Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

OPTIMIZATION AND DETERMINATION OF INORGANIC ARSENIC BY LIQUID EXTRACTION AND INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY IN FOOD MATRIX

(Pengoptimuman dan Penentuan Arsenik Tak Organik Melalui Pengekstrakan Cecair dan Spektrometri Jisim-Plasma Gadingan Aruhan dalam Sampel Makanan)

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Received: 28 August 2021; Accepted: 28 October 2021; Published: xx December 2021

Abstract

Various kinds of food are at risk of arsenic exposure and this can lead to potential influences on human health. However, the toxicity of arsenic depends on its different forms, i.e., inorganic arsenic species (i.e., arsenite and arsenate) are more toxic than organic compounds (i.e., monomethylarsonic acid, dimethylarsinic acid, etc.), and total arsenic contents may provide limited information. In this study, a low-cost analytical method for analyzing inorganic arsenic (iAs) in various food matrices was optimized and validated. The sample preparation was conducted by acidic hydrolysis, arsenic reduction, extraction to the organic phase, then back-extraction to the aqueous phase to obtain the solutions prior to measurement on the inductively coupled plasma mass spectrometry (ICP-MS). The food matrices were hydrolyzed by 30% (v/v) HCl at 90 ^{o}C within 120 minutes to obtain the sample liquid containing iAs. The inorganic species in the sample liquid were reduced by N_2H_4/HBr, then extracted by $CHCl_3$ to organic phase, back-extracted into 2% HNO_3 solution, and quantified by ICP-MS with He as the collision gas. Various parameters related to the sample preparation were investigated and evaluated by different food matrices as well as the rice proficiency testing scheme 07273 provided by Fapas. The results showed no significant interconversion between inorganic and organic arsenic species when HCl was utilized as the hydrolysis agent. The limit of detection (LOD) and limit of quantification (LOQ) values were estimated as 1.7 and 5.0 µg kg^{-1}, respectively. The repeatability and reproducibility were assessed by calculating RSD_r (below 4.4%) and RSD_R (below 10%), favorable with Appendix F. AOAC (2016). The recoveries for all spiked samples ranged from 88 to 115%. The analytical method was applied to determine and assess the variations of iAs contents in anchovy, herring, white rice, brown rice, and fish sauce. All available samples met the requirements performed in Codex Stan 1993-1995, Amended in 2015, Commission Regulation (EU) 2015/1006, and Vietnam National technical regulation 8-2:2011.

Keywords: inorganic arsenic, inductively coupled plasma mass spectrometry, food matrices, extraction

Abstrak

Pelbagai jenis makanan boleh berisiko terhadap kehadiran arsenik dan ia akan membawa kesan terhadap kesihatan manusia. Namun, ketoksikan arsenik bergantung kepada spesies yang berbeza seperti spesies arsenik tak organik (i.e. arsenit dan arsenat) lebih toksik berbanding sebatian organik (i.e. asid monometilarsonik, asid dimetilarsinik dan lain-lain), dan kandungan jumlah arsenik juga mempunyai maklumat yang terhad. Melalui kajian ini, kaedah analisis kos rendah bagi analisa arsenik tak organik (iAs) di dalam pelbagai matriks makanan telah di optimum dan di tentusahkan. Penyediaan sampel dijalankan melalui hidrolisis berasid, penurunan arsenik, pengekstrakan pada fasa organik, kemudian pengekstrakan semula pada fasa akues untuk mendapatkan larutan sebelum pengukuran menggunakan spektrometri jisim plasma gandingan aruhan (ICP-MS). Matriks makanan telah di hidrolisis menggunakan 30% (v/v) HCl pada 90 ^{o}C selama 120 minit untuk mendapatkan sampel cecair mengandungi iAs. Spesies tak organik di dalam sampel cecair kemudian diturunkan oleh N_2H_4/HBr, dan diekstrak mengunakan $CHCl_3$ pada fasa organik, kemudian pengekstrakan semula ke dalam larutan 2% HNO_3, dan pengkuantitian oleh ICP-MS bersama He sebagai gas pelanggaran. Pelbagai parameter yang berkaitan penyediaan sampel dikaji dan dinilai mengunakan matriks makanan berbeza dan skim 07273 ujian kemahiran terhadap beras oleh Fapas. Hasil kajian menunjukkan tiada perbezaan diantara spesies organik dan tak organik apabila HCl digunakan sebagai agen hidrolisis. Nilai had pengesanan (LOD) dan pengkuantitian (LOQ) masing-masing ialah 1.7 dan 5.0 µg kg^{-1}. Kebolehulangan dan penghasilan semula dinilai melalui pengiraan RSDr (di bawah 4.4%) dan RSD_R (di bawah 10%), sesuai berdasarkan Apendiks F. AOAC (2016). Perolehan semula bagi semua sampel dipaku pada julat 88 hingga 115%. Kaedah analisis kemudian digunapakai bagi penentuan dan penilaian variasi kandungan iAs di dalam ikan bilis, hering, beras putih, beras perang dan sos ikan. Semua sampel mematuhi keperluan di bawah Codex Stan 1993-1995, Pindaan 2015, Peraturan Suruhanjaya (EU) 2015/1006, dan peraturan teknikal Kebangsaan Vietnam 8-2: 2011.

Kata kunci: arsenik tak organik, spektrometri jisim plasma gandingan aruhan, matriks makanan, pengekstrakan

Introduction

Arsenic (As) contamination in food has been reported globally, especially in South and Southeast Asia [1, 2]. Scientists have shown that there is the uptake of As from water and soils by various plants, marine animals, fruits, and vegetables, then to food products with specific studies [3]. Plants specifically take up arsenate, which is the main As species in aerobic soils, through phosphate transporters. Meanwhile, arsenite, which is predominant in flooded paddy soils, is mostly taken up through aquaglyceroporins in microbe [4, 5]. The reducing environments of paddy soils lead to the mobilization of arsenite into soil solutions and the bioavailability to rice plants. The arsenite is absorbed through the silicon pathway to root cells and efflux towards the xylem. Moreover, the arsenate present in root cells could be reduced to arsenite, which is then complexed by thiol peptides or transported to shoots [5]. Animals on the other hand, are exposure to inorganic arsenic (iAs) has been present in drinking water and feed. This especially occurs for feed of plant origins, through biotransformation, in which iA is converted into organic arsenic (oAs) [6].

Total arsenic (tAs) and inorganic arsenic (iAs) are classified as "Carcinogenic to humans" (Group 1) by the International Agency for Research on Cancer because they cause cancers of the skin, bladder, and lungs. Limited evidence are available on their ability to cause cancers of the kidney, liver, and prostate [7]. Various organizations in the world have established the maximum level (ML) of tAs and iAs in many kinds of food. The World Health Organization (WHO) regulated the ML of tAs in foods allowed as 0.1 mg kg^{-1} [8]. The European Union (EU) published Regulation 2015/1006 on the ML values of iAs in foodstuffs, typically rice and rice-based products, ranging from 0.10 to 0.3 mg kg^{-1} [9]. Besides, the Australia New Zealand Food Standard Code showed the ML of 1 mg kg^{-1} for seaweed and mollusks, while iAs is not permitted to exceed 2 mg kg^{-1} for crustacean and fish [10]. Moreover, according to Food and Agriculture Organization, FAO/CODEX guideline, a previous screening by tAs determination and speciation analysis should be carried out in case of tAs > 0.2 mg kg^{-1}; however, this guide is only for polished rice [11]. Many other food categories have been reported to contain relatively high amounts of tAs of

0 - 1.9 mg kg^{-1} (corns, *Zea mais as* cereals) [12-14], 13 mg kg^{-1} (lettuce leaf as vegetables) [15], 22.4 µg L^{-1} (apple juices) [16], 42.6 µg L^{-1} (beef broth as animal products) [17], and 98 mg kg^{-1} (freshwater algae as seafood products) [18]. Additionally, rice was reported to perform the highest As concentration with the maximum reported as 267.7 mg kg^{-1} [19]. This could be explained due to the cultivation conditions and geographical factors that rice plants are grown in the flooded soil areas, mostly in South and Southeast Asia, considered to have the highest As concentration in the world [1, 20-22]. Thus, individuals who eat a lot of rice and rice-related products as their main diets would have a much higher risk of As exposure [23].

The toxicity of As depends on its species, in which inorganic arsenic (iAs) are more toxic than organic arsenic (oAs) due to the binding ability to the protein thiol groups (−SH) [24, 25]. This phenomenon causes protein activity inhibition and damaging the cellular structure. It is noteworthy that As in the dissolved mineral form of As_2O_3 (arsenic trioxide) is easily absorbed through the intestinal wall. The LD_{50} of As(III) and As(V) are 34.5 mg kg^{-1} and 41 mg kg^{-1}, respectively [26]. LD_{50} values showed that the toxicity of two inorganic species was similar, and the control of iAs is perfectly reasonable. International Organizations such as FAO and EU impose threshold limits on iAs in food (total content of As(III) and As(V)) rather than tAs [9, 27] . Under the current global free-trade conditions, the potential risk of As exposure is not only for those living in the polluted areas but also in other parts worldwide. Therefore, the determination of the specific As species and obtain information about the inorganic arsenic/total arsenic (iAs/tAs) ratios, particularly in the food, was interested by scientists during the last decade.

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For determining inorganic species of arsenic, certain separation techniques should be carried out prior to measurement, especially iAs is present at remarkably low concentrations in food samples. The high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) is known as the reference technique for determining iAs due to its favorable sensitivity and effectiveness [28, 29]. However, such systems are expensive and complicated, and hence they are not commonly equipped and used in many laboratories. Moreover, the operation requires skilled techniques and high running costs. Several alternative analytical methods such as solvent extraction combined with hydride generation-atomic absorption spectrometry (HG-AAS) [26], solid phase extraction (SPE) combined with HG-AAS [11], gas chromatography-tandem mass spectrometry (GC–MS/MS) after derivatization with Dimercapto-1-propanol (British Anti-Lewisite reagent) [30] could be applied to serve both the separation and analyte quantification. However, these alternatives have an average sample preparation time of up to about 12 to 16 hours; therefore, it is necessary to investigate and develop a more simple, cheaper, but faster analytical method for iAs determination in various food matrices. Notably, the quality and commercial value of perishable foods such as seafood, vegetables, and fruits would be degraded if the storage and waiting period for analysis is prolonged.

In Vietnam, the maximum levels of several heavy metals in foods are based on National Technical Regulation, QCVN 8-2:2011/BYT [31] and Decision 46/2007/QD-BYT [32], but for As, the regulated values only refer to tAs. Therefore, the objectives of this study were to (i) investigate several parameters related to a low-cost and time-saving sample preparation procedure based on liquid extraction after sample hydrolysis and measuring on the inductively coupled plasma mass spectrometry (ICP-MS) specified for elemental analysis, (ii) evaluate the optimized analytical method performance for iAs quantification in food matrices, and (iii) determine and assess the variability of iAs contents in various foods purchased in Vietnamese local markets around Ho Chi Minh City. This is among the initial studies to build up a database to serve the future control regulations in Vietnam.

Materials and Methods

Instrumentation and apparatus

The Thermo Scientific™ iCAP Q ICP-MS (USA) was used for As measurement (Table 1). The device is placed at Warrantek Joint Stock Company-Testing Center, Can Tho City, Vietnam.

The microwave digestion system MARS 6 (CEM, USA) was employed for sample preparation. All laboratory apparatus and glassware had to be cleaned by soaking in 2% (v/v) HNO_3 overnight, then washed three times using deionized distilled water, and allowed to dry before using.

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Table 1. Instrumental conditions for ICP-MS quantification

Parameter	Value
RF power	1550 W
Plasma-generating argon flow rate	14.0 L min^{-1}
Auxiliary air flow	0.8 L min^{-1}
Spray air flow	1.0 L min^{-1}
Peristaltic pump speed	40 rpm
Sampling depth	5.0 mm
Collision gas flow	5.0 mL min^{-1}
Spray chamber temperature	2 °C
Dwell time	0.2 s
Sweeps	10 cycles

Reagents and standards

Deionized water (DIW), 18 MΩ.cm, was obtained from a Milli-Q water system (Molsheim, France). Other chemicals and reagents, including 65 % nitric acid (HNO_3), 38% hydrochloric acid (HCl), 48% bromic acid (HBr), 99% hydrazine sulfate ($(N_2H_5)HSO_4$), methyl isobutyl ketone or MIBK ($(CH_3)_2CHCH_2COCH_3$) ≥ 99.9%, benzene (C_6H_6), toluene ($C_6H_5CH_3$), ethyl acetate ($CH_3COOC_2H_5$) ≥ 99.9%, chloroform ($CHCl_3$) ≥ 99.9%, iso-propanol ($CH_3CHOHCH_3$) ≥ 99.9%, hexane ($CH_3(CH_2)_4CH_3$) ≥ 99.9%, 30% hydrogen peroxide (H_2O_2) and sodium sulfate (Na_2SO_4), were of analytical grade and bought from Merck (Germany). Arsenate (H_3AsO_4) standard stock solution (1000±3 mg L^{-1}) was purchased from Merck (Germany). An internal standard solution of ^{89}Y (10 µg mL^{-1}) was provided by Accu Standard. Tune B iCAP Q solution (Ba, Bi, Ce, Co, In, Li và U) of 1.0 µg L^{-1} was supplied by Thermo Fisher. Multi-element solution was Thermo iCAP Q (0.035 mg L^{-1} Be; 0.020 mg L^{-1} Zn; 0.015 mg L^{-1} Cu, Ni; 0.010 mg L^{-1} Al, Ga, Mg; 0.008 mg L^{-1} Co, Li, Sc; 0.006 mg L^{-1} Ag, Mn; 0.005 mg L^{-1} Sr; 0.004 mg L^{-1} Ba, Tl (Thallium); 0.003 mg L^{-1} Bi, Ce, Cs, Ho, In, Rh, Ta, Tb, U, Y in 2 % HNO_3).

Sample collection

The study was carried out on several specific food matrices in Vietnam with highly potential risks of iAs exposure such as cereals (rice, brown rice), fish, and seafood products (fish, fish sauce). Various types of foods, including rice (n=3), brown rice (n=3), herring (n=3), anchovy (n=3), commercial Vietnamese fish sauce (n=3) were obtained from the local markets in Ho Chi Minh City. About 500-1000 g of shrimp, fish, and cereal samples, kept in a polyethylene (PE) bag with a folding edge and an entire bottle of fish sauce product. Fish samples were removed of their head, viscera, and tail. The samples were crushed and well homogenized in a mill. The resulting samples were stored in a decontaminated tube and stored in a freezer until analysis. Cereal samples were crushed and homogenized to a fine texture in a mill, and stored at room temperature (20 °C) until analysis. Fish sauce was stored at room temperature (20 °C), as recommended on the product's label.

Determination of total arsenic by ICP-MS

The tAs concentration was determined according to the method described in AOAC 2015.01 [33]. Briefly, a sample quantity of (1.000±0.001) g for seafood, 0.500±0.001 g for rice, and 0.200±0.005 mL for fish sauce) was weighed into a PTFE digestion tube, in which 4.0 mL of 65% HNO_3 and 1.0 mL 30% H_2O_2

were added. The digestion process was completed by the microwave digestion system MARS 6. Finally, the digested solution was diluted to the calibration mark by DIW in a 50 mL volumetric flask before the measurement by ICP-MS.

Determination of inorganic arsenic by ICP-MS

A sample (1.000±0.001 g) was weighed into a sealed reaction tube, in which 4.0 mL of DIW was added, and the tube was shaken for 5 minutes. Next, 18.0 mL of 30% (v/v) HCl was added, and the mixture was incubated at 90 °C for 120 minutes for hydrolysis. Next, 1 mL of 1.5% N_2H_4, 2 mL of 38% HBr, and 5 mL of $CHCl_3$ were added into the tube, followed by 5 minutes of shaking after each chemical was added. Phase separation was allowed to occur before centrifugation at a minimum speed of 5000 rpm. The bottom phase of $CHCl_3$ was transferred to another reaction tube to repeat the extraction in $CHCl_3$ for two more times. All organic phases were then combined and filtered through a 0.45 µm PTFE membrane. Next, 5 mL of 2% (v/v) HNO was added to the organic filtrate, followed-by centrifugation at 5000 rpm for 5 minutes for the back-extraction of iAs into the aqueous acid phase. The upper phase of the HNO_3 was transferred to a 50 mL volumetric flask and extraction in HNO_3 was repeated two more times. All extracted HNO_3 phases were collected and diluted to the calibration mark by DIW, prior to measurement by ICP-MS.

Method validation and data processing

Based on the procedure of the Nordic Committee on Food Analysis [34], statistical evaluation was carried out using Microsoft Excel (2019) with conformity to ISO 5725-2 [35]. The calibration curve was established based on the linear relationship between the analyte concentrations of 0.10, 0.20, 0.30, 0.50, 1.0, 1.5, 2.5, 5.0, 7.5, 10, 15, 20, and 25 µg L^{-1} and a specific ratio between the intensities of analyte and internal standard at each concentration ($^{75}As^{+}/^{89}Y^{+}$). The limit of detection (LOD) and limit of quantification (LOQ) were determined at three and ten times the standard deviation (SD) at in-house validation conditions, respectively. The intra-day and inter-day data were used to determine the in-house relative standard deviation of repeatability (RSD_r) and reproducibility (RSD_{iR}), respectively. The accuracy of the method was tested by a recovery test of the spiked iAs in actual food samples of rice, fish, and fish sauce (n = 8). The iAs concentrations in these samples were estimated, and the concentrations were mimicked using a standard solution. The samples were spiked, whereby the amount of spiked iAs equalled the amount of iAs in each matrix samples. Recoveries were calculated using the following equation 1:

$$\text{Recovery} = \frac{C_{\text{spiked-determined}} - C_{\text{non-spiked}}}{C_{\text{spiked-desired}}} \quad (1)$$

where $C_{\text{spiked-determined}}$ and $C_{\text{non-spiked}}$ represent for the amount of iAs determined in spiked and non-spiked samples; $C_{\text{spiked-desired}}$ is the actual amount of iAs desired to be spiked in the sample.

In a collaborative trial, the method performance Fapas proficiency testing program of rice flour sample (ID 07273) through z-score, in which $|z - \text{score}| \leq 2$ would exhibit satisfactory performance [36]. The calculation of z-score is shown as in the following equation 2:

$$z - \text{score} = \frac{C_{\text{lab}} - C_{\text{ref}}}{\sigma} \quad (2)$$

where C_{lab} is the measurement results reported by the participant laboratory in the proficiency testing program, C_{ref} is the assigned value provided by the proficiency testing program and σ is the standard deviation for proficiency assessment, provided and fixed by the proficiency testing program organizer.

Results and Discussion

The sample preparation involved for determining iAs in food matrices was carried out based on the liquid extraction technique using organic solvent after sample hydrolysis in an acidic medium and As(V) reduction to As(III), by a proper reductant. The iAs in the organic phase was then back-extracted to the acidic aqueous phase to be quantified on the ICP-MS. Furthermore, the influence of several parameters towards the sample preparation procedure, particularly on the sensitivity of the analytical method, were investigated. These parameters were the types of acid reagents used for sample hydrolysis, hydrolysis time and temperature,

reducing agents and organic solvents for liquid extraction, and back-extraction conditions to obtain aqueous solutions for the ICP-MS measurement.

Effects of acid reagents on the hydrolysis

The iAs (i.e., arsenite) binds to protein molecules via ligands of thiol sulfide (i.e., −SH−As) [37]. Before further steps in the liquid extraction process, the sample matrices were hydrolyzed in an acidic medium (combined with heat). The acid treatment aid in the breaking of bonds between iAs and sample matrices, and obtains liquid samples containing iAs species. Incomplete or low-efficiency hydrolysis would lead to a remarkable quantity of iAs to remain in the matrices, leading to a lower detection of intensities. It is also crucial to avoid the conversion between the iAs and oAs species during the hydrolysis period.

nitric acid: missing number "3" in the chemical equation

HCl and HNO have different oxidation properties that make them beneficial for utilization as hydrolysis reagents for food matrices [11, 38-40]. In this study, 30% (v/v) HCl and 2% (v/v) HNO_3 were used as the hydrolysis reagents to investigate their effects on the measured iAs intensities (Figure 1). However, concentrations of the acid were guided from previous studies which used favorable conditions, specifically those from Muñoz et al. [26], Fiamegkos et al. (2015) [39] for HCl and dos Santos et al. (2017) [11], Kang et al. (2016) [38] for HNO_3. It was also estimated that the lower concentration of HNO_3 might help to minimize the unexpected reactions between the residual HNO_3 and other reactants due to its strong oxidation properties (discussed later). Meanwhile, Cl^- is relatively inactive in acidic medium and the excess H^+ from HCl might also promote the reduction reaction of As(V) to As(III) after sample hydrolysis ($H_3AsO_4 + 2H^+ + 2e^- \rightleftharpoons HAsO_2 + 2H_2O$, $E^o = 0.560V$) [41].

The results shown in Figure 1 indicates that the efficiency of using HCl was about 3 times higher than that of HNO_3 for the three sample matrices. This was comprehended from the measured intensities of 3094 *vs*. 724, 1057 *vs.* 214, and 988 *vs.* 314 for rice, fish, and fish sauce, respectively. The lower intensities obtained from HNO_3 could be explained by the acid's strong oxidizing property and competitive reactiveness with N_2H_4 as the reducing agent. The unexpected reaction between the residual HNO_3 from the hydrolysis and N_2H_4 as the reducing agent could compete for the reduction reaction to form As(III) from As(V), thus decrease the reaction's performance. Therefore, lower intensities of iAs were obtained when HNO_3 was applied as the hydrolysis reagent, compared to HCl. Additionally, in several sample matrices with high oAs, such as fish and fish sauce, the application of HNO_3 might cause positive errors, due to the decomposition of oAs by HNO_3, particularly at the acid's high concentrations. Meanwhile, some common oAs species such as DMA and MMA were proved to be relatively stabler in both acidic and neutral media. Both oAs species were not present in any critical conversion into iAs when HCl was used as the hydrolysis reagent [42]. Therefore, HCl was used as the hydrolysis reagent for further investigations and experiments in this study.

Effects of temperature and time on hydrolysis

The effects of the hydrolysis temperature and time on the intensities of iAs were recorded in various food matrices (fish, fish sauce, and rice). The sample's matrix hydrolysis required the combination of an acidic medium and high temperature within a certain hydrolysis time. HCl is a common H^+ ion provider to form an acidic medium for sample hydrolysis before further reduction and liquid extraction. However, the use of HCl usually requires a higher temperature and/or a longer time to completely and quantitatively hydrolyze entire sample matrices. The hydrolysis temperature was previously reported to be performed at 85 to 95°C [11, 26, 42] or ambient condition [26].

spacing between number and unit

However, remarkably longer hydrolysis time of up to 12-15 hours might be required in the case of lower temperatures [26]. Therefore, this study did not apply very low temperature and limited the surveyed temperature from relatively high (50 °C) to the highest referenced values of 95 °C in order to evaluate the effects of temperature on hydrolysis efficiency.

In this study, the reaction time varied from 50 to 210 minutes. The conditions with the highest measured intensities were applied for further experiments. Based on Figure 2, the hydrolysis temperature was dependent on the sample matrices. A temperature of under 60 °C

was insufficient to completely hydrolyze all samples although hydrolysis time was increased up to 210 minutes. This means that the measured intensities did not reach their maximum. In particularly, food matrices (rice, brown rice in Figure 2c) with a higher starch or cellulose content required a higher hydrolysis temperature (up to 90ºC). Meanwhile, the protein-rich matrices (fish, fish sauce) required lower temperatures (80 and 70ºC for fish and fish sauce in Figure 2a and 2b, respectively).

The hydrolysis time above 120 minutes showed the highest and most stable intensities for all matrices. Therefore, for the wide range of food, 90ºC and 120 minutes would be used as the hydrolysis temperature and time, to assure the highest intensities. The optimization of temperature on hydrolysis has reduced the analysis time compared to several previous studies [11, 26, 28].

The effects of solvents on the extraction efficiency

Extraction is an important step to recover iAs. In complicated matrices, As(III) and As(V) exist simultaneously, therefore, it is necessary to use an appropriate reducing agent to convert all forms of iAs to As(III). The most commonly used reducing agent is I^- in KI [11, 43]. The methylated compounds of As(V), such as MMA and DMA reacted with KI to form CH_3AsI_2 or $(CH_3)_2AsI$. These species were then extracted by the less polar solvents (benzene, toluene, or chloroform). Moreover, AsI_3 has a large nonpolar covalent bond strength (electronegativity difference of 0.44), that to break this bond at the organic phase, using I^- was more difficult than Br^-. A previous study [44] had showed that Br^- was the more favorable halide forming agent for As(III) in the matrix. This was in the presence of oAs and the extraction efficiency by benzene, toluene, or chloroform was in the order of As(III) > As(V) >> oAs [40]. However, one disadvantage is that Br^- has a weak reducing strength. Thus, the combination of HBr and another reducing agent, N_2H_4 was employed to enhance the reduction performance [34, 39].

The iAs in the liquid samples endured liquid extraction by an organic solvent after hydrolysis. Various lesser polar solvents, including chloroform, methyl isobutyl ketone or MIBK, ethyl acetate, iso-propanol, and hexane, were investigated during the extraction of $AsBr_3$ compounds (electronegativity difference of 0.78). The effects of solvent on the extraction efficiency were evaluated through the recovery test of the spiked fish, fish sauce, and rice samples. The iAs concentrations in the real samples were estimated, and the standard solution was spiked to the sample (the amount of spiked iAs equal to the amount of iAs in the real samples for each matrix).

For each extraction solvent, the recoveries of three sample matrices (fish, fish sauce, and rice) did not exhibit any remarkable variations. The average recoveries were calculated from the results obtained from these three sample matrices (Figure 3). The result showed that the chloroform performed the highest extraction efficiency with the highest recovery (97.2%). Therefore, chloroform was used as the extraction solvent for further investigations and experiments to assure the highest sensitivity due to low iAs contents in food matrices. The application of chloroform as the extraction solvent was also reported in the publications of O. Muñoz et al. [26], Chappell et al. [45], Maria et al. [46]. The emulsification did not occur during the extraction, and the recovery of iAs depended on the solubility of $AsBr_3$ into the organic phase.

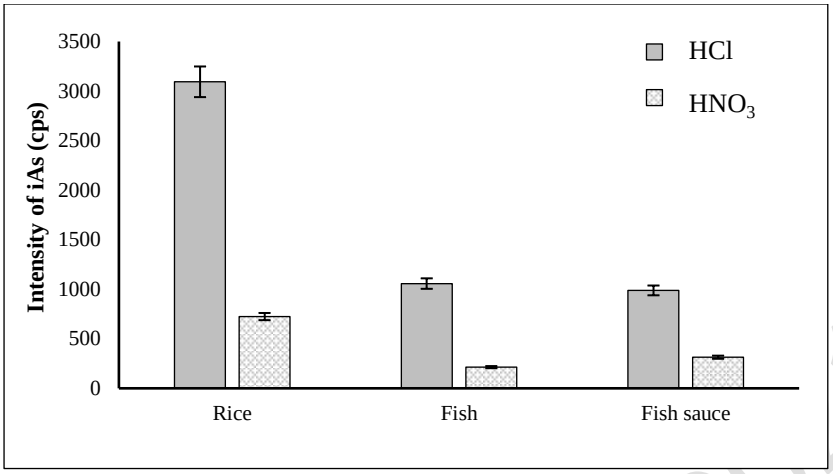


Figure 1. Effects of acid reagents on the hydrolysis efficiency

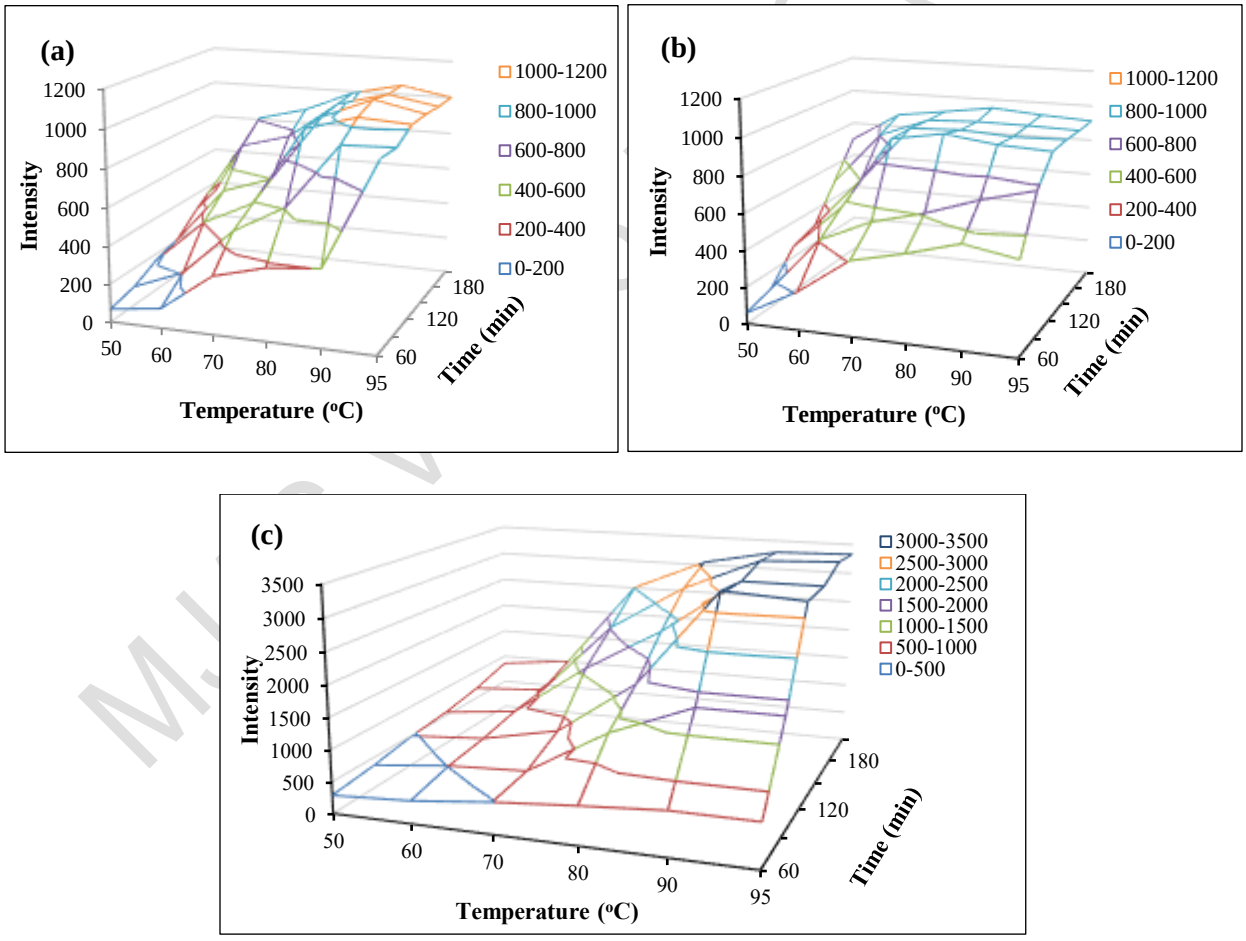


Figure 2. Effect of temperature and time on the hydrolysis efficiency of (a) fish, (b) fish sauce, (c) rice (the color representing for the different intensity magnitude)

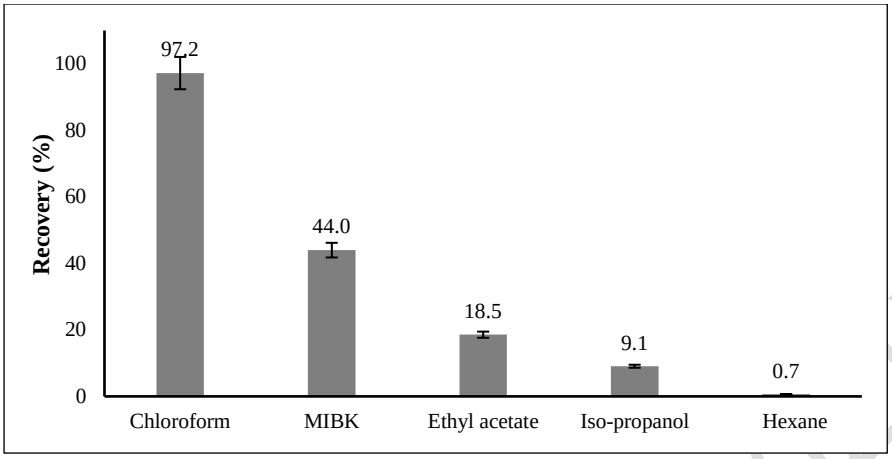


Figure 3. Effects of acid reagents on the hydrolysis efficiency (MIBK: Methyl Isobutyl Ketone)

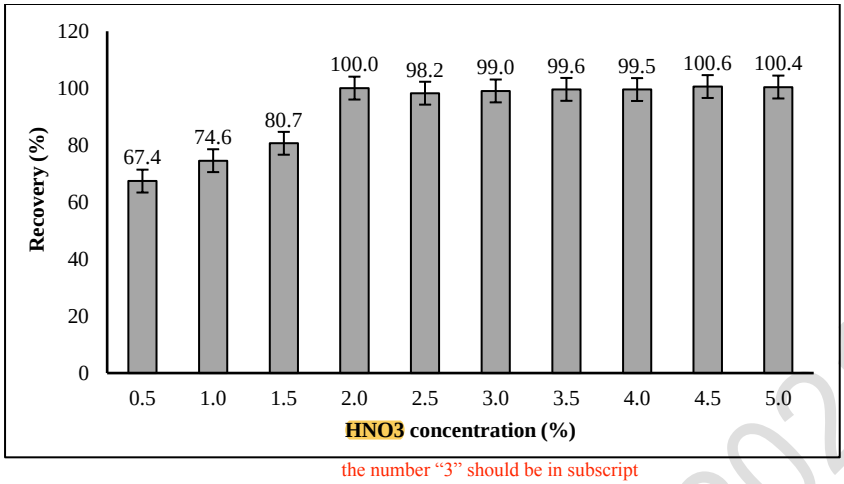
Back-extraction into the aqueous phase

oAs-Br (MMA, DMA) and iAs(III)-Br were simultaneously extracted into the organic phase, so it was necessary to use a suitable agent to break As-Br bonds in oAs-Br and iAs-Br. In this study, 1% (v/v) HCl and 2% (v/v) HNO_3 were used as the acid reagents for back-extraction. The intensities (repeatability, %RSD) during the back-extraction by HCl and HNO_3 were 840.23 (RSD = 26%) and 1179.30 (RSD = 4.0%). When HCl solution was used, $AsBr_3$ was dissociated according to the equation 3:

$$AsBr_3 + 3Cl^- \rightleftharpoons AsCl_3 + 3Br^- \text{ (*)} \quad (3)$$

According to Le Chatelier's principle, when Cl^- concentrations increased, the equilibrium (*) was towards the formation of $AsCl_3$, and $AsCl_3$ was a weaker covalent compound than $AsBr_3$ (due to the ion radius difference between Br^- and Cl^- ions). Therefore, it was difficult to extract $AsCl_3$ into less polar organic solvents. Moreover, the application of HCl for back-extraction and introduction in the ICP-MS was not appropriate because HCl created polyatomic interferences, typically $^{40}Ar^{35}Cl^+$ (m/z = 75), which coincides with the m/z of As. Due to its strong oxidizing properties, HNO_3 easily broke the $AsBr_3$ molecule bond by oxidizing As(III) to As(V). As(V) compounds tended to exist in the forms of oxygen bonds under conditions where the concentration of halogen ions was not high enough. Therefore, As(V) was distributed into the HNO_3 phase according to the reaction (**), resulting in higher recoveries and repeatability (Figure 4). For the ICP-MS, the acid concentration was as low as possible. The concentrations of 2% HNO_3 were possible to prevent memory effects.

$$AsBr_3 + 2HNO_3 + 2H_2O \rightarrow H_3AsO_4 + 2NO_2 + 3HBr \text{ (**)} \quad (4)$$



the number "3" should be in subscript

Figure 4. The effects of HNO_3 concentrations on the recoveries

Analytical method evaluation for the determination of inorganic arsenic in food

The regression equation was y = 0.0137x + 0.0003, whereas x and y stand for standard concentrations and intensity ratios between the analyte and internal standard, respectively. The squared correlation coefficient, R^2 = 0.9995, indicated the goodness of linearity. The calibration curve was established from 0.10 to 25 µg L^{-1}, suitable for wide ranges of foodstuffs with various iAs concentrations. The limit of detection (LOD) and limit of quantification (LOQ) were 1.7 µg kg^{-1} and 5.0 µg kg^{-1}, respectively. These estimated LOD and LOQ values were very low compared to the actual concentrations of iAs in real food samples. The repeatability and reproducibility were assessed through the calculation of RSD_r (< 4.4%) and RSD_R (< 10%), in agreement with Appendix F. AOAC [47]. The recoveries of spiked food samples ranged from 88 to 115%. The method performance was evaluated by rice proficiency testing scheme 07273 provided by Fapas (Table 2). The determined arsenic results showed that the developed method was equivalent to the arbitration method on the LC-ICP-MS. The concentrations of iAs, oAs, tAs with |Z-score| ≤ 2 complied with the required performance criteria for methods used for official food control purposes as decided by the European Commission [48]. The results obtained also indicated that the studied method did not perform a conversion between iAs and oAs. Therefore, it was possible to selectively extract iAs in the matrices containing oAs.

Evaluation of the inorganic arsenic in food

The optimized method was evaluated for accuracy through the data in Table 2. The determination of arsenic on the ICP-MS was set up according to the parameters mentioned in Table 1. The optimized method was applied to evaluate the inorganic arsenic in anchovy, herring, white rice, brown rice, and fish sauce (Table 3).

As can be seen from Figure 5, when the samples were animal tissues or plant products, typically rice, the ratios of iAs/tAs were relatively high, while the samples of interest were animals or their products; these ratios were smaller. The brown rice products presented their remarkably higher iAs/tAs ratios (51.1-97.9%), which was consistent with the report of Hassan et al. [49]. The ingredients used for fish sauce production performed the lowest iAs/tAs (0.31% to 0.58%); however, these ratios got higher for fish sauce samples (3.82-4.54%). The experimental results indicated that iAs contents were all met the requirements shown in Codex Stan 1993-1995 [27], Commission Regulation (EU) 2015/1006 [9], and Vietnam National technical regulation 8-2:2011 on the limits of heavy metals contamination in food [31].

Table 2. Comparison of assigned arsenic values and determined results

	Assigned Value (μg kg^{-1})	Result (μg kg^{-1})	Z-score
iAs	162	160	0.0
tAs	220	226	0.1
oAs[a]	58	66	0.1

[a] [oAs] = [tAs] – [iAs]

Table 3. Variations of arsenic contents in food (average ± standard deviation for three replicates)

Food sample	iAs (μg kg^{-1})	tAs (μg kg^{-1})	iAs/tAs (%)
Herring-1	16.43 ± 0.47	3165 ± 85	0.5
Herring-2	10.67 ± 0.19	2130 ± 69	0.5
Herring-3	22.08 ± 0.63	3788 ± 99	0.6
Anchovy-1	8.13 ± 0.27	2610 ± 87	0.3
Anchovy-2	10.82 ± 0.76	3146 ± 88	0.4
Anchovy-3	14.99 ± 0.99	4138 ± 96	0.4
Rice-1	93.0 ± 5.9	109.2 ± 3.6	85.2
Rice-2	98.6 ± 6.4	122.1 ± 3.4	80.7
Rice-3	110.5 ± 1.3	118.9 ± 7.6	92.9
Brown rice-1	167.9 ± 1.8	171.5 ± 7.8	97.9
Brown rice-2	120.5 ± 2.7	130.3 ± 9.5	92.5
Brown rice-3	196.8 ± 6.8	385 ± 20	51.1
Fish sauce-1	44.66 ± 0.30	1168 ± 49	3.8
Fish sauce-2	29.9 ± 1.1	661 ± 25	4.5
Fish sauce-3	52.1 ± 3.3	1195 ± 54	4.4

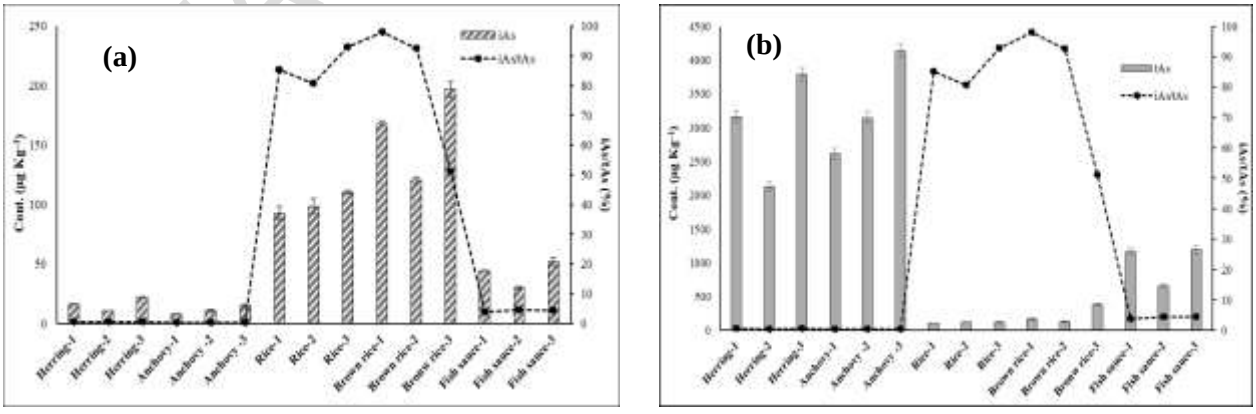


Figure 5. iAs concentration and iAs/tAs ratio (a) iAs and (b) tAs

Conclusion

ICP-MS method for the determination of iAs achieved good selectivity without any significant interconversion between inorganic and organic species. The limit of detection (LOD) and limit of quantification (LOQ) were 1.7 µg kg^{-1} and 5.0 µg kg^{-1}, respectively. The repeatability RSD_r (%) and reproducibility RSD_R (%) were lower than 4.4 and 10%, respectively. The recoveries ranged from 88 to 115% for all spiked samples. This analytical method was applied to assess the inorganic arsenic in anchovy, herring, white rice, brown rice, and fish sauce with high reliability. The iAs contents in various foods were analyzed by the optimized method at 10.67-22.08 µg kg^{-1} for herring, 8.13-14.99 µg kg^{-1} for anchovy, 93.0-110.5 µg kg^{-1} for rice, 120.5-196.8 µg kg^{-1} for brown rice, and 29.9-52.1 kg^{-1} for fish sauce. The concentration of iAs in the rice and rice products was lower than the limits established by FAO and EU for rice products for adult consumption. In most samples of the rice products, iAs was approximately 51.1% to 97.9% of the total content of As present. However, no legislation is currently available for iAs in fish and fish sauce and it is believed that the present study will contribute to the establishment of a threshold concentration. The developed method is suitable for routine analysis in the context of many laboratories only equipped with the ICP-MS instead of modern and high-cost HPLC-ICP-MS.

Acknowledgment

The authors would like to express our thanks and appreciation to Warrantek Joint Stock Company-Testing Center, Can Tho City, Nguyen Tat Thanh University (NTTU), and the University of Science, Vietnam National University Ho Chi Minh City (US-VNU HCMC), Vietnam, for their assistance and support during this study.

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