products, and less available on the market, other products described as "bio", "eco" – available only in health food stores, and with a higher price. Each chosen product has been bought in triplicate to eliminate an accidental batch. Certified reference material: Rice Flour SRM 1568b (National Institute of Standards and Technology – NIST) was used for the validation of elaborated procedures.

2.2. Reagents

For digestion and extraction process 65% HNO_3 (p.a. Sigma, purified by sub-boiling point distillation), 40% HF (suprapure, MERCK), H_2O_2 (p.a., FLUKA), methanol (for HLPC, Sigma Aldrich) were applied. The standard of arsenic used for total content analysis by ICP-MS and NAA was supplied by Perkin Elmer as stock standard solutions of 1 mg mL^{-1}. Standards solution of As(III) and As(V) with certified value 1000 μg/mL ± 5 μg/mL were delivered by SPEX CertiPrep. Solutions of the organic: MMA and DMA, were prepared by dissolution in water monosodium methyl arsenate ($CH_4AsNaO_3 \geq 98.5\%$) and dimethylarsinic acid ($(CH_3)_2AsO(OH) \geq 99\%$). Organic arsenic species reagents were purchased from Sigma-Aldrich. Individual working solutions of for arsenic species were stored at 4 °C in the dark.

2.3. Apparatus

The microwave digestion system (Anton Paar Multiwave 3000, USA) equipped with temperature and pressure regulation was used for the digestion of the samples. ICP-MS instrument ELAN DRC II (Perkin Elmer) with crossflow nebulizer with a Scott double-pass spray chamber and Ni cones was used. Perkin Elmer HPLC system Series 200 was used for arsenic species separation. Hamilton PRP-X100 HPLC Column (5 μm particle size, L × I.D. 25 cm × 4.6 mm, PEEK hardware) was used for arsenic species separation with Hamilton PRP-X100 HPLC guard cartridge. Chromera software (Perkin-Elmer) ensured the data collection.

To perform gamma-ray spectroscopic measurements the following detectors were used:

- 255 cm^3 HPGe well-type detector (Canberra) coupled to a multichannel analyzer and spectroscopy software Genie-2000 (Canberra),
- Broad Energy Ge BE3830 detector (Canberra) coupled to a multichannel analyzer and spectroscopy software Genie-2000 (Canberra).

2.4. Analytical procedures

2.4.1. Radiochemical NAA (RNAA) for total As

The samples of biological material, elemental standards, CRMs and blank were placed in (PE) containers, irradiated in the nuclear reactor MARIA, for 50 min at a thermal neutron flux of 10^{14} cm^{-2} s^{-1} and cooled for appropriate cooling time. Then, the samples were acid digested using a high-pressure microwave system. After decomposition, the samples were subjected to separation procedure. The details of the radiochemical procedures were published previously (Chajduk & Dybczyński, 2010). The count rate of the separated ^{76}As was measured by gamma-ray spectrometry and compared with that of As standard.

2.4.2. HPLC-ICP MS

A sample mass of 1 g was weighted, mixed with 10 mL of 0.5% HNO_3 and shaken for 3 h in 85° C. Next the solution was centrifuged for 20 min. at 14000 rpm and filtered on syringe filter 0.2 μm and stored at 4° C prior to the analysis. The As species were separated using anion-exchange chromatography, and 0.15 mM tartaric acid (pH − 3) as a mobile phase, isocratic elution, flow rate 1 mL min^{-1}. The following measurement conditions were used: RF power − 1000 W, plasma gas flow − 13.0 L min^{-1}, nebulizer gas flow − 0.98 L min^{-1}, lens voltage −6.0 V, working mode – DRC, gas NH_3.

2.4.3. HPLC-NAA

The separation conditions were the same as described above. The chromatographic column was the same as used with ICP-MS spectrometer, but the individual fractions of effluent were collected manually. To improve the detection limit for arsenic species determination by HPLC-NAA, three elutions were carried out and appropriate fractions containing the same species were combined. Next, each separated and collected arsenic specie was evaporated to dryness in the irradiation vials. Separated fractions, arsenic standards and blank were irradiated for 1 h at thermal neutron flux of 10^{14} cm^{-2} s^{-1}. After 1–2 days of cooling time, samples were measured; time of measurements varied between 3600 and 10000 s.

2.5. Data analysis

Statistical calculations of the obtained data was analyzed using a statistical software tool PQStat (PQStat Software) at a significance level of $p < 0.05$.

Table 1
The determination of total arsenic in cereals (porridges) for infants > 4 months obtained by RNAA.

material	As content	material	As content
Soya-based milk	50.6 ± 1.5	Oatmeal[1]	15.2 ± 0.5
Corn	17.0 ± 0.5	Buckwheat[1]	< 7
Rice 1	47.8 ± 1.4	Millet[1]	13.8 ± 0.5
Rice 2	44.3 ± 1.3	Spelt[1]	20.2 ± 0.6
Rice 3	46.2 ± 1.4	Tapioka[1]	15.7 ± 0.5
Hypoallergenic product	72.2 ± 2.2		

[1] Products with EU organic production logo.

3. Results

3.1. Total content of arsenic

Determination of total As content was done by RNAA method. In the case of the analysed materials, the instrumental mode of NAA could not be applied due to high activity of the irradiated sample, low content of As in the samples and short half live of ^{76}As formed. Elaborated previously primary reference measurement procedure (PRMP) by RNAA allows to determine As at single ppb level with expanded uncertainties of 2–3% what is comparable to ID-MS methods (VIM (International Vocabulary of Metrology – Basic and General Concepts and Associated Terms), (2012), 2012). However RNAA procedure is the only PRMP which can be used for the determination of trace amounts of mono-isotopic elements like arsenic. The obtained results of the analysis of tested infant formulas are shown in Table 1. As can be seen, the highest As content is in hypoallergenic product, soy-based milk and rice-based porridges. In other samples its content does not exceed 21 ng/g. It could be mentioned, that all samples with higher arsenic level were classified as “the most popular brands”. The results show that arsenic content varied statistically among the analysed samples depending on the used plant. There was no significant difference between total arsenic content in rice porridges.

3.2. Optimization of extraction procedures and selection of HPLC mobile phase

Water, water-methanol mixtures and 0.1–1% HNO_3 were used for extraction of arsenic species from SRM Rice Flour 1568b. Extractable arsenic determinations were done by ICP-MS. In all cases, the yield of As extraction was above 90% (Table 2). The quantitative extraction was obtained for nitric acid in higher temperature. Shaking for three hours with 0.5% HNO_3 in 85 °C, has been chosen for use in this study.

Several chemicals can be applied as a mobile phase in the speciation of arsenic in food samples by anion-exchange chromatography, eg. solutions of nitrates, phosphates. In the case of NAA determination, mobile phase should not contain elements with high neutron cross section. Although the use of phosphates gives a good separation of arsenic peaks; this system cannot be used in the case of detection with NAA because of high background of ^{32}P. In order to eliminate this phenomenon, phosphates should be replaced by tartaric acid in separation process.

Table 2
Extraction efficiency of arsenic species from infant food.

Reagent	Shaking for 24 h, room temperature	Shaking for 3 h, 85 °C
H_2O	90 ± 1%	96 ± 1%
H_2O-methanol	92 ± 1%	95 ± 1%
0.1% HNO_3	93 ± 1%	96 ± 1%
0.5% HNO_3	95 ± 1%	99 ± 1%
1% HNO_3	92 ± 1%	99 ± 1%

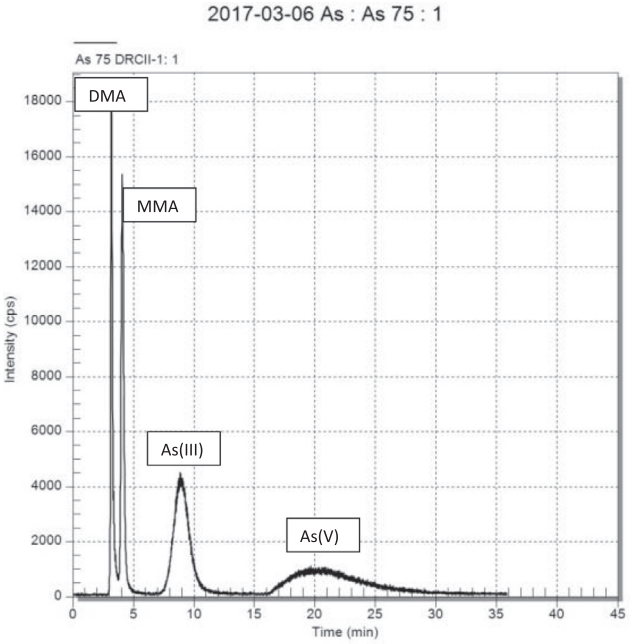

Fig. 1. HPLC-ICP-MS chromatogram of a mixed solution of arsenic species obtained under the described separation conditions.

3.3. Chromatographic separations and determination

Fig. 1 presents a HPLC-ICP-MS chromatogram showing separated peaks associated with the four arsenic species at 50 ng/g each. The retention time for DMA is 3.3 min, for MMA – 4.2 min, 8.9 min and 20 min for As(III) and As (V), respectively. Fig. 2 shows a chromatogram obtained by HPLC-ICP MS for the soy-based milk. As can be seen, the determination of separated As species with good accuracy is hampered – and only DMA can be quantitatively determined. Similar chromatograms were obtained for other tested samples. In the case of HPLC-NAA, fractions of single isolated species were collected manually (based on the timing measurements of arsenic standards), evaporated to dryness, irradiated in reactor and measured by γ-spectrometry. The

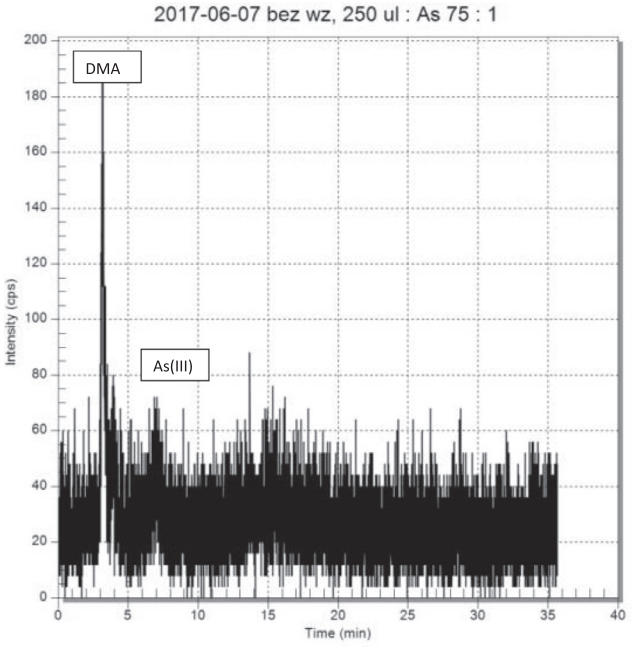

Fig. 2. HPLC-ICP MS chromatogram of an extract from the soya based milk.

Table 3
Results of the determination of arsenic and its species in Rice Flour 1569b, μg/g, n = 3.

	Total content	DMA	MMA	Inorganic As
Certified values	0.285 ± 0.014	0.180 ± 0.012	0.0116 ± 0.0035	0.092 ± 0.010
ICP-MS	0.288 ± 0.029			
HPLC-ICP-MS		0.164 ± 0.025	n.d.	n.d.
RNAA	0.280 ± 0.008			
HPLC-NAA		0.176 ± 0.009	0.0100 ± 0.0015	0.100 ± 0.010

Table 4
Results of the determination of arsenic and its species in infant formulas by HPLC-NAA.

Infant formula	n	As content in particular species [ng/g]		
		DMA	MMA	Inorganic As
Soya-based milk	5	30.2 ± 2.0	< 10	24.9 ± 2.7
Corn	3	< 10	< 10	< 10
Rice 1	5	13.5 ± 1.4	< 10	37.4 ± 3.7
Rice 2	5	< 10	< 10	38.9 ± 3.9
Rice 3	5	12.2 ± 1.2	< 10	34.3 ± 3.4
Hypoallergenic product	5	12.1 ± 1.2	< 10	65.0 ± 6.5
Oatmeal[1]	3	< 10	< 10	10.1 ± 1.0
Millet[1]	3	< 10	< 10	< 10
Spelt[1]	3	< 10	< 10	< 10
Tapioka[1]	3	10.3 ± 1.0	< 10	< 10

[1] Products with EU organic production logo.

results of the analysis SRM Rice Flour 1569b obtained by ICP-MS and NAA are compared in Table 3. Taking into account the total As content, both techniques give accurate results. The differences between those methods are observed for the validation parameters. The expanded uncertainty U (k = 2) was estimated at 10% for ICP-MS and 3% for RNAA. The limit of detection for total As determination, was calculated at 120 ng/g for ICP-MS measurements, and 7 ng/g for RNAA.

DMA, as a predominant compound is SRM 1569b, was determined with good precision both by HPLC-ICP-MS and HPLC-NAA. In the case of MMA, only determination by HPLC-NAA was possible. Determination of total inorganic arsenic (inAs) by HPLC-ICP-MS was impossible. Whereas a distinct analytical signal for As(III) was observed, the signal from As (V) was very low, closed to the background noise. The results obtained by HPLC-NAA for inAs were in good agreement with the certificate values. Also, in the case of very low content of MMA determined by NAA, obtained results and certified values were in good agreement.

Table 4 presents results for infant formulas analysed in this work by HPLC-NAA, except buckwheat. The total content of As in the latter material was below the detection limit. As can be seen, for rice-based samples, the biggest contribution was from inAs – mostly from As(III). In the case of soy-based milk, the content of DMA and inorganic As were at a similar level. For corn, millet and spelt cereals, the concentration of all species were below 10 ng/g. Due to low content of arsenic in grain porridges, only DMA and inorganic As (III, V) could be determined with good accuracy. The MMA content was below detection limit in all analysed materials. Detection limit for HPLC-NAA was calculated according to Roger's convention and amounts to 10 ng/g (Rogers, 1970).

Results obtained in this work demonstrate, that using of chromatographic separation coupled with off-line NAA detection, enables to determine selected chemical forms at low concentration level with good accuracy.

4. Conclusions

The highest content of inAs was in hypoallergenic product (rice as main constituent) and rice porridges. In the case of organic products, inAs content was at 10.1 ng/g and lower. Assuming (according to manufacturers' recommendations) that the daily intake of hypoallergenic cereal for a six-month-old infant is 40 g, the estimated inorganic As per serving is 2.6 μg. The obtained value is twice higher as estimated by the EFSA (EFSA, 2014). Obtained results show that HPLC-NAA is a complementary method to HPLC-ICP-MS when determining As species in food. This is particularly important for samples with low analyte content. Increasing the number of chromatographic injections for 3–5 times improved the detection limit of HPLC-NAA up to 10 ng/g. Results obtained using the NAA are independent on chemical form of elements. There are no chemical matrix effects that can occur in analytical techniques based on atomic properties and their changes. It should be strongly mentioned, that this advantage is particularly important for the speciation analysis – when no chemical standards for individual species are available.

A proposed complementary method enables the verification of results obtained with HPLC-ICP-MS, especially for samples with low As content. Also, presented results have confirmed the possibility of using HLPC-NAA in the certification of As species in new certified reference materials with low arsenic content, even below 100 ng/g.

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Compliance with ethical standards

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent: Not applicable.

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