ARSENIC SPECIATION IN FISH BY HPLC-ICP-MS

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Approval of the thesis:

ARSENIC SPECIATION IN FISH BY HPLC- ICP-MS

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ABSTRACT

ARSENIC SPECIATION IN FISH BY HPLC-ICP-MS

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Arsenic speciation in fish samples on the market was performed using isocratic elution with cation exchange column high performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) detection. Total As concentrations were found by ICP-MS using samples digested by nitric acid-hydrogen peroxide solution using microwave oven digestion; the results were in the range of 1.15-12.6 μg/g. Separation of organic arsenicals, namely arsenobetaine (AB), dimethylarsinic acid (DMA) and monomethylarsonic acid (MA), have been achieved in 12 minutes. Freeze-dried samples were extracted by deionized water with a shaker system; the concentrations of AB and DMA in the extract was then determined using HPLC-ICP-MS. The accuracy of the method for determining AB concentration was confirmed using certified reference material (CRM), DOLT 4 (dog fish liver); for this CRM only preliminary data are available for AB. The arsenic compounds in 6 fish muscle samples were investigated. The predominant arsenic compound found in extracts was AB; the concentrations were in the range of 0.86-12.0 μg/g. DMA concentration was 0.40±0.03 μg/g in one of the samples; in the others it was below the limit of quantation (0.21 μg/g).

Keywords: Arsenic, speciation, arsenobetaine, HPLC-ICP-MS

ÖZ

HPLC-ICP-MS
İLE BALIKTA ARSENİK TÜRLENDİRMESİ

Özcan, Şefika Eroğlu
Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. O. Yavuz Ataman

Eylül 2010, 103 sayfa

Çeşitli marketlerden elde edilen balık örneklerında katyon değiştirici kolon bağlı HPLC-ICP-MS ve izokrotik elüsyon kullanılarak arsenik türlendirme çalışması yapıldı. Nitrik asit ve peroksit karışımı kullanarak mikrodalga etüvde örnek çözülerek toplam As konsantrasyonu tayin edildi. Toplam As sonuçları.15-13 µg/g arasında bulundu. Arsenik türlendirme süresi toplam 12 dakika olarak belirlendi. Bu süre içinde monometil arsonik asit MA(V), dimetil arsinik asit DMA(V) ve Arsenobetain (AB) ayrıştırıldı. Balıklar için bulunan AB değeri sertifikalı referans madde olan CRM DOLT 4 (Köpekbalığı ciğeri) ile karşılaştırıldı. Her balık çeşidi için arsenik türlendirme çalışması yapıldı. Elde edilen AB konsantrasyonları 0.86-12.0 µg/g aralığında bulundu Balık örneklerinde bulunan arsenik miktarının çoğunu AB olduğu belirlendi. Bir örnekte AB'nin yanısıra DMA(V) da (0.40±0.03 µg/g) tayin edildi. Diğer balık örnekleri için DMA(V) tayin sınırının (0.21 µg/g) altında bulundu.

Anahtar Kelimeler: Arsenik, türlendirme, arsenobetain, HPLC-ICP-MS

To my husband Serdar ÖZCAN and my parents

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ABBREVIATIONS

AB	Arsenobetaine
ATG	Arsenic triglutathione
CE	Capillary Electrophoresis
CRM	Certified Reference Material
CX	Cation Exchange
DMA(III)	Dimethylarsinous acid
DMAV)	Dimethylarsinic acid
GSH	Reduced glutathione
HG-AAS	Hydride Generation Atomic Absorption Spectrometry
HPLC	High Performance Liquid Chromatography
ICPMS	Inductively Coupled Plasma Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MA(III)	Monomethylarsonous acid
MA(V)	Monomethylarsonic acid
MADG	Monomethylarsonic diglutathione
MP	Mobile Phase
RM	Reference Material
SAM	S- adenosyl-L- methionine
TMA	Trimethyl arsine
TMAO	Trimethyl arsine oxide

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INTRODUCTION

1.1 Information about Arsenic

1.1.1 Properties of Arsenic

Arsenic is a semimetallic element. It has an atomic weight of 74.92. Pure arsenic is seldomly found in nature; it exists in three allotropic forms: yellow (alpha), black (beta), and gray (gamma). Arsenic compounds generally occur in trivalent and pentavalent forms. Ttrivalent forms are arsenic trioxide and sodium arsenite, and pentavalent forms are arsenic pentaoxide and various arsenates. Arsenic and arsenic compounds can occur in crystalline, powder, amorphous, or vitreous forms. Elemental arsenic has a specific gravity of 5.73, sublimes at 613 ºC and has a very low vapor pressure of 1 mmHg at 373 ºC. Many of the inorganic arsenic compounds occur as white, odorless solids with a specific gravity ranging from 1.9 to 5 [1].

Arsenic is found throughout the environment and may cycle between the atmosphere, soil, water and sediments. However, most As reserves are associated with the regolith materials; anthropogenic activities (like mining) increase the biogeochemical cycling of As in the environment. Arsenic is the 47th most abundant element in nature (1.8 mg/kg). [2] Moreover it is widely distributed in more than 200 minerals. These minerals include

elemental arsenic, arsenides, sulfides, oxides, arsenates and arsenites. The most common ores of arsenic are arsenopyrite (FeSAs), orpiment (As_2S_3), and realgar (AsS) [3].

1.1.2 Occurrence of Arsenic

Arsenic has two sources one of them is natural and the other is man made. Natural sources include; earth crust, soil, water, air and living organisms whereas anthropogenic sources include man-made ones such as fertilizers, drugs, feed additives, insecticides, wood preservatives and herbicides. Significant anthropogenic sources include combustion of fossil fuels, leaching from mining wastes and landfills, mineraling, metal production, timber treatment, cattle and sheep dips and arsenical pesticides. Precipitation from the atmosphere and application of a range of agricultural byproducts such as poultry manure, can also contribute to large quantities of arsenic contamination on land [4].

1.1.2.1 Arsenic in Soil

Arsenic contamination source in soil is the parent rock from where the soil is obtained. As concentrations in soil ranges between 5-10 mg/kg; it may vary with the geological history of the region. Cullen and Reimer [5] examined a wide range of soils and reported an average of 5-6 mg/kg for uncontaminated soils. Sandy soil and granites has the lowest concentration of arsenic. On the other hand alluvial and organic soils have higher concentrations of As [3,6] .

1.1.2.2 Arsenic in Atmosphere

High temperature processes such as coal-fired power generation, smelting, burning vegetation and volcanism results in emission of Arsenic into the atmosphere. Naturally occurring low temperature biomethylation of arsenic species and microbial reduction process also release arsenic to the atmosphere. In this process microorganisms forms volatile methylated derivatives of arsenic under both aerobic and anaerobic conditions [7].

Also microorganisms can reduce these methylated compounds to release arsine gas. Arsenic is released to the atmosphere primarily as As_2O_3 or less frequently as one of several volatile organic compounds [7]. In air, arsenic exists predominantly absorbed on particulate matters, and is usually present as a mixture of arsenite and arsenate, with being of negligible importance except in areas of arsenic pesticide application or biotic activity [8].

1.1.2.3 Arsenic in Water

Arsenic is the 10th most abundant element in seawater with an average value of 2.0 µg/L. In general concentration of As is relatively stable in seawater, but some seasonal variations can occur due to biological uptake of surface seawater. Inorganic As(V) is the major As species because arsenic can be stabilized as a series of pentavalent oxyanions H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-} at high redox potential conditions. However under most reducing (acid and mildly alkaline) conditions and lower redox potential, the trivalent arsenite species (H_3AsO_3) predominate. As(0) and As(3-) are rare in aquatic environment. As(III), MA and DMA are present at lower levels, usually accounting to 10% of the total As concentration [9].

In groundwater, inorganic As commonly exists as As(V) and As(III), the latter is considered to be more mobile and toxic for living organisms. In aqueous environments prokaryotes and eukaryotes biomethylate inorganic

As to DMA and MA reductively [10] but the toxicity of these methylated forms is low. Biomethylation is a more subtle but persistent process and it may affect mobility and transport of As in groundwaters.

1.1.2.4 Arsenic in Food

Trace elements are important but they are more important in food. Because they can have adverse health effects. Arsenic, one of the trace element has no known biological function an human body higher levels may cause cancer in Arsenic, if in its inorganic form, can damage DNA and it is carcinogenic. However, arsenic present in food is in less harmful (organic) forms [11]. Inorganic arsenic species found in food is no more than 1 to 3 percent of the total arsenic presents [12].

Levels of arsenic are higher in the aquatic environment than in most areas of land as it is mostly water-soluble and it could be washed out of arsenic-bearing rock and sea foods arsenic enters into the daily consumption of human being.

1.1.3 Biomethylation of Arsenic

The definition of biomethylation is the formation of both volatile and nonvolatile methylated compounds of metals [13]. The major volatile arsenic compounds formed by biomethylation have the structure $(CH_3)_n AsH_{3-n}$; for n =1, 2, and 3, the products are mono-, di-, and trimethylarsine, respectively. The major nonvolatile arsenic compounds are methylarsonate and dimethylarsinate. Biomethylation of arsenic happens not only in microorganisms but also in algae, plants, animals, and humans.

Biomethylation of arsenic is the major metabolic pathway for the metabolism of inorganic arsenic humans and other mammals [14]. The full pathway was shown in Figure 1.2. Many fungi involves the reduction of a pentavalent arsenic species to a trivalent arsenic species followed by the addition of a methyl group to the trivalent arsenic [15,17]. Specifically, as shown in Figure 1.2 inorganic arsenate As(V) is reduced to arsenite As(III) and then As(III) is oxidatively methylated to monomethylarsonic acid, MA(V). MA(V) is then reduced to monomethylarsonous acid, MA(III) and further methylated to dimethylarsinic acid, DMA(V). Similarly, further steps produce dimethylarsinous acid, DMA(III) and trimethylarsine oxide, TMAO, which can be reduced to trimethylarsine TMA(III).

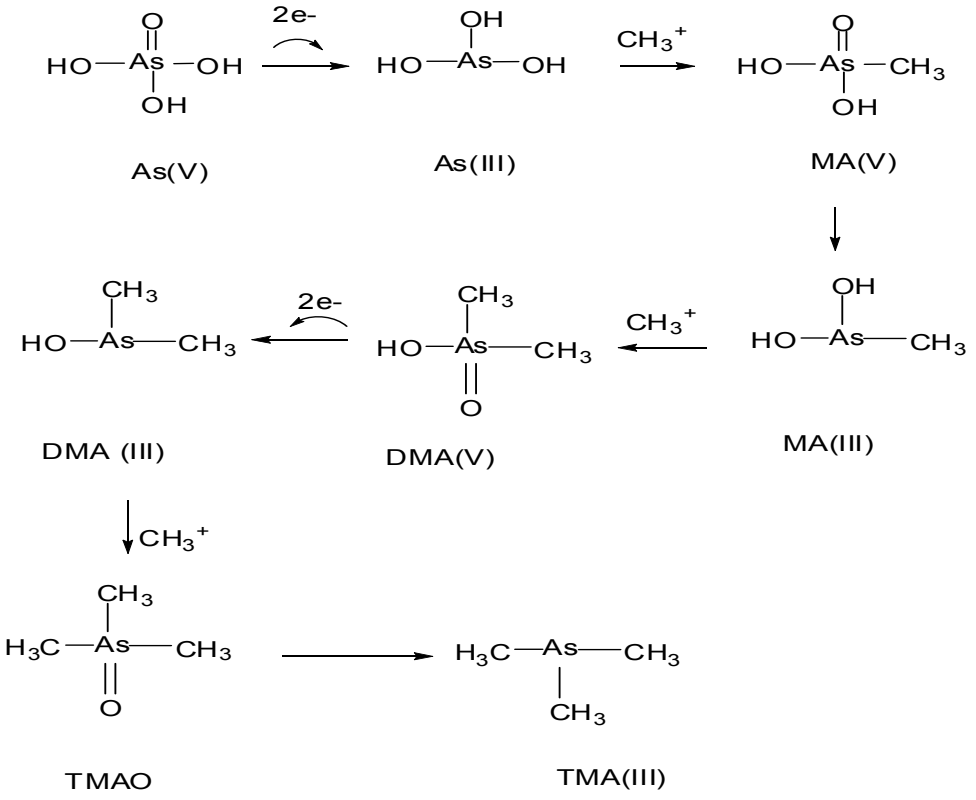


Figure 1.1 Pathway of arsenic methylation, showing alternate steps of two-electron Reduction (2e-) and oxidative addition of a methyl group (CH_3^+)[15,17].

In past it has been considered that the metabolic pathway did not usually continue beyond the dimethylarsenic species in humans and most animals [4]. Thus biomethylation of arsenic has been considered as a detoxification process. However, this suggestion was changed with the findings of trivalent methylation metabolites, MA (III) and DMA (III) in human urine [18-21]. The numerous studies showed these metabolites have higher toxicity than the inorganic arsenic species [22-26]. It is now believed that the methylation of inorganic arsenic may not be a detoxification mechanism and could be an activation process [27, 28]. As a result, much attention has been paid to studies of toxicology of these metabolites. Recently it has been suggested that arsenic-glutathione (As-GSH) complexes are directly involved in the methylation of inorganic arsenic metabolites [29]. Therefore, a new metabolic pathway is given in Figure 1.3 where trivalent metabolites are converted to the less toxic pentavalent species.

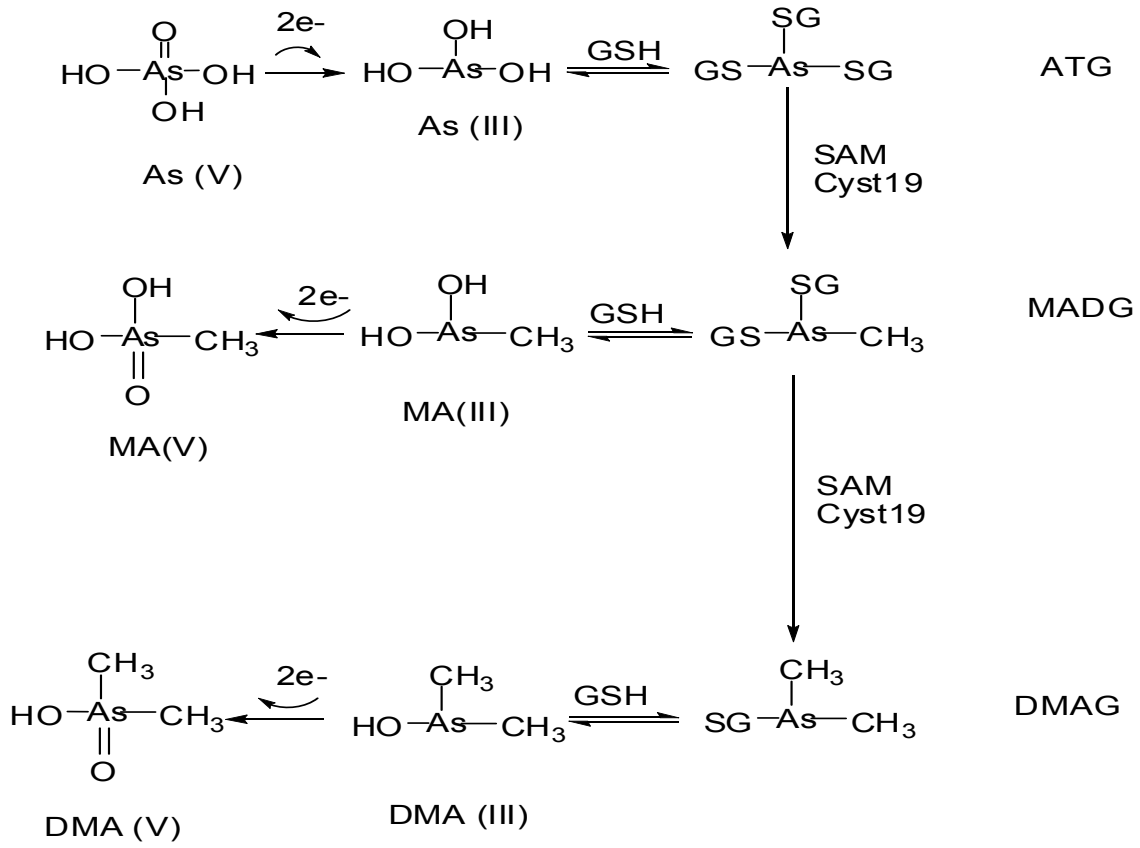


Figure 1.2 A new metabolic pathway of inorganic arsenic via arsenic–GSH complexes [28].

This new pathway is in agreement with the concept that oxidation of arsenic is detoxification. In brief, in the new pathway, both arsenic triglutathione (ATG) and monomethylarsonic diglutathione (MADG) are substrates for human arsenic methyltransferase Cyt19. Arsenite is metabolized and converted to DMA (V) by Cyt 19 via formation of As-GSH complexes.

1.1.4 Toxicity of Arsenic Species

Inorganic arsenic is more toxic than organic arsenic. The toxicity of inorganic arsenic compounds depend both on the oxidation state of arsenic (-3, +3, and +5) the physical and the chemical properties of the compound. As(III) and As(V) are considered to be most toxic forms and classified as human carcinogen substances. Moreover, they are the predominant forms found in water. One of the most toxic arsenic compounds is arsine gas, AsH_3, with -3

oxidation state. Organic arsenic species such as MA and DMA classified as cancer promoters while Arsenobetaine, AB, Arsenocholine, AC, and Trimethylarsine oxide, TMAO, is considered to be harmless. AB is being excreted from human urine with un-metabolized chemicals. The most toxic arsenic species found in foods are the inorganic species, As(III) and As(V), followed by TETRA (tetramethylarsonium ion), MA (V), and DMA (V). Generally, in literature the toxicity of arsenic species summarized as AsH_3 > As III> As V > MA > DMA > AB, AC, TMAO. On the other hand as described in 1.1.3, recent studies have shown that MA (III) and DMA (III) are more acutely toxic and more genotoxic than their parent pentavalent compounds. In other words these trivalent arsenic species are more toxic than inorganic As(V), MA(V), and DMA(V) *in vitro* [24, 25, 30]. This may be related to more efficient uptake of trivalent methylated arsenic species than of pentavalent arsenic species by micro vessel endothelial cells and CHO (Chinese Hamster Ovary) cells [31, 32]. The toxicity of trivalent arsenic is related to its high affinity for the sulfurydyl groups of biomolecules such as glutathione (GSH) and lipoic acid and the cysteinyl residues of many enzymes [33]. The formation of inorganic As (III)–sulfur bonds results in various harmful effects by inhibiting the activities of enzymes such as glutathione reductase, glutathione peroxidases, thioredoxin reductase, and thioredoxin peroxidase [34]. The higher toxicity of MA (III) than inorganic As (III) may be caused by a higher affinity of MA (III) for thiol ligands in biological binding sites than As(III)–thiolate complexes [35]. Moreover DMA (III) forms complexes with sulfur-rich proteins [36].

1.1.4.1 Toxicity in Food

The most toxic arsenic species found in foods are the inorganic species, As (III) and As(V) , followed by TETRA, MA (V), and DMA (V). On the other hand some organic arsenic species found in food as major or trace constituent like AC, AB, TMAO, and arsenosugars are considered practically harmless. Assays of cytotoxicity performed with trivalent methylated species, recently detected in the urine of exposed populations [19,20], this have shown that

the mono [MA(III)] and dimethylated [DMA(III)] trivalent species are more toxic than the inorganic species [23,29]. So far, however, they have not been found in any kind of food, possibly because of lack of suitable extraction methods.

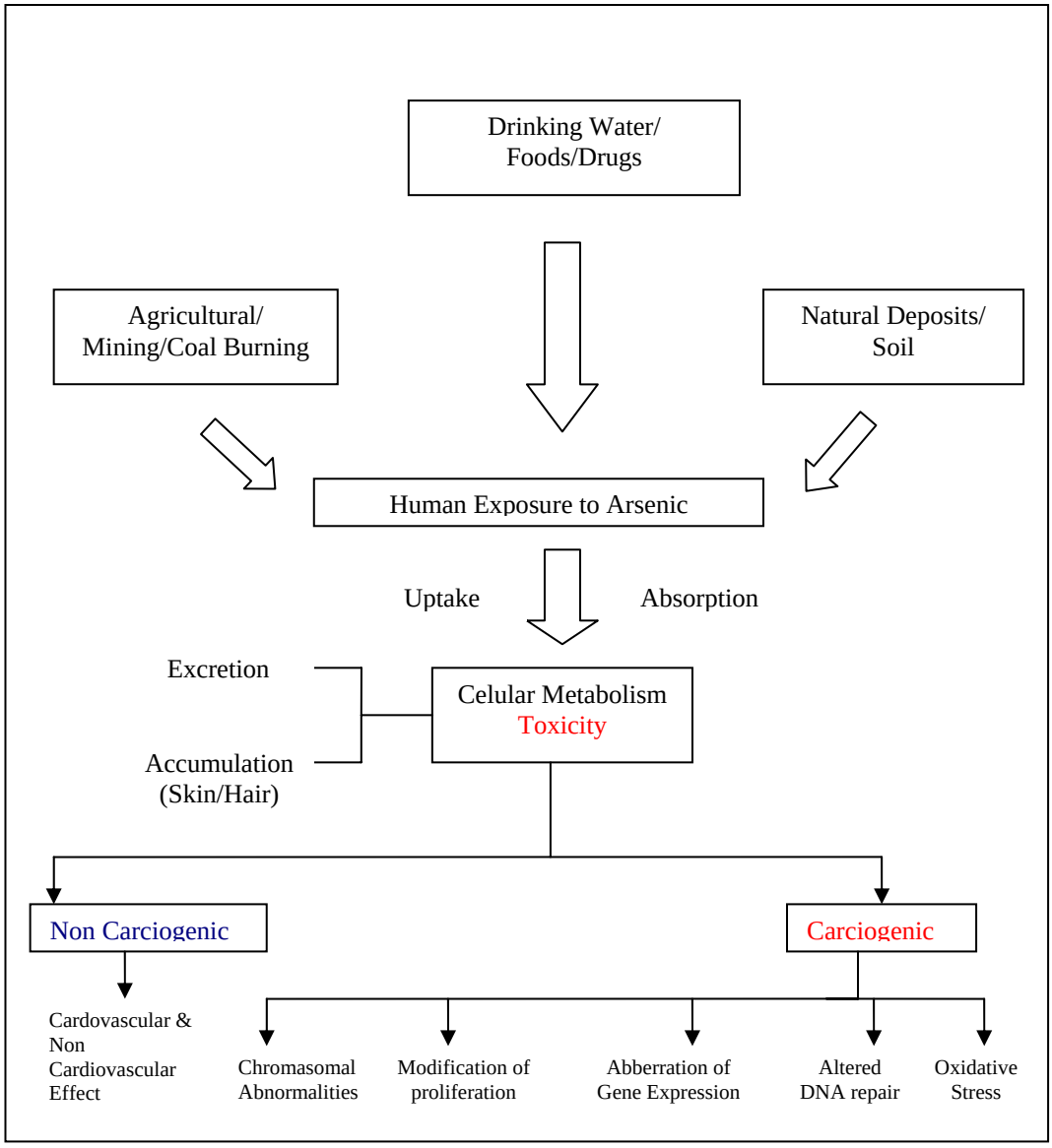


Figure 1.3 Sources of human exposure to arsenic and resulted toxicity. (Modified from ref [37])

1.1.5 Standards and Guidelines for Arsenic

1.1.5.1 Soil Standard

The soil guideline and health criteria for arsenic are 12 and 20 mg/kg for residental soil and 500 mg/kg for commercial or industrial soil [38,39]

1.1.5.2 Water Standard

In 1993 the WHO, in 1975 USEPA established a provisional guideline value for arsenic in drinking water of 10 µg/L , which was described as the 'practical quantification limit' by the former one .

1.1.5.3 Food Standard

Arsenic in food is mostly present in less harmful (organic) forms [11]. Inorganic arsenic species found in food typically account for no more than 1 to 3 percent of the total arsenic present [12]. There is no Europe-wide regulation of arsenic in food.

1.2 Arsenic in Fish

1.2.1 Uptake, Transport, Accumulation and Excretion in Fish

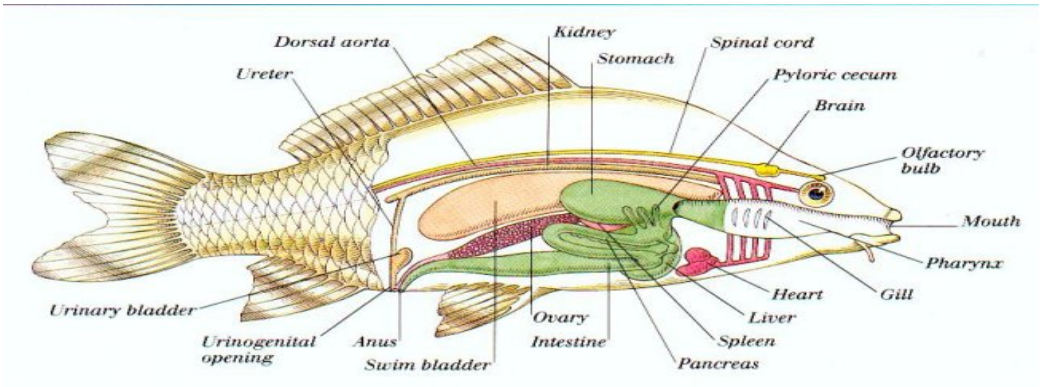


Figure 1.4 Internal Anatomy of Fish [40].

Fish has two paths for metal intake. The first one is by waterbone exposurein whicmeans of gills. Second one is by body surface or digestive tract [41]. Transportation across the epithelium provides dissolved metals enter the gills [42]. The other way for fish to take metal is its diet. The sources of diet can be a metal contamination in sediment, or other organisms. The digestive tract is the major uptake organ for this diet route [43].

Transportation of metals to the other organs are achieved by blood. In fish, storage of the metal occurs in fat tissues,liver and muscles.metals are stored in fat tissue, liver, kidney or muscle [42].

Metals in the blood of fish are transported through different organs in different amounts. The reason for different amount of metal content of the body organ is different affinity of the metal and uptake / excretion rate of the organ for the metal. Uptake time and dose of the metal affects the accumulation of it in fish [44]. In fish, some organs are more likely to store metals. Examples are gills, liver, and kidneys as these organs have metal-binding proteins. [45].

The excretion of metals in fish occurs in the gills, skin, digestive tract, liver, and kidneys by the processes of exocytosis after lysosomal digestion in digestive cells and haemocyte diapedesis across gill, kidney and digestive epithelia [42].

1.3 Arsenic Compounds Found in Seafood

Table 1.1 Name, abbreviation and structures of As species

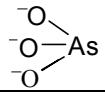
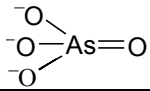
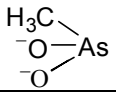
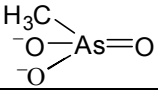
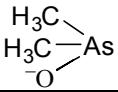
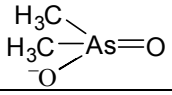
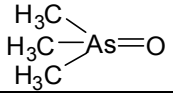
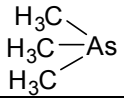
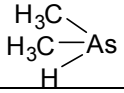
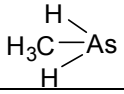
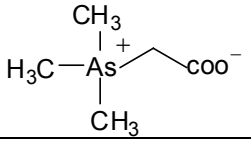
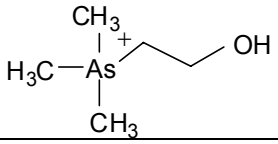
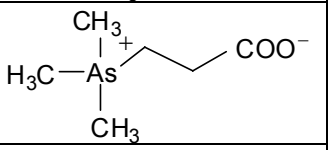
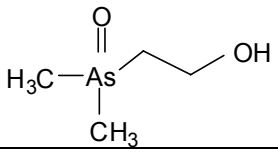
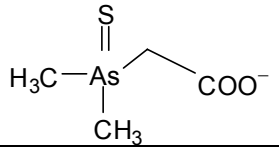
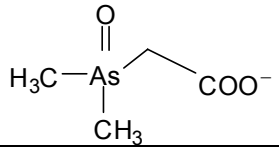
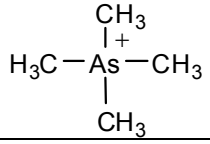
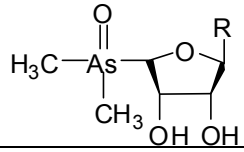
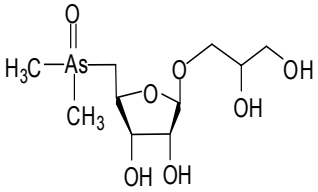
Name	Abbreviation	Structure
Arsenite	As (III)	
Arsenate	As (V)	
Methylarsonite	MA (III)	
Methylarsonate	MA (V)	
Dimethylarsinite	DMA (III)	
Dimethylarsinate	DMA(V)	
Trimethylarsine oxide	TMAO	
Trimethylarsine	TMA	
Dimethylarsine		
Methylarsine		
Arsine		
Arsenobetaine	AB	
Arsenocholine	AC	
Trimethylarsoniopropionate	(TMAP)	
Dimethylarsinoylethanol	DMAE	

Table 1.1 Name, abbreviation and structures of As species, continues.

Dimethlyarsinothioylacetate		
Dimethylarsinoylacetate	DMAA	
Tetramethylarsonium ion	TETRA	
Dimethylated Arsenosugars		
Trimethylated Arsenosugars		

1.3.1 Inorganic Arsenic

Inorganic arsenic, found mainly as arsenate As(V) and to a much lesser extent as arsenite As(III), is the predominant form of arsenic in seawater [4,46]. but inorganic compounds covers only a small proportion of total seafood arsenic. An analysis of five types of ocean finfish and also shrimp resulted in that inorganic arsenic was less than 0.1% of total arsenic in all sample [47]. More recent survey report levels reported inorganic As level as less than 1% [48]. Statistical reanalysis of data from 10 primary studies revealed that the proportion of inorganic arsenic was about 1% of total arsenic at very low total arsenic concentrations, and about 0.5% at total arsenic levels of 720 mg/kg [41].

1.3.2 MA and DMA

Generally little or no MA or DMA is found in seafood; detectable levels have been reported in mainly fatty types of fish. For example, “trace” levels of MA were detected by means of high-performance liquid chromatography coupled to atomic absorption spectrometry (HPLC-AAS) in *mackerel* and *herring*, but not in *tuna*. It has also not been found in *crab* [49]. MA was not detectable in a more recent HPLC-AAS analysis of *white herring* [50]. DMA has been detected at low levels (i.e., µg As/kg) in *mackerel* and *herring* by means of HPLC with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and in *prawns* by means of arsine-generator flameless AAS, but it was not detectable in *cod, dab, haddock, sole, plaice, tuna or whiting* fish analyzed by HPLC-ICP-MS or HPLC-AAS [49-51].

1.3.3 Arsenobetaine

In contrast to the small amounts of inorganic arsenic in most *finfish* and *shellfish*, arsenobetaine represents the vast majority of total arsenic in

marine animals [46]. It has been proposed that arsenobetaine represents an “end point of the arsenic cycle in the marine ecosystem” [4]; because it is largely inert, non-toxic and rapidly excreted. Sedimentary microorganisms can degrade arsenobetaine, but it is generally not transformed in humans and other mammals, in which it is excreted essentially unchanged [4,52]. With respect to potential toxicity, arsenobetaine was not mutagenic in *in vitro* tests, was not cytotoxic and had no transforming activity in mammalian cells and was not immunotoxic [53].

1.3.4 Arsenocholine

Arsenocholine has been reported at only trivial levels [4,11,54]. Arsenocholine is a metabolic precursor of arsenobetaine in marine animals [55]. Following administration of labeled arsenocholine, it is rapidly absorbed and transformed to arsenobetaine with little or no degradation to inorganic arsenic, MA or DMA. Although studies of arsenocholine toxicity are limited, it is regarded as essentially non-toxic [53].

1.3.5 Arsenosugars

At least 15 different arsenosugars have been identified in marine algae, almost all containing a pentavalent As(V) arsenic bound to two methyl groups [11]. Amoung these, there are four principal arsenosugars. Those sugars found in particular algal strains differ in relatively characteristic ways with specific patterns [54]. Principal dietary source of arsenosugars is *seaweed*. Other sources include herbivorous marine mollusks such as *oysters, mussels*, and *clams* [20]. There have been only limited studies of arsenosugar toxicity. In mammalian cells, a synthesized arsenosugar was not cytotoxic at micro molar levels;

paradoxically, enhanced viability was seen in some cell types at much higher levels [55]. A more recent study compared the *in vitro* toxicity of two synthetic arsenosugars, one trivalent and the other pentavalent [56]. The trivalent sugar was positive for cytotoxicity, but inactive for *Salmonella* mutagenicity. The pentavalent sugar was not active in any of those tests.

1.3.6 Trimethyl arsine oxide

Trimethyl arsine oxide (TMAO) has been identified in some species of marine animals as a minor arsenic constituent, rarely detected except at "trace" levels [4,54]. Levels are higher in stored, frozen fish than in fresh fish [4]. It is virtually non-toxic.

1.3.7 Tetramethyl arsonium ion

The tetramethyl arsonium ion (TETRA) is generally a minor specie of arsenic in finfish, on the other hand it may be a major species in some mollusks [4,46,54]. TETRA was reported up to 0.17 µg/g dry weight in *anchovies*, from 0.05–1.0 µg/g dry weight in some *crustaceans* [57] and levels ranging from 0.2 to 16 µg/g were found in various organs of some clams [34]. Freezing and cooking can enhance the level of TETRA usually at temperatures >160°C, especially in charred meat, As there are limited amounts of TETRA in commercially sold fish, acute poisoning by TETRA is unlikely: the highest reported levels of TETRA in grilled or roasted fish were 0.571 µg/g wet basis and 1.79 µg/g dry basis [58].

1.4 Arsenic Speciation Analysis

1.4.1 Definition and Need for Speciation

The elements exist in different oxidation states and in different chemical form in the environment. These forms of the elements are sometimes called as species. An element can have different species and these species can have different property and toxicity between each other. The doses of these elements are also important. In general higher doses cause toxicity and lower doses cause deficiency. As a result, total concentration of an element does not give enough information abot its toxicity, biotransformation and other biochemical properties of the element to be analyzed [60].

In literature it is reported that "Speciation is essential to understand the distribution, mobility, toxicity and bioavailability of chemical elements in natural systems. When evaluating interactions with the environment or assessing absorption, binding mechanisms, reactivity and excretion of the elements in humans, speciation can provide far more information than the analysis of elemental tools" [56]. To illustrate this idea one can give the example of As(III). For example As(III) can bind the thiol (-SH) group of protein, whereas As(V) can displace phosphate during oxidative phosphorylation and disrupt respiration [60].

Speciation of arsenic is also an important consideration because toxicity is mainly species-dependent and not well correlated with the total As concentration. Therefore, determination of total As in a sample is of limited value because the result does not usually reflect the true level of hazard of that element. Hence, speciation of As is highly relevant in providing meaningful risk assessment data to asses the appropriate hazard level [60].

Distribution of an element among its various species will affect the behavior of a particular element greatly. And this is accepted by many by science fields and authorities. Now by means of new developments in analytical instrumentation and methodology make us to identify and measure the species present in a particular system.IUPAC recommends that the term speciation should be used to indicate "the distribution of species in a particular sample or matrix" [61].

Consequently, biotransformation of elements in aquatic systems, quantification of individual species of an element should be known for the real analysis of the toxicity of the element. The fundamental requirement in element speciation is the need to determine each of the forms of a given element quantitatively, independently and without interference from the other forms. From this point of view, "an ideal element speciation method is one that can provide the desired information without altering the original sample and the quantification of different metal species is rather difficult because the concentration of heavy metals in the environment is generally very low relative to the detection limits of the available analytical techniques". [62].

1.4.2 Sampling and Sample Pretreatment for Speciation

Sample preparation or sampling is the first and crucial step in any analytical procedure. Weather conditions and nature and seasonal affects should be considered during sample collection from naturele. The sampling itself need to be planned, designed for the specific sample, documented and controlled to assure quality effectively [63].

Biological samples should be kept at low temperatures as bacteria can deform the structure of the tissue or the sample to be analyzed, so prevention of the sample is an importantant issue. There exists a

different way of preservation which is removal of water by freeze drying, which can concentrate our sample. Mass measurement before and after freeze-drying is important will allow calculating the moisture in the sample. After freeze-drying process, the sample is dry and is ready to be homogenized. The sample should be homogeneous because it is important to minimize errors resulting from moisture in calculationsvalue [64].

1.4.3 Extraction

The extraction of biological tissues requires efficient procedure while lowering the extractant volume as larger volumes of extractant. Minimizing the extraction period is also important in extraction procedures. Water samples can generally be analyzed without any extraction procedure but all solid samples must be extracted prior to speciation analysis, because ″in situ″ determination of As is currently impossible at low concentrations. The extraction step is very crucial. And it is necessary that no transformation of species occur during extraction, moreover one should be sureab out that all As species present in a sample is extracted. Since no chemical extraction is currently ideal, a combination of various extractants is often required to reach this goal [65].

In solid matrices it is more difficult to extract analyte. Hence optimization of extraction is a need.Commonly methanol/water mixture is used as an exractant the analyte is often difficult to extract; it is necessary to optimize the procedure for each matrix investigated. Until quite recently most researchers used methanol/water (or methanol) as the extraction solvent for As speciation [66]. Almost certainly, methanol was used because it extracts fewer non-arsenical compounds and it is easy to remove by evaporation. Methanol, however, is a poor solvent

for extracting inorganic arsenic species [67] and hence is not suitable for samples containing such species. In general it is thought that organic arsenic species will prefer methanol to water as an extraction solvent because they are organic. But the organic arsenicals are polar so they can prefer water too. For these reasons, the best general solvent for extracting arsenic species is probably water, provided it can penetrate the sample matrix. Moreover, in literature almost no report exists for extraction of nonpolar arsenic species (arsenolipids). Using methanol as an extraction solvent may not be practical as reported methods using methanol, which might extract some non-polar arsenic, include a step to remove the methanol and redissolve the residue in water prior to analysis. Non-polar arsenic species would be lost at this stage.

1.4.3.1 Common Methods of Extraction

1.4.3.1.1 Solvent Extraction

Solvent extraction is easy and provides robustness. This method can be applied to complex matrices. Moreover it can be applied to non-filtered samples as well. [68].

1.4.3.1.2 Enzymatic Extraction

Enzymes can be used to determine a bioavailable fraction of species by mimicking living environments, such as gastric digestion processes Proteases, lipases and amylases are examples of extraction material used in this type of extraction.

1.4.3.1.3 Solid Phase Extraction

Solid phase extraction (SPE) is similar with liquid–liquid extraction. In this methods two solutes are partitioning between the phases. The theory behind this method is explained as follows "The basic approach involves passing the liquid sample through a column, cartridge, tube or disk containing an adsorbent that retains the analytes and subsequent recovery upon elution with an appropriate solvent then the mechanism of retention depends on the nature of the sorbent. The advantage of this method is its components can be removed and changed easily. Moreover it is easy to apply.

1.4.3.1.4 Pressurized Liquid Chromatography

This technique depends on. In t two main things. One of them is analyte the other is matrix. As it is mentioned " The process is based on applying increased temperatures, accelerating the extraction kinetics, and elevated pressure, keeping the solvent below its boiling point, hence enabling safe and rapid extractions" [68].

1.4.3.1.5 Microwave Assisted Extraction

Microwave is an efficient method for analyte extraction. It can be applied to various matrices during sample preparation. A low power microwave can be applied for the extraction of organometallic compounds. The aim of using low power is not to break carbon-metal bond during the process. This method can be coupled with separation systems and online procedures can easily be applied [68].

1.4.3.1.6 Ultrasound Probe Extraction

In order to fasten the mixing process, ultrasonic probe extraction method can be used. This method provides removal of particles from the substrate itself. At first glance it is simple and efficient but it has disadvantages too. This method has the main disadvantage of non uniformity in mixing resulted from bubbling in water. The other disadvantage is high local temperature and pressure [68].

1.4.4 Methods of Separation

1.4.4.1 Liquid Chromatography

Liquid chromatography (LC) is another method used in As speciation. Thismet5hod is more popular than others as it provides separation of organic and inorganic forms of As. Among the various LC technique.In LC a column exists theough which a mobile phase flows. Mobile phase is generally for analyte transports. Some of the analyte specie retains in the mobile phase and the others on the stationary phase and hence separation occurs. LC technique has the advantage of simplicity because the analyte should be in liquid form. Extracted solutions can easilt be detected with LC technique coupled with various detection systems.

1.4.4.2 Gas Chromatography

Gas chromatography (GC) is an uncommon method for As speciation because As compounds are not all easily volatilized. The separation is typically carried out at elevated temperature and therefore temperature-

stable species can be analyzed properly. This method is rarely used in biological samples.

1.4.4.3 Capillary Electrophoresis

Capillary electrophoresis (CE) has the advantages of high efficiency of separation. Also it is not time consuming. It requires only small volumes of samples and its cost is low. In this method, once the proper buffer and pH is optimized separation based on charge to size ratio and it can easily be achieved. [69].

1.4.5 Methods of Detection

As in most speciation studies, As speciation has 3 main step. First step is extraction, second step is separation and the last step is detection. Derivatization can be thought as a combined separation and pre-concentration method In literature, hydride generation (HG) is the most common technique used for As derivatization [69].

As has both organic and inorganic forms. The behavior and the properties of these forms are different from each other. As a result separation of these different forms is quite difficult. In order to overcome such an obstacle combination of various separation systems with different detectors can be applied. As hDue to different chemical properties of As compounds, a reliable separation within one single run is not possible. Therefore a combination of various separation procedures must be employed. Hydride Generation (HG), liquid chromatography (LC) will be discussed in this thesis.

1.4.5.1 Hydride Generation

Hydride generation is usually used as asample introduction techniques for As determination at trace level [64]. This method includes the production of volatile arsines either by zinc/hydrochloric acid or sodium borohyride/acid mixtures. After formation of the volatile arsebic species they are transported by argon to the detection systems.

Hydride generation system has two main advantages. One of them is that it is inexpensive, the other is that it can be coupled with different types of detection systems. Tproves the sensitivity and hence lowers the detection syshe main advantage of hydride generation is that it improves sensivity [64]. Hoydride generation system has the ability of lowering the interferences as only gaseous hydrides are introduced to the detector. However it is applicable only to hydride-active arsenic species. This method is suitable for water and urine samples because these contain mainly As(III), As(V), MA and DMA which are all hydride-active. Hyphenated techniques applied with hydride generation atomic absorption spectroscopy (AAS) system is shown in Table 1.2

Table 1.2 Hyphenated techniques with HG-AAS for As speciation in several matrices.

Analyte	Matrix	HPLC Conditions	Detection Limits (ng/mL)	Reference
AB, As(III), DMA, MA, As (V)	Biological Tissues (certified material TORT-1 and fresh bivalve tissues)	Hamilton PRP X-100 strong anionic exchange column with phosphate buffers (10 mM and 100 mM at pH 5.8)	AB: ND As (III): 1.1 DMA: 2.0 MA: 1.9 As (V): 3.9	[70]
As (III), As (V)	Tsunami deposits left on land	Shimadzu liquid chromatograph (LC-10A) equipped with a pump (LC-10AT), vacuum degasser unit (GT-104), and a thermostated column oven (CTO-10AAvp) anion-exchange column LC-SAX1 with phosphate buffer (50 mmol/L Na_2HPO_4 and 5 mmol/L $KH_2PO_4.2H_2O$)	As (III): 0.5 As (V): 0.7	[71]
As (III), As (V), (MA), (DMA) and (TMAO)	Human Urine	PRP-X100 anion-exchange column with a polystyrene divinylbenzene stationary phase and trimethylammonium as functional group (Hamilton), mobile phase A: 10 mM $Na_2B_4O_7.10 H_2O$/20 mM NaCl, pH 9; mobile phase B: 50 mM $Na_2B_4O_7.10 H_2O$/30 mM NaCl, pH 9.	As (III): 2.4 As (V): 2.6 MA: 2.4 DMA: 2.3 TMAO: 1.1	[72]
As (III), As (V), MA, DMA	Fresh Water	Anionic column (Hamilton PRP-X100), mobile phase (17 mmol/L H_2PO_4-/HPO_4^{2-} pH: 6.0)	As (III): 0.1 As (V): 0.6 MA: 0.3 DMA: 0.2	[73]
As (III), As (V), MA, DMA	Iron oxide rich sediment	Hamilton (Reno, NV, USA) PRP X-100 strong anion-exchange column. Phosphate buffers at pH 5.8, mobile phase A and B [10 and 100 mM KH_2PO_4 and $K_2HPO_4.3H_2O$ (Merck)]	-	[74]
As (III), As (V), MA, DMA	Throat earls and industrial licorice extract	Hamilton PRP X-100 anion-exchange column (Hamilton, Reno, NV, USA). Mobile phase, 10 mM K_2HPO_4/KH_2PO_4 adjusted to pH 6.0 (isocratic)	As (III): 1.7 As (V): 1.5 MA: 1.1 DMA: 1.4	[75]

1.4.5.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Elemental determinations can be easily achieved by ICP-MS instrument. As it is mentioned in the paper "ICP-MS has many advantages over other elemental analysis techniques such as atomic absorption and optical emission spectrometry, including ICP Atomic Emission Spectroscopy (ICP-AES), some of them have the ability of ICP-MS to obtain isotopic information, to handle both simple and complex matrices with a minimum of matrix interferences due to the high temperature of the ICP source " [76].

1.4.5.2.1 ICP Source

ICP-MS instrument has a plasma source; this plasma temperature is very high. In the plasma atoms are converted to ions. When the ions leave the plasma they are separated and detected by means of mass spectrometer.

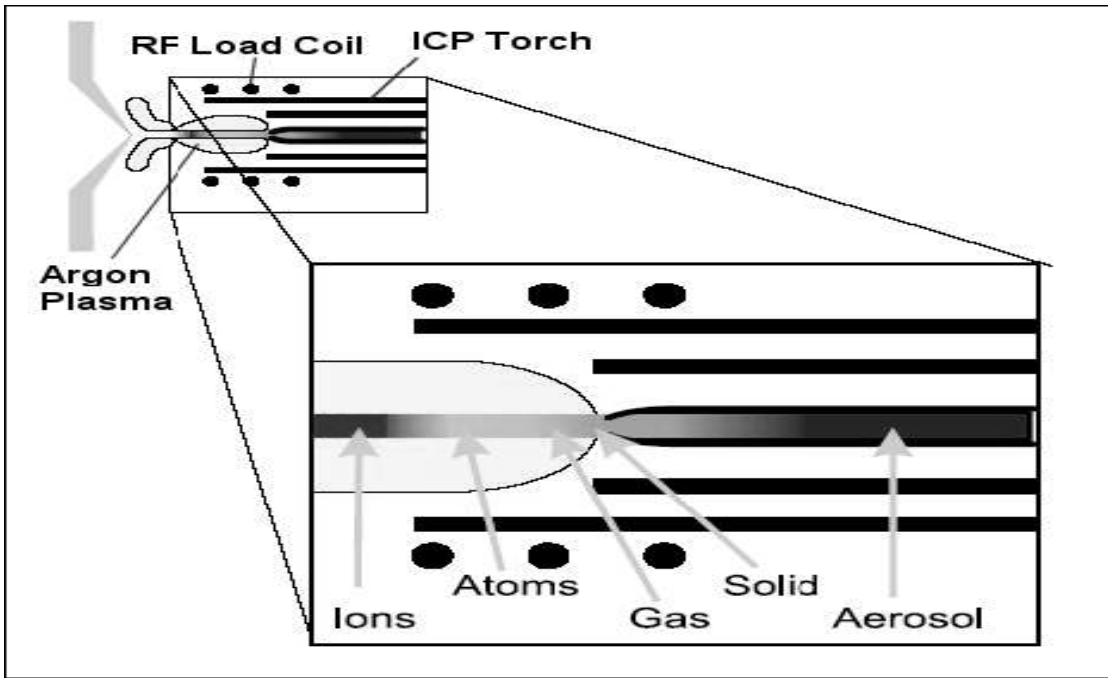


Figure 1.5. The ICP torch showing the sample flow [76].

ICP-MS has torch by means of applying magnetic field and spark to this torch, electrons removed from argon atoms resulting in a positively charged argon ions. These ions collide with other argon atoms which forms argon plasma.

Sample introduction to the ICP-MS system is achived by aerosol formation of the sample. When the sample aerosol is reached into the ICP torch, it is desolvated and firstly the elements in the aerosol are converted to gaseous atoms after this process they are ionized in the plasma.

As soon as the elements in the sample are converted into the electrons, interface cones mke them pass to the mass analyzer. Figure1.7 shows the interface region of an ICP-MS system. The sampler and skimmer cones are made up metals and they have a small hole (~1mm) in the center. The aim of using these cones is to sample ion beam coming from the torch. Since sampler and skimmer cones have small holes in their center, it limits ICP-MS about the total dissolved solids in the sample introduced to the instrument,

ICP-MS has some limitations as to the amount of total dissolved solids in the samples.

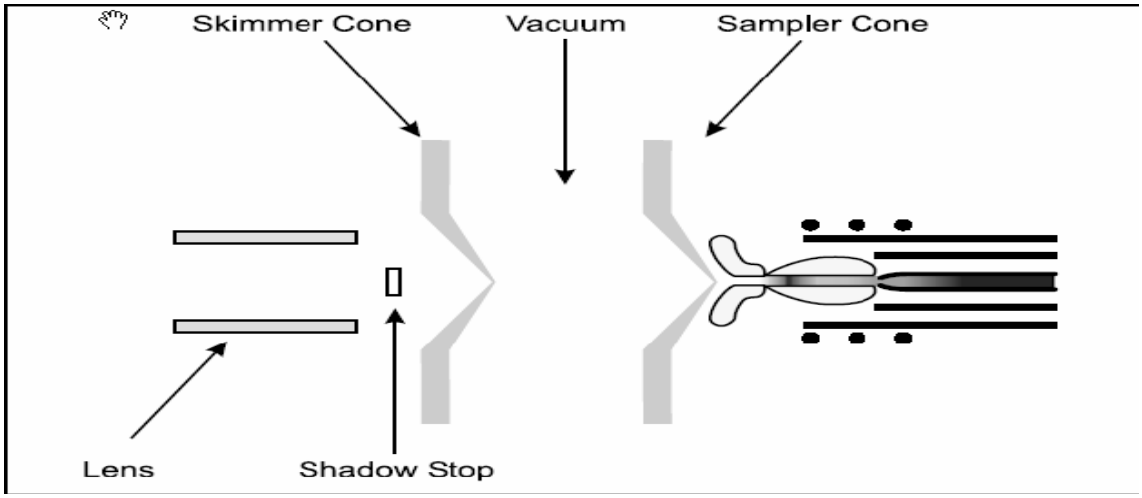


Figure 1.6 The interface region of an ICP-MS [76].

The ions coming from the plasma ce are then focused by the electrostatic lenses in the ICP-MS syster the mam. These ions are positively charged and the lenses serves as slit foRemember, the ions coming from the system are positively charged, so the mass analyzer.

As soon as the ions come to the mass analyzer they are separated in accordance to their mass to charge ratio. One example to the mass analyzer is the quadrupole mass filter. It is also a widely mass analyzer.Once the ions enter the mass filter. In the quadrupole mass filter, there exists 4 rods. These rods are arranged as shown in Figure 1.8. According to the paper the theory behindthe quadrupole mass filter lies on alternating voltages that creates a n RF-field.This fields allow the ions to pass through in accordance to their single mass to charge ratio. We can say that the mass filter is a sequential one and it has the ability of separating up to 2400 amu per second. In the paper it is concluded that "Because of this speed, the quadrupole ICP-MS is often considered to have simultaneous multi-elemental analysis properties. The ability to filter ions on their mass-to-charge ratio allows ICP-MS to supply

isotopic information, since different isotopes of the same element have different masses " [76].

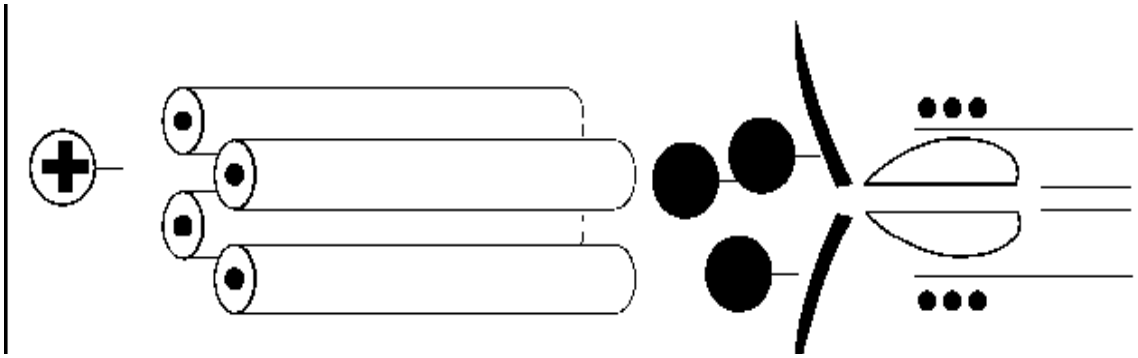


Figure 1.7 Schematic of quadrupole mass filter [76].

1.4.5.3 Application of ICP-MS System in Speciation Studies

In recent years, most environmental scientists were interested in trace analysis of environmental samples. Currently, various elements, which were not used in past, are consumed in modern industries for production of new materials. New types of environmental pollution must be resulted due to disposal of industrial products. Therefore, there is a need for analytical techniques that are capable of rapid, multielement and simultaneous analysis such as ICP-MS. ICP-MS is a combination of two analytical tools, inductively coupled plasma (ICP) and mass spectrometry (MS). This combination resulted in an instrument with powerful potential for multielement trace analysis. When compared to other methods, ICP-MS have the advantages of having higher sensitivity, high selectivity also it provides rapid multielement analysis and has wide dynamic range, low background, and the ability to measure specific isotopes [77]. Using this technique, it is possible to detect all arsenic species with essentially uniform response which greatly facilitates quantification of the various species.

However spectral and non spectral interference may occur in this method. but there are variety of methods to get rid of these interferences. One disadvantage of ICP-MS coupling with HPLC is that it is tolerable to low organic content mobile phases. Moreover determination of non polar arsenic

speices has not been studied with HPLC-ICP-MS technique. Besides these drawbacks ICP-MS is still a widely used technique for As speciation. In Table 1.3. Arsenic speciation with HPLC-ICP-MS in several matrices is summarized.

Table 1.3 As speciation in different matrices by using HPLC-ICP-MS

Column	Mobile Phase	pH	Flow mL/min	Matrix	Species Rt (min)	Ref
Supelcosil PRP X100	Mobile Phase A : 5 mM NH_4NO_3 + 5 mM $(NH4)_2CO_3$ Mobile Phase B: 50 mM NH_4NO_3 + 5 mM $(NH4)_2CO_3$	9,0	1,0	DORM 2	AB + AC (3,9); As(III) (5,8); DMA (7,9); MA (9,9);As(V) (11,1)	[78]
Supelcosil PRP X100	Mobile Phase A: 15 mM $(NH4)_2CO_3$ Mobile Phase B: 50 mM (NH4)2CO3	8,5	0,8	TORT 2	AB (1,8); AC (1,9); As(III) (3,0); DMA (5,5); MA (17,0); As(V) (26,0)	[79]
Hamilton PRP X100	Mobile Phase A:15 mM $(NH4)_2CO_3$ + 2% MeOH Mobile Phase B: 50 mM $(NH4)_2CO_3$+ 2% MeOH	9,0	1,0	Urine, Dorm 2, fish	AB (2,0); As(III) (3,3); DMA (4,3); MA (6,8); As(V) (8,0)	[80]
Dionex Ionpac AS4A4	Mobile Phase A: 0,4 mM HNO_3 Mobile Phase B: 50 mM HNO_3	3,4 1,3	1,2	DORM 2, Fishery Products, Algae	As(III) (1,2); MA (1,9); DMA (4,4); As(V) (5,7); AB (8,0); TMAO (9,7); AC (10,3); TETRA (11,8)	[81]
Dionex Ionpac AS7	Mobile Phase A :0,5 mM CH_3COOH^- CH_3COONa Mobile Phase B: 25 mM HNO_3	4,8 1,6	0,5	DORM 2 Fishery Products	As(III) (2,5); DMA (5,8); MA (7,5); As(V) (10,0); AB (12,5); TMAO (18,5); AC (21,0); TETRA (22,0)	[82]

Table 1.3 As speciation in different matrices by using HPLC-ICP-MS, continues

Dionex Ionpac AS7	Mobile Phase A: 2,5 mM NH_4H_2PO4 Mobile Phase B: 50 mM $NH_4H_2PO_4$	2,6 1,3	1,0	Water	AB (1,5); DMA (1,8); As(III) (3,0); MA (8,8); As(V) (14,8)	[83]
Supelcosil PRP X100	12,5 mM $(NH4)_2CO_3$ 50 mM $(NH_4)_2CO_3$	8,5	0,8	Apples	AB (1,8); AC (1,9); As(III) (3,0); DMA (5,5); MA (19,0); As(V) (26,0)	[84]
Hamilton PRP-X100 Agilent Zorbax 300	20 mM $NH_4H_2PO_4$ 10 mM C_5H_5N	6,0 2,3	1,5	Fish Sauce	As(III) (2,0); DMA (2,7); MA (3,5); As(V) (7,5) DMA (1.6); AB (2.0); TMAO (3.2); AC (4.9); TETRA (5.5)	[85]
Hamilton PRP-X100	10 mM HPO_4^{2-}/ $H2PO4^-$	8.5	1.5	Rice, SRM 1568, chicken, fish	AB (1.5); As(III) (1.9); DMA (2.6); MA (4.0); As(V) (10.5)	[86]
Asahipak ES-502N 7C	15 mM $C_6H_8O_7$	2.0	1.0	Urine, hair, nail, SRM 2670	AC (2.3); AB (2.7); DMA(V) (3,0); MA(V) (3.7); As(III) (4.0); DMA(III) (5.0); MA(III) (6.0); As(V) (8.2)	[87]
Hamilton PRP X100 Supelcosil LC SCX	Mobile Phase A:20 mM $NH_4H_2PO_4$ Mobile Phase B:20 mM C_5H_5N	4.5-5.6 2.4	1,5	Fİsh, DORM 2, TORT 2	As(III) + AB (1.5); DMA (2.0); MA (3.5); As(V) (6.3) ;AB (3,5); TMAO (4.9); AC (5.6); TETRA(6,8)	[88]

1.5 Aim of This Study

The aim of this study is to develop a sensitive method for the determination of arsenic, to eliminate and monitor possible interferences for the total arsenic concentration determination and to optimize extraction of arsnic species for fish matrix. Moreover, separation and detection of arsenic species with HPLC-ICP-MS were aimed in extracted fish tissues. The difference of this study from the others in literature is that the samples were collected from Black Sea. In addition, the possible spectral and non spectral interferences for Arsenic prior to analysis will be studied in detail.

CHAPTER 2

EXPERIMENTAL

2.1. Reagents and Apparatus

All the chemicals used in all parts of this study were of analytical grade or high purity. Standard solutions containing 1000 mg/kg As each from the following compounds were used for the identification and quantification of arsenic species: As(V) stock solution (High Purity), As(III) stock solution (High Purity), DMA(V) from dimethylarsinic acid sodium salt (Sigma), MA(V) from disodium methyl arsonate hexahydrate salt(ChemService Inc), and arsenobetaine stock solution (BCR 627 1033 g/kg). HNO_3 (Merck), H_2O_2 (Merck) were used for total arsenic studies. For the interference studies, LiCl (Riedel), CsCl (Merck), HCl (Merck) $NaNO_3$ (Riedel), $LiNO_3$(Riedel), NaCl (Riedel) and $CsNO_3$ were used. For the further dilutions of all these standards, de-ionized pure water from a Milli-Q Water Purification System was used. All the standards were prepared daily. Argon gas used in ICP-MS for the production of plasma was supplied from Habaş Industrial and Medical Products, Ankara. Clean test tubes were used at each time. For calibrating standards dilution by mass rather than volume dilution was preferred.

In the HPLC studies, formic acid (Riedel), hydrochloric acid (Merck), ammonia (Merck), methanol (Merck), pyridine (Riedel, RH 16037), citric acid (Sigma, SIAL 251275) were used as reagents in mobile phases.

In this study, NIST RM 50 Albacore Tuna (total As 3300 ± 70 ng/g) and CRM DOLT-4 (total As 9.60 ± 0.62 mg/kg). National Research Center of Canada, Ottawa, Canada) were used as references for accuracy check.

2.2. Instrumentation

A Heto FD 8 freeze-drying unit was used to freeze-dry samples in the sample preparation step. In the procedure samples were kept at -85 ^{0}C for 24 hours before lyophilization process; in the next step samples were introduced to freeze-drying instrument where adjusted temperature was -55^{0}C. Lyophilization process continued for 48 hours.

Dionex HPLC system equipped with a binary HPLC pump was used in separation of arsenical step.The HPLC system was connected to ICP-MS using 85.0 cm tubing having 1.06 mm i.d. and 1.7 mm o.d. This tubing connected the output of HPLC column and the nebulizer of ICP-MS. Cation exchange (Spheris S5 SCX) columns were used for the separation of 3 arsenic species, MA(V), DMA(V) and AB.

Arsenic species were analyzed using Thermo X Series ICP-MS system without collision cell technology for the elimination of spectral interferences on $^{75}As^{+}$ caused by $^{75}ArCl^{+}$ ($^{40}Ar^{35}Cl^{+}$). However the software uses an interference equation where spectral interference is corrected mathematically and it is shown in chapter 3 that no collision cell is needed and interference equation is valid for low concentration of chlorine and salt bearing matrices.

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Ethos Plus Milestone microwave oven system equipped with temperature controller unit was used to digest the samples for the determination of total arsenic in fish samples.

Millipore Stirred ultrafiltration cell (8400 Model) was used to filtrate extracts. This cell contains a cap (Nylon), membrane holder (Polysulfone), body (Polysulfone), magnetic stirrer assembly (Acetal, polysulfone), retaining stand (Nylon), O-rings (Silicone rubber) and tube fitting assembly (Nylon). Cell capacity is 400 mL. Maximum operating pressure should be lower than 75 psi (5.3 kg/cm^{2}). In this study 10 psi was used as an operating pressure. Pure argon gas was used as a carrier. 10.0 KDa ultrafiltration membrane made up

of polyethersulfone (Filter Code: YM10 Dia: 63.5 mm, 28.7 cm^2) was used in extraction of arsenic species step to filtrate supernatant from residue.

In addition to these instruments, Elma, Elmasonic S 40 H brand sonication instrument and shaker and ultrasonication probe were used in the extraction studies. Sigma 2-16 (D-37520 Osterode am Harz, Germany) brand ultracentrifuge instrument was used to separate supernatant.

2.2.1 HPLC System

A Dionex HPLC pump coupled with Thermo X Series ICP-MS instrument is used through out the whole study.

Chromatographic conditions including flow rate of mobile phase, mobile phase composition, pH of mobile phase were optimized to obtain a good separation of analytes. Cation exchange (Spheris S5 SCX) HPLC system was used for the separation of arsenic species, mainly MA, DMA and AB. Pyridine in different concentrations buffered to different pH values were tried as a mobile phase composition in cation exchange HPLC system.

2.2.2 Optimization Parameters for ICP-MS System

Auto optimization specific to element was done weekly before analysis or when sensitivity loss was observed (~30%). In this study for As, mass 75 was used and all of the conditions were optimized as arsenic is monoisotopic element. In ICP-MS system, optimized conditions for arsenic are as follows. For interference effect of $^{75}ArCl$, $^{77}ArCl$ and ^{82}Se, ^{83}Kr was also monitored throughout the analysis.

Table 2.1 Optimized conditions for ^{75}As Determination

Parameter	Optimum Value
Extraction Lens Voltage, V	-137
Lens 1 Voltage, V	-0.6
Lens 2 Voltage, V	-14.9
ocus Lens Voltage, V	12.9
1. Diffraction Aperture Voltage, V	-40.0
2. Diffraction Aperture Voltage, V	-135
Quadrupole Voltage, V	-2.0
Hegzapole Voltage , V	1.6
Argon Flow Rate in Nebulizer, L/min	0.76
Lens 3 Voltage, V	-200.0
Horizontal Pozition of Torch	64
Vertical Pozition of Torch	625
3. Diffraction Aperture Voltage, V	-36.9
Argon Flow Rate to Cool Torch, L/min	12.0
Argon Flow Rate to Produce Plasma, L/min	0.78
Sampling Depth	17
Forward Power, W	1500

Singly charged cations of selenium isotopes and krypton are also monitored in this study as interference correction based on the m/z ratio of them. ^{75}As, $^{77}ArCl$, ^{78}Se ^{82}Se and ^{83}Kr were monitored during all measurements. In all calculations throughout this thesis, peak area; integrated counts per second

(ICPS) values were used. Corrections using these masses are done by the instrument software.

2.2.3 Microwave Digestion System

Microwave digestion cell consists of 10 PTFE vessels one of which is the reference vessel, temperature at the inside of reference cap is measured using thermocouple to control the inside temperature. Temperature program and acid mixture used were optimized to obtain an effective digestion. HNO_3-H_2O_2 (1+1, volume of concentrated reagents) were used as acid mixture. 8.0 mL of acid mixture is added to the 0.20 g of sample in PTFE vessel. After digestion process, the contents were diluted to 10.0 mL exactly with deionized water. Temperature program used in digestion is given in Table 2.2.

Table 2.2 Temperature program of microwave digestion system used for fish samples.

Period, min	Temperature, ºC
5	↑100
10	→100
5	↑150
10	→150
5	Ventilation

2.3 Sample Collection and Preperation

Fish samples analyzed for this study were collected from local Market in two shopping locations of Ankara where most people prefer to get fish which are in Kızılay and Ulus. Markets here sell fish coming daily or weekly from Black Sea region directly. Stock fish are kept at -20 ºC until fish on the shows consumed up. Anchovy is collected from Bursa. Carp was caught up from Kızılırmak River from which water supplement of Ankara citizens was

provided for a certain period in 2009: this supply known to be containing As with a concentration occasionally higher than allowed limit of WHO and USEPA (10.0 ng/g in drinking water). In Table 2.3, the names of fish samples analyzed in this study are shown. The photos of fish samples used in this study are also shown in Figure 2.1 to 2.7

Fish samples collected from markets were kept at -40 ºC until analysis. Then they were washed with deionized water, inner organs were discarded and tissues were gently removed. Removed muscle tissues were washed with deinozed water again. Tissues were blended with a blender at room temperature for 5.0 min. After blending, they were placed in agar plates in the form of a thin film. The samples were kept at -80 ºC prior to lyophilization process. After freeze drying process each sample was homogenized by grinding. The dried and homogenized samples were kept at -4 ºC until the day of analysis.

For the total As studies, 0.20 g of homogenized fish tissue was weighed. Then they were digested with acid mixture of volume 8mL (1/1,v/v) HNO_3/H_2O_2 by microwave acid digestion system. At least 2 replicates for each sample were treated at each step. After acid digestion for each replicate 2 parallel standard additions were applied to minimize matrix effects. Three point standard addition method was applied to each sample In this study direct calibration method was also tried but the experimental results did not match with the certified results. For the accuracy check of the method CRM Dolt-4 was used and when standard addition method was used the experimental results were checked with CRM Dolt 4 which were in good agreement with the certified one. Hence, standard addition method was used through out the study. Total As analysis were done with flow injection ICP-MS system using loop volume of 1.0 mL were used.

For extraction studies NIST RM 50 Reference Material was chosen as a candidate since its total concentration was known and referenced in the literature. At each step of the extraction method development, 2 parallel samples of reference material were weighed into a clean test tube and the

procedure in Extraction Method 2 was applied. After obtaining an extracted solution, 3 point-standard addition was applied.

For speciation studies, extracted solutions were treated with 3 point-standard addition by addition of DMA(V) and AB standard solutions with 2 replicates. After all, the solutions spiked with standards were injected to CX-HPLC-MS system for speciation analysis.

In order to find out the relative AB extraction efficiencies, first of all each fish samples were extracted according to Extraction Method 2 After obtaining a clear extracted solution, this solutions were spiked with AB and injected to the ICP-MS system for the determination of total AB after extraction. All the calculations were based on no smoothed peak area. And standard addition was done with pure AB stock solution at each step and total AB after extraction process. Extraction efficiencies of each fish sample was calculated as concentration of AB (obtained from CX-HPLC–ICP-MS)/total As concentration after acid digestion (obtained from ICP-MS)x100

In extraction part, separation and also speciation part, for the preparation of standard solutions of As species calculated concentrations based on As in that specie. As(As specie). For example 10 ng/mL AB refers to 10 ng/mL As in AB. Likewise 50 ng/mL DMA(V) refers to 50 ng/mL As in DMA(V).

Table 2.3 Names of analyzed fish samples in Turkish, English and Latin

Turkish	**English**	**Latin**
Barbunya	Red Mullet	*Mullus Barbatus*
Kefal	Gray Mullet	*Mugil Cephalus*
Mezgit	Whiting fish	*Melangius Merlangus*
Sazan	Carp	*Cyprinus carpio*
Hamsi	Anchovy	*Engraulis encrasicholus*
Somon	Salmon	*Salmo Salar*
Akorkinoz Ton balığı (RM)	Albacore Tuna (RM)	*Thunnus alalunga*
Köpek balığı (SRM)	Dog Fish (CRM)	*Squalus acanthias*

|----------|----------|----------|----------|----------|----------|----------|

0cm **45cm**

Figure 2.1 *Gray Mullet* (Black Sea)

|----------|----------|

0cm **12cm**

Figure 2.2 *Whiting Fish* (Black Sea)

|----------|----------|----------|----------|----------|----------|----------|----------|

0cm **75cm**

Figure 2.3 *Salmon* (Black Sea)

I----------I----------I

0cm **10cm**

Figure 2.4 *Anchovy* (Black Sea)

I----------I----------I

0cm **15cm**

Figure 2.5 *Red Mullet / Barbun*

CHAPTER 3

RESULTS AND DISCUSSION

PART 1

3.1 Arsenic Interference Studies

3.1.1 Interferences in ICP- MS

While quadrupole-based ICP-MS is an immensely powerful multi-element analytical technique, it does suffer from some well-documented spectral and non-spectral interferences. Some spectral interference can be corrected mathematically by the software used, provided that the relative contribution of the interference peak to the analyte peak is not severe.

The main sources of spectral interferences in ICP-MS are:

1. Direct overlap from a different element with an isotope at the same nominal mass is known as an isobaric interference. For example, $^{114}Sn^{+}$ overlapping $^{114}Cd^{+}$ signals.
2. Overlap from a polyatomic ion formed from the combination of species in the plasma, sample solvent and/or sample matrix. For example $^{40}Ca^{16}O^{+}$ signal overlapping $^{56}Fe^{+}$ signal.
3. Doubly-charged species resulted from ions created by the loss of two electrons instead of one. Because the quadrupole separates ions based on m/z (mass over charge ratio), a doubly-charged ion (M^{2+}) will appear at mass m/2. An example of a doubly-charged interference would be the $^{136}Ba^{2+}$ signal overlapping on $^{68}Zn^{+}$ signal

There are many ways to remove or correct for spectral interferences. The easiest way to avoid a direct isobaric overlap is to choose another

(interference free) isotope of the element of interest, if available. For example, $^{114}Cd^{+}$ is interfered by the presence of $^{114}Sn^{+}$, so $^{111}Cd^{+}$, which has no isobaric overlap, can be used. The drawback is that the detection limit may be degraded due to the low abundance of ^{111}Cd (12.80%) whereas the ^{114}Cd isotope abundance is 28.73%.

Oxides (MO^{+}) and doubly charged species (M^{2+}) can be significantly reduced through proper tuning of the plasma and torch conditions, and by a good plasma design. Oxides are far more problematic in ICP-MS than doubly charged species, since there are very few elements that generate significant levels of doubly charged species, and these can be easily avoided by choosing the alternate isotope of the element

3.1.2 Correction of Some Spectral Interference by ICP-MS Software.

Interference equations are mathematical equations that are used to correct elemental, polyatomic and doubly charged isobaric interferences in ICP-MS analysis. They are based on the fact that the relative abundances of the naturally occurring isotopes of almost all elements are fixed in nature and are not changed through any sample preparation or analysis techniques. Because natural isotopic abundances are known and constant, isobaric overlaps are predictable and, where an alternative, uninterfered isotope is either unavailable or too small in abundance, mathematical correction can be used to correct for isobaric spectral overlaps. Although this type of correction can also be used for polyatomic interferences, the intensity of polyatomic species can vary with tuning, and many corrections need to go through more than one level of measurement and calculations to obtain a concentration value for the target element causes some uncertainty in the result. A common example is ^{75}As as shown in Figure 3.1 where the interference correction equations are given.

$^{75}As = {}^{75}M^{+} - 3.12 \times {}^{77}ArCl$

$^{77}ArCl = {}^{77}M^{+} - 0.860 \times {}^{82}Se$

$^{82}Se = {}^{82}M^{+} - 1.001 \times {}^{83}Kr$

Figure 3.1 Software Correction Equations for ^{75}As.

First, instrument acquires data at masses 75, 77, 82 and 83 and then it assumes the signal at mass 83 is from $^{83}Kr^{+}$ only and uses this to estimate the signal from $^{82}Kr^{+}$ .Then it subtracts the estimated contribution from $^{82}Kr^{+}$ from the signal at mass 82; the residual should be the value for $^{82}Se^{+}$. After this, software uses the estimated $^{82}Se^{+}$ signal to predict the size of the signal from $^{77}Se^{+}$ on mass 77. After subtracting the estimated $^{77}Se^{+}$ contribution from the total signal at mass 77; the residual should be from $^{40}Ar^{37}Cl^{+}$. After all these calculations, software uses the calculated $^{40}Ar^{37}Cl^{+}$ signal to estimate the contribution on mass 75 from $^{40}Ar^{35}Cl^{+}$. Now, subtracts the estimated contribution from $^{40}Ar^{35}Cl^{+}$ on mass 75; the residual intensity should be due to $^{75}As^{+}$.

3.1.2.1 Evaluation of Interfere Equation

The most serious limitation of this type of correction equation is that it can not deal with the common situation where interference appears at one of the intermediate masses used in the calculation. In the As example provided, the presence of Br in the sample gives rise to a $^{81}Br^{1}H^{+}$ interference at mass 82, which leads to an error in the calculated concentration of ^{82}Se, which then propagates a further error in the calculated concentration of As. These errors can be very difficult to identify, since they can lead to either over- or under-reporting of the target analyte concentration. Hence, analyst should have proper information about matrix components. But possibility of such interference is pretty low. For fish extracts or acid digests there were no Br

contamination in samples hence no over or under correction of the $^{75}As^{+}$ signals is expected due intermediate interference of $^{81}Br^{1}H^{+}$.

In this study effects of HCl, LiCl, NaCl, CsCl, $LiNO_3$, $NaNO_3$ and $CsNO_3$ were checked with or without software interference correction.

3.1.3 Non Spectral Interference Effect of HCl, LiCl, NaCl, $NaNO_3$ and $CsNO_3$

In order to observe salt effect, space charge effect and chlorine isobaric effects on As, several concentrations of group 1A chloride and nonchloride salts were prepared in 20 ng/mL As standard solutions. Necessary dilutions of the solutions were done by de-ionized water. Most of the stock solutions and standard solutions are prepared in 1 M HNO_3 for longer stability of solutions. However, in this case addition of nitric acid or any other chemical would certainly change the matrix; this is undesired as only effect of one chemical on As signal is to be investigated to understand the interference phenomena. Therefore, each solution in desired matrix was prepared in H_2O daily; for sample introduction, flow injection ICP-MS (FI-ICP-MS) was used with injection volume of 1.0 mL for plasma stability.

3.1.3.1 Effect of HCl on As signal

In order to investigate both chlorine correction check and acid effect on As signal, 20 ng/mL of As in different concentration of HCl is prepared. As it can be seen in Figure 3.2 and Figure 3.3, chlorine interference seems to be successfully corrected. In addition no serious suppression effect of the proton was observed. Slight changes were observed; this may due to change in aerosol generation, analyte transport, changes in excitation, ionization process in the plasma.

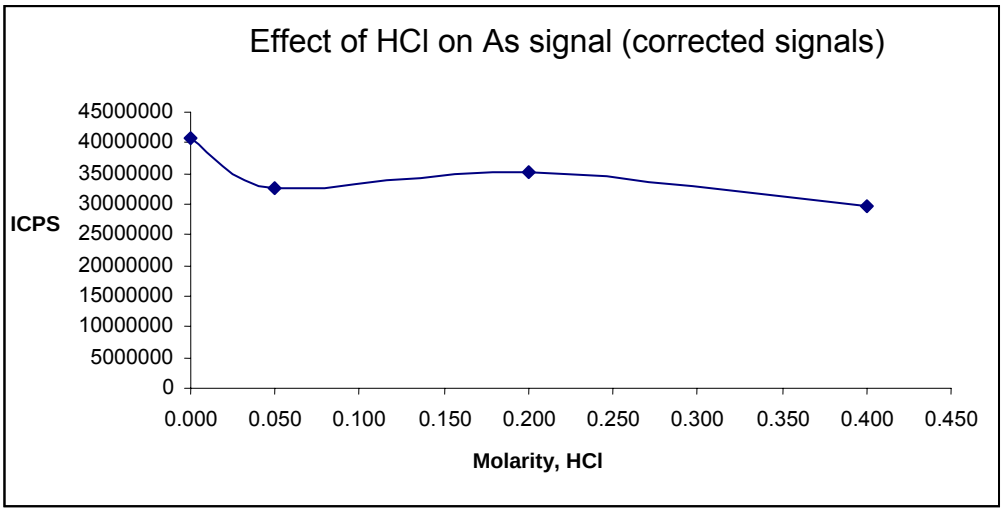

Figure 3.2 Effect of HCl on 20 ng/mL As signal (with software correction).

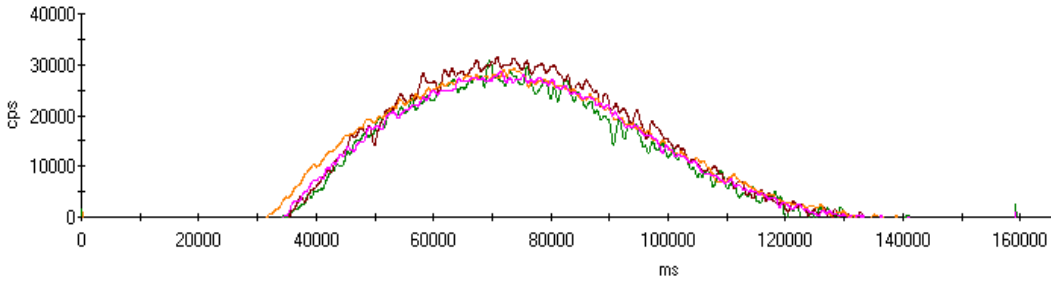

Figure 3.3 ICP-MS signals for 20 ng/mL As in different HCl concentrations (pink 20 ng/mL As, yellow+0.05 M HCl, brown+0.20M HCl, green +0.40 M HCl using ICP-MS method having loop volume of 500 µL.

When the option of interference equation is removed, chlorine interference effect is obviously seen. As concentration of HCl is increased, ^{75}As signal is increased. Since software correction is removed this enhancement in the signal is due to ^{75}ArCl interference. On the other hand increase in acid content suppresses the As signal due to change in aerosol droplet size. However since the ^{75}ArCl interference is more severe, the net interference effect is positive. In our country salt ratio for Black Sea, Mediterranean and Aegean Sea is 0.18, 0.39 and 0.33% (m/V), respectively. It is important to know tolerance of analytical system to chlorine and other species present in sea water.

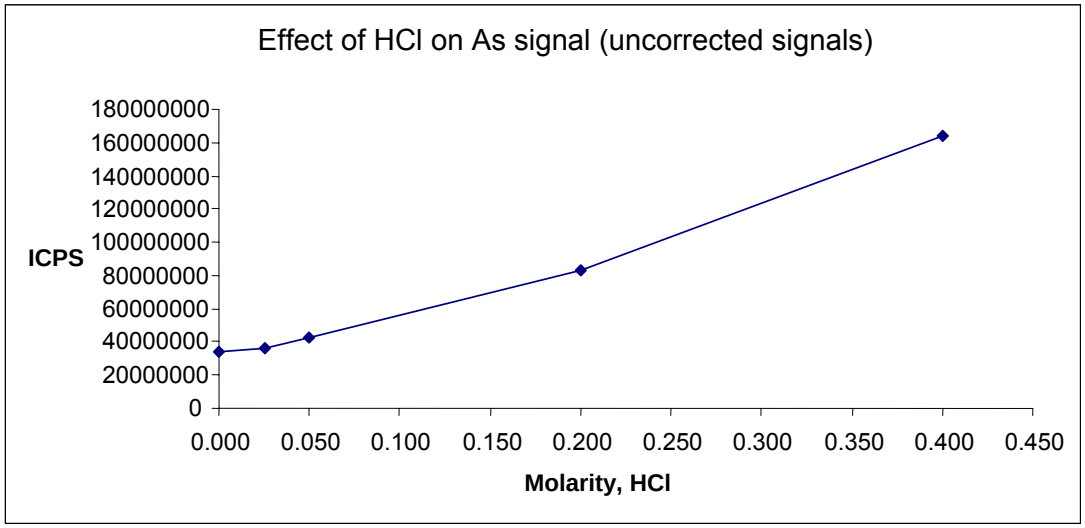

Figure 3.4 Effect of HCl on 20.0 ng/mL As signal (without software correction).

3.1.3.2 Effect of LiCl on As signal

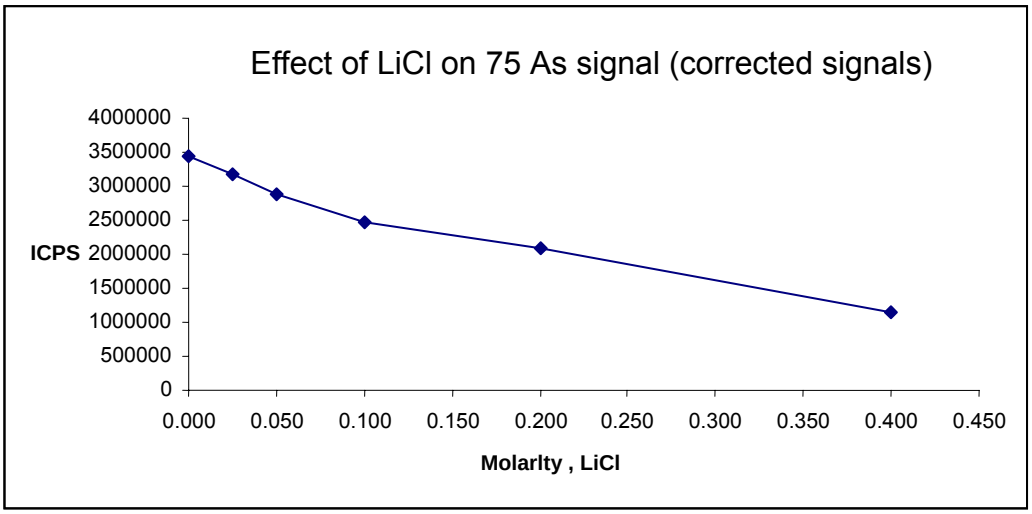

Figure 3.5 Effect of LiCl on 20.0 ng/mL As signals (with software correction).

It was observed that the presence of LiCl suppressed As signals.This may due to both ionization effect and mathematical correction method done by instrument. Since ionization potential of Li (5.39 eV) is lower than As (9.81 eV), in the plasma temperature (7000K-7500K) Li ions dominate (percent ionization is 99.7%) where As is ionized up only 16.4%. Hence, As ions is

suppressed not only in the presence of Li ion but also interference correction done by the instrument itself. However, mathematical correction of instrument is done by the software automatically if preffered. In this case, correction $^{75}As^{+}$ signals are corrected according to $^{40}Ar^{37}Cl^{+}$ signals. From this point masses nearest to 75; that $^{77}Se^{+}$, $^{78}Se^{+}$ and $^{82}Se^{+}$ are monitored to validate interference effect. As seen in Figure 3.5 suppresion of As signal is observed due to chlorine correction in LiCl. Besides chlorine correction, ionization effect of Li results in double suppression on 75 mass.

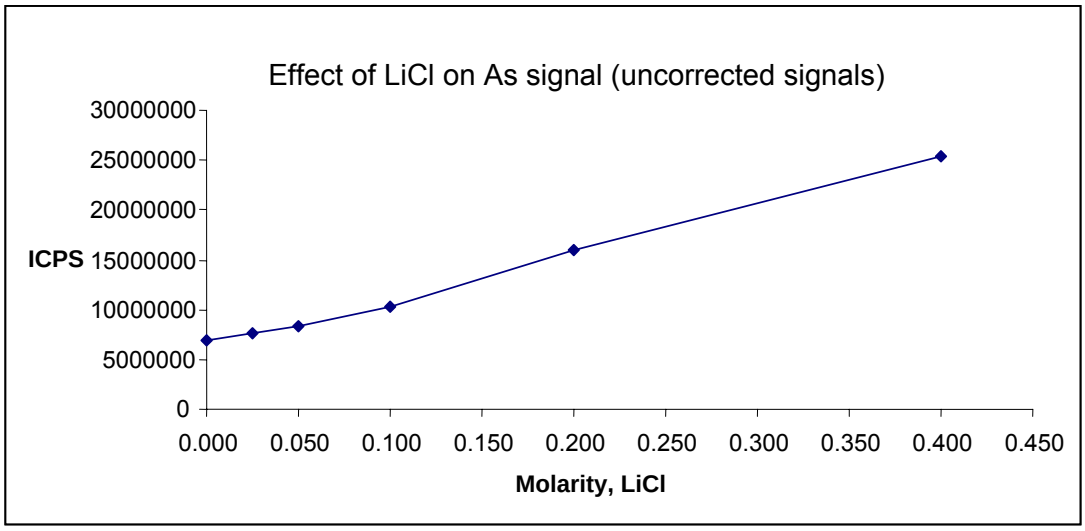

Figure 3.6 Effect of LiCl on 20.0 ng/mL As signals (without software correction).

ICP-MS interference correction mode was removed in order to see the behavior of instrument against chlorine source of Li. As the results are given in Figure 3.6, $^{75}As^{+}$ signals increased due to $^{75}ArCl^{+}$ spectral interference. Spectral effect was significantly higher than suppressing effect of Li ionization; therefore the net interference effect is positive

3.1.3.3 Effect of $LiNO_3$ on As signal

In order to see only the Li suppression effect, 20 ng/mL of As in different concentrations of $LiNO_3$ is tested (Figure 3.7 and Figure 3.8). In this medium, spectral effect of $^{75}ArCl^{+}$ does not exist. As it is expected, suppression effect

of Li on As is observed again no matter interference equation is used or not. In order to explain this effect firstly one should consider contribution of

energy that is needed to break N=O bond (6.1 eV highly energetic) to the overall energy consumption. Secondly contribution of decomposition product to the efficient transfer of energy to the central channel of the plasma through the production of species with thermal conductivities greater than that of Ar must be considered. In the presence of NO_3^- more energy might be consumed up which lowers thermal conductivity of central channel and this results a decrease in local temperature. As a result ionization of As^+ ions more difficult in such a medium, so the suppression is inevitable.

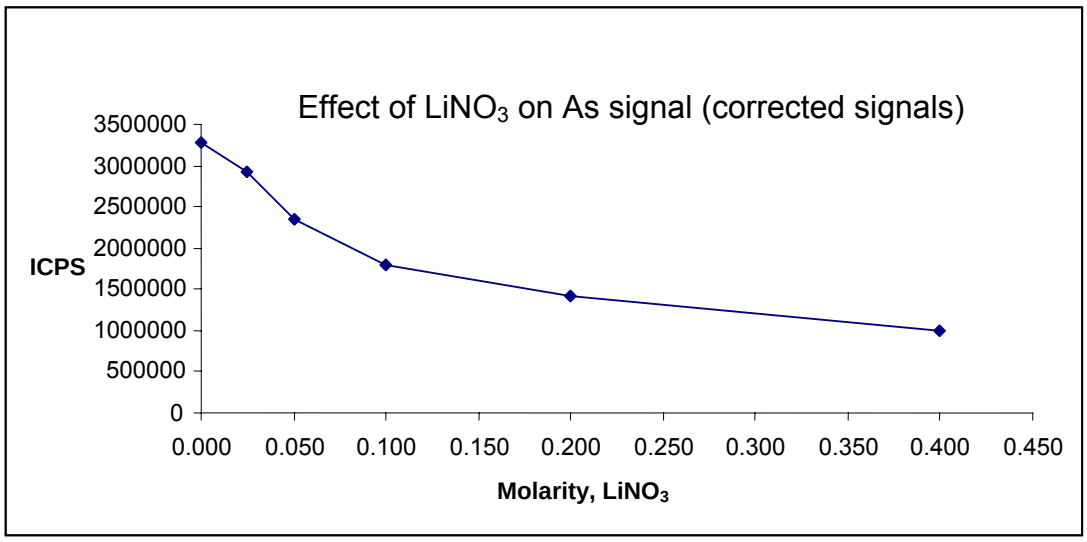

Figure 3.7 Effect of $LiNO_3$ on 20.0 ng/mL As signals (with software correction).

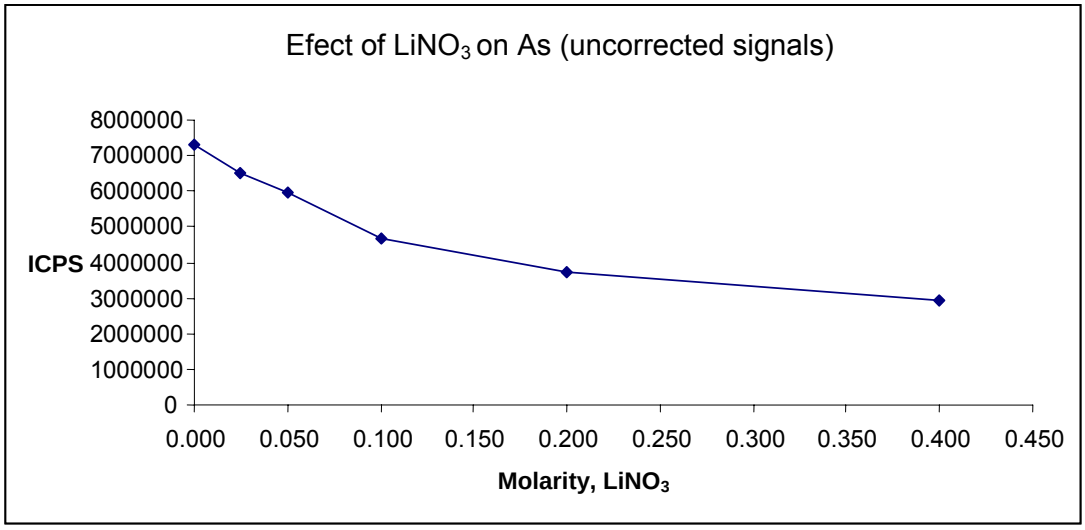

Figure 3.8 Effect of $LiNO_3$ on 20.0 ng/mL As signals (without software correction).

3.1.3.4 Effect of NaCl on As signal

In order to see the interference effect of NaCl, on 20.0 ng/mL of Arsenic in different concentration of NaCl salt was prepared. Na, having a ionization potential of 5.14eV, is expected to have a greater suppression affect than Li. The shift in the ionization equilibrium is a simple explanation for create very high electron density so electrons that are created by Na and Li is negligible.[89], On the other hand it is reported that "measurements using Thompson and Rayleigh scattering [90] have shown that there is a significant local increase in electron density along the central axis of the plasma in the presence of this kind of elements". Moreover the localized increase in electron density may be result of an aerosol droplet containing elements such as Na, Li which shifts the ionization equilibrium back to the atomic form when compared to a droplet which does not contain such elements This may be most important in lower locations in the plasma where the temperature is lower. Moreover, it has been reported that "with the first ionization potential of the matrix elements, where those having the lowest ionization potential caused the greatest amount of suppression" [91,9,43]. In Figure 3.9 suppression of As signal is severe due to correction of ^{75}As signals over $^{77}ArCl$ ones.

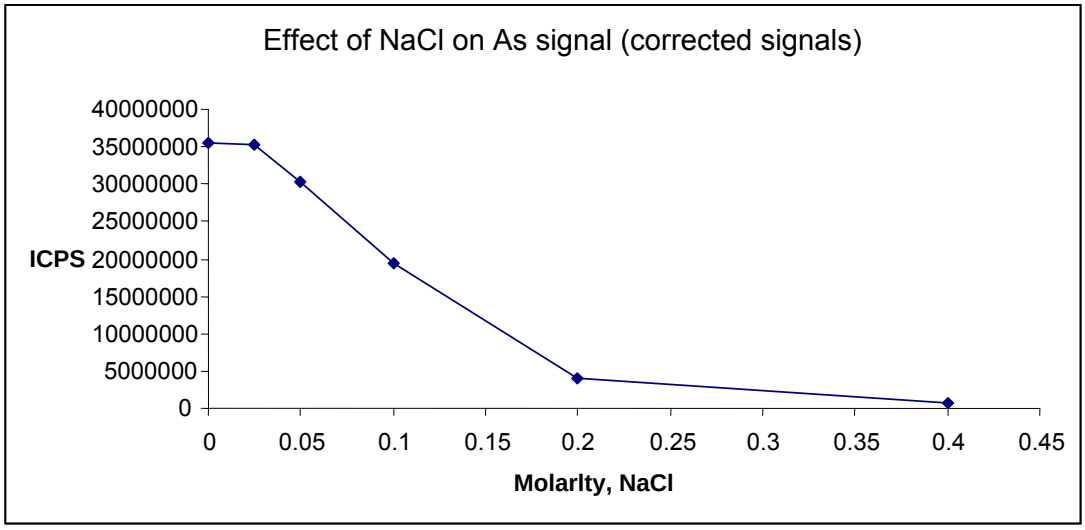

Figure 3.9 Effect of NaCl on 20.0 ng/mL As signals (with software correction)

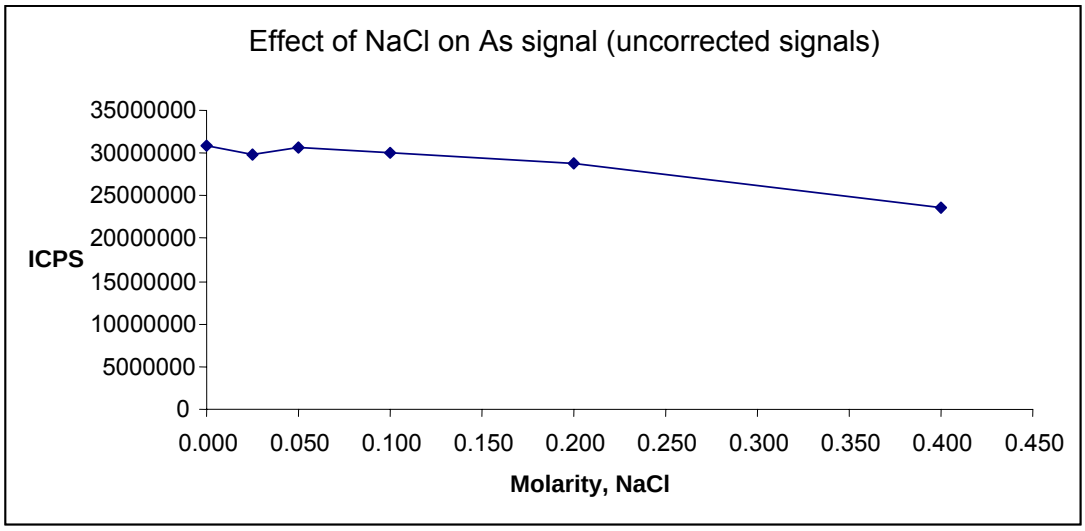

Figure 3.10 Effect of NaCl on 20.0 ng/mL As signals.(without software correction).

When correction mode is removed suppression effect of Na is nearly balanced with $^{75}ArCl^{+}$ spectral interference on $^{75}As^{+}$ up to 0.40 M NaCl content. When 0.40 M is reached, Na suppression is predominated although stoichiometric ratio of chlorine to Na is 1 and chlorine concentration and hence the $^{75}ArCl^{+}$ interference effect was also increased. This may be due to inefficient aerosol transport of the analyte.

3.1.3.5 Effect of $NaNO_3$ on As signal

Graph obtained from uncorrected and corrected signal in the presence of $NaNO_3$ matrix found out to be same with $LiNO_3$ as expected. (Figures 3.11 and 3.12) Suppression effect of Na is observed in both cases. It is easier for Na to give out an electron in a charge transfer reaction between Ar than one with a higher ionization potential. The presence of such kind of atoms present as a matrix component will give more reactions and lowers the Ar^+ ions available for ionization of the analyte If there is a limited number of Ar^+ ions available, which may be the case in the central regions of the plasma, then this would leave fewer Ar^+ available to ionize analyte elements in this case As. This would result in fewer analyte ions becoming ionized and the analyte signal being suppressed. In evidence to the idea, in the paper it is mentioned as "A matrix element with a higher ionization potential would not deplete the Ar^+ population to the same extent as Na^+ leaving more available Ar^+ species to ionize the analyte species and this possibility could be explored by looking at matrices that are likely and not likely to undergo charge transfer with Ar^+ " [95].

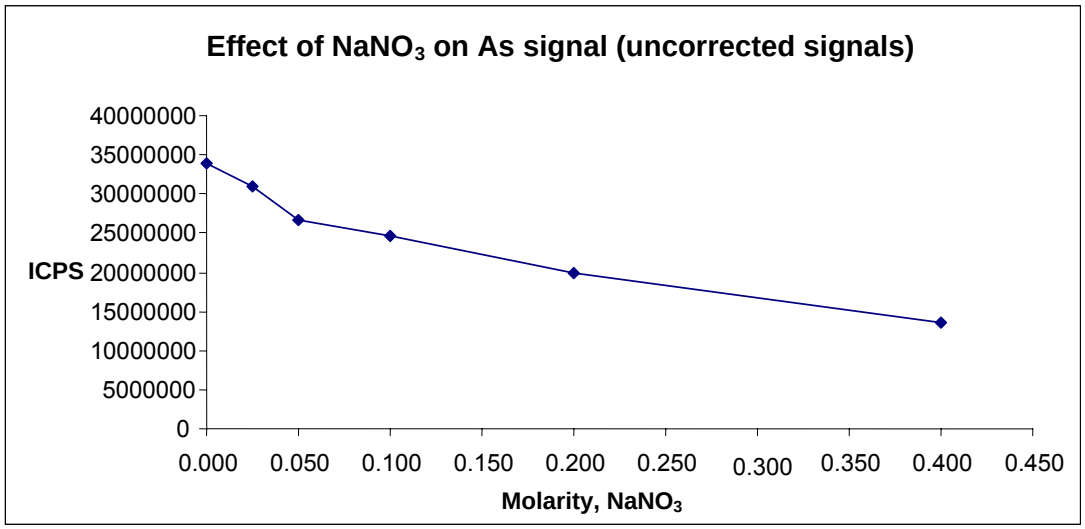

Figure 3.11 Effect of $NaNO_3$ on 20 ng/mL As signals (without software correction).

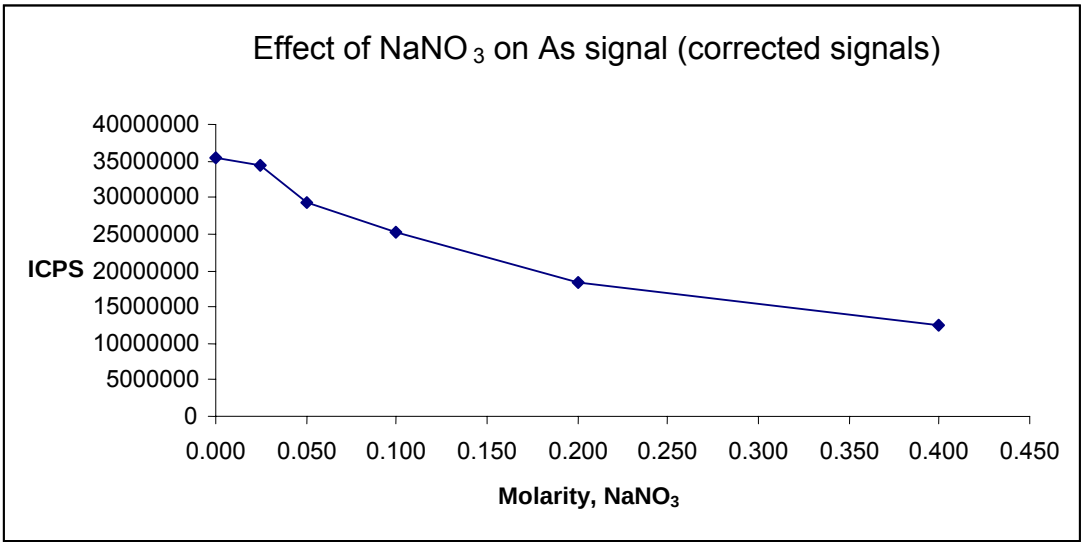

Figure 3.12 Effect of $NaNO_3$ on 20 ng/mL As signals (with software correction).

3.1.3.6 Effect of CsCl on As signal

In the case of Cs the magnitude of the suppression is more severe as the mass of the Cs is higher relative to the analyte ion, As. (Figure 3.13) Severe suppression effect is seen when As in CsCl matrix is injected. This has two reasons one is due to space charge affect described in Part 3.7.1 and the other is interference correction equation used for Cl in the matrix

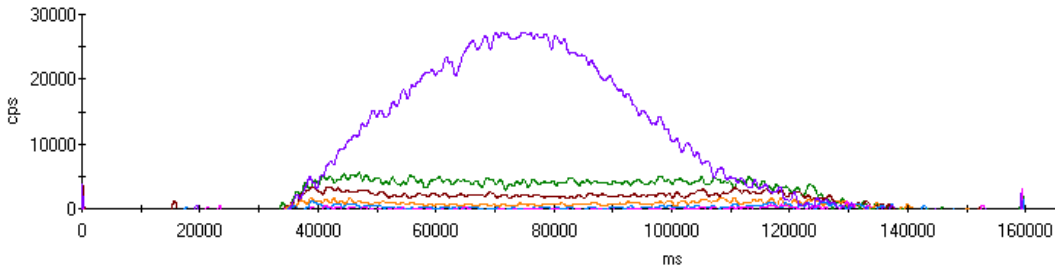

Figure 3.13 ICP-MS signals of $^{75}As^{+}$ for 20 ng/mL As in different concentration of CsCl (purple 20 ng/mL As, green +0.025 M CsCl, brown+0.05 M CsCl, yellow+ 0.1M CsCl, orange + 0.1 M CsCl, blue + 0.4M CsCl using ICP-MS having loop volume of 1mL. Injected volume of solvent reached plasma at around 35 s and left at 120 s (with software correction).

In the case of Cs the magnitude of the suppression is more severe because the mass of the matrix ion increased relative to the analyte ion. As it is mentioned before "Mass dependent decreases in the transport efficiency from the plasma to the mass spectrometry detector are consistent with space-charge effects" [96]. Severe suppression effect is seen when As in CsCl matrix is injected. This has two reasons one is due to space charge affect described in Part 3.7.1. and the other is interference correction equation used for Cl in the matrix

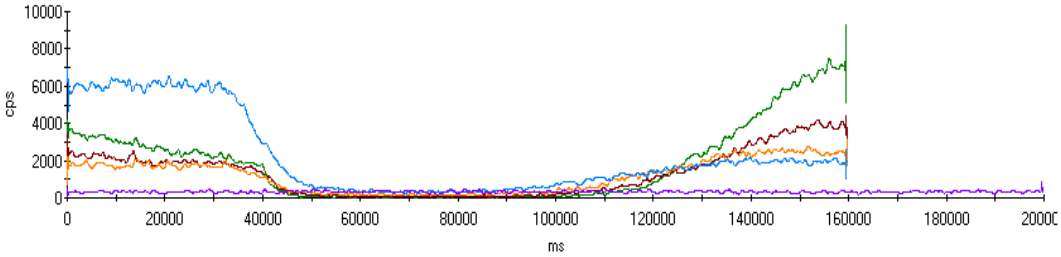

Figure 3.14 ICP-MS response of $^{77}ArCl^{+}$ for 20 ng/mL As in different concentration of CsCl (purple 20 ng/mL As, green +0.025 M CsCl, brown +0.05 M CsCl, yellow +0.10 M CsCl, orange +0.20 M CsCl, blue +0.40 M CsCl using ICP-MS having loop volume of 1.0 mL. Injected volume of solvent reached plasma at around 40 s and left at 120 s (with software correction).

Cs suppression effect severity can further be observed in $^{77}ArCl^{+}$ signals. Before flow injection counts were started > 2000 CPS. At injection time 5th s. drastic decrease in the signal observed due Cs is even greater molecule than $^{77}ArCl$ and pushes molecules lighter than itself in skimmer cone (Figure 3.14).

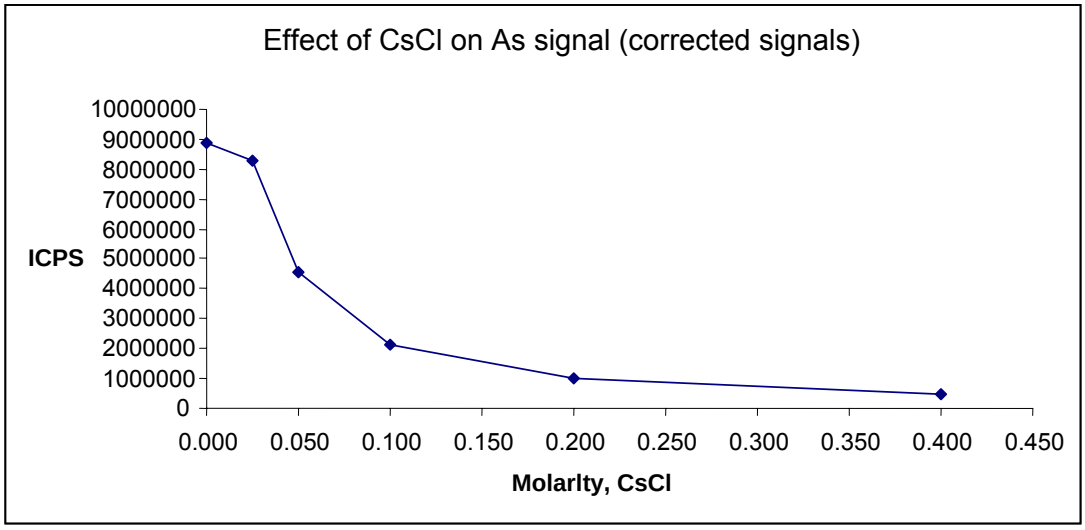

Figure 3.15 Effect of CsCl on As signals (with software correction).

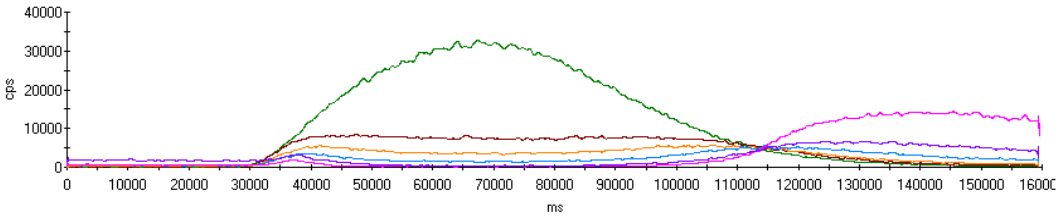

Figure 3.16 ICP-MS response of ^{75}As for 20 ng/mL As in different concentration of CsCl (purple 20 ng/mL As, green +0.025 M HCl, brown +0.050 M CsCl, yellow +0.10 M CsCl, orange +0.20 M CsCl, blue +0.40 M CsCl using ICP-MS having loop volume of 1.0 mL. Injected volume of solvent reached plasma at around 30 s and left at 115 s (without software correction).

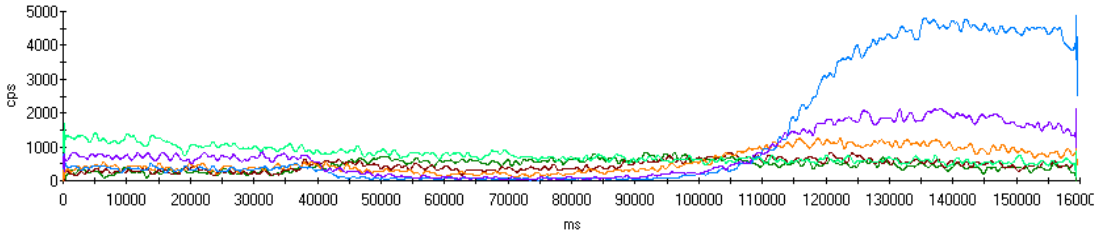

Figure 3.17 ICP-MS response of $^{77}ArCl$ for 20 ng/mL As in different concentration of CsCl (purple 20 ng/mL As, green +0.025 M CsCl, brown +0.050 M CsCl, yellow +0.10 M CsCl, orange +0.20 M CsCl, blue +0.4M CsCl using ICP-MS having loop volume of 1.0 mL. Injected volume of solvent reached plasma at around 40 s and left at 110 s (without software correction).

Figure 3.13 and 3.16 shows that no matter interference equation is used or not signal suppression is observed; this means that space charge effect of Cs^+ is dominant in this case. Figure 3.14 and 3.17 proves the severity of suppression as even $^{77}ArCl^+$ signals also reduced with or without using correction equation.

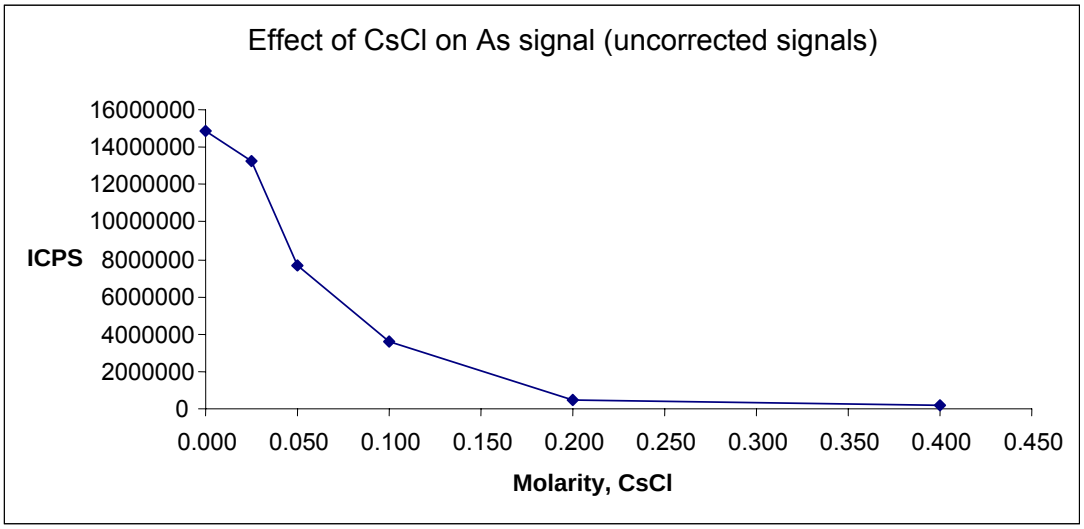

Figure 3.18 Effect of CsCl on As signals (without software correction).

3.1.3.7 Evaluation of Cs Space Charge Effect

The topic of space charge effect in ICP-MS can be understood when one can take into account the various processes that takes place when ions are sampled from the plasma and transported to the detector. First, a″quasineutral″ plasma consisting of neutral atoms, ions, and electrons, comes from atmospheric pressure and go through a sampler–skimmer interface are pumped into the mass spectrometry vacuum chamber. As the plasma flows through the interface, mobile electrons will be separatedfrom bulk flow. This results in positive ion beam made up of neutral atoms and positive ions. There place where the space chThe exact locationarge occurs remain uncert of the charge separation remains unknown; hoain t is thought to be occurring within the skimmer cone [96].

3.1.3.8 Effect of $CsNO_3$ on As signal

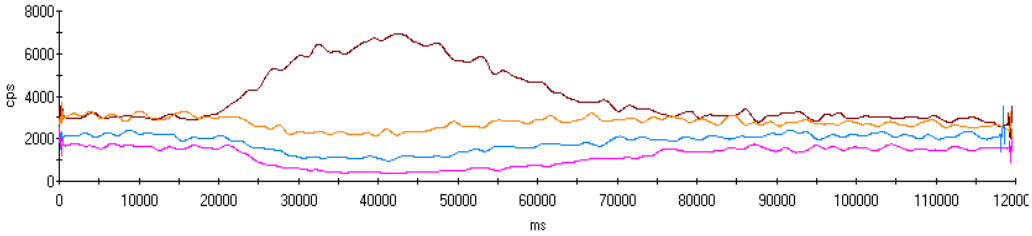

Figure 3.19 ICP-MS response of ^{75}As for 20 ng/mL As in different concentration of $CsNO_3$ (brown 20 ng/mL As, orange +0.025 M $CsNO_3$, blue +0.050 M $CsNO_3$, pink +0.10 M $CsNO_3$ using ICP-MS having loop volume of 1mL. Injected volume of solvent reached plasma at around 20.0 s and left at 80.0 s (without software correction).

Space charge effect of $CsNO_3$ was observed without chlorine interference. In these plasma conditions, suppression of Cs^+ with chlorine free matrix is recorded. Degree of suppression in Figure 3.19 is different from that given in Figure 3.16. Reduction of signal is higher in this case; this may due to analyte transport, aerosol droplet effects occurred before reaching plasma as

there is NO_3^- source besides the Cs atoms. In the plasma the energy will be portioned to break the bonds of NO_3^- and also it affects the aerosol physical structure thus resulting in over suppression on mass 75.

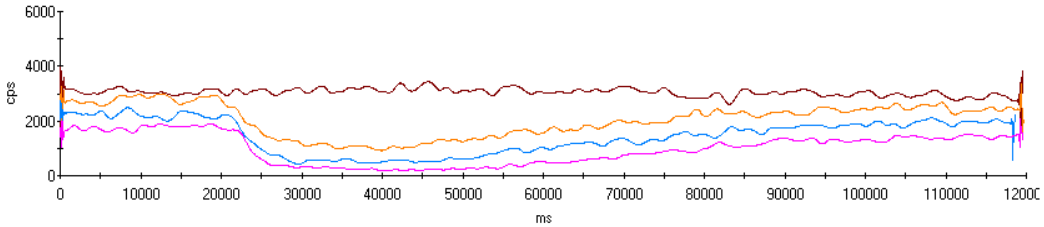

Figure 3.20 ICP-MS response of $^{77}ArCl$ for 20.0 ng/mL As in different concentration of $CsNO_3$ (brown 20 ng/mL As, orange +0.025 M , blue +0.050 M $CsNO_3$, pink +0.10M $CsNO_3$ using ICP-MS having loop volume of 1.0 mL. Injected volume of solvent reached plasma at around 25 s and left at 80.0 s (without software correction)

As in the CsCl case, $CsNO_3$ suppresses the $^{77}ArCl^+$ signal no matter software correction is used or not.

In this part of the study, when As in $CsNO_3$ matrix is determined with software correction, over correction is observed and background of the signal is moved to negative side.

PART 2

SPECIATION STUDIES

3.2 Extraction of Water Soluble Arsenic Species

3.2.1 Choice of Extraction Solvent

The aim of choosing a proper extraction solvent is to extract arsenic species with no changes in the species present in the matrix. When we compare arsenic extraction with other metals in fish samples, it is easier to extract arsenic species than others thanks to arsenobetaine which is a major constituent in fish samples. Arsenobetaine is a stable molecule and is soluble both in methanol and water. In literature if these two solvents are used together extraction efficiency greater than 90% commonly was reached. Methanol, however, is a poor solvent for extracting inorganic arsenic species [96]**.** It may seem that methanol is a proper solvent as it is an organic molecule hence organoarsenic species would prefer the organic solvent. However arsenobetaine is a zwitterion and other naturally occurring arsenic species are polar and very water soluble. From this point of view, naturally occurring arsenic species would prefer water over methanol. Moreover methanol affects the ICP-MS plasma. As methanol concentration is increased, ICP-MS plasma goes out during the studies. ICP-MS plasma can tolerate up to 10% methanol content. As a result, water rather than methanol is chosen to be the extractant in this study.

3.2.2 Optimization of Extraction Device

The extraction of biological tissues has developed over the past decade with focus directed towards increased efficiency with lower solvent volumes and reduced extraction periods. In solid tissues, the analyte is often difficult to extract; it is necessary to optimize the procedure for each matrix investigated.

The quantitative and reproducible extraction of As species, especially from solid samples, is the weakest step in the sequence of speciation analysis. Extraction recoveries depend on the matrix, species present, types of solvents and extraction.

For the extraction of arsenic species from fish samples, ultrasonication bath, sonication probe, and shaker were used in order to increase the extraction efficiency.

3.2.2.1. Sonication Probe

0.20 g of NIST RM 50 Albacore Tuna fish tissue weighed into clean 50.0 mL of centrifuge tube. 40.0 mL of water as extraction solvent was added. Firstly each replicate solution was mixed for 5.0 min with vortex mixing method. In this extraction step, sonication probe was chosen as extraction device. Each sample was sonicated for 10 min. Temperature control was provided by ice bath around the sample. Samples were centrifuged at 4500 rpm for 5.0 min then filtrated with a 10.0 KDa ultrafiltration membrane. And then standard addition technique with 3 points applied for Arsenobetaine, AB. Result calculated as total As concentration and it was found as 2027 ± 71 ng/g (N=2). The referenced result for NIST-RM 50 is 3300 ± 71 ng/g

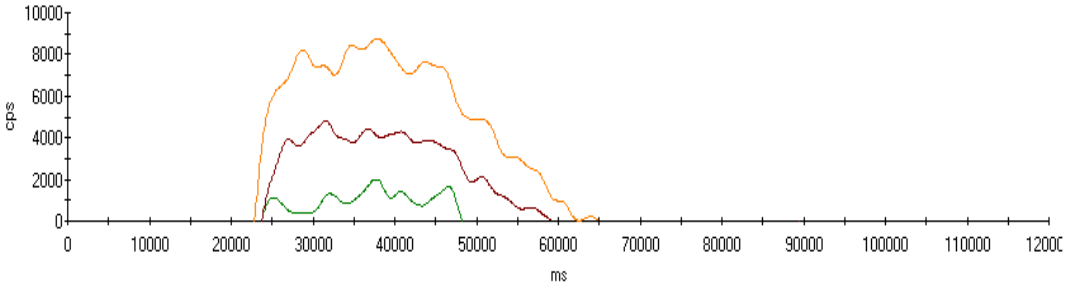

Figure 3.21 ICP-MS signals for extracted NIST RM 50 solution obtained by sonication probe. Green +0, red +10.0 ng/mL, orange +20.0 ng/mL AB.

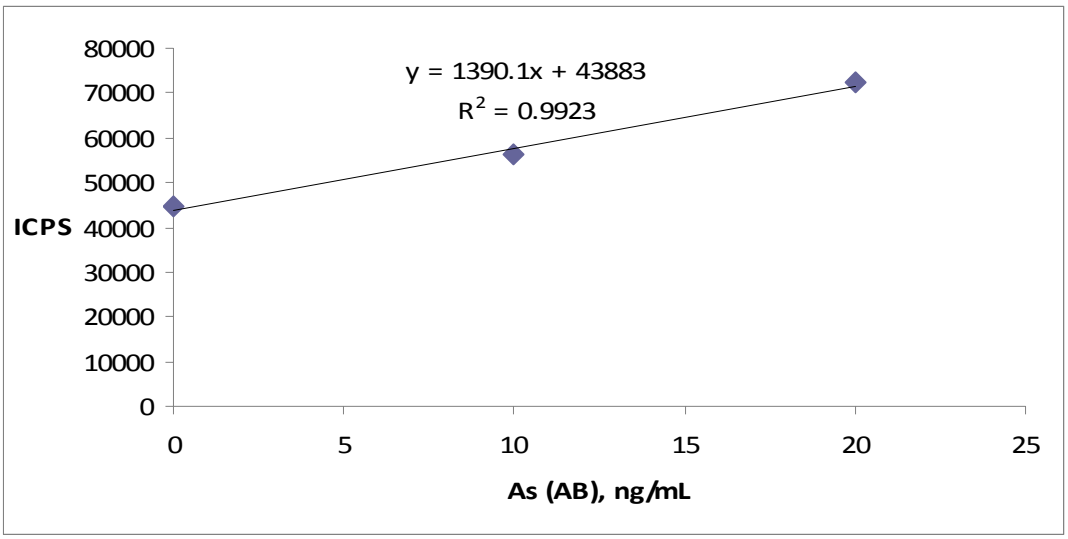

Figure 3.22 Standard addition (after extraction) calibration plot by extraction with sonication probe for NIST RM 50.

3.2.2.2 Ultrasonication bath

0.20 g NIST RM 50 Albacore Tuna fish tissue was weighed into a clean centrifuge tube. After adding 10.0 mL of water, the contents are sonicated in Emma Sonic Ultrasonification bath for 30.0 min. Then the mixture was centrifuged for 5.0 min at 4500 rpm. Separation of supernatant from mixture was done by decantation. The supernatant further filtrated using 10.0 KDa ultrafiltration membrane and analyzed by ICP-MS on total As concentration using 3 point standard addition with AB. The result was 2350 ± 82 ng/g As, (N=2).

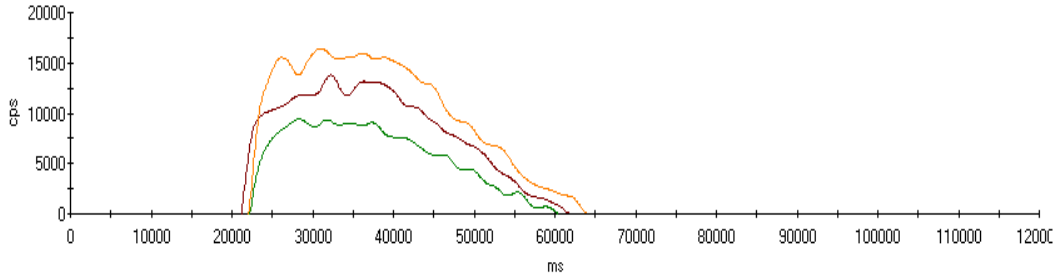

Figure 3.23 ICP-MS signals for extracted NIST RM 50. solution obtained by ultra sonication bath green +0, red +10 ng/mL, orange +20 ng/mL AB.

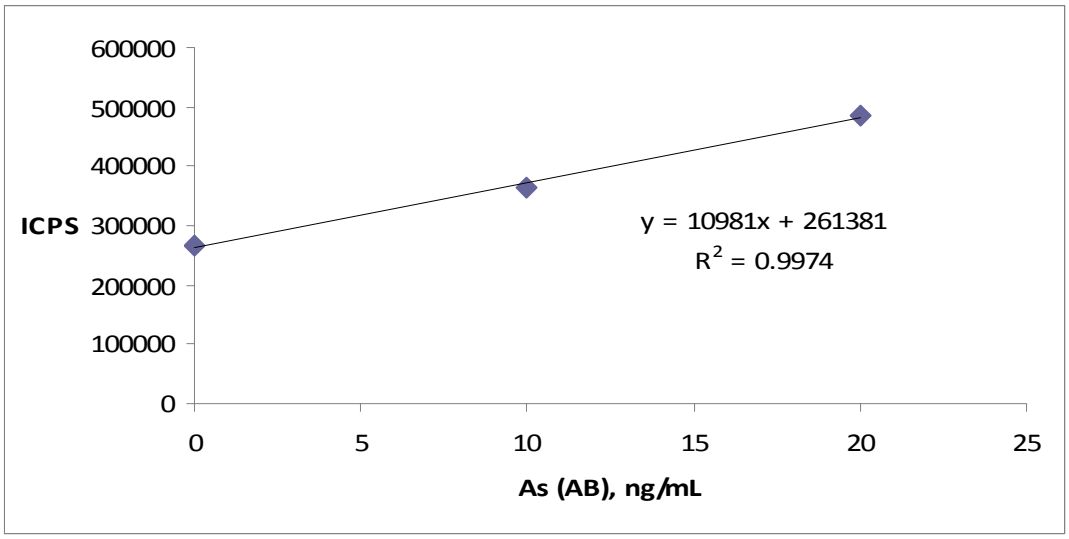

Figure 3.24 Standard addition (after extraction) calibration plot by extraction with ultrasonication bath for NIST RM 50.

3.2.2.3 Evaluation of Ultrasonication Bath/Probe Method

In literature it is mentioned that "the use of ultrasonication bath is a well known tool in analytical chemistry and It is used to achieve removal of particles from a substrate (in general glassware) or to accelerate mixing processes." From this one can conclude that ultrasonication method is easy to use. But its mixing process is not same thorough out the whole process. Driving force of sonochemical action is the acoustic cavitation, provoked by bubbles formed by the sound wave in a liquid that continuously compresses and decompresses. This results in higher local temperatures and pressureswhich makes infirmity in mixing [98]. The other drawback of using

ultrasonication method bath/probe is difficulty in temperature controlling and both applicable to large solvent containing mixtures. In this study at each extraction step 10.0 mL of extraction solvent was used. Hence another extraction device; a mechanical shaker which had been used for Se extraction at an early study in our group was tried for As extraction from fish samples for further studies.

3.2.2.4 Mechanical Shaker

0.20 g of NIST RM 50 Albacore Tuna fish tissue was weighed into clean centrifuge tube. 10.0 mL of water is added as an extraction solvent. Then it was vortex mixed for five minutes before shaken in shaker for 30.0 min at room temperature. Centrifugation duration was 5.0 minutes as in other extraction procedures. Then extracted solution was filtrated throughout 10.0 KDa ultra filtration membrane. Before instrumental analysis 3 point standard addition with AB was applied to the extracted solution. The result found out to be 2853 ± 98 ng/g As. And the referenced results for NIST RM 50 is 3300 ± 70 ng/g.

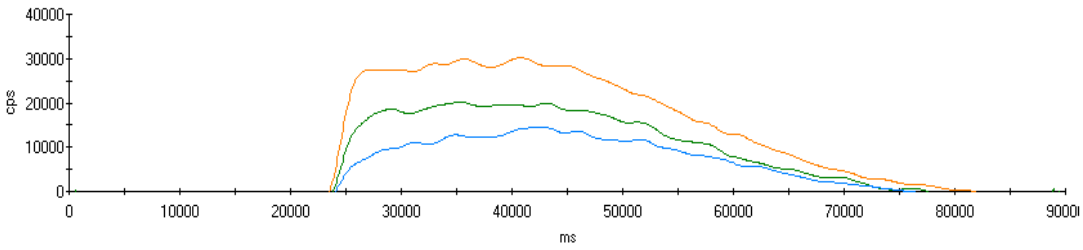

Figure 3.25 ICP-MS signals for extracted NIST RM 50. solution obtained by mechanical shaker green +0, red +10.0 ng/mL, orange +20.0 ng/mL AB.

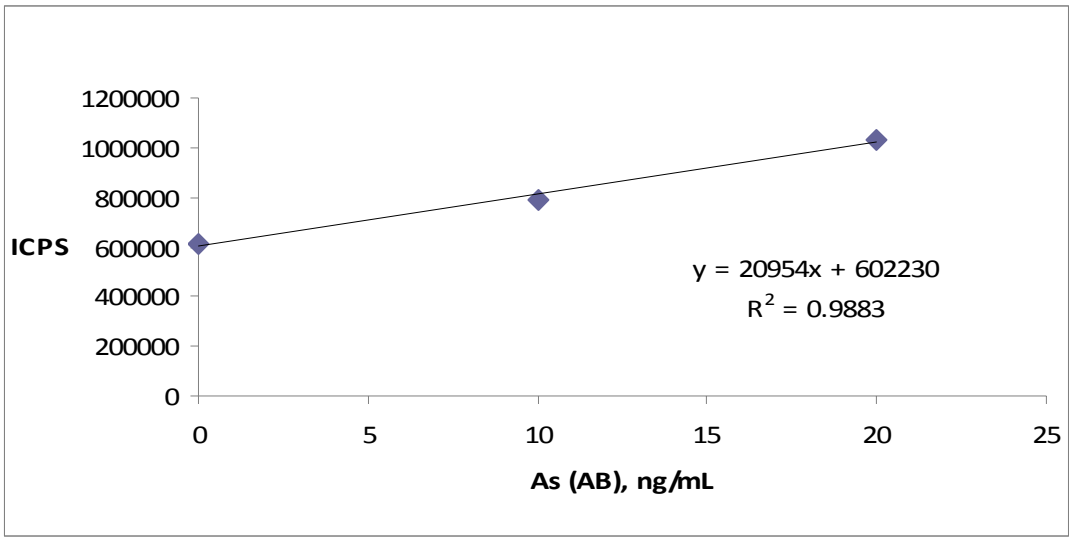

Figure 3.26 Standard addition (after extraction) calibration plot by extraction with mechanical shaker for NIST RM 50.

NIST RM 50 Albacore tuna reference material was reported to contain 3300 ± 70 ng/g As. According to this result, calculated efficiencies between for different extraction devices are 61.4%, 71.2% and 86.8% for sonication probe, ultrasonic bath and mechanical shaker, respectively.

The extraction efficiency of the shaker was found to be higher than others. In addition sonication methods have drawbacks such as temperature controlling and difficulty in handling. Shaker is chosen to be the optimum extraction device for this study. Extraction method development was continued using shaker.

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3.2.3 Optimization of Extraction Period

In the extraction study, extraction period was also optimized to obtain the highest extraction efficiencies for the species of interest. In general, extraction step is known as the most time consuming step in speciation studies. In this study, the aim was not only to find the best extraction period but also to minimize the time consumption. For this aim, about 0.20 g sample of NIST RM 50 was taken and 10.0 mL of extractant were added to the sample. Samples were placed in the shaker. Tubes were shaken for 5.0, 10.0, 20.0, 30.0, 60.0 and 120.0 minutes. At the end of each period, dublicate of the samples were taken and then extraction solutions were filtrated using

10.0 KDa ultrafiltration membrane and Millipore Stirred ultra filtration cell (8400 Model) in order not to only reduce the matrix content but also obtain a clear solution. After the filtration, clear solutions were analyzed using ICP-MS for their total arsenic contents. The results obtained for total As content of NIST RM 50 were 2930 ± 102 ng/g, 3139 ± 109 ng/g, 3156 ± 110 ng/g, 3314 ± 115 ng/g, 3394 ± 118 ng/g, and 3109 ± 108 for 5.0, 10.0, 20.0, 30.0, 60.0 and 120.0 min extraction periods, respectively, as also shown in Figure 3.26

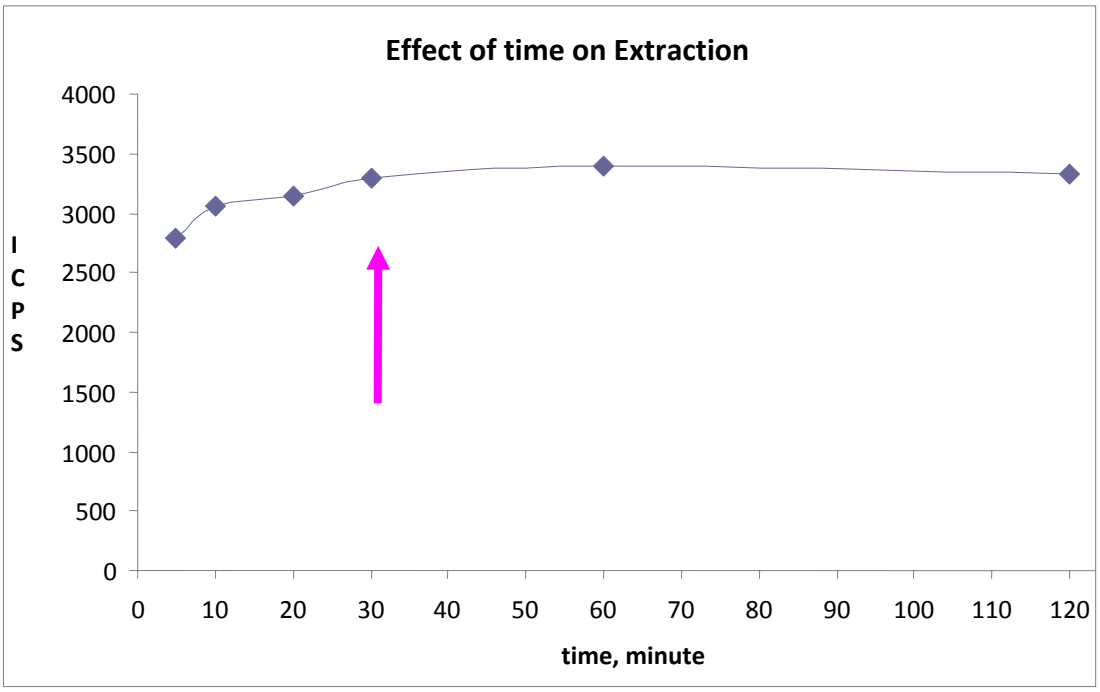

Figure 3.27 Effect of extraction period on extraction efficiency.

According to the results shown above, optimum extraction period is 30.0 minutes for Albacore Tuna Reference Material. From this point on extraction period used is 30 minutes in all cases.

3.2.3.1 Extraction Method 1

Approximately, 0.20 g of NIST RM 50 Albacore Tuna powder was placed in a 50.0 mL centrifuge tube and 10.0 mL of water were added as the extraction solvent. After 30.0 min of shaking in shaker, the samples were centrifuged for 15.0 min at 6000 rpm, the extract was then removed using decantation and the residue was re-extracted following the former procedure. The two

extracts were filtered through a 10.0 kDa filter membrane and analyzed separately.

The results have shown that some As was also obtained from the second extraction. The signals are given in Figure 3.28. Therefore it was decided to perform 3 consecutive steps in order to extract the arsenic in the second step. This following procedure was used.

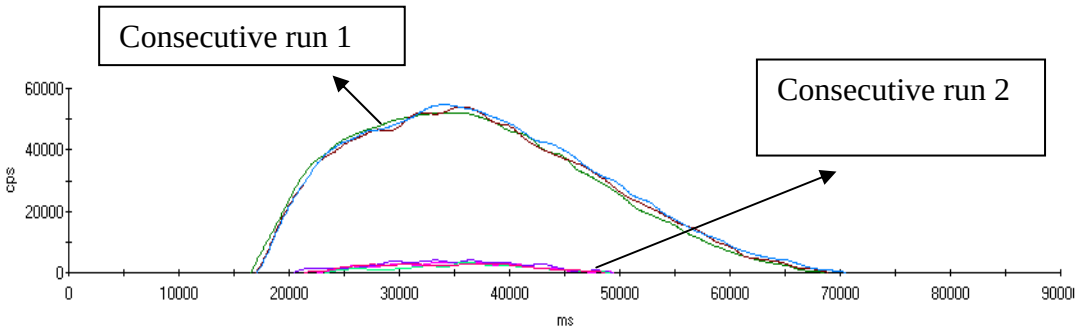

Figure 3.28 ICP-MS signals for 30 min shaking NIST-RM 50 (brown & green+0, blue & orange +12 ng/mL, purple& pink +24 ng/mL AB).

3.2.3.2 Extraction Method 2

Approximately, 0.20 g of NIST RM 50 Albacore Tuna powdered fish tissue was placed in a 50.0 mL centrifuge tube and 10.0 mL of water were added as the extraction solvent. After 30.0 min of shaking in shaker, the samples were centrifuged for 15.0 min at 6000 rpm, the extract was then removed using decantation and the residue was re-extracted following the former procedure. In the third step residue from second extraction is further extracted starting with 5 min vortex mixing then with 10.0 mL of extractant solvent in shaker for 30 min. After decantation and filtration processes, the three combined extracts were mixed and filtered through a 10.0 kDa ultrafiltration membrane. For the next step 3 point standard addition was applied and each solution with two replicate was injected to the ICP-MS system for the determination of total AB recovered in extraction method 2. The result was 3412 ± 169 ng/g As(AB). The certified result was 3300 ± 70 ng/g.

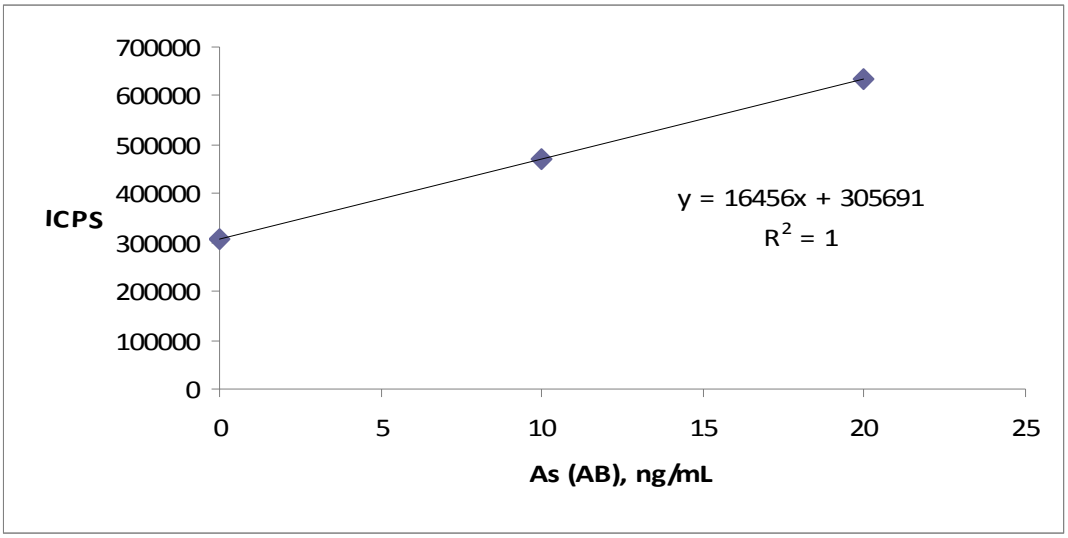

Figure 3.29 Standard addition (after extraction) calibration plot by extraction using 3 step consequtive extraction shaker for NIST RM 50.

According to the results above we choose Extraction Method 2, containing 3 consecutive steps as the best method. When three consecutive extraction of the sample was performed, in the third run no arsenic signal was obtained. Therefore there was no need to run the 4th step extraction.

3.2.4 Optimization of Separation Conditions

3.2.4.1 Optimization of HPLC Parameters

Optimization of the chromatographic parameters was carried out with respect to pH, ion-pairing reagent, and percentage of the methanol in mobile phase and flow rate of the mobile phase. The method was then applied to the analysis of fish samples.

3.2.4.2 Optimization of Mobile Phase

The separation of arsenic species by HPLC is pH-dependent [99]. For example, at neutral pH, arsenate (pKa1 = 2.3), MA(V) (pKa1 = 3.6), and DMA(V) (pKa = 6.2) are present as anions; arsenocholine (AsC) and the tetramethylarsonium ion (Tetra) as cations; arsenobetaine (AB) as a

zwitterion; and arsenous acid (pKa1 = 9.3) as an uncharged species. In other words, the pH of the mobile phase should have a remarkable effect on the retention time of analytes Therefore, it is necessary to optimize the pH of mobile phase for separating desired arsenic species. Optimum pH value for a column is between 2 and 8 so pH values ranged 2 to 6 were studied. Table below show the relative pKa values of Arsenic species mostly found in marine samples.

For the preparation of standard solutions of As species both in separation and speciation part, concentrations were calculated as As(As specie). In example, 50 ng/mL DMA(V) refers to 50 ng/mL As in DMA(V) compound.

Table 3.1 Predominant form of each species as a function of pH and pKa values.

Species	**pKa Values**
As(III)	9.2
$As^{(V)}$	2.3/6.7/11.6
MA	3.6 / 8.2
DMA	6.3
AB	2.2
AC	-

3.2.4.2.1 Mobile Phase CX-MP1

In this system, 20.0 mM formic acid adjusted by NH_3 to pH 2.8 is used as a mobile phase. Optimized parameters can be seen in Table 3.2

Table 3.2 Isocratic chromatographic separation conditions for cation exchange HPLC-ICP-MS using Cation Exchange-MP1.

Parameter	Value
Column	Spheris S5SCX cation exchange column
Mobile phase	20.0 mM of formic acid, pH 2.8
Flow rate	1.0 mL/min
Loop Volume	182 µL

The chromatogram obtained from each arsenic species injected to the cation exchange column separately are shown in Figure 3.30.

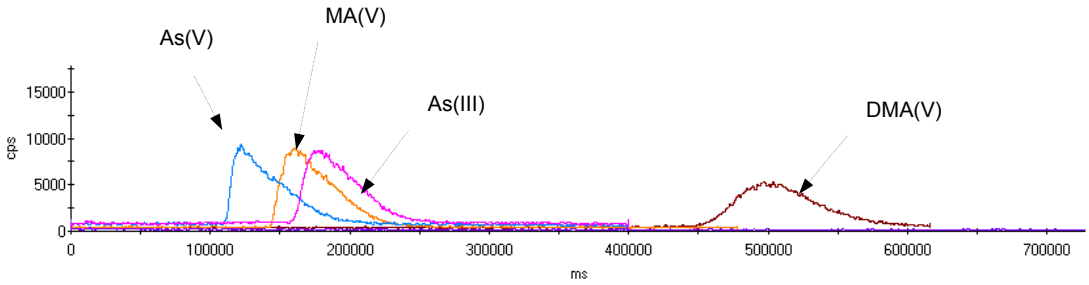

Figure 3.30 Signals obtained using separate solutions containing 100.0 ng/mL As(V), MA(V), As(III), DMA(V) and AB species injected to cation exchange-HPLC-ICP-MS system by using CX-MP1 at 1.0 mL/min flow rate, conditions are given in Table 3.2

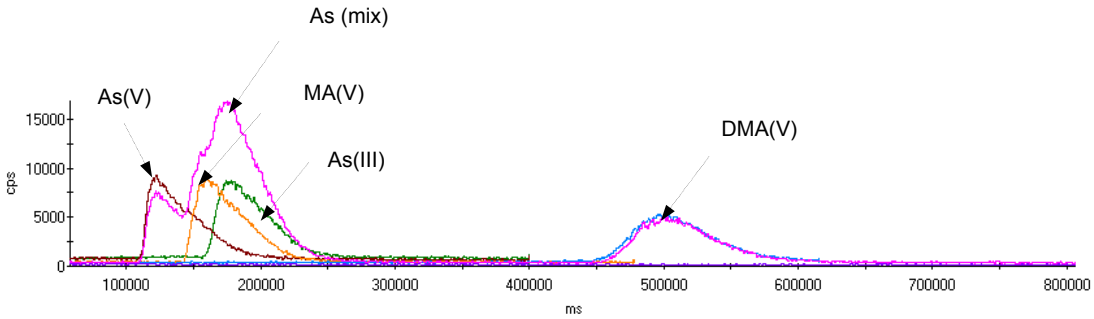

Figure 3.31 HPLC-ICP-MS chromatogram of mixed arsenic standard containing 100.0 ng/mL of As(V), MA(V), As(III), DMA(V) and (AB) (pink) using CX-MP1 at 1.0 mL/min flow rate and these species alone (corrected signal).

The retention times of As(V), As(III), MA(V) and DMA(V) in the cation exchange column using Mobile Phase CX-MP1 were found to be 110.0, 143.0, 156.0 and 445.0 seconds, respectively, as shown in Figure 3.31. But AB was eluted easily so that even its signal cannot be seen at these conditions. Although the separation of MA(V) and DMA(V) was achieved, separation is poor between MA(V) and inorganic species. AB could not be retained at this pH so different mobile phases at different pH values tried.

3.2.4.2.2 Mobile Phase CX-MP2

In this mobile phase, 20.0mM of formic acid is used (pH 5.0). For pH adjustment, NH_3 was chosen. All parameters for Mobile Phase 2 system can be seen in Table 3.3

Table 3.3 Isocratic chromatographic separation conditions for cation exchange HPLC-ICP-MS using CX-MP2.

Parameter	Value
Column	Spheris S5SCX cation exchange column
Mobile phase CX-MP2	20.0 mM ammonium formate, pH 5.0
Flow rate	1.5 mL/min
Loop Volume	182 µL

Under the conditions given in Table 3.3, an arsenic standard of different

species was injected to HPLC-ICP-MS system and chromatogram seen in Figure 3.32 was obtained.

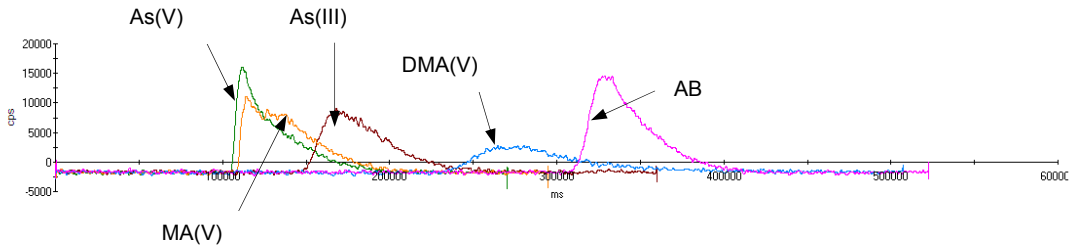

Figure 3.32 Signals obtained using 100.0 ng/mL As(V), MA(V), As(III), As DMA(V), and (AB) species injected to cation exchange-HPLC-ICP-MS system by using CX-MP2 at 1.5 mL/min flow rate conditions are given in Table 3.3 (corrected signal).

When the corrected signals were used in the chromatogram background is moved to negative values. This may be due to change in pH resulted in over correction of signals since the interference equation is applied in this experiment. Since there is no chlorine source in the standards,

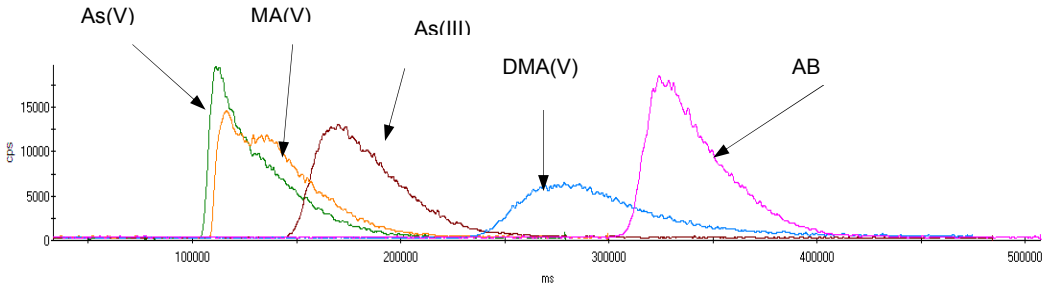

Figure 3.33 Signals obtained using 100.0 ng/mL of As(V), AsMA(V), As(III), DMA(V), and AB species injected to cation exchange-HPLC-ICP-MS system by using CX-MP2 at 1.5 mL/min flow rate, conditions are given in Table 3.3 (uncorrected signal).

At the beginning, each arsenic species was injected to system one by one to find out retention time of each species. AB was eluted at 540 s. Retention times of other species shift to smaller retention times. In all cases separation of MA(V) from AB is achieved. On the other hand, separation of inorganic species As(III) and As(V) from MA(V) and separation of DMA(V) from AB could not be achieved.

3.2.4.2.3 Mobile Phase CX-MP3

Mobile phase CX-MP3 was also applied to CX-HPLC-ICP-MS. In this system, 20.0 mM of formic acid adjusted to pH 6 was used as the mobile phase. The parameters for Mobile Phase CX-MP3 are shown in Table 3.4 In this system, three different flow rates were tried. The flow rates tested for separation was 0.75 mL/min, 1.0 mL/min and 1.5 mL/min The resolution of As(DMA) and As(AB) was improved as flow rate increased but it was not enough to solve the separation problem between AB and DMA.

Table 3.4 Isocratic chromatographic separation conditions for cation exchange HPLC-ICP-MS using CX-MP3.

Parameter	Value
Column	Spheris S5SCX cation exchange column
Mobile phase CX-MP3	20.0 mM of ammonium formate pH 6.00
Flow rate	0.75,1.0, 1.5 mL/min
Loop Volume	182 µL

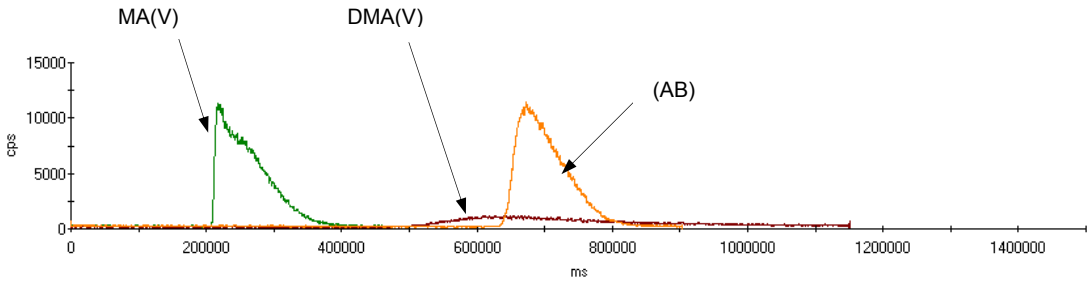

Figure 3.34 HPLC-ICP-MS chromatogram of each arsenic species solution containing 100 ng/mL MA(V), DMA(V) and AB, using 0.75 mL/min flow rate and parameters given in Table 3.4, CX-MP3 (uncorrected signals).

Separation of DMA(V) and AB is even poorer at this flow rate as peak broadening of DMA(V) took place. Hence, other flow rates were checked to improve separation

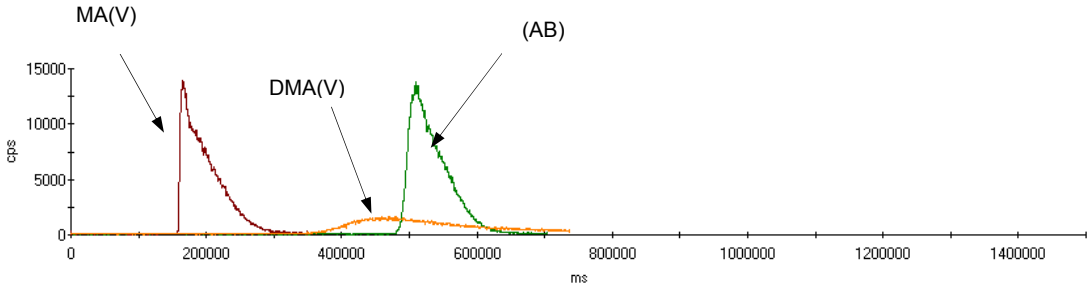

Figure 3.35 HPLC-ICP-MS chromatogram of each arsenic species solution containing 100 ng/mL of MA(V), DMA(V) and AB, using 1.0 mL/min flow rate and parameters given in Table 3.4, CX-MP3 (uncorrected signals)

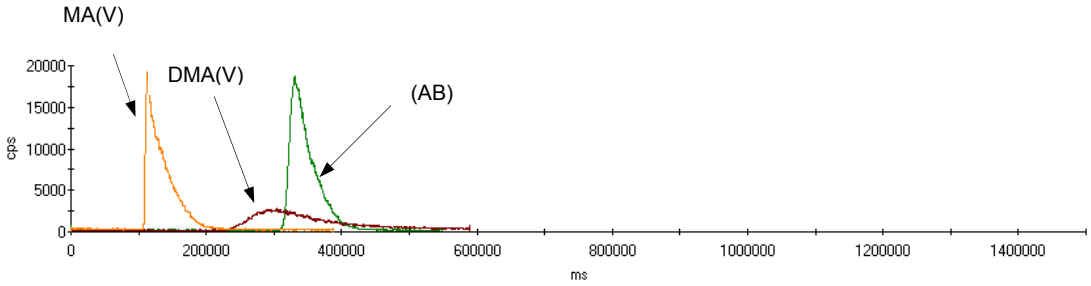

Figure 3.36 HPLC-ICP-MS chromatogram of each arsenic species solution containing 100 ng/mL of As MA(V), DMA(V) and AB, using 1.5 mL/min flow rate and parameters given in Table 3.4, CX-MP3 (uncorrected signals).

As flow rate increased DMA(V) peak broadening was reduced but was not enough to achieve separation from AB. The flow rates were tested, in the range of 0.75-1.5 mL/min. Smaller or greater than these values were not tried because of the effect pressure of pump on nebulization efficiency. As it is shown in Figure 3.34, 3.35 and 3.36, flow rate did not affect separation of arsenic species significantly.

During separation studies of several As species, besides $^{75}As^{+}$ signals, $^{77}ArCl^{+}$ signals were also monitored to see if there is any interference effect. $^{77}ArCl^{+}$ signals were found to be stable during the chromatogram at different flow ratesof 0.75, 1.0 and 1.5 mL/min and maximum and their maximum value was 750 cps where the related $^{75}As^{+}$ signals have an average peak height of 10000 cps. Therefore it can be concluded that in this mobile phase composition and with these As standards it is safe to use uncorrected signals as there is no important chlorine source to need correction.

3.2.4.2.4 Mobile Phase CX-MP4

Knowing that pKa of AB is 2.00, in this system; 20.0 mM pyridine adjusted by HCl to pH 2.00 is used as a mobile phase. Optimized parameters are given in Table 3.5.

Table 3.5 Isocratic chromatographic separation conditions for cation exchange HPLC-ICP-MS using Cation Exchange-MP4.

Parameter	Value
Column	Spheris S5SCX cation exchange column
Mobile phase	10.0 mM of pyridinium chloride , pH 2.00
Flow rate checked	1.0 mL/min, 1.5 mL/min
Loop Volume	182 µL

The chromatogram obtained from each arsenic species injected to the cation exchange column separately are shown in Figure 3.37 and 3.38.

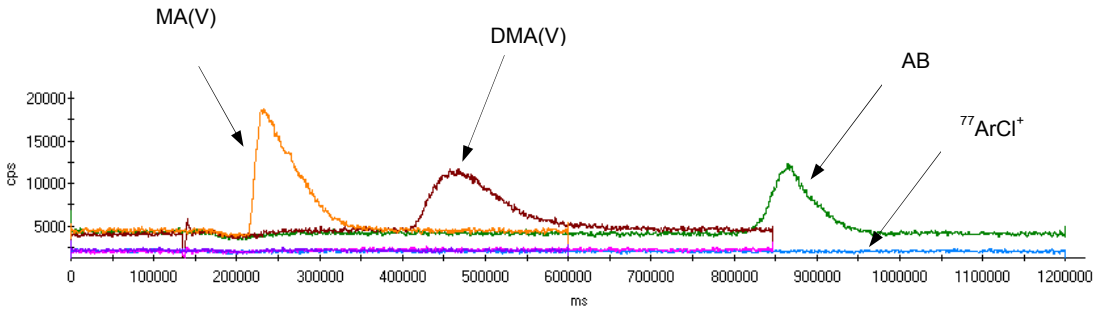

Figure 3.37 HPLC-ICP-MS chromatogram of each arsenic species solution containing 100 ng/mL of As MA(V), DMA(V) and 43 ng/mL and (AB), using 1.0 mL/min flow rate and parameters given in Table 3.5, CX-MP4 with $^{77}ArCl^{+}$ signals of each species below the peaks (uncorrected signal).

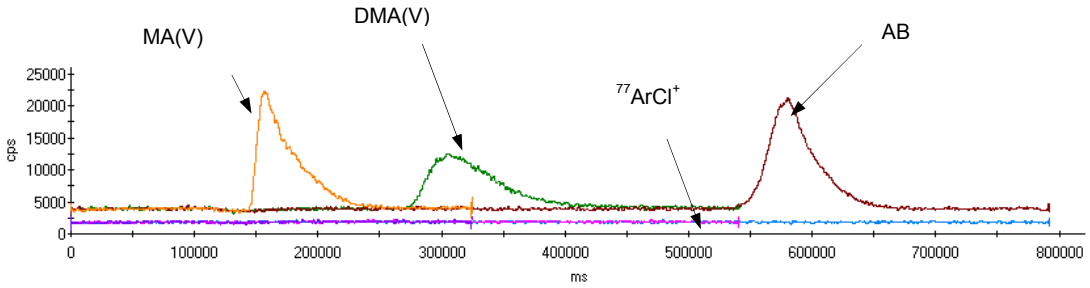

Figure 3.38 HPLC-ICP-MS chromatogram of each arsenic species solution containing 100 ng/mL of As MA(V), As DMA(V) and AB, using 1.5 mL/min flow rate and parameters given in Table 3.5, CX-MP4 with $^{77}ArCl^{+}$ signals of each species below the peaks (uncorrected signal).
Separation of DMA(V) and AB was achieved in this system. As a flow rate, 1.5 mL/min is chosen for shorter analysis period. Maximum chromatogram duration was about 12 min.

In the next alternative for mobile phase, CX-MP5 was used;only difference from CX-MP4 was that it contains 5% MeOH in addition to 20.0 mM pyridine pH 2.00, in order to reduce the peak broadening, mobile phase CX-MP5 was tested.

3.2.4.2.5 Mobile Phase CX-MP5

Each arsenic species was injected to system one by one to determine retention time of each species. There were no changes in the retention times of arsenic species in the mixture and pure solutions. As seen in Figure 3.39 and 3.40 DMA(V) and AB can be separated from MA(V) and inorganic types and each other. Hence, this system is proper to make qualitative and quantitative measurements of DMA(V) and AB former being minor latter being the major constituent in fish samples.

Table 3.6 Isocratic chromatographic separation conditions for cation exchange HPLC-ICP-MS using Cation Exchange-MP5.

Parameter	Value
Column	Spheris S5SCX cation exchange column
Mobile phase	10.0 mM of pyridinium chloride , pH 2.05, 5% (v/v) MeOH
Flow rate checked	1.0 mL/min, 1.5 mL/min
Loop Volume	182 µL

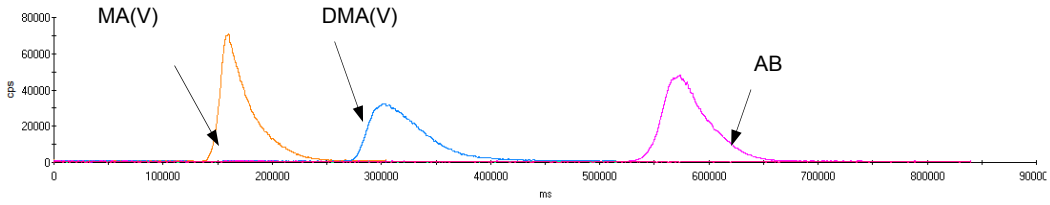

Figure 3.39 HPLC-ICP-MS chromatogram of each arsenic species solution containing 100 ng/mL of MA(V), DMA(V) and AB, using 1.5 mL/min flow rate and parameters given in Table 3.6, CX-MP5 with $^{77}ArCl^{+}$ signals of each species below the peaks (uncorrected signal).

In the mobile phase system, addition of methanol prevents the broadening of the peaks to some extent; but this is not enough. Since too much increase in the methanol content would result in ICP-MS plasma shutdown, methanol content could not be further increased to prevent peak broadening. As it can be seen in Figure 3.40, separation of three organic arsenic species is achieved, the peak of the AB seemed to be greater than the peak of DMA(V) although their As concentration was same, possibly because of carbon enhancement affect. AB is rich in C atoms; carbon enhances the signals of As signals.

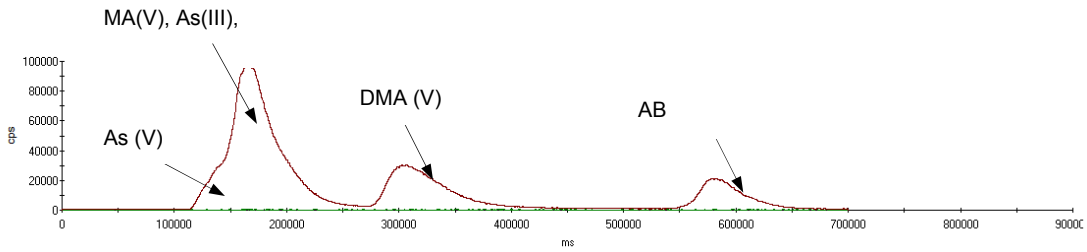

Figure 3.40 HPLC-ICP-MS chromatogram of mixed arsenic standard containing 100.0 ng/mL of As(V), MA(V), As(III), DMA(V) and 50.0 ng/mL AB using CX-MP5 at 1.5 mL/min flow rate. $^{77}ArCl^{+}$ signals of each species below the peaks.

3.2.4.3 Analytical Performance of DMA(V) and AB in Cation Exchange-HPLC-ICP-MS System using Mixed Standard Solution

1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/mL of mix standard solution containing As(III), As(V), MA(V), DMA(V) and 0.5 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 ng/mL of AB is injected in Cation Exchange HPLC-ICP-MS. Chromatograms obtained are shown in Figure 3.41 Linear calibration plot was constructed using peak area of these solutions. Retention times of all concentrations were found to be same. This shows that stability of cation exchange column is satisfactory.

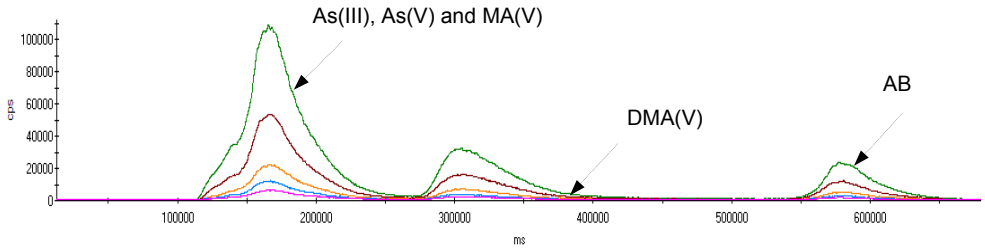

Figure 3.41 HPLC-ICP-MS chromatogram of mixed arsenic standards containing 100.0, 50.0, 20.0, 10.0 ng/mL of DMA(V), MA(V), As(III), As(V) and 50.0, 25.0, 10.0, 5.0 ng/mL of AB and using the parameters given in Table 3.6, CX-MP5.

Linear calibration plot for DMA(V) and AB obtained can be seen in Figures 3.42 and 3.43 As seen in figures, linearity of calibration plots is sufficient.

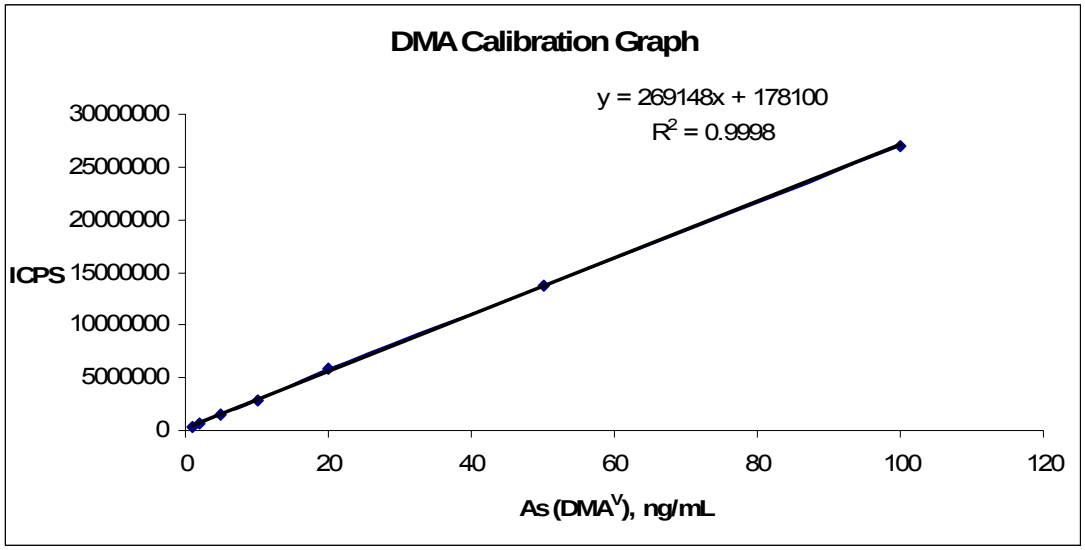

Figure 3.42 Linear calibration plot of DMA(V) obtained using Cation Exchange-HPLC-ICP-MS.

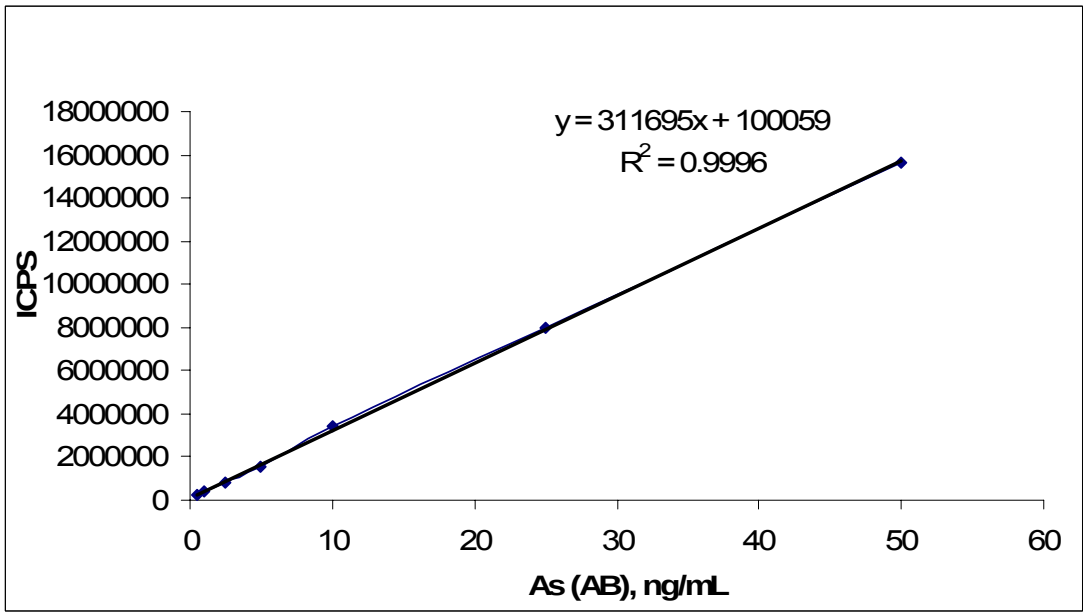

Figure 3.43 Linear calibration plot of AB (V) obtained using CX-HPLC-ICP-MS.

For the LOD and LOQ calculation, a mixed standard solution containing 2.0 ng/mL of As(V), As(III), DMA(V), MA(V) and 1.0 ng/mL of AB was injected to system 5 times. Peak areas were used in all calculations (Figure 3.44).

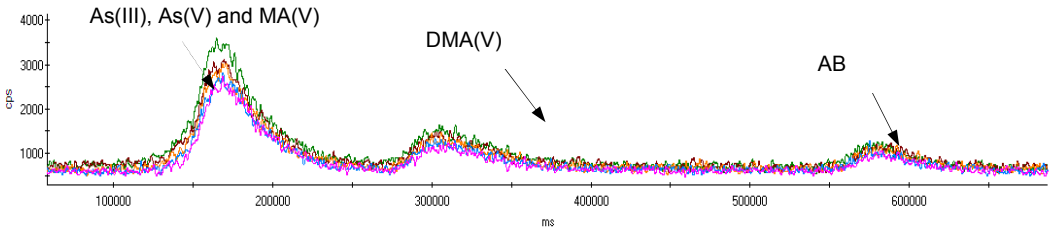

Figure 3.44 Replicate signals of 2.0 ng/mL of mix standard solution containing 2.0 ng/mL As(III), As(V), MA(V), DAM(V) and 1.0 ng/mL of AB injected to Cation Exchange HPLC-ICP-MS system using parameters given in Table 3.6, CX-MP5.

In LOD calculation 3s/m and in LOQ calculation 10s/m were used. LOD and LOQ calculations standard deviations of 5.0 ng/mL As was used. Standard deviations were obtained using the areas of signals (ICPS) and slope of the given graphs for DMA(V) and AB were used. LOD and LOQ results of DMA (V) and AB can be seen in Table 3.7.

Table 3.7 LOD and LOQ results of AB, DMA (V) and in CX-HPLC-ICP-MS system using mixed standard solution.

	AB	DMA(V)
Limit of Detection, LOD, ng/mL	0.20	0.33
Limit of Quantitation, LOQ, ng/mL	0.58	1.11
Dynamic range, ng/mL	0.5-100	1-100

As seen in Table 3.7, LOD and LOQ values for As(AB) and As(DMA^V) are sufficiently low to make quantitative measurements.

3.2.4.4 Column Recovery Study

Recovery of arsenic species from the column was investigated. For this aim, As mix standard solution (containing AB, As(III), As(V), DMA(V and MA(V)) was injected to HPLC-ICP-MS under the optimum conditions given in Table 3.6. After the column, eluent was collected throughout the elution. The same experiment was also done without column. As mix standard solution (containing AB, As(III), As(V), DMA(V and MA(V)) was also injected to HPLC using the same loop, and eluent was collected

before the column. Both solution with and without column were injected to ICP-MS, and it was observed that there is no difference in the results obtained from with and without column experiments (lower than 2%, N=2). This proves that the column recovery is about 99% and this chromatographic method is proper for quantitative determination of AB, DMA(V) and MA(V).

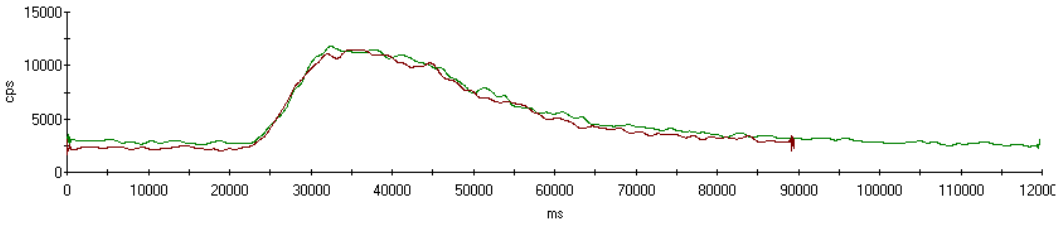

Figure 3.45 Recovery test for cation exchange column. Green 100.0 ng/ mL As (mix) standard solution with cation exchange column.Dark red 100.0 ng/mL As (mix) standard solution without column. Signals were obtained with ICP-MS.

3.2.5 Speciation of As in Fish Samples

Collected fish samples from markets originating from Black Sea, Kızılırmak River and Balıkesir were analyzed (see Table 2.3) for the determination of total As. Names of the fish samples were given in Table 2.3. Sample pretreatment steps such as washing the fish, tissue removal, blending, freeze drying and grinding were explained in experimental part of this study. After sample pretreatment, 0.20 g of fish muscle is weighed into PTFE vessels with their parallels. Two parallel samples were prepared from each fish sample and acid digested using the microwave acid digestion program given in Chapter 2 Table 2.2 after obtaining a clear solution of the samples; 3 point-standard addition method was applied to the samples. In the standard addition method, As(V) was used because all the arsenic species should be converted to highest oxidation state after HNO_3 digestion procedure. For each sample 2 parallel standard additions were applied. The reason for standard addition method was that the slope of standard addition method was different from slope of direct calibration plot during the analysis. Hence, standard addition method had to be applied to samples to eliminate interferences coming from matrix. Actually matrix interference originates from

acidity difference between acid digested samples and standard solutions. Samples acidity was 6.0 M (HNO_3/H_2O_2) after acid digestion. On the other hand standard solutions were prepared in 1.0 M HNO_3. Since the suppression of nitric acid on As signal increase as the concentration of acid increase, standard addition method was applied to eliminate this effect.

Extraction of arsenic species from fish samples were done using the Extraction Method 2 described before. Two sample replicates were used for each fish sample. Extraction solutions of fish samples were injected to Cation Exchange-HPLC-ICP-MS system with CX-MP5 for the determination of AB, MA(V) and DMA(V); $^{75}As^+$, $^{77}ArCl^+$, $^{82}Se^+$, $^{78}Se^+$ and $^{83}Kr^+$ were monitored throughout the study.

3.2.5.1 Gray Mullet / Kefal

For the total As determination, Gray mullet sample was prepared according to the procedure mentioned in Part 3.2.5. After addition of the standards to the samples, each sample was injected to the ICP-MS by flow injection system for the total analysis of As. The loop volume used in the flow injection system was 1.0 mL. Non smoothed peak area was used in all calculations.

In order to make speciation analysis of arsenobetaine in the Gray Mullet, extraction solution of gray mullet with 3 point standard addition after extraction were injected to CX-HPLC-ICP-MS system with CX-MP5

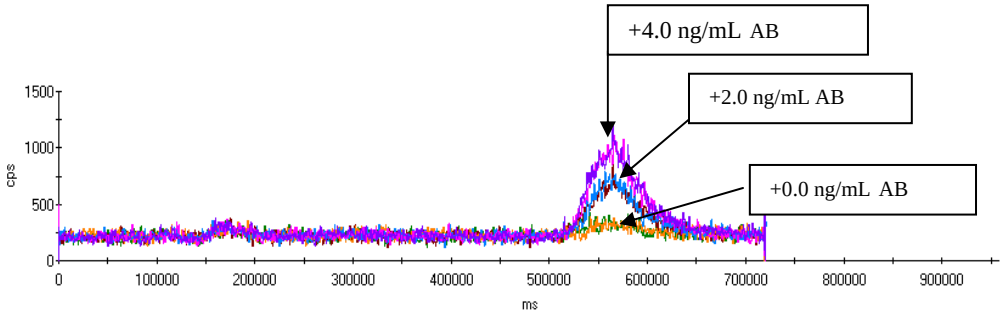

Figure 3.46 HPLC-ICP-MS chromatograms of 2 replicates of Gray Mullet using parameters in Table 3.6, CX-MP5.

As seen in the figure, retention times of all signals are exactly the same and

the peaks are sharp and symmetric. There is no other signal observed in the chromatograms except the Arsenobetaine (AB) peak. The peak like shape at 150th s is due to background movement when the extracted solution reached to the plasma. No smoothed peak area was used in the calculations of AB content. Chromatograms of gray Mullet with 3 point standard addition after extraction are shown in Figure 3.46.

3.2.5.2 Red Mullet / Barbunya

For the total As determination in Red Mullet, Gray the was prepared according to the procedure mentioned in Part 3.2.5. After addition of the standards to the digested fish sample, each sample was injected to the ICP-MS by flow injection system for the total analysis of As. The loop volume used in the flow injection system was 1.0 mL. Non smoothed peak area was used in all calculations.

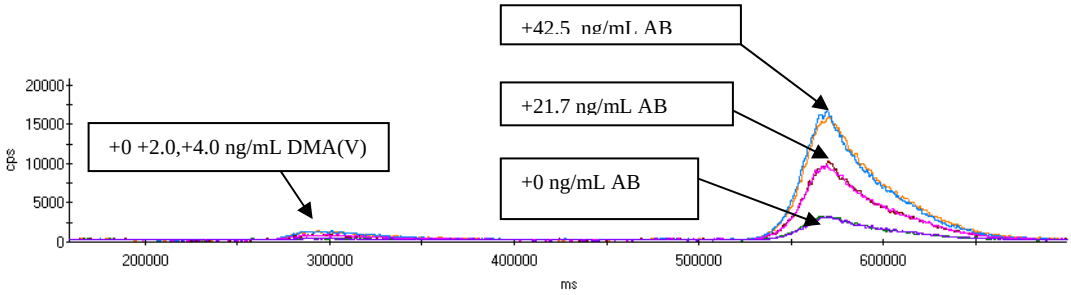

Figure 3.47 CX-HPLC-ICP-MS chromatograms of +0 Red Mullet, +21.7 ng/mL of AB and +2.0 ng/mL of DMA(V) added Red Mullet and also +42.5 ng/mL of AB and +4.0 ng/mL of DMA(V) added Red Mullet using the parameters given in Table 3.15, CX-MP5.

For the investigation of AB and DMA(V) concentrations in red mullet, 3 point standard addition of AB and DMA(V) in extracted red mullet solution was prepared. The solution prepared so as to contain 2 ng/mL DMA(V) and 21.7 ng/mL AB, 4 ng/mL DMA(V) and 42.5 ng/mL AB. After preparation of the standard added solutions, each sample was injected to CX-HPLC-ICP-MS

system with CX-MP5 according to the conditions in Table 3.6. No smooth peak area used in calculations. 2 replicate samples with 2 parallel standard additions. As it can be seen in the chromatogram AB and DMA(V) peaks shape and retention times are good enough to make quantitative standard addition measurement.

3.2.5.3 Salmon / Somon

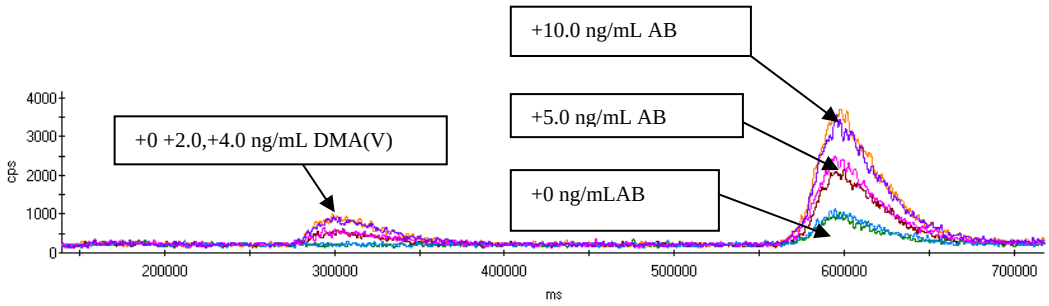

Figure 3.48 CX-HPLC-ICP-MS chromatograms of +0 Salmon, +5.0 ng/mL of AB and +2.0 ng/mL DMA(V) added Salmon and also +42.5 ng/mL of AB and +4.0 ng/mL DMA(V) added Salmon using the parameters given in Table 3.6, CX-MP5.

Salmon is a fatty fish so the etraction of fish tissue of Salmon is rather dificult. And in literature generally fatty parts are removed by organic solvents after methanole extraction. In this study fatty parts were not removed, only water extaction was aplied. This may result in lower recovery results of AB in Salmon. In the chromatogram, Figure 3.48 AB and DMA(V) added extraction solution was indjected to the system. The retention times of the analyes did not change and quantitative masurement can be done for both specie. However DMA(V) was not detected in Salmon.

3.2.5.4 Carp Fish / Sazan

Carp fish was obtained from *Kızılırmak* river. Kızılırmak river is important because water supply for Ankara provided from there. And it was reported that As content in water from Kızılırmak exceeds the allowed value by both WHO and USEPA which is 10 ng/mL As per day.

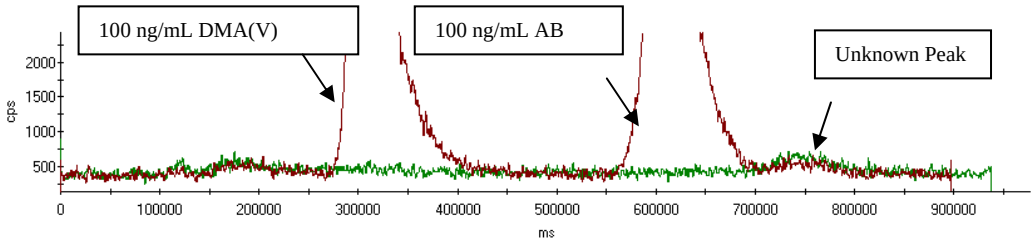

Figure 3.49 HPLC-ICP-MS chromatogram of Carp fish using parameters in Table 3.15, CX-MP5.

When the extacted carp fish solution was injected to the sstem, no peak was observed until 750th s. The retention time of this small pak was not consistent with either AB or DMA(V). At the same time, a mix standard that contains both 100ng/mL of AB and DMA(V) standard solution was injected to the system. When two chromatograms brought together as it is seen in Figure 3.49, the peak observed was neither AB nor DMA(V) but an unknown peak. This may due to the act that Carp Fish was not a sea water fish but river fish. And the resultted peak may be arsenosugar or TMAO which is common in freshwater fish samples. Since there have been only the standard solutions of MA(V), DMA(V) and AB, it was not decided that which specie is the unknown peak.

3.2.5.5 Whiting Fish / Mezgit

For the investigation of AB and DMA(V) concentrations in Whiting Fish, 3 point-standard addition of AB and DMA(V) in extracted whiting fish solution was applied. The solutions were prepared so as to contain 2 ng/mL DMA(V) and 21.7 ng/mL AB, 4 ng/mL DMA(V) and 42.5 ng/mL AB. After preparation of the standard added solutions, each sample was injected to CX-HPLC-ICP-MS system with CX-MP5 according to the conditions in Table 3.6. No smoothing was used for peak area calculations; replicate samples with 2 parallel standard additions were tested during the analysis.

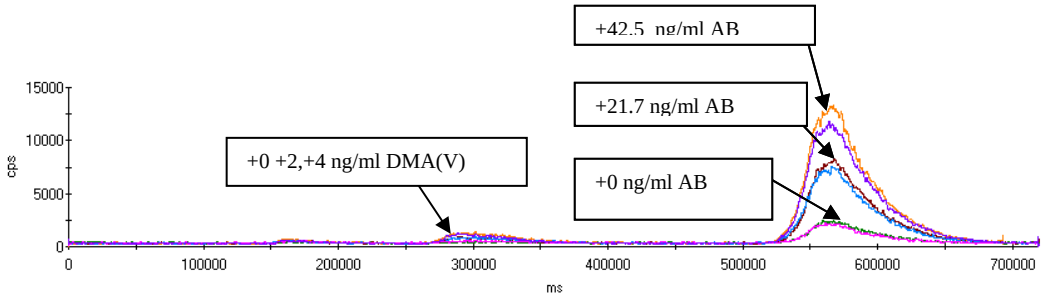

Figure 3.50 CX-HPLC-ICP-MS chromatograms of +0 Whiting Fish, +5 ng/mL of As(AB) and +2 ng/mL DMA(V) added Whiting Fish and also +42.5 ng/mL of As(AB) and +4 ng/mL DMA(V) added Whiting Fish using the parameters given in Table 3.15, CX-MP5.

As it can be seen in Figure 3.50, AB peaks shape and retention times are good enough to make quantitative standard addition measurement. No DMA(V) signal was detected.

3.2.5.6 Anchovy / Hamsi

Anchovy Fish was obtained from Balıkesir for another study where boron and arsenic contents were determined. After 2 replicate-extraction of the anchovy fish tissues according to the Extraction Method 2. Then DMA(V) and AB mix solution was added to this extracts. For each standard addition calibration line point, average values of 2 replicates were used. After preparation of the standard added solutions, each sample was injected to CX-HPLC-ICP-MS system with CX-MP5 according to the conditions in Table 3.6. No smoothed peak area was used in calculations.

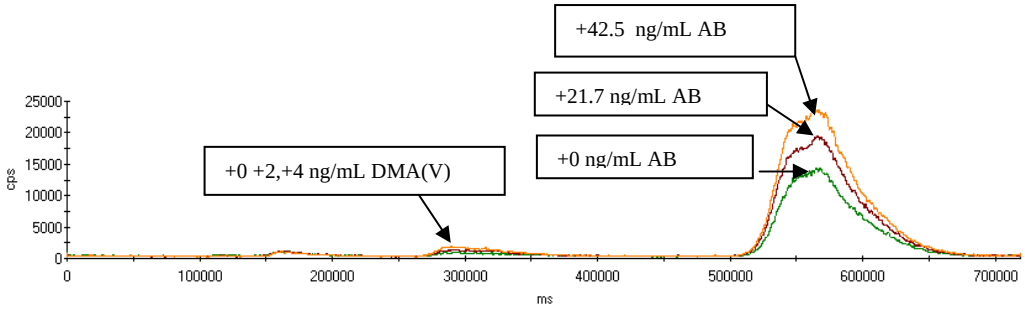

Figure 3.51 CX-HPLC-ICP-MS chromatograms of +0 Anchovy, +5 ng/mL of AB and +2 ng/mL DMA(V) added Anchovy and also +42.5 ng/mL of AB and +4 ng/mL As(DMA^{V}) added Anchovy using the parameters given in Table 3.15, CX-MP5.

As it can be seen in Figure 3.51, AB peaks are broader than usual. This may be high AB content of Achovy. Like in the other fish species, no signals for DMA(V) could be detected.

3.2.5.7 CRM DOLT-4

In this study to validate the AB content of the fish samples, CRM DOLT-4 was used as it contains remarkable amounts of As and AB (uncertified value). It is important for speciation analysis that CRM contains sufficient amounts of the analyte for convenient detectability even by systems of low sensitivity.

DOLT-4 (Dogfish Liver Certified Reference Material for Trace Metals) was prepared from dogfish by National Council of Canada, Institute for National Measurement Standards. According to the report "Cold vapor atomic absorption spectrometry, inductively coupled plasma mass spectrometry, electrothermal vaporization atomic absorption spectrometry, isotope dilution gas chromatography mass spectrometry, hydride generation atomic absorption spectrometry" are some of the methods that have been used in the certification of this CRM [100]. Certified values of the elements for DOLT-4 are given in Table 3.8

Table 3.8 Information about CRM- DOLT-4 [100]

	Certified Values, mg/kg
Element	DOLT-4
Aluminum	
Arsenic	9.66 ± 0.62
Cadmium	24.3 ± 0.8
Cobalt	
Chromium	
Copper	31.2 ± 1.1
Iron	1833 ± 75
Lead	0.16 ± 0.04
Mangnese	
Mercury	2.58 ± 0.22
Nickel	0.97 ± 0.11
Selenium	8.3 ± 1.3
Silver	0.93 ± 0.07
Thallium	
Tin	
Zinc	116 ± 6
Methylmercury (as Hg)	1.33 ± 0.12
Arsenobetaine (as As)	4.29 ± 0.38 (uncertified results by HPLC-ICP-MS method)
Tetramethylarsonium (as As)	

For the validity of the results, CRM-DOLT 4 was used. Firstly, the CRM sample was acid digested to validate total arsenic concentration. Moreover, 2 replicate samples were extracted according to Extraction Method 2. After preparation of the standard added solutions, each sample was injected to CX-HPLC-ICP-MS system with CX-MP5 according to the conditions in Table 3.6. No smoothed peak area was used in calculations. The obtained chromatogram can be seen in Figure 3.52

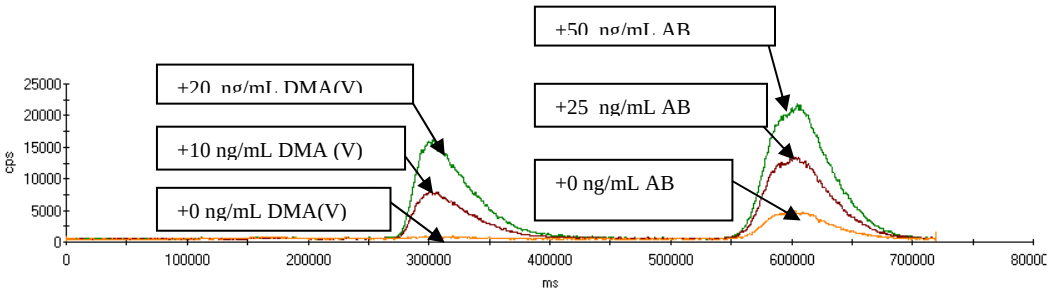

Figure 3.52 CX-HPLC-ICP-MS chromatograms of +0 DOLT-4, +25 ng/mL of AB and +10 ng/mL DMA(V) added DOLT-4 and also +50 ng/mL of AB and +20 ng/mL As(DMA^V) added DOLT-4 using the parameters given in Table 3.15, CX-MP5.

3.2.5.8 NIST RM-50

Reference Material NIST-RM 50 Tuna Fish was used in both total As concentration part to validate the results and and also in extraction method development part to find out most efficient extraction method. Firstly, the RM sample was acid digested to validate total arsenic concentration. Moreover, 2 replicate samples were extracted during all extraction method development steps. In the speciation part, it was re-extracted according to the extraction method 2 before injection to the CX-HPLC-ICP-MS system. After preparation of the standard added solutions, each sample was injected to CX-HPLC-ICP-MS system with CX-MP5 according to the conditions in Table 3.6. No smoothed peak area was used in calculations. The obtained chromatogram can be seen in Figure 3.53.

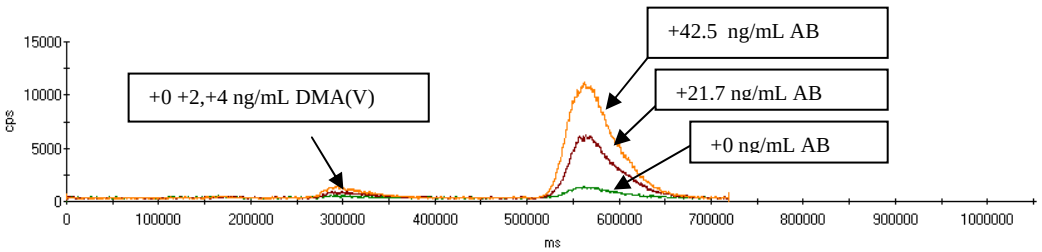

Figure 3.53 CX-HPLC-ICP-MS chromatograms of +0 NIST-RM 50, +25 ng/mL of AB and +10 ng/mL DMA(V) added NIST-RM 50 and also +50 ng/mL of AB and +20 ng/mL DMA(V) added NIST-RM 50 using the parameters given in Table 3.15, CX-MP5.

As it can be seen from Figure 3.53 AB retention times and peak shapes are appropriate enough to make quantitative analysis. Although the DMA(V)

peaks are not clear, NIST RM contains trace amounts of DMA(V) and it was barely detectable.

For the fish samples and reference materials the results are shown in Table 3.9, 3.10 and 3.11 respectively

3.2.5.9 Concentration of MA(V), DMA(V) and AB in Fish Samples

Table 3.9 Analytical Results for Fish Samples

Sample	As found in fish sample, Mean ± S.D., ng/g (dry mass)					
	Total As	Total As(AB) after Extraction	MA(V)	DMA(V)	Arsenobetaine (AB)	Extraction Efficiency* (for AB (%)
Red Mullet	2914 ± 298	2500 ± 152	N.D.	N.D.	2035±61	70
Salmon	1154 ± 120	861±39	N.D.	N.D.	855± 36	74
Whiting	2382 ± 83	2142±128	N.D.	N.D.	1986±72	83
Anchovy	12585± 599	-	N.D.	N.D.	11991± 512	95
Gray Mullet	1430± 154	442 ± 41	N.D.	N.D.	433± 39	30
Crap	914 ± 31	-	N.D.	N.D.	N.D.	-

*In this study extraction efficiency was calculated as a ratio of obtained AB concentration after injection to CX-HPLC-ICP-MS using CX-MP5 to total As concentration of each fish samples after acid digestion.

Extraction efficiency which is calculated as AB concentration obtained after CX-HPLC-MS study/ Total As concentraion after acid digestionx100 was found out to be different for each fish sample. Maximum extraction efficiency was found for Anchovy. The possible reason fort the different extraction efficiencies of each fish sample may due to matrix difference. Fish muscle structure in terms of fattiness, water solubility can be different for each fish sample. For example Salmon muscle is far fattier than the other fish samples.

In addition, since our extraction solvent was water, arsenolipids and other arsenic species might not extracted through water.

For the fish samples in this study, DMA(V) was not detected. Moreover the possibility to contain inorganic As species is relatively low as the column recovery was found out to be 99%in the system used for speciation.

3.2.5.10 Concentration of Total As and AB in CRM DOLT-4 and NIST RM-50

CRM DOLT-4 was certificated for both total As concentration and certification of AB study peformed in the PhD thesis of Dr. Bakırdere in Natural research Center, Canada, Ottowa and the unpublished result was found out to be 4.10 ± 0.20 mg/kg AB (concentratios were calculated as As in AB). And the result found in this study was in good agreement with that one which was 4.29 ± 0.38 mg/kg. DMA(V) was not detected in DOLT-4. Extraction efficiency which is calculated as AB concentration obtained after CX-HPLC-MS study/ Total As concentraion after acid digestionx100 was foud out to be 44.8%. this result may due to matrix of the sample. Fish liver matrix is different than fish muscle matrix. Moreover speciation analysis of arsenolipids, arsenosugars and other organic arsenic species like TMAO was not performed in the study. Since the column recovery was found out to be 99%. Possibility of CRM DOLT-4 to contain inorganic arsenic species is low.

Table 3.10 Analytical Results for CRM DOLT- 4

Sample	Certified Results (mg/kg)		Experimental Results (mg/kg)	
	Total As	Arsenobetaine (AB)	Total As	Arsenobetaine (AB)
DOLT-4	9.66 ± 0.62	4.29 ± 0.38	9.14 ± 0.64	4.10 ± 0.20

Referenced value for NIST- RM 50 was only total As concentrion one. So this RM used in all optimization parts of this study. Moreover total As concentration found experimentally was in good agreement with the referenced one. Beside the total As concentration, NIST RM -50 Albacore Tuna analyzed for the AB and DMA(V) content. The results are shown in Table 3.11.

Table 3.11 Analytical Results for NIST RM 50.

Sample	Certified Results (mg/kg)	Experimental Results (mg/kg)		
	Total As	Total As	Arsenobetaine (AB)	DMA (V)
NIST-RM 50	3.30 ± 0.70	3.35 ± 0.25	2.64 ± 0.08	0.4 ± 0.02

Extraction efficiency for NIST- RM 50 was found out to be 90%. Major As specie in this RM was found out to be AB. DMA(V) content of the RM was not high. However it can be concluded that NIST RM 50 does not contain inorganic arsenic species since the column recovery was found out to be 100%.

CHAPTER 4

CONCLUSION

4.1 Evaluation of the Results

Interference study conducted in this thesis has proven that software correction for the $^{77}ArCl^{+}$ does works. Moreover for the suppression effect of easily ionized elements and space charge effect of Cs was observed and hence for matrices containing this type of elements should be spiked or standard addition method should be applied for As which was perfomed in this study.

Arsenobetain is the most abundant specie of arsenic found in marine animals [101]. In this study it is found out that main water extractable arsenic species was AB which is consistent with literature. Almost in all fish samples AB found was between 70% and 95% of the total arsenic content. And the main arsenic component of the tissue was As(AB). DMA(V) was found in only NIST RM 50 in tuna fish. In the fish samples used in this study no inorganic As species observed. Column recovery test was done to prove this fact. If any inorganic As species retained in the column then the column recovery would not be 99% However arsenolipids which are not water soluble could not be detected in this study and this may be reason for low extraction efficiency of some fish samples. Moreover speciation of other organic As species was not perfomed. These may be reasons for low extraction efficiency results for some of the samples. For extraction of As species in fish samples methanol–water may be used for further extraction of other As species like MA ,TMAO and AC.

4.2 Future Studies

For future study, fish samples from one reagon like Black Sea or Kızılırmak should be collected to evaluate arsenic problem of that area. Mass, length and other properties of the fish samples will be well noted. For extraction method development different solvents like methanol, hexane and water or their mixtures may be used to extract all As species besides AB. Automated extraction devices is easy to use for large number of samples and for their replicates. For the speciation analysis part HPLC-ES-MS may be useful for further information about speciation study besides HPLC-ICP-MS.

REFERENCES

[1] ATSDR(Agency for Toxic Substances and Disease Registry), U.S. Department of Healthand Human Services, Case Studies in Environmental Medicine, http://www.atsdr.cdc.gov Retrevied 05. 06. 2010.

[2]http://en.wikipedia.org/wiki/Abundance_of_elements_in_Earth's_crust Retrieved 13/07/2010.

[3] Smedly P.L., Kinniburg D.G., A review of the source, behaviour and distribution of arsenic in natural water, *Appl. Geochem*., 2002, **17**, 517-568.

[4] Christen K., "Chickens, manure, and arsenic", *Environ. Sci. Technol*., 2001, **35**, 184A.

[5] Cullen W. R., Reimer K. J., Arsenic speciation in the environment, *Chem Rev.,* 1989, **89**, 713– 764.

[6] Mandal B.K., Suzuki K.T., Arsenic round the world: A review, *Talanta.,* 2002, **58**, 201-235.

[7] http://www.inchem.org/documents/ehc/ehc/ehc018.htm. Retrevied 08. 06. 2010.

[8] Davidson C.I., Goold W.D., Mathison T.P., Wiersma G.B., Brown K.W., Reilly M.T., Airborne trace elements in Great Smoky Mountains, Olympic and Glacier National Parks, *Environ. Sci. Technol.,* 1985, **19,** 27-35.

[9] Le C.X., (2002) *Environmental chemistry of arsenic* (ed WT Frankenberg), , Dekker, New York, 95-106.

[10] Kramer J.R., Allen H.E., *Metal Speciation: theory, analysis, and application*, 1988, Lewis Publishers, Chelsea, MI.

[11] Edmonds J.S., Francesconi K.A., Arsenic in seafoods: human health aspects and regulations *Marine Poll. Bull.*, 1993, **26**, 665-674.

[12] Food Standards Agency (2004). 1999 Total Diet Study: Total and inorganic arsenic in food. *Food Surveillance Information Sheet* **51/04**.

[13] Bentley R., Chasteen T.G., Microbial Methylation of Metalloids: Arsenic, Antimony and Bismuth, *Microbiol. Mol. Biol. Rev.,* 2002, **66**, 250-271.

[14] Lu X., Arnold L., Cohen S. M., Cullen W.R., Le X.C., Speciation of Dimethylarsinous Acid and Trimethylarsine Oxide in Urine from Rats Fed with

Dimethylarsinic Acid and Dimercaptopropane Sulfonate, *Anal Chem, 2003,* **75**, 6463-6468.

[15] Challenger F., Biological methylation, *Chem Rev*, 1945, **36**, 315-361.

[16] Cullen W.R., McBride B.C., Manji H., Pickett A.W., Reglinski, J. The metabolism of methylarsine oxide and sulfide. *Appl Organomet Chem.*, 1989, **3**, 71-78.

[17] Cullen, W.R., McBride B.C., Reglinski J.,The reaction of methylarsenicals with thiols: Some biological implications, *J Inorg Biochem.,* 1984, **21**, 179-194.

[18] Aposhian H.V., Gurzau E.S., Le X. C., Gurzau A., Healy S.M., Lu X., Ma M., Yip L., Zakharyan R.A., Maiorino R. M., Dart R. C., Tirus M. G., Gonzalez-Ramirez D., Morgan D. L., Avram D., Aposhian M. M., Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic, *Chem Res Toxicol.,* 2000, **13**, 693-697.

[19] Sampayo-Reyes A., Zakharyan R.A., Healy S.M., Aposhian H.V., Monomethylarsonic acid reductase and monomethylarsonous acid in hamster tissue,*Chem. Res. Toxicol.,* 2000, **13**, 1181-1186.

[20] Le X.C., Lu X., Ma M., Cullen W.R., Aposhian H.V., Zheng B., Speciation of key arsenic metabolic intermediates in human urine, *Anal Chem*., 2000, **72,** 5172–5177.

[21] Del Razo L. M., Styblo M., Cullen W.R., Thomas D.J., Determination of Trivalent Methylated Arsenicals in Biological Matrices. *Toxicol Appl Pharmacol*., 2001, **174**, 282-293.

[22] Styblo M., Thomas D. J., Binding of arsenicals to proteins in an *in vitro*methylation system, *Toxicol Appl Pharmaco*l., 1997, **147,** 1–8.

[23] Lin S., Del Razo L. M., Styblo M., Wang C., Cullen W. R., Thomas, D.J., Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. *Chem Res Toxicol,* 2001, **14**, 305–311.

[24] Petrick J.S., Ayala-Fierro F., Cullen W.R., Carter D.E., Aposhian H.V., Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. *Toxicol Appl Pharmacol.,* 2000, **163**, 203–207.

[25] Mass M.J., Tennant A., Roop B.C., Cullen W.R., Styblo M., Thomas D.J., Kligerman A.D., Methylated trivalent arsenic species are genotoxic, *Chem Res*

Toxicol., 2001, **14**, 355–361.

[26] Andrews P., Kitchin K.T., Wallace K., Dimethylarsine and Trimethylarsine are potent Genotoxins In Vitro, *Chem Res Toxicol.,* 2003, **16**, 994-1003

[27] Styblo M., Drobna Z., Jaspers I., Lin S., Thomas D.J., The Role of Biomethylation in Toxicity and Carcinogenicity of Arsenic: A Research Update, *Environ Health Perspect.,* 2002, **110**, 767-771.

[28] Hughes M.F., Arsenic toxicity and potential mechanisms of action, *Toxicol Lett.,* 2002, **133**, 1-16.

[29] Hayakawa T., Kobayashi Y., Hirano X.C.S., A new metabolic pathway ofarsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19., *Arch Toxicol.,* 2005, **79**, 181-191.

[30] Styblo M., Del Razo L.M., Vega L., Germolec D.R., LeCluyse E.L., Hamilton G.A., Wang C., Cullen W.R., Thomas D.J., *Arch. Toxicol.,* 2000, **74,** 289-299.

[31] Dopp E., Hartmann L.M., Florea A.M., Recklinghausen U., Pieper R., Rettenmeier A.W., Hirner A.V., Obe G., *Toxicol. Appl. Pharmacol.,* 2004, **201**, 156-165.

[32] Hirano S., Kobayashi Y., Cui X., Kanno S., Hayakawa T., Shraim A., The accumulation and toxicity of methylated arsenicals in endothelial cells: important roles of thiol compounds. *Toxicol Appl Pharmacol.,* 2004, **198**, 458-467.

[33] Aposhian H.V., Aposhian M.M., Arsenic Toxicology: Five questions, *Chem. Res. Toxicol.,* 2006, **19**, 1-15.

[34] Schuliga M., Chouchane S., Snow E.T., Upregulation of glutathione-related genes and enzyme activities in cultured human cells by sublethal concentrations of inorganic arsenic, *Toxicol. Sci.,* 2002, **70**, 183-192

[35] Spuches A.M., Kruszna H.G., Rich A.M., Wilcox D.E., Thermodynamics of the As(III)-thiolinteractions: arsenite and monomethylarsenite complexes with glutathione,dihydrolipoic acid, an dothe rthiol ligands, *Inorg Chem.,* 2005, **44**, 2964–2972

[36] Naranmandura H., Suzuki N., Suzuki K.T., Trivalent arsenicals are bound to proteins during reductive methylation, *Chem. Res. Toxicol.,* 2006, **19**, 1010–1018.

[37] Roy P., Saha A., Metabolism and toxicity of arsenic: A human carcinogen, *Curr.Sci.,* 2002, **82**, 38-45.
[38] DEFRA, Soil guideline values for arsenic contamination, (2002), Department for Environment Food and Rural Affair, UK
[39] EC, Canadian Soil Quality Guidelines for Arsenic, (1999), Scientific Supporting Document, National Guidelines and Standards Office, Environmental Canada, Ottawa.
[40] http://universe-review.ca/R10-33-anatomy.htm&usg Retrieved 1/08/2010
[41] Iger Y., Lock R.A.C., Van der meij J.C.A., Wendelaar-Bonga S.E., Effects of water-borne cadmium on the skin of common carp (*Cyprinus carpio*). *Arch. Environ. Con. Tox*., 1994, **26**, 342-350.
[42]http://www.ehu.es/europeanclass2003/biological_aspects_of_metal_accu .htm Retrieved 22/08/2010
[43] Pedlar R.M., Ptashynski M.D., Wautier K.G., Evans R.E., Baron C.L., Klaverkamp J.F., The accumulation, distribution, and toxicological effects of dietary arsenic exposure in lake whitefish (*Coregonus clupeaformis*) and lake trout (*Salvelinus namaycush*), *Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol,* 2002, **131**, 73-91.
[44] George S.G., Young P., The time course of effects of cadmium and 3-methylcholanthrene on activities of enzymes of xenobiotic metabolism and metallothionein levels in plaice, *Pleuronectes platessa*, *CompPharmacol. Toxicol. Endocrinol.,* 1986, **83**, 37-44.
[45] Lauren D.J., McDonald D.G., Acclimation to copper by rainbow trout, *Salmo gairdneri* physiology, *Can. J. Fish. Aquat. Sci.,* 1987, **44**, 105-111.
[46] Francesconi K.A., Edmonds J.S., Arsenic Species in Marine Samples* *Croactica. Chemica. Acta.,* 1998, **71**, 343-359.
[47] Schoof R.A., Yost L.J., Eickhoff J., Crecelius E.A., Cragin D.W., Meacher D.M., Menzel D.B., A market survey of inorganic arsenic in food, *Food. Chem. Toxicol*., 1999, **37**, 839-846.
[48] Sloth J.J., Larsen E.H., Julshamn K., Survey of inorganic arsenic in marine animals and marine certified reference materials by anion exchange high-performance liquid chromatography—inductively coupled plasma mass spectrometry, J. Agric. Food Chem., 2005, **53**, 6011–6018.

[49] Arbouine M.W., Wilson H.K., The effect of seafood consumption on the assessment of occupational exposure to arsenic by urinary arsenic speciation measurements, *J. Trace Elem. Electrolytes Health Dis*.,1992, **6**, 153–160.

[50] Heinrich-Ramm R., Mindt-Prufert S., Szadkowski D., Arsenic species excretion after controlled seafood consumption, *J. Chromatogr B.,*2002, 263–273.

[51] Branch S., Ebdon L., O'Neill P., Determination of arsenic species in W*sh by directly coupled high-performance liquid chromatographyinductively coupled plasma mass spectrometry. J. Anal. Atom Spectrom., 1994, **9** , 33–37.*

[52] Brown R.M., Newton D., Pickword C.J, Sherlockl J.C., Human metabolism of arsenobetaine ingested with fish, *Hum. Exp. Toxicol.*,1990, **9**, 41–46.

[53] Sakurai T., Kojima C., Ochiai M., Ohta T., Fujiwara K., Evaluation of *in vivo* acute immunotoxicity of a major organic arsenic compound arsenobetaine in seafood, Int. Immunopharmacol., 2004., **4**, 179–184.

[54] Francesconi K.A., Edmonds J.S., In: Nriagu J.O. (1994) Arsenic In The Environment. Part I: Cycling and Characterization, John Wiley, New York, , 221–261.

[55] Sakurai T., Kaise T., Ochi T., Saitoh T., Matsubara C., Study of in vitro cytotoxicity of a water soluble organic arsenic compound, arsenosugar, in seaweed, *Toxicology.,* 1997, **122**, 205-212.

[56] Andrewes P., Demarini D.M., Funasaka K., Wallace K., Lai Vivian W.M., Hongsui S.Cullen W.R., Kitchin K.T., Do arsenosugars pose a risk to human health? The comparative toxicities of a trivalent and pentavalent arsenosugar., *Environ. Sci. Technol.,* 2004, **38**, 4140–4148.

[57] Almela C., Algora S.,. Benito V, Clemente M. J., Devesa V., Suner M. A., Velez D., Montoro R., Heavy Metal, Total Arsenic, and Inorganic Arsenic Contents of Algae Food Products., *J. Agric. Food Chem.*, 2002, **50**, 918-923.

[58] Devesa V., Su´n˜er M.A, Algora S., Ve´lez D., Montoro R., Jalo´n M., Urieta I., Macho M.L., Organoarsenical species contents in cooked seafood, *J. Agric. Food Chem*., 2005, **53**, 8813-8819.

[59] Apostoli P., Bartoli D., Alessio L.,.Buchet J.P, Biological monitoring of occupational exposure to inorganic arsenic. *Occup. Environ. Med*, 1999, **56** , 825–832.

[60] Ng, J. C., Kratzmann S. Q., Crawley H., Chiswell B., Moore M. R., "Speciation and absolute bioavailability. Risk assessment of arsenic-contaminated sites in a residential suburb in Canberra.", *Analys,t* 1998, **123** 889-892.

[61] Templeton D.M., Ariese F., Cornelis R., Danielsson L.G, Muntau H., Leeuven H.P., Obinzki R.L., IUPAC Recommendations, *Pure Appl. Chem.,* 2000, **72**,1453-1470.

[62] Jain C.K., Ali I., Arsenic: occurrence, toxicity and speciation techniques, *Wat. Res.*,2000, **34**, 4304-4312.

[63] Wagner G., Klein R., Quevauviller P., (1995).Quality Assurance in Environmental Monitoring: Sampling and Sample Pretreatment, VCH, Weinheim, Germany,

[64] Maier A., Quevauviller P., Maier E.A., Griepink B., (1995) Quality Assurance for Environmental Analysis, Elsevier, Amsterdam, The Netherlands.

[65] Goessler W., Kuehnelt D., *Environmental Chemistry of As*, Dekker, New York , 2001, 27-50.

[66] Shibata Y., Morita M., Speciation of arsenic by reversed-phase high performance liquid chromatography–inductively coupled plasma mass spectrometr**y,** *Anal. Sci.*, 1989, **5**, 107-109.

[67] Edmonds J.S., Shibata Y., Prince R.I.T., Francesconi K.A., Morita M., *J. Mar. Biol. Assoc. UK*, 1994, **74**, 463-466.

[68] Dietz C., Sanz J.,.Sanz E, Munoz-Olivas R., Camara C., Current perspectives in analyte extraction Strategies for tin and arsenic speciation *J. Chromatogr. A.,* 2007, **1153**, 114-129.

[69] Naidu R., Smith J., McLaren R.G., Stevens D.P., Sumner M.E., Jackson P.E., *Soil. Sci. Soc. Am. J*. **64**, 122.-128

[70] Gomez-Ariza J.L., Sanchez-Rodas D., Beltran R., Giraldez I., *Int. J. Environ. Anal.Chem.*, 1999, **74**, 203-213.

[71] Kozak L., Niedzielski P., Szczucinski W., The methodology and results of determination of inorganic arsenic species in mobile fractions of tsunami

deposits by a hyphenated technique HPLC-HG-ASS, Int. *J. Environ. Anal. Chem*., 2008, **88**, 989–1003.

[72] Sur R., Dunemann L., Method for the determination of five toxicologically relevant arsenic species in human urine by liquid chromatography-hydride generation atomic absorption spectrometry, *J Chromatography (part B)* .2004, **807**, 169-176.

[73] Gomez M., Camara C., Palacios M.A., Lopez Gonzalvez A., *Fresenius J. Anal.Chem.* 1997, **357**, 844-849.

[74] Gomez-Ariza J. L., Sanchez-Rodas D., Giraldez I., *J. Anal. At. Spectrom*, 1998, **13**, 1375.

[75] Signes A., Burlo F., Casinos T., Carbonell-Barrachina A.A., Speciation of Arsenic in Licorice Confectionery Products and Estimation of Health Risks *Food Sci. and Technol Int.*, 2007, **13**, 41-47.

[76] http://www. minerals.cr.usgs.gov/icpms/What_is_ICPMS.pdf. Retreived 10.04.2010

[77] Meyers R.A., (2000) Encyclopedia of Analytical Chemistry. Volume 3: InductivelyCoupled Plasma Mass Spectrometry in Environmental Analysis (ed. Yamasaki, S.I.), JohnWiley & Sons Ltd., 2672-2692.

[78] McKiernan J.W., Creed J.T., Brockhov C.A., Caruso J.A., Lorenzana R.M., "A comparison of automated and traditional methods for the extraction of arsenicals from fish.", *J.Anal. At. Spectrom,* 1999, **14**, 607-613.

[79] Brisbin J.A., Caruso J.A., "Comparison of extraction procedures for the determination of arsenic and other elements in lobster tissue by inductively coupled plasma mass spectrometry.", *Analyst,* 2002, **127**, 921-929.

[80] Wang R.Y., Hsu Y.L., Chang L.F., Jiang S.J., "Speciation analysis of arsenic and selenium compounds in environmental and biological samples by ion chromatographyinductively coupled plasma dynamic reaction cell mass spectrometer.", Anal. Chim. Acta, 2007, **590**, 239-244.

[81] Karthikeyan S.H., Iyer, C. S. P. "Determination of arsenic species bymicrowave-assisted extraction followed by ion-pair chromatography-ICP-MS : analysis ofreference materials and fish tissues.", *Int. J. Environ. An. Ch*. 2004, **84**, 573-82.

[82] Foster S., Maher W., Krikowa F., Apte S., "A microwave-assisted sequential extraction of water and dilute acid soluble arsenic species from marine plant and animal tissues", *Talanta,* 2007, **71**, 537-549.

[83] Ronkart S.N., Laurent V., Carbonnelle P., Mabon N., Copin A., Barthélemy J.P., "Speciation of five arsenic species (arsenite, arsenate, MMAA, DMAA and AsBet) in different kind of water by HPLC-ICP-MS.", *Chemosphere*, 2007, **66**, 738-745.

[84] B'Hymer C., Caruso J.A., "Evaluation of HPLC Systems for the Separation andQuantification of Arsenic Compounds from Apple Extracts." *J. Liq. Chromatogr. Relat.Technol*., 2002, **25**, 639-653.

[85] Rodriguez I.B., Raber G., Goessler W., "Arsenic speciation in fish sauce samples determined by HPLC coupled to inductively coupled plasma mass spectrometry", *Food. Chem*., 2008, **16**, 49-54.

[86] Sanz E., Munoz-Olivas R., Camara C., "Evaluation of a focused sonication probefor arsenic speciation in environmental and biological samples."*J. Chromatogr. A.,* 2005, **1097**, 1-8.

[87] Mandal B.K., Ogra Y., Suzuki K.T., Speciation of arsenic in human nail and hair from arsenic-affected area by HPLC-inductively coupled argon plasma mass spectrometry, *Toxicol. Appl. Pharmacol*., 2003, **189**, 73– 83.

[88] Kirby J., Maher W., Chariton A., Krikowa F., "Arsenic concentrations and speciation in a temperate mangrove ecosystem, NSW, Australia.", *Appl. Organometal. Chem., 2002,* **16,** 192**-**201.

[89] Vaughan M.A., Horlick G., Effect of sampler and skimmer orifice size on analyte and analyte oxide signals in inductively coupled plasma mass spectrometry *Spectrochim. Acta Part B*., 1990, **45**, 1289-1299.

[90] Hanselman D.S., Sesi N.N., Huang M., Hieftje G.M., The effect of sample matrix on electron density, elec-tron temperature and gas temperature in the argon inductively coupled plasma examined by Thomson and . Rayleigh scattering, *Spectrochim. Acta Part B*., 1994, **49**, 495-451.

[91] Olivares J.A.,. Houk R.S, Suppression of analyte signal by various concomitant salts in inductively coupled plasma mass spectrometry, *Anal. Chem*., 1986, **58**, 20-25.

[92] Beauchemin D., McLaren J.W., Berman S.S., Study of the effects of concomitant elements in inductively coupled plasma mass spectrometry, Spectrochim. *Acta Part B.,* 1987, **42**, 467-490.

[93] Gr´egoire D.C., The effect of easily ionizable concomitant elements on non-spectroscopic interferences in inductively coupled plasma-mass spectrometry, *Spectrochim. Acta Part B*., 1987, **42**, 893-903.

[94] Vandercasteele C., Nagels M., Vanhoe H., Dams R., Suppression of analyte signal in inductively-coupled plasmarmass spectrometry and the use of an internal standard, *Anal. Chim. Acta.,* 1988, **211**, 91-98.

[95] Farnsworth P.B., Woolley A., Omenetto N., Matveev O., Experimental studies of charge transfer reactions between argon and the third row metals calcium through copper in the inductively coupled plasmaSpectrochim Acta Part B., 1999, **54**, 2143.

[96] Gangqiang L., Yixiang D., Hieftjie G.M., Space-Charge Effects and Ion Distribution in Plasma Source Mass Spectrometry *J. Mass. Spectrom.,*1995, **30**, 841.

[97] Edmonds, Y. Shibata, R. Prince I. T., Francesconi K. A., Morita M., Arsenic compounds in tissues of the leatherback turtle, Dermochelys coriacea *J. Mar. Biol. Assoc. UK*, 1994, **74**, 463-466.

[98] Manson J.T., (1999), Sonochemistry, Oxford University Press, Oxford, UK

[99] Le X.C., Ma M., Wong N.A., Speciation of Arsenic Compounds Using High-Performance Liquid Chromatography at Elevated Temperature and Selective Hydride Generation Atomic Fluorescence Detection, *Anal. Che.,*1996, **68**, 4501-4506.

[100]National Research Council, Canada, http://www.nrc-cnrc.gc.ca/obj/inms-ienm/doc/crm-mrc/eng/DOLT-4_e.pdf. Retreived 10.06.2010

[101] Francesconi K.A., Edmonds J.S., "Arsenic and marine organisms", *Adv. Inorg.Chem*., 1997, **44**, 147-189.