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Total arsenic in fish as determined by ETAAS following acid digestion or tetramethylammonium hydroxide solubilization

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Abstract. Simple and robust methods for arsenic determination are described. First approach is based on pressurized microwave decomposition of fish tissues in the presence of nitric acid and hydrogen peroxide followed by electrothermal atomic absorption spectrometric (ETAAS) measurement by using modifiers: Pd (1 μg) + Mg (0.6 μg) as the most suitable modifier ensuring pretreatment temperature up to 1300 °C and optimal atomization temperature of 2300 °C. Second approach is based on microwave assisted solubilization of fish tissue by using tetramethylammonium hydroxide (TMAH) as reagent alkaline hydrolysis. In this case most efficient modifier which ensures leveling off responses of different arsenic species presented in fish samples is Pd (1 μg). Loss free pretreatment temperature is 1400 °C and optimal atomization temperature is 2000 °C. Calibration based on standard addition method by using As-betaine as representative standard should be used for As quantification for both proposed procedures. Detection limit achieved for approaches is 0.5 μg g^{-1} and relative standard deviation varied is in the range 7-12 % for the As content in the fish between 0.5-30 μg g^{-1}. The accuracy of both developed methods is verified by the analysis of certified reference materials.

Key words: arsenic, fish, ETAAS, slurry determination

1. Introduction

It is well known fact that marine organisms accumulate arsenic and convert it into organoarsenicals. Practically all the arsenic found in edible seafood species is present either as small cationic organic molecule (e.g. arsenobetaine and arsenocholine) or as phospholipid complexes. The predominance of these forms is significant because they are extremely stable, both chemically and metabolitically, and practically nontoxic [1]. Determination of total. As in seafood is still necessary in many cases as a complement to speciation studies or for food control when only total As is needed. Conventional procedure for total As determination consist of two steps sample digestion followed by instrumental measurement, practically all spectrometric techniques could be used for this purpose – HG AAS [2], ICP-AES [3], ETAAS [4-6] and ICP-MS [7]. Arsenic has traditionally been considered as difficult element when determined because of both: difficult decomposition of organoarsenicals together with high volatility of the element which mean eventual losses during digestion and spectral and chemical interferences during the instrumental measurement. Therefore another approach based on sample solubilization achieved by microwave assisted hydrolysis in organic base such as tetramethylammonium hydroxide (TMAH) is accepted as a convenient and suitable technique for trace As determination. This sample pretreatment can overcome some of the drawbacks inherent in sample decomposition procedures, it is less time and reagents consuming. Nevertheless careful optimization of the whole analytical procedure is required in order to be obtained accurate and reliable results. The main goal of the present work is to develop analytical methodologies for trace arsenic determination in fish based on (i) high pressurized microwave acid digestion or (ii) sample solubilization by using microwave assisted hydrolysis of fish tissues in TMAH followed by accurate As measurement by transverse heated ETAAS with longitudinal Zeeman

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background correction. Variables in the microwave assisted alkaline hydrolysis such as concentration of reagents, mass-to-volume ratio, temperature program for microwave assisted hydrolysis are optimized. As a second step optimization of the instrumental parameters for ETAAS measurement of As is performed e.g. optimal pretreatment and atomization temperature, suitable modifier for efficient isoformation of different arsenic species, suitable calibration procedure taking into account observed matrix interferences are presented.

2. Experimental

Instrumentation

The measurements were carried out with Perkin Elmer Model AAnalyst 600 atomic absorption spectrometer equipped with a transverse heated graphite atomizer (THGA), longitudinal Zeeman effect background corrector and an AS-800 autosampler. THGA graphite tubes with integrated platforms were used as atomizers. Instrumental parameters for AAS are presented in Table 1. The measurements were in peak area mode. Sample aliquots of 20 µl and 5 µl modifier injections were performed successively.

An Ethos Touch Control pressurized microwave digestion device (Milestone, Italy) with a rotor for 12 Teflon digestion vessels was used for Pressurized microwave acid decomposition. A specially designed vessel, which allows a temperature sensor and a pressure sensor to be connected and the progress of the digestion to be monitored, is substituted for one of the 12 vessels. CEM Model Marsx microwave solvent extraction system (CEM Corporation Mathews USA) was used for microwave assisted hydrolysis of fish tissues. The temperature and pressure were monitored and controlled.

Reagents

Stock standard solutions used for As measurements were: 1000 µg ml^{-1} As(III) (atomic absorption spectroscopy standard solution, Fluka 11082); organoarsenics, 1000 µg ml^{-1} dimethylarsinate (DMA) prepared by dissolving sodium cacodylate, $C_2H_6O_2AsNa\cdot 3H_2O$ (Carlo Erba) in doubly distilled water and BCR (Community Bureau of reference) CRM 626 Arsenobetaine calibrated solution 1031 µg ml^{-1} as As-betaine. The working solutions were prepared weekly and kept refrigerated at 4 °C. Stock standard solution of palladium 1000 µg ml^{-1} in 5 % HCl, stock standard solution of Mg 1000 µg ml^{-1} in and stock standard solution of iridium 1000 µg ml^{-1} in 20% HCl were used for modifier solution preparation. Tetramethyl ammonium hydroxide (TMAH, 25 % *V/V*, Merck, p.a.) was used for sample stabilization. Ultrapure nitric acid prepared by subboiling distillation and high purity H_2O_2 prepared by ion exchange was produced by the Institute for high purity substances, Faculty of Chemistry, Sofia. Double distilled water was used in all operations. The following CRMs certified for total As content were used for the validation purposes: Oyster tissue (Standard reference material 1566a Oyster tissue) was purchased from the National Institute of Standards and Technology (NIST) and BCR CRM 627, forms of As in tuna fish.

Table 1. The optimal THGA temperature program for ETAAS determination of As

Step	Parameter	Acid digests	Slurry in TMAH
Drying 1	Temperature, °C	110	110
	Ramp time, s	10	30
	Hold time, s	20	20
	Argon, ml min	250	250
Drying 2	Temperature, °C	130	150
	Ramp time, s	10	15
	Hold time, s	10	20
	Argon, ml min	250	250
Pyrolysis	Temperature, °C	1250	1400
	Ramp time, s	20	30
	Hold time, s	20	20
	Argon, ml min	250	250
Atomi-zation	Temperature, °C	2050	2200
	Ramp time, s	0	0
	Hold time, s	3	5
	Argon, ml min	0	0
Clean	Temperature, °C	2450	2450
	Ramp time, s	1	1
	Hold time, s	3	3
	Argon	250	250

Sample preparation

Pressurized acid digestion in a microwave oven: Lyophilized fish tissue or certified reference material ca. 0.2 g were placed in a teflon digestion vessels, 3 ml conc. HNO_3 and 1.5 ml 30 % H_2O_2 were added, the vessels are capped closed, tightened and placed in the rotor of microwave oven. The

digestion was carried out with the digestion programs: 150 W/10 min; 0 W/2 min; 300 W/5 min; 0 W/2 min; 500 W/10 min. Finally the vessels were cooled, carefully opened and digests quantitatively transferred in 25 ml calibrated flasks.

Microwave assisted hydrolysis and sample solubilization in TMAH: Lyophilized fish tissue or certified reference material ca. 0.1 g were placed in a teflon vessels, 0.3 ml TMAH (25%, *V/V*) and 0.5 ml doubly distilled water are added, the vessels are capped, and placed in the rotor of microwave extraction oven. The hydrolysis of fish tissue was performed by using ramp time of 10 min, hold time 5 min and temperature of 50 °C. Finally the vessels are cooled and hydrolyzates quantitatively transferred in 25 ml calibrated flask.

3. Results and discussion

Arsenic determination in fish after pressurized wet digestion in a microwave oven

It is well known that accurate transformation of As-betaine major arsenic species in fish tissues to As(V) is possible only under very harsh conditions e.g. 300 °C for 90 min, not always achievable with commercially available microwave ovens especially when using medium pressure teflon vessels [8, 9]. This mean that in the sample solution, after pressurized wet digestion of fish tissues in a microwave oven, still exists some amount of As-betaine which is not completely mineralized. However it is also known that not all modifiers equalize thermal behaviour of all As species and also different sensitivity of their ETAAS determination could be expected. From this point of several widely proposed and used modifiers for As thermal stabilization are tested for accurate determination of As(V) and As-betaine in fish digests. In order to construct pre-treatment and atomization curves for As(V) and As-betaine, acid digests obtained after digestion of fish tissues with very low As level were spiked with known amounts of investigated As species. Modifiers used in the optimization studies are: 3 μg Pd, 3 μg Pd + 100 μg citric acid and 1 μg Pd + 0.6 μg Mg. Results obtained showed that all investigated modifiers ensured thermal stabilization of both As species. However the most effective is the combination of 3 μg Pd + 1 μg Mg which allows thermal stabilization to the same pre-treatment temperatures of 1300 °C as well as which ensures identical characteristic masses for both investigated arsenic species. The loss-free pre-treatment temperatures, optimal atomization temperatures as well characteristic masses for As(V) and As-betaine achieved with studied modifiers are summarized in Table 2.

Table 2. Loss free pretreatment temperatures (T_{pr}, °C), optimal atomization temperatures (T_{at}, °C) and characteristic masses (m_0, pg) for As in fish acid digests obtained with different modifiers.

As species	Parameter	Pd	Pd+Mg	Pd+citric
As(III)	T_{pr}	1300	1400	1200
	$T_{at,}$	2200	2300	2200
	m_0	28	28	29
DMA	T_{pr}	1200	1400	1200
	T_{at}	2200	2300	2300
	m_0	27	28	28
As-betaine	T_{pr}	1300	1400	1100
	T_{at}	2200	2300	2200
	m_0	27	28	27

Matrix interferences were evaluated through the slopes of calibration graphs obtained in the presence of aqueous standards and matrix digests as well as by the recovery studies using certified reference materials. Results obtained undoubtedly showed strong matrix interferences evidently due to the increased phosphate content in fish tissue. Therefore standard addition calibration method should be used for As quantification.

Arsenic determination after sample hydrolysis in TMAH

Various arsenic species presented in fish sample hydolyzate after alkaline solubilization may exhibit different behaviour in the graphite atomizer during thermal pretreatment and atomization, resulting in pre-atomization losses and species depend response. Therefore preteratment and atomization curves are constructed with As-betaine, dimethylarsinate (DMA) and As(V) as representative As standards in the presence of 0.3 % TMAH and in the fish solubilizates in TMAH with modifiers: 1 μg Pd, 1 μg Pd + 0.6 μg Mg and 1 μg Pd + 100 μg citric acid. From the results summarized in Table 3 it is evident that 1 μg Pd is the most efficient modifier in this case. It ensures 1400 °C loss free pretreatment for all investigated As species. Unexpectedly optimal atomization

temperature is 2000 °C - higher sensitivity and signals with better absorbance profiles were detected.

Table 3. Loss free pretreatment temperatures (T_{pr}, °C), optimal atomization temperatures (T_{at}, °C) and characteristic masses (m_0, pg) for As in fish solubilizate in TMAH obtained with different modifiers

As species	Parameter	Pd	Pd+ Mg	Pd+citric acid
As(III)	T_{pr}	1400	1400	1300
	$T_{at,}$	2000	2200	2100
	m_0	27	26	29
DMA	T_{pr}	1400	1400	1300
	T_{at}	2000	2200	2100
	m_0	27	25	27
As-betaine	T_{pr}	1400	1400	1100
	T_{at}	2000	2200	2100
	m_0	27	24	22

Matrix interferences were evaluated through the recovery experiments with certified reference materials by using aqueous standard calibration curve based on As-betaine standard, standard curve in 3% TMAH also based on As-betaine standard and standard addition method with As-betaine additions. Results obtained are presented in Table 4. As can be seen strong matrix interferences were detected therefore standard addition method should be used for As quantification in fish solubilizates in TMAH.

Table 4. Arsenic recoveries achieved in certified reference materials by using different calibration procedures

CRM	Recoveries, %		
	Aqueous standards	Standards in 0.3 % TMAH	Standard Addition
Oyster 1566 a	72 ± 4	92 ± 3	96 ± 3
BCR	66 ± 5	79 ± 4	92 ± 2

Accuracy and precision. For both described analytical procedures for As determination in fish detection limit defined as three times standard deviation of the blank is 0.5 μg g^{-1} As and the between-batch precision expected as relative standard deviation varied in the range 7-12 % for the As content in the fish between 0.5-30 μg g^{-1}. Accuracy of the both methods is confirmed by the analysis of certified reference materials.

4. Conclusion

Simple sample solubilization in TMAH is robust and reliable approach for sample pretreatment for As determination in fish tissue. Detection limits and RSD values achieved are comparable with those obtained by more tedious and time consuming high pressurized acid digestion.

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