DETERMINATION OF ARSENIC, SELENIUM AND CADMIUM IN SOME TURKISH SPICES BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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DETERMINATION OF ARSENIC, SELENIUM AND CADMIUM IN SOME TURKISH SPICES BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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ABSTRACT

DETERMINATION OF ARSENIC, SELENIUM AND CADMIUM IN SOME TURKISH SPICES BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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The major source of all the nutrient elements for human body is the daily diet which is affected by age, sex, and health status, geographic and climatic conditions. It is important to analyze food items in order to determine their elemental contents, estimate their dietary intakes and compare with recommended or tolerated intake values in order to limit or increase their consumption. Spices are taken from different parts of plants and widely used in cooking processes. They are convenient samples to investigate the distribution of elements among different parts of plants and to evaluate their daily intakes. Arsenic and selenium are both essential and toxic elements whereas cadmium is considered to be toxic to human health. The recommended dietary allowance (RDA) level of selenium ranges from 50 to 200 μg/day. The RDA level of arsenic is set to be 100-200 μg/day for adults to meet the requirements. The weekly tolerable intake of cadmium and arsenic are 7 and 15 μg/kg, respectively whereas selenium is toxic when intake is greater than 750 μg/day .

In this study the analysis of a variety of Anatolian spices including *daisy (Chamomillae Vulgaris),* bay leaf *(Folium Lauri)*, mint *(Folium Menthane)*, rosehip *(Rosae Caninae),* sage *(Folium Salviae Officinalis),* thyme *(Herba Thymi),* cumin *(Fructus Cummuni),* sumac *(Folium Rhois Coriariae),* linden flower *(Flos Tilliae*)

and black pepper *(Piper Nigrum)* were performed and As, Cd and Se levels were determined by using inductively coupled plasma mass spectrometry (ICP-MS). The results obtained were evaluated together with the results of previous studies for determination of Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Hg, Pb and Mn by inductively coupled plasma optical emission spectrometry (ICPOES) and atomic absorption spectrometry (AAS). Samples were digested in microwave oven in optimized concentrations of HNO_3 and H_2O_2. microwave temperature program was optimized to maximize digestion efficiency. Samples were analyzed by using direct calibration method for cadmium and standard addition method for arsenic and selenium considering the effect of HNO_3 concentration on ICP-MS signals. The accuracy of the methods was checked by using Oyster Tissue 1566b SRM for cadmium and arsenic and BCR Human Hair, 397 SRM for selenium. The mean arsenic and selenium levels were found to be in the range of 100-500 μg/ kg whereas cadmium levels were relatively lower falling in a range of 10-100 μg/ kg with few exceptions. In order to investigate the effects of spectral and nonspectral interferences on arsenic signals interference studies were performed by using HCl, NaCl, $NaNO_3$, CsCl, $CsNO_3$, LiCl and $LiNO_3$. Statistical evaluations were performed on data in order to detect on significant trends.

Keywords: ICP-MS, selenium, arsenic, cadmium, spices

ÖZ

BAZI TÜRKİYE BAHARAT ÖRNEKLERİNDE ENDÜKTİF EŞLEŞMİŞ PLAZMA KÜTLE SPEKTROMETRİ İLE ARSENİK, SELENYUM VE KADMİYUM TAYİNİ

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İnsan vücudu için faydalı çok sayıda elementin kaynağı günlük diyettir. Günlük diyet yaş, cinsiyet, sağlık durumu, iklim ve coğrafya koşullarına bağlı olarak farklılıklar gösterir. Gıda maddelerini analiz ederek, içerdikleri element derişimlerini tayin etmek, bu elementlerin günlük alım değerlerini belirlemek ve bulunan değerleri önerilen alım değerleriyle karşılaştırmak, gıda maddelerinin tüketimlerini arttırmak ya da sınırlandırmak açısından büyük önem taşır. Baharat bitkilerin çeşitli bölümlerinden elde edilir. Bu nedenle bitkilerin çeşitli bölümlerinin içerdiği elementleri tayin etmek için kullanılabilir. Arsenik ve selenyum insan sağlığı için gerekli ve/veya zehirli elementlerdir; ancak kadmiyum zehirlidir. Selenyumun önerilen günlük alım değeri (RDA) 50-200 µg'dır. Bu değer arsenik için yetişkinlerde 100-200 µg'dır. Arsenik ve kadmiyum için haftalık tolere edilebilen alım değerleri sırasıyla 7 ve 15 µg/kg iken selenyum günde 750 µg'dan fazla alındığında zehirlidir.

Bu çalışmada çeşitli Anadolu baharatları As, Cd ve Se tayini için ICP-MS yöntemiyle analiz edilmiştir. Analiz edilen baharatlar, *papatya (Chamomillae Vulgaris)*, defne yaprağı *(Folium Lauri),* nane *(Folium Menthane),* kuşburnu *(Rosae Caninae)*, adaçayı *(Folium Salviae Officinalis),* kekik *(Herba Thymi)* kimyon *(Fructus Cummuni),* sumak *(Folium Rhois Coriariae),* ıhlamur *(Flos Tilliae*) ve karabiber *(Piper Nigrum)*' dir. Sonuçlar daha önceki çalışmalarda ICPOES ve AAS

yöntemleriyle tayin edilen Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Hg, Pb ve Mn sonuçlarıyla birlikte değerlendirilmiştir. Örnekler, mikrodalga etüv içinde özütlenmiş, HNO_3 ve H_2O_2 derişimi ve sıcaklık programı optimize edilmiştir. Baharat örnekleri Cd tayini için doğrudan kalibrasyon, As ve Se tayini için standart katma yöntemi kullanılarak analiz edilmiştir. Çözme sırasında kullanılan HNO_3 ve H_2O_2 derişiminin sinyaller üzerine etkisi ve As tayini sırasında oluşabilecek spektral ve spektral olmayan girişimler araştırılmıştır. Kullanılan yöntemlerin doğruluğu Cd ve As için 1566b Oyster Tissue CRM; Se için 397 BCR Human Hair CRM kullanılarak irdelenmiştir. Analizler sonucunda baharat örneklerinde As ve Se derişimi 100- 500 µg/ kg olarak tayin edilirken, Cd derişimi göreceli olarak daha düşük olup 10-100 µg/kg olarak belirlenmiştir. Arsenik sinyalleri üzerine etki edebilecek spektral ve spektral olmayan girişimler HCl, NaCl, $NaNO_3$, CsCl, $CsNO_3$, LiCl ve $LiNO_3$ kullanılarak araştırılmıştır. Veriler üzerinde istatistiksel değerlendirme yapılarak önemli olabilecek özellikler incelenmiştir.

Anahtar Kelimeler: ICP-MS, arsenik, kadmiyum, selenyum, baharat

Dedicated to My Mother...

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X-Ray Fluorescence Spectrometry	XRF

CHAPTER 1

INTRODUCTION

There are two groups of elements needed in small amounts for human health. The *trace microelements* are those having concentrations lower than 100 mg/kg such as iron, copper and zinc. On the other hand the concentrations of *ultratrace microelements* are lower than 1 mg/kg. Chromium, manganese, fluorine, iodine, cobalt, selenium, silicon, arsenic, boron, vanadium, nickel, cadmium, lithium, lead and molybdenum are included in this second group [1, 2]. These elements may be divided into three groups as *essential*, *toxic* and *both essential and toxic*. For those referred as *essential* recommended dietary allowance (RDA) values have been listed [2]. Provisional tolerable daily or weekly intakes have been stated in order to prevent large exposures.

The major source of all the nutrient elements is daily diet which is affected by age, sex, and health status, geographic and climatic conditions. It is important to analyze food items to determine their elemental contents, estimate their dietary intakes and compare with recommended or tolerated intake values in order to limit or increase their consumption [3].

To make general discussions about trace elements and design a diet involving all essential nutrients in recommended amounts chemical and biological interactions

should be taken in consideration. Many of the trace minerals have more than one oxidation state. Living cells select the elements with respect to these states.

Spices are taken from different parts of plants and widely used in cooking processes. They are convenient samples to investigate the distribution of elements among different parts of plants and to evaluate their daily intakes [4].

The aim of this study is to perform the analysis of a variety of spices including *daisy (Chamomillae Vulgaris),* bay leaf *(Folium Lauri),* mint *(Folium Menthane),* rosehip *(Rosae Caninae),* sage *(Folium Salviae Officinalis),* thyme *(Herba Thymi),* cumin *(Fructus Cummuni),* sumac *(Folium Rhois Coriariae),* linden flower *(Flos Tilliae*) and black pepper *(Piper Nigrum)* and to determine As, Cd and Se levels by using inductively coupled plasma mass spectrometry (ICP-MS).

The results obtained are to be evaluated together with the results of previous studies for determination of Na, K, Mg, Ca, Li, Zn, Fe, Cu, B and Mn by inductively coupled plasma optical emission spectrometry (ICPOES) and atomic absorption spectrometry (AAS) as performed in our laboratory [5].

1.1 Spices

Spices are aromatic plant materials coming from woody shrubs, vines, aromatic lichens, parts of trees and the roots, seeds or fruits of herbaceous plants [6]. There have been many definitions for ‘spices’ over time which are often used interchangeably with ‘herbs’. Spices are defined as the aromatic parts of any plants that are used to add flavor to food. This definition includes herbs. But herbs can be defined separately. They are the aromatic leaves of any plant that can add flavor to

food. The origins of herbs are leaves of plants that have soft stems or are shrubs but not trees [7]. In general spices are defined as unleafy-dried substances whereas herbs are leafy-undried substances [8].

Spices have a long history. In ancient Egypt dried *mint leaves*, *onions* and *garlic* were commonly used in addition to *cardamon* and *cinnamon* traded from Ethiopia. Although ancient Greek and Roman herbs were used more often than spices, spices are still the central part of the dietary pattern of Mediterranean countries [9]. It is known that in India, where the traditional medicine evolved over 5000 years ago, herbs and spices are still used for health [10]. Traditionally Chinese integrate food nutrition and health by adding herbs and spices to specially prepared soups, dishes or beverages for health benefits [9].

In terms of its flora Turkey is one of the richest countries in Europe and Middle East due to its climate and geographical location. There are more than 9000 plant species about 3000 of which are endemic and 1000 are used as medicine and spice. Wild edible plants have been used as sources of food, flavoring agents, spices, pharmaceuticals and biological agents since ancient times [11, 12].

There are many possible explanations why spices should have an important place in dietary guidance systems. Spices add variety, flavor, color and aroma to everyday diets and increase the uptake of nutrients and bioactives necessary for health. They synergistically increase the health related properties of other foods. They also reduce the intake of fats and decrease their health risks. In order to increase the nutritional quality and attractiveness of daily diet specific attention should be given to spices regardless of their small serving sizes [8].

Botanically spices are part of the vegetable and fruit food group including bioactives and phytochemicals which have antioxidant capacity. In addition to antioxidation

capabilities some of these phytochemicals are associated with the reduction of cholesterol, low density lipoprotein (LDL) cholesterol whereas some others are known to decrease the risk of colon cancer [11]. Since using spices reduce the intake of salts and fats in diet they may also decrease cardiovascular risk. Certain spices are known to have anti-carcinogenic properties and have effects on the reduction of tumor risk.

In order to evaluate the convenience of foods in diets, metal levels of food samples can be used as nutritional guidance. Spices are used worldwide as complements for flavor in cooking processes or to preserve food; yet they are also important sources of trace elements. Food is our most important source of chemical pollution. Food reaches the consumer after a long chain of preparation and processing as an end product during which it can be contaminated by trace metals [13]. On the other hand some of the spices can be loaded with elements to improve their color or taste up to a toxic level. Spices contain heavy metals over a wide range of concentrations. Some of these elements are considered to be essential whereas some others have toxic roles in human body [14]. Toxic trace heavy metals are known to pose a variety of health risks. The content of essential elements in plants is conditional. The characteristics of soil and the ability of plants to accumulate some metals determine the metal contents. The main sources of contamination of plants are environment in developing countries, pollution in irrigation water, atmosphere, soil, sterilization methods and storage conditions. Rainfall, traffic density, use of oil or fossil fuels for heating, atmospheric dusts, plant protecting agents and fertilizers are the main sources of metals for plants [15].

It has been stated by Food and Health Organization that the worldwide import of spices which was 209,293 tonnes in the year 2000 has increased to 226,293 tonnes in 2004. As a result of the increase of the awareness on importance of trace elements in foods and their speciation, the demand for more sensitive determinations has also increased [16].

1.2 Methods for the Determination of Trace Elements

Accuracy and precision are the two most important analytical figures of merit for trace element determination in biological materials. In many applications the methods need to be suitable for automation and routine applications and the sample consumption should be as low as possible [17].

Atomic absorption techniques (AAS) using flame (FAAS), electrothermal atomic absorption spectrometry (ETAAS), atomic fluorescence spectrometry (AFS) and inductively coupled plasma techniques (ICP) are the most commonly used techniques by the greatest number of researchers for trace element determination in seasoning products. On the other hand instrumental neutron activation analysis (INAA), proton-induced X-ray emission (PIXE), X-ray fluorescence spectrometry (XRF) and voltammetry techniques including adsorption differential pulse voltammetry (ADPV), cathodic stripping voltammetry (CSV) and differential pulse anodic stripping voltammetry (DPASV) are other methods known to be used scarcely in this field [16].

1.3 Sample Treatment

Sample treatment is an important step in order to separate the analyte from the matrix and to avoid organic matter which may react with the metal ions or chemical reagents and interfere with the analyte during measurements.

The most commonly used methods for the sample treatment of spices are (1) dry ashing, (2) wet digestion, (3) ultrasound-assisted extractions and (4) microwave-assisted treatment. Instrumental nuclear techniques require no sample treatment, since solid samples can be used directly [16].

1.3.1 Dry Ashing

Digestion of the organic matter done by ashing at atmospheric pressure is known as dry ashing; programmable furnaces may be used for this purpose. The commonly applied temperature is around 450 ^{0}C. Fresh or dried samples are weighed in suitable crucibles and placed in the furnace. Temperature is elevated up to 450 ^{0}C following a heating programme. The resulting inorganic residue known as ash is dissolved in a suitable acid to obtain a solution to be analyzed. Results are expressed on fresh or dry weight depending on the initial condition [18].

Dry ashing offers the advantage of complete elimination of the organic matter leading to high pre-concentration factors. However it suffers from possible losses of volatile analytes such as As, Se, Cd and Hg, analyte reactions with the crucible material and sample contamination from combustion residues [16, 19].

1.3.2 Wet Digestion

Wet digestion is performed by using concentrated acids including nitric acid, perchloric acid, hydrogen peroxide and mixture of acids in open or closed vessels. Incomplete digestion of the matrix is a common problem. In such a case the mixture of sulphuric, perchloric or nitric acids is one of the best means to dissolve organic components. For plant species including varying amounts of silicates, hydrogen fluoride is used for complete digestion [16, 20].

It is possible to apply convective or microwave heating during wet digestion. When open vessels are used loss of volatile analytes and contamination may occur and they require constant operator attention [16].

1.3.3 Ultrasound- Assisted Extraction

Ultrasound is commonly used for biological, environmental and agricultural solid sample pre-treatment because the energy provided accelerates some steps such as dissolution, fusion and leaching.

The chemical effects of ultrasound are due to cavitation which generates local high temperatures and to mechanical action between solid and liquid interfaces. The efficiency of analyte extraction depends on temperature, viscosity, presence of solid particles, height of water column, frequency and the position of vessels used for extraction [21].

Ultrasound-assisted extraction represents the advantage of quantitative dissolution of the analyte to be determined without complete matrix dissolution. Thus the original form of the elements concerned are preserved which is required in speciation analysis [16].

1.3.4 Microwave (MW)-Assisted Treatment

Nowadays microwave heating is the most commonly used technique for treatment of a variety of samples. It has proved to be the most suitable digestion method for complex matrices including oxides, silicates and organic substances. It decreases digestion times, increases analyte recoveries also for volatile elements and reduces cross contamination and consumption of reagents leading to improvement in detection limits and overall accuracy of analysis [16, 22].

1.3.4.1 Closed Vessel MW Systems

Closed vessel MW systems consist of bombs, usually constructed of a fluorinated polymer such as polytetrafluoroethylene (PTFE) or perfluoroalkoxy (PFA) in which the samples are placed.

The major advantage of this system is its high heating efficiency. Heating decomposes the sample matrix and causes the evaporation of the gases produced during decomposition and of the digestion acids. This increases the pressure inside the bomb leading to an increase in the boiling point of the reagents which in turn increases decomposition efficiency. However, excessive build up of the pressure can lead to the rapture of sealed vessels. For this reason most systems are equipped with pressure relief valves designed to open at very high pressures in order to maintain safety [23].

1.3.4.2 Open Vessel MW Systems

Open vessel MW systems operate under atmospheric pressure. The system does not suffer from pressure build up and allows flexibility in control of the treatment such as delivery of digestion reagents at any stage of the procedure without cooling or opening of the vessels. The potential loss of the volatile species and the requirement for high boiling point acids for complete decomposition of the sample matrix are the main problems encountered with this type of systems [23].

1.4 Inductively Coupled Plasma Mass Spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) was commercially introduced in 1983. The low limits of detection (ng/L in most cases), its multi-element capability, the wide linear dynamic range, high sample throughput, relatively simple spectra, the ability to obtain isotopic information, the ease with which it can be used with alternative sample introduction techniques have increased the popularity of ICP-MS for trace element determinations in a variety of sample matrices.

The majority of the samples analyzed with ICP-MS are aqueous solutions, therefore in its standard configuration the instrument is equipped with a pneumatic nebulizer for sample introduction with a quadruple mass analyzer because of their simplicity, stability and low costs [24].

Although ICP-MS is a powerful analytical technique it has some limitations; (1) trace elements at a mass to charge ratio (m/z) smaller than 80 suffer from spectral interferences as a result of limited mass resolution at heavy matrices, (2) solid samples have to be taken into solution, (3) no information on the chemical form of the analyte can be obtained since all the species introduced into the plasma are atomized, (4) dissolved solid levels must be controlled carefully, typically no higher than 0.2% (usually limited to 2000 mg/L) in order to prevent deposition on spectrometer interface, (5) when compared to ICPOES, ICP-MS has a limited capability in the determination of very high analyte concentrations, (6) for quadruple mass spectrometers polyatomic and doubly charged ion interferences cause difficulties for the determination of certain elements. However many polyatomic interferences may be overcome by using high resolution inductively coupled plasma mass spectrometry (HRICPMS) which provides high resolution and sensitivity with low background results and detection limits [25-27].

However these limitations may be overcome to a large extent with instrumental modifications, new detector technology and by the use of alternative sample introduction or separation systems. Sample dilution in order to decrease matrix effects of non-spectral interferences and isotopic correction for spectral interferences are some other approaches to the limitations. For elements at μg/L level coupling ICP-MS to a flow injection system may reduce the sample preparation time, sample volume and risk of sample contamination while improving the precision of the measurement [25-28].

1.4.1 Optimization

A simplified diagram of the process that occurs during the passage of a sample aerosol droplet through ICP is shown in **Figure 1.1**. The sample aerosol which is carried by the nebulizer or carrier gas flow passes through the center of the plasma, punches through the center of the plasma discharge and gains energy mainly by the radiation from the hotter region of the plasma, located inside the outer wall of the torch.

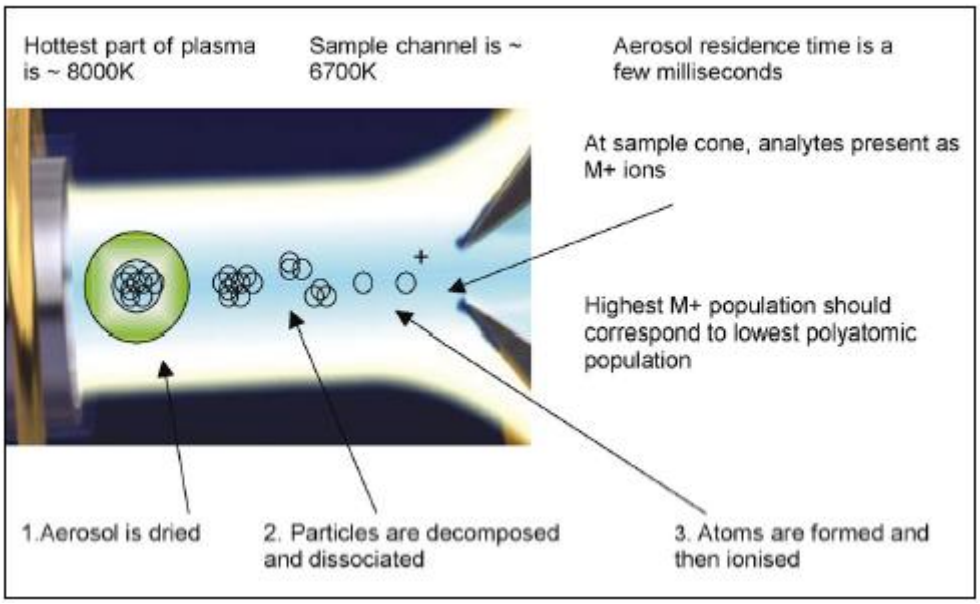

Figure 1.1 The passage of a sample aerosol droplet through ICP [25]

The residence time of the sample aerosol in the plasma is a few milliseconds. In this time the plasma must dry, decompose, dissociate, atomize and ionize the sample to achieve, as far as possible, 100% conversion of the sample into singly charged positive ions.

Plasma temperature, sample residence time, sample aerosol density and water vapor content of the sample aerosol are the main factors that will affect the efficiency of this process. Some of these parameters are under the control of instrument operator whereas others depend on instrumental design. In both cases the fundamental requirement is to obtain a high plasma temperature.

Degrees of ionizations for a set of elements based on different plasma central channel temperatures are shown in **Table 1.1**. It is obvious that reducing plasma temperature has an important effect on the ionization of many elements especially on those with high first ionization energies.

Spray chamber temperature control and the design of plasma RF generator, ion lenses and plasma torch affect the performance of ICP-MS. Several optimization parameters including the plasma RF forward power, sampling depth, carrier gas flow rate and sample uptake rate affect the matrix decomposition and interference removal capability [25].

Simple adjustment of all these parameters is necessary to improve the performance of the instrument when the impact of the optimization on other performance characteristics of the instrument is considered [26].

Table 1.1 First Ionization potentials of various elements at several plasma temperatures [24]

		Ar Plasma Temperature, K			
Element	**1st IP(eV)**	**5000**	**6000**	**7000**	**8000**
Cs	3.89	**99.4%**	**99.9%**	**100.0%**	**100.0%**
Na	**5.14**	**90.0%**	**98.9%**	**99.8%**	**99.9%**
Ba	5.21	88.4%	98.7%	99.8%	99.9%
Li	**5.39**	**83.4%**	**98.2%**	**99.7%**	**99.9%**
Al	5.98	56.2%	94.5%	99.1%	99.8%
Pb	7.42	4.3%	51.2%	91.1%	98.3%
Mg	7.64	2.6%	40.7%	87.7%	97.7%
Cd	**8.99**	**0.1%**	**4.8%**	**43.2%**	**85.7%**
Se	**9.75**	**0.0%**	**1.1%**	**17.8%**	**66.6%**
As	**9.81**	**0.0%**	**1.0%**	**16.4%**	**64.6%**

1.5 Interferences in Inductively Coupled Plasma Mass Spectrometry

Although inductively coupled plasma mass spectrometry has a number of advantages over other techniques for trace element analysis it suffers from interferences.

The interferences encountered in ICP-MS are divided into two categories. Although there is a considerable overlap between these two groups some of the methods used to overcome them are different [28].

1.5.1 Spectral Interferences

The sources of spectral interferences are the atomic or molecular ions having the same nominal mass as the analyte of interest thus causing erroneous enhancement in the signal. Spectroscopic interferences are divided into two categories depending on the origin of the interference.

Overlapping of signals may be caused by the isotopes of different elements. A number of elements have only one naturally occurring stable isotope. However there are no spectral isobaric overlaps on these elements. Also almost all of the elements have one interference-free isotope although it is not always the most abundant isotope. Isobaric overlaps are predictable and natural abundance values are tabulated so they can be easily overcome by using different isotopes of the analyte of interest or by utilizing elemental correction equations for analysis [29].

Molecular or polyatomic ions may also cause overlapping. The possible sources of these interferences are the precursors in (1) plasma gases, (2) entrained atmospheric gases, (3) water and acids used for dissolution and (4) sample matrix [28]. Argon, oxygen and hydrogen combined with other elements present in sample matrix are

thus the basic components of polyatomic ions [30]. Most of these overlaps are originally identified. There exist extensive tables of spectral interferences of ICP-MS formed by Horlick and co-workers [31].

1.5.1.1 Methods to Overcome Spectral Interferences

When an element is introduced into the plasma the only species observed in the final mass spectrum is not the monoatomic singly charged analyte ion (M^+). Elements having low second ionization energies will be doubly ionized (M^{2+}) whereas elements having a high MO bond strength will form oxide (MO^+) and hydroxide ions (MOH^+) [32].

The intensities of these species can be reduced by adjusting the instrumental design and operating conditions such as nebulizer gas flow rate, plasma potential, spacing between load coil and sampler cone if the origins of the interferences are due to the plasma and entrained atmospheric gases. Alternative gas and mixed gas plasmas, alternative plasma sources, high resolution instruments or multivariate correction methods can be used to recognize and overcome these interferences.

However, the situation becomes more unpredictable when water and acids are used for dissolution. In this case alternative sample preparation methods or sample introduction methods are applicable.

1.5.2 Nonspectral Interferences

Unlike spectral interferences, non-spectral interferences cause reduction or enhancement in analyte signal by forming a number of factors affecting (1) sample transport, (2) ionization in the plasma, (3) ion extraction or (4) ion throughput in the resultant ion beam.

The nature and concentration of sample matrix have a direct effect on the magnitude of these interferences.

1.5.2.1 Sample Introduction and Transport

Factors affecting sample introduction and transport are: (1) design of the nebulizer, (2) nebulizer gas flow rate, (3) physical properties of the sample and solvents such as viscosity, surface tension, density and evaporation rate [33]. So matrix matched samples and standards with respect to the solvent are required to obtain similar nebulization and transport characteristics [28].

1.5.2.2 Effect on Ionization in the Plasma

Sample matrix may have a profound effect on plasma temperature which in turn changes atomization, excitation and ionization characteristics. In particular organic solvents and excess of easily ionizable elements present in sample matrix have the most severe effects. Organic solvents decrease plasma temperature and electron density as a result of solvent load and energy requirement to dissociate molecular species such as C_2 [28]. On the other hand easily ionizable elements cause ionization suppressions of poorly ionized elements leading to reduced sensitivity [25].

1.5.2.3 Salt Build-up on the Cones

Deposition of salts on sampler and skimmer cones may cause the suppression of analyte signals by clogging orifices and substantially affecting sampling processes. Flow injection which allows minimal sample consumption compared to normal nebulization, decreases the effects of orifice clogging [28, 34].

1.5.2.4 Matrix Induced Suppression in the Ion Beam

Heavy, easily ionizable element or elements in the matrix may suppress or in some cases enhance the analyte signal. If there are heavy elements with low ionization potentials in the matrix the effects are severe. Light analytes having high IPs are most severely affected. The magnitude of these effects depend on plasma operating conditions and the absolute amount of the matrix element rather than the molar ratio of the matrix element to analyte since the effects can be reduced by dilution of the sample [28].

1.5.2.5 Methods to Overcome Matrix-Induced Suppression in the Ion Beam

(1) Internal standardization, (2) isotope dilution, (3) separation methods and (4) flow injection are most commonly used to overcome matrix induced interferences.

Internal standardization is an effective means of compensating matrix induced suppressions. Atomic masses and ionization potentials of the analyte and the internal standard should be close to each other to achieve successive corrections [35].

The most widely used methods to separate matrix from analyte are precipitation, solvent extraction and on-line separation methods including ion exchange and chromatographic methods [28].

1.6 Arsenic

1.6.1. Occurrence, Properties

Arsenic occurs in environment as a result of several sources containing the element in organic and inorganic forms. It is a semi metallic element with an atomic weight of 74.92. It is rarely found in free-state. It mostly occurs in combination with sulphur, oxygen and iron. There is one naturally occurring isotope of arsenic which is ^{75}As. Arsenic and arsenic compounds occur in crystalline, powder, amorphous or vitreous forms. Many of inorganic arsenic compounds occur as white, odorless solids.

Arsenic is used in the production of lead alloys used in lead-acid batteries. It can be added to the alloys of bearings, type metals or to brass to improve corrosion resistance. High purity arsenic is used in a number of semiconductor applications such as solar cells, light emitting diodes, lasers and integrated circuits [36].

1.6.2 Toxicity and Health Hazards

The general source of arsenic exposure is consumption of foods. Most foods contain 7 μg/g dry weight As. The levels range up to 80 μg/ kg dry weight in seafood. The daily arsenic intake for adults is estimated to be in the range of 100 to 200 μg although intake of 12-25 μg/day arsenic is probably enough to meet the requirements [1, 2]. Seafood, rice, rice cereal, mushrooms and poultry are claimed to contain the highest levels of arsenic. Drinking water contaminated with arsenical pesticides, natural mineral deposits or arsenical chemicals is another source of arsenic exposure.

Arsenic in soil can be released to ground or surface water through natural processes [36].

Many metallic ions are found in many forms in the environment. They differ from each other by their chemical and physical properties. The bioavailability and toxicity of an element depend on the degree of oxidation.

The oxidation states of arsenic are as follows;

As (-III), As (0), As (III), As (V)

Commonly present inorganic forms of As(V) and As(III) are arsenate, AsO_4^{3-}, and arsenite, AsO_3^{3-}, ions. Monomethylarsonic acid, MMAA, dimethylarsinic acid, DMAA, and arsenobetaine, AsB, are common organic species [37].

Arsenic is considered to be both a toxic and essential element. The toxicity of different arsenic species is in the following order;

Arsenite > arsenate > monomethylarsenate (MMA) > dimethylarsinate (DMA)

As (III) is around 60 times more toxic than As (V) and inorganic As compounds are about 100 times more toxic than organic As compounds. When arsenic is taken into human body, around 50% of As is transferred into urine. The rest is found in feces, skin, hair, nails and lungs in smaller portions.

Skin lesions, various diseases such as cardiovascular diseases, disturbance in the peripheral vascular and nervous systems, skin cancer and gangrene are long term effects of As toxicity [38].

The recommended safe exposure limit of arsenic for adults is 15 μg/ kg of body weight per week [1]. Lethal dose 50% (LD_{50}) values represent the estimated doses of toxicants causing death of 50% of the universal population of the species exposed to toxicity tests and are frequently used to express the potency of the toxicants [39]. In humans lethal dose of inorganic arsenic is estimated to be in the range of 1-3 mg As/kg [40].

1.6.3 Analytical Methods for Arsenic Determination

Hydride generation is a popular technique which was initially developed for AAS whereby sodium or potassium tetrahydroborate is used as reduction reagent for arsine, AsH_3, production [40]. As(III) and As(V) give AsH_3, monomethylarsonic acid, MMAA ($CH_3AsO(OH)_2$), gives MMA (CH_3AsH_2), dimethylarsinic acid, DMAA ($(CH_3)_2AsO(OH)$) gives DMA ($(CH_3)_2AsH$). The formation of arsines is pH dependent. This indicates that arsenic species must be fully protonated before reduction to corresponding arsine so As(III) reacts with tetrahydroborate at a higher pH than As (V). The procedure can be used for differential determination of As (III) and As (V) [41, 42].

When hydride generation is used, transition metals might interfere with the determination of arsenic by the reaction with tetrahydroborate and the formation of precipitates which capture and catalytically decompose evolved hydrides. Flow injection technique whereby the concentration of reductant is usually lowered, may eliminate the transition metal interferences [41].

Graphite furnace atomic absorption spectrometry (GFAAS) or electrothermal atomic absorption spectrometry (ETAAS) is based on the absorption of free atoms produced from the sample deposited in a small graphite tube which is resistively heated to high temperatures. The most important disadvantage of the technique for arsenic determination is the requirement for pre-concentration in order to increase sensitivity [41].

The ICP technique is based on the use of Ar plasma to ionize the components after the sample is acidified and nebulized into the plasma. High temperature plasma is capable of ionizing all forms of arsenic. However it suffers from spectral and non-spectral interferences such as the formation of $^{40}Ar^{35}Cl^{+}$ which has the same nominal mass with $^{75}As^{+}$[41].

Instrumental neutron activation analysis (INAA) is one of the most sensitive analytical techniques whereby target nuclides in the sample are activated with the formation of radioactive nuclides which decay by beta particle and gamma ray emission. Produced gamma rays are detected by a high resolution gamma ray spectrometer [41].

Electrochemical methods play an important role in trace metal determinations. For arsenic determination differential pulse polarography (DPP) provides relatively high sensitivity and improved limits of detection [43].

Voltammetric methods including cathodic stripping voltammetry (CSV) and anodic stripping voltammetry (ASV) whereby As(III) is first deposited onto a working electrode and then stripped are also applicable.

CSV analysis of arsenic are based on arsenic pre-concentration in acidic media and then scanning in cathodic direction to obtain peak as a result of arsine formation whereas in ASV analysis As is deposited on electrode surface and anodic stripping is applied [41].

1.7 Selenium

1.7.1 Occurrence, Properties

Selenium is both essential and toxic with a narrow range of intake between the two. In nature, selenium is found in combination with lead, copper, mercury and silver. These combinations are known as selenides. Selenium has at least 29 isotopic forms among which only six are found in nature: ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se. It has three valance states; Se(II), Se(IV) and Se(VI) [2] The common species of Se(IV) and Se(VI) are selenite, SeO_3^{2-}, and selenate, SeO_4^{2-}, ions [44].When Se reacts with sulphur and oxygen it forms selenomethionine, selenocystine, methylselenocystine and dimethylselenide [2].

Selenium is present in different amounts in different foods such as vegetables, nuts, fruits and milk. It is rich in high protein foods including eggs, meats, entrails, seafood. Selenium is accumulated in liver and kidney meat. The amount of Se in different foods depends on geographical origin. Selenium content in fruits and vegetables are lower than animal based foods since there is a strong correlation between protein content and selenium and plants do not require Se to grow whereas it is an essential element for the survival of animals [2].

1.7.2 Toxicity and Health Hazards

The recommended dietary allowance for selenium is 55 μg/ day for adults. The minimum Se concentration enough to combat with diseases is 30-70 μg/ day for adults while the maximum level is 400 μg/ day [37]. Selenium is toxic when its intake is greater than 750 μg/ day [2]. The median lethal dose (LD_{50}) values in rats for some selenium compounds are: sodium selenite, 7 mg/kg; dimethylselenide, 1600 mg/kg; trimethylselenonium chloride, 49 mg/kg; and elemental selenium, 6700 mg/kg [45].

Since the range between toxic and essential levels of Se is narrow accurate determinations in food samples are required [46].

Selenium is very toxic at the mentioned high levels and may cause selenium poisoning (selenosis) in humans and animals. Toxicity of selenium depends on both chemical form and quantity of the element consumed and a variety of other factors including species, age, physiological state, nutrition and dietary interactions. The levels of selenoproteins in blood plasma are good indicators for selenium status in humans. The bioactivities of these selenoproteins are affected by diet and food intakes which are the main sources of selenium [38].

1.7.3 Analytical Methods for Selenium Determination

There are a number of methods for determination of selenium in biological samples especially in foods. In selenium determination, chemical action of reagents used in decomposition and digestion steps, the resistance of selenium species to oxidation,

volatilities of selenium species present or formed and low concentration limits are the main sources of incorrect results [47].

Fluorometry, graphite furnace atomic absorption spectrometry (GFAAS), hydride generation atomic absorption spectrometry (HGAAS), instrumental neutron activation analysis (INAA), radiochemical neutron activation analysis (RNAA), isotope dilution mass spectrometry (IDMS), X-ray fluorescence (XRF), inductively coupled plasma optical emission spectrometry (ICPOES) and inductively coupled plasma mass spectrometry (ICP-MS) are well known techniques for selenium determination in biological samples.

Although GFAAS allows the determination of a wide range of elements including selenium at μg/kg level, matrix interferences particularly from phosphates and significant volatilization losses during atomization affect accuracy of the results for Se determination in biological samples. HGAAS is a suitable technique for the determination of Se, having volatile hydrides, with minimal matrix interferences. FAES is far less sensitive than AAS for Se determination. If flame is replaced by plasma (ICPOES) it is possible to obtain improved detection limits, accuracy and precision. However it is still not sufficient in terms of sensitivity for Se determination in biological and clinical samples. ICP-MS combines high sensitivity of ETAAS with simultaneous multi-element analysis capacity of ICPOES leading to a highly sensitive analysis at very low levels of concentrations [48].

1.8 Cadmium

1.8.1 Occurrence, Properties

Cadmium can be extracted from lead and zinc ores as a byproduct in which it occurs as a constituent. In nature all cadmium exists as seven stable isotopes and one radioactive isotope. The seven stable isotopes are ^{106}Cd, ^{108}Cd, ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{114}Cd and ^{116}Cd. ^{113}Cd is radioactive. Emissions from industrial and waste incineration plants and coal fired power plants are the main sources of Cd distribution over land. About one third of Cd produced is used in manufacture of the batteries, plastics, paints and metal plating processes. It is also used as an anticorrosive element for some metals. It is a highly accumulative element having a biological life of about 20-30 years in humans.

1.8.2 Toxicity and Health Hazards

Cadmium is a serious environment and soil pollutant and it is highly toxic even at low concentrations. If the growth medium contains high levels of cadmium, crop plants easily capture it through the roots.

Presently the main sources of Cd are; Cd rich phosphate fertilizers, atmospheric events, sewage sludge, industrial and mining wastes [49].

According to World Health Organization (WHO) and Food and Agriculture Organization (FAO), the tolerable limit of Cd is 100µg/ kg in cereals and food and it is 100 and 300 µg/kg in medicinal plants [50].

Cereals and other vegetables especially grown in countries with extensive industrial activities account for almost 50% of cadmium intake which is 2-25 µg/day in children and 10-50 µg/day in adults. According to WHO the maximum tolerable weekly intake of cadmium is 7 µg/kg body weight [1, 2]. The LD_{50} value of $CdCl_2$ ranges from 47 to 109 mg/kg in rats depending on age [51]. Weekly tolerable intake values of Cd is listed in **Table 1.2** together with those of As and Se and RDA values.

Table 1.2 RDA and weekly and daily intake values of As, Se and Cd

	RDA	Tolerable Intake Value
As	12-25 µg/ day	15 µg/ kg body weight per week
Se	55 µg/ day	□ 750 µg/ day
Cd	-	7 µg/ kg body weight per week

1.8.3 Analytical Methods for Cd Determination

FAAS is a very simple technique with high sample throughput and low operation cost for Cd determination. However even in contaminated areas toxic trace constituents are generally found at concentrations far below the FAAS quantification limits.

GFAAS has been a powerful tool for the determination of Cd for over 30 years in terms of high sensitivity and selectivity for specific elements. At present it is most

commonly used since the procedure is relatively easy, fast and detection limits are low enough for analysis of most environmental and biological samples especially when one or a few samples are to be analyzed.

ICP-MS is superior to conventional GFAAS, especially when multi-element analysis with a large number of samples is required. Its excellent detection limits, simple spectra and high analysis throughput are the main advantages [52, 53].

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and Reagents

All reagents during the experiments were of analytical grade. The standard solutions were prepared by appropriate dilutions of the stock solutions (1000 mg/L, Merck) by using de-ionized water obtained from Millipore (Molsheim, France) Milli-Q water purification system. The system is fed by using the water produced by Millipore Elix 5 electrodeionization system. For the acidification of standard and analyte solutions distilled analytical grade 65% HNO_3 (Merck) was used.

All the glassware and plastic apparatus were kept in 2.0 M HNO_3 before and after use to remove all possible contaminations.

During Microwave digestions, mixtures of distilled analytical grade 65% HNO_3 (Merck) and 35% H_2O_2 (Merck) were used.

During interference studies solutions of interfering ions, Na^+, Li^+ and Cs^+, were prepared from their salts in deionized H_2O, using NaCl, $NaNO_3$, LiCl, $LiNO_3$, CsCl and $CsNO_3$. Analytical grade 37% HCl (Merck) was used as a source of chlorine which is known to have spectral interference during arsenic determinations.

For accuracy check, NIST 1573a Tomato Leaves and NIST 1566b Oyster Tissue standard reference materials were used for Cd, As and Se.

2.2 Instrumentation

Selected spice samples were digested in a closed vessel microwave digestion system (ETHOS, Plus, Milestone) equipped with fiber optic temperature and pressure sensors. The system consists of 10 Polytetrafluoroethylene (PTFE) digestion bombs.

BSB-939-IR, Berghof acid distillation system was used for the distillation of HNO_3. It was used for digestion of samples and the preparation of standards.

The spice samples were analyzed by using a Inductively Coupled Plasma Mass Spectrometer, Thermo X Series, equipped with a quadruple mass analyzer and a flow injection system with a loop volume of 500 µL for sample introduction.

The optimization parameters for ICP-MS for Cd, As and Se are listed in **Table 2.1.** These parameters were not used during all analysis. Before each analysis the sensitivity of the instrument was checked by using standard solutions and the parameters were optimized if necessary.

Table 2.1 Optimization parameters of ICP-MS for Cd, As and Se

Parameter	**Optimum Result As, Se**	**Optimum Result Cd**
Extraction Lens Voltage, V	-443	-137
Lens 1 Voltage, V	-186.8	-0.6
Lens 2 Voltage, V	-24.3	-14.9
Focus Lens Voltage, V	15.5	12.9
1. Diffraction Aperture Voltage, V	-57.3	-40.0
2. Diffraction Aperture Voltage, V	-201	-135
Quadruple Voltage,	-1.8	-2.0
Hegzapole Voltage , V	1.3	1.6
Argon Flow Rate in Nebulizer, L/min	0.70	0.76
Lens 3 Voltage, V	-194.5	-200.0
Horizontal Position of Torch	60	64
Vertical Position of Torch	688	625
3. Diffraction Aperture Voltage, V	-62.7	-36.9
Argon Flow Rate to Cool Torch, L/min	12.0	12.0
Argon Flow Rate to Produce Plasma, L/min	1.19	0.78
Sampling Depth	8	17
Forward Power, w	1600	1600

2.3 Procedures

2.3.1 Samples and Sample Preparation

For this analysis 10 different spice samples purchased from three different markets and two different bazaars were collected. The names of the spices selected are given in **Table 2.2**.

Table 2.2 Types of spice samples analyzed in research

English	**Latin**	**Turkish**
Black pepper	*Piper Nigrum*	Karabiber
Cumin	*Fructus Cummuni*	Kimyon
Thyme	*Herba Thymi*	Kekik
Mint	*Folium Menthane*	Nane
Rosehip	*Rosae Caninae*	Kuşburnu
Sage	*Folium SalviaeOfficinalis*	Adaçayı
Daisy	*Chamomillae Vulgaris*	Papatya
Bay Leaf	*Folium Lauri*	Defne yaprağı
Sumac	*Folium Rhois*	Sumak
Linden Flower	*Flos Tilliae*	Ihlamur

Samples were kept in closed vessels and packages were firstly grinded in porcelain mullers to obtain homogenous powder form. Without applying any kind of drying procedures 0.2 g of each sample were put in PTFE digestion bombs and after the addition of 3.0 mL of distilled concentrated HNO_3 and 3.0 mL of concentrated H_2O_2 they were digested by applying microwave heating. The microwave temperature program was optimized before the analysis for more efficient digestions with minimal digestion reagent consumption. The optimized MW temperature program is shown in **Table 2.3**. At the end of the digestion process, PTFE digestion bombs were cooled to room temperature and the contents of digestion vessels were transferred into firstly PTFE vessels and then into glass volumetric flasks having a volume of 10.0 mL. Digested samples were brought to a volume of 10.0 mL by using deionized water.

Table 2.3 Optimized MW temperature program for sample digestion

Period, Min.	**Temperature, ^{0}C**
5	25-100
15	100
5	100-150
20	150
5	150-180
15	180
15	Ventilation

2.3.2 Analytical Determinations

50 spice samples each having a parallel sample solution, were analyzed by using ICP-MS, Thermo X Series, equipped with a flow injection system. Deionized water was used as the carrier solution for all determinations. The sensitivity of ICP-MS was checked by using standard solutions and operating parameters were optimized before each analysis if necessary.

Standard solutions used for drawing of calibration plots were prepared in 1.0 M HNO_3. For Cd determination, 0.5, 1.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/ mL Cd standards prepared in 1 M HNO_3 were analyzed and direct calibration method was applied. During the analysis $^{111}Cd^+$ signals were monitored although the abundance of ^{111}Cd is lower than those of ^{112}Cd and ^{114}Cd, because of possible interference problems.

For As determination, As standards having concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/ mL prepared in 1.0 M HNO_3 were used direct calibration method was found to be not applicable for As determination with ICP-MS unless the

matrices of sample and standard solutions are matched so standard addition method was applied for the determinations. The final volume of each spice sample was 10.0 mL so standard addition method was applied with three points after suitable additions and dilutions of 1.0 mL of each sample to 2.0 mL.

Since As has one naturally occurring isotope $^{75}As^{+}$ signals were monitored during the analysis. Interference studies indicated that for the spice samples analyzed there were not possible spectral and non-spectral interferences for As since both the chlorine and salt content of spice samples were found to be sufficiently low to exhibit any kind of interference effect.

For the determination of Se, similar to As standard addition method was applied in the same way explained above. 2.0, 5.0, 10.0, 20.0 50.0 and 100.0 ng/ mL Se standard solutions prepared in 1 M HNO_3 were used to obtain calibration plots however direct calibration method was found to be not applicable for Se determination with ICP-MS without matrix matching.

During the analysis $^{82}Se^{+}$ signals were monitored since $^{78}Se^{+}$ and $^{80}Se^{+}$ signals suffer from spectral interferences arising from the presence of argon as the carrier gas.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Optimization of Microwave Digestion Temperature Program

Sample preparation is a crucial step in any kind of analysis. Depending on the properties of the samples to be analyzed, any of wet ashing, dry ashing or microwave digestion technique is applied to extract the target analytes from sample matrix as much as possible.

For the analysis of spices, microwave digestion technique is commonly used since wet and dry ashing techniques are time consuming and have no advantage in terms of digestion efficiency with exception of freedom of using large sample sizes. MW digestion is a sufficiently good, safe and clean method for sample preparation [54].

Based on this evidence, MW digestion technique was found to be applicable for sample preparation. Then suitable reagents for digestion were chosen.

Nitric acid is one of the cleanest digestion reagents available and is considered to be the best acid medium for ICP-MS. There exist plenty of nitrogen, oxygen and hydrogen in air and water so polyatomic interferences are not increased by the presence of HNO_3 [55]. It is also possible to distill HNO_3 resulting in lower

contamination and blank levels. To check the effect of distillation on ICP-MS signals a simple experiment was carried out. Cadmium signals obtained from a concentrated nitric acid solution is compared with that of obtained from the same solution after distillation and found to be approximately 40 times higher. H_2O_2 is commonly used in combination with HNO_3 for the digestion of plant samples. It is a strong oxidizing agent and converts organic compounds into CO_2 and H_2O [56]. However it is not possible to distill H_2O_2 so it increases blank levels leading to the need for the optimization of its volume to reduce contamination.

To develop a sample preparation procedure for spices suitable for ICPMS analysis two parameters were optimized; (1) the volumes of concentrated HNO_3 and H_2O_2 solutions used for digestion and (2) the MW temperature program.

Based on the older procedures present in literature 0.20 g of Tomato Leaves 1573a SRM samples were digested in a variety of combinations of HNO_3 and H_2O_2. Four different temperature programs were applied for each combination. Digested samples were transferred into firstly PTFE vessels and then into glass volumetric flasks and diluted to a volume of 10.0 mL by using deonized water. The best digestion reagent combination and MW temperature program was determined by monitoring $^{111}Cd^{+}$ signals.

The four tried MW digestion temperature programs are listed in **Table 3.1**. The results obtained with these programs are summarized in **Table 3.2**.

Table 3.1 MW digestion temperature programs for optimization

MW Program	Period, min	Temperature ^{0}C	MW Program	Period, min	Temperature ^{0}C
MW-1	5.0	25-100	**MW-3**	5.0	25-100
	10.0	100		5.0	100
	5.0	100-150		5.0	100-150
	15.0	150		15.0	150
	5.0	150-180		5.0	150-180
	10.0	180		15.0	180
MW-2	5.0	25-100	**MW-4**	5.0	25-100
	15.0	100		5.0	100
	5.0	100-150		5.0	100-150
	20.0	150		10.0	150
	5.0	150-180		5.0	150-180
	15.0	180		20.0	180

Table 3.2 Comparison of MW digestion programs for optimization by using 0.20 g of 1573a Oyster Tissue SRM

MW Program	Volume of Concentrated HNO_3, mL	Volume of Concentrated H_2O_2, mL	Experimental Results, mg/kg Cd	Certified Value, mg/kg Cd
MW-1	2.0	2.0	1.26±0.02	**1.52±0.04**
	3.0	3.0	1.31±0.03	
MW-2	2.0	2.0	1.34±0.03	
	3.0	3.0	1.52±0.01	
MW-3	2.0	2.0	1.26±0.04	
	3.0	3.0	1.34±0.01	
MW-4	2.0	2.0	-	
	3.0	3.0	1.85±0.01	

When **Table 3.2** is investigated it is seen that using 2.0 mL of HNO_3 with 2.0 mL of H_2O_2 is not enough to digest 0.20 g samples when any of the digestion programs above was applied. All the results are lower than the certified value. When 3.0 mL of HNO_3 and 3.0 mL of H_2O_2 mixture is used the results are better indicating more efficient digestion. However they are still not in good agreement with the certified value unless digested by using MW-2; therefore this program was chosen for further studies.

After choosing MW-2, 3.0 mL of concentrated HNO_3 and 3.0 mL of concentrated H_2O_2, in order to reduce the blank readings due to H_2O_2, an effort was spent by using 1.0 and 2.0 mL of concentrated H_2O_2. However experimental values of 1573a Tomato Leaves SRM results were lower than those of certified values for Cd determination with direct calibration method. It was then decided to use 3.0 ml of H_2O_2 with other mentioned parameters above.

The results showed that it is best to digest spice samples in a mixture of 3.0 mL of HNO_3 with 3.0 mL of H_2O_2 for the determination of cadmium by using direct calibration method.

To investigate if the optimized MW digestion program, MW-2, and the digestion reagent combination, 3.0 mL of concentrated HNO_3 mixed with 3.0 mL of concentrated H_2O_2, are suitable for arsenic and selenium determination, NIST Oyster Tissue 1566b SRM was digested under optimum conditions shown in **Table 3.1** and **Table 3.2** and analyzed by using standard addition method. The results are shown in **Table 3.3**. and indicate that themethod is suitable.

Table 3.3 NIST Oyster Tissue 1566b SRM results for As and Se with standard addition method

Oyster Tissue 1566b SRM	Certified Value, mg/kg	Experimental Value, mg/kg
As	7.65±0.65	7.35±0.01
Se	2.06±0.15	2.11±0.16

3.2 Effect of HNO_3 and H_2O_2 Concentration on $^{75}As^+$, $^{82}Se^+$ and $^{111}Cd^+$ Signals

Dilute acid matrices are commonly used for analysis with ICP-MS since acids are powerful reagents for sample digestion. However they are also sources of interferences which have serious effects on precision and accuracy of ICP-MS analysis.

Acid effects are classified as *steady state* and *transient*. The suppression in analyte signal with increasing acid concentration is referred as steady state acid effect whereas time dependent changes in aerosol properties and analyte signals depending on different acid concentrations are expressed as transient acid effects [56].

Steady state effects depend on both the type and concentration of acids. The effect of the acid on (1) aerosol generation, (2) analyte transport and (3) plasma conditions leading to excitation and ionization processes change analyte signals which indicates that it is not possible to obtain accurate results unless the matrices of samples and standards are properly matched for direct analysis [56].

To investigate the effect of HNO_3 concentration on $^{75}As^+$, $^{82}Se^+$ and $^{111}Cd^+$ signals, a mixture containing 50.0 ng/mL of each analyte is prepared in increasing HNO_3

concentrations. Four different standards prepared in 1.0, 2.0, 4.0 and 6.0 M HNO_3 were analyzed starting with the one with the most diluted matrix. Sufficient length of time was allowed between each analysis to study under the same plasma conditions, for this purpose deionized water was aspirated into the plasma for a few minutes. And the condition of the plasma was checked by using standard solutions.

It was found that increasing HNO_3 concentrations of matrices have suppression effect on both arsenic, selenium and cadmium signals. ICP-MS signals are shown in **Figure 3.1** for $^{75}As^{+}$, $^{82}Se^{+}$ and $^{111}Cd^{+}$ similar patterns were observed.

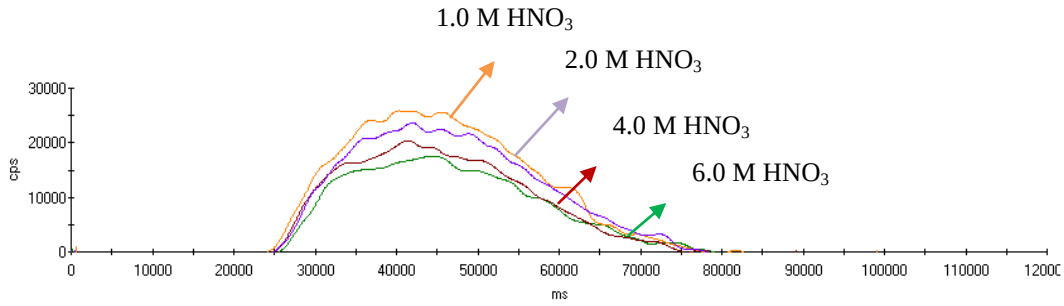

Figure 3.1 50.0 ng/mL $^{75}As^{+}$ Signals in 1.0, 2.0, 4.0 and 6.0 M HNO_3

According to the **Figure 3.2**, **Figure 3.3** and **Figure 3.4**, HNO_3 concentrations have almost the same suppression effect on arsenic and selenium signals whereas the effect is much less pronounced for cadmium. The reason why the effect of same concentrations of HNO_3 on each element is not the same is the difference between their ionization potentials. The first ionization potentials of arsenic, selenium and cadmium are 9.81, 9.75 and 8.99 eV respectively [57].

Having the lowest ionization potential cadmium ionizes more easily than arsenic and selenium of which the ionization potentials are almost the same. When plasma is brought in touch with high acid content cooling in plasma temperature occurs as a

result of aerosol and vapor loading, leading to a decrease in ionization and atomization efficiency of the plasma [56]. This behavior is observed with arsenic and selenium. However, the ionization potential of cadmium is sufficiently low to minimize this suppression effect.

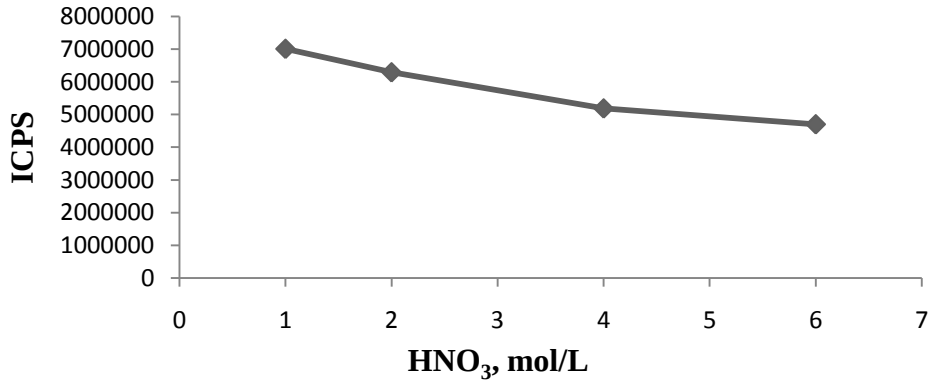

Figure 3.2 Effect of HNO_3 concentration on $^{75}As^+$ signals

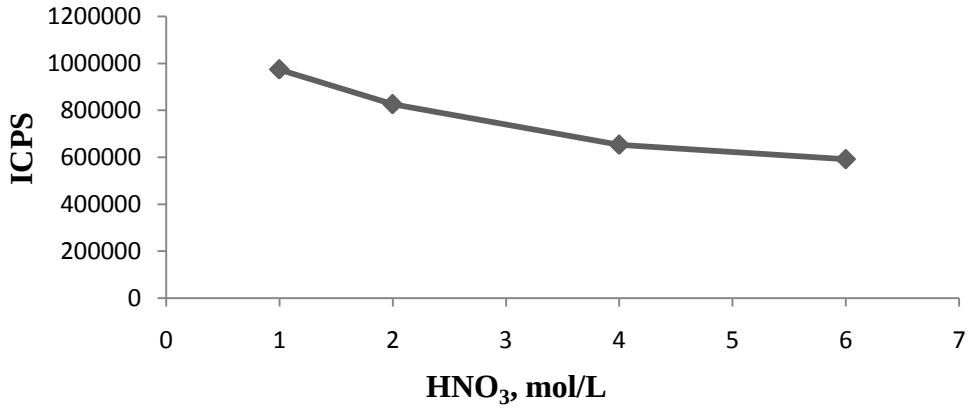

Figure 3.3 Effect of HNO_3 concentration on $^{82}Se^+$ signals

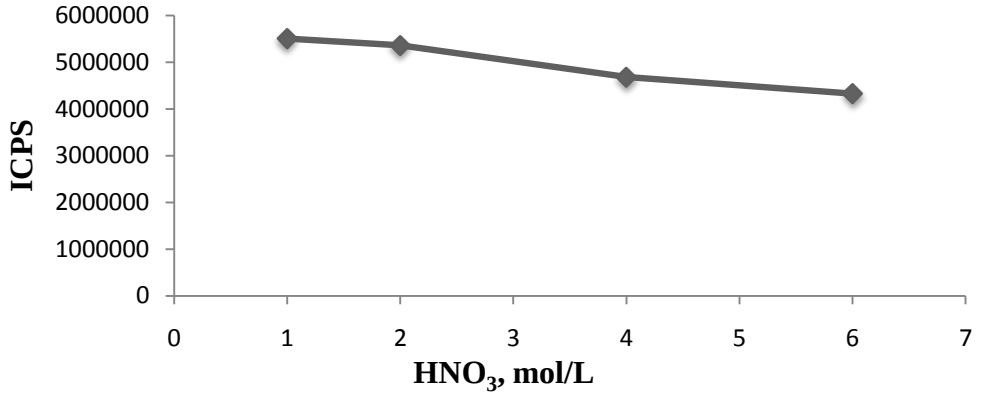

Figure 3.4 Effect of HNO_3 concentration on $^{111}Cd^{+}$ signals

H_2O_2 is a widely used reagent for MW digestion of samples to decrease solid residue and residual carbon content. Moreover high amounts of H_2O_2 are effective in pressure buildup leading to effective organic matter oxidation at lower temperatures [58].

Similar to HNO_3 case, in order to investigate the effect of H_2O_2 concentration on ICP-MS signals, a mixture containing 50.0 ng/mL of As, Cd and Se was prepared in 1.0, 2.0, 4.0 and 6.0 M H_2O_2. The effect of H_2O_2 concentration on signals of each element was different. Increasing H_2O_2 concentrations suppressed As signals whereas higher amounts of hydrogen peroxide enhanced Cd and Se signals. The effect of hydrogen peroxide is not totally known. However it is known to decrease the precision of analysis as a result of out-gassing of peroxide solutions in peristaltic pump tubing before reaching the plasma [55].

The best way of minimizing the suppression effects of acids is to run the instrument under robust conditions. Robustness is the capability of the plasma to keep its conditions stable when the concentration of acid matrix is changed. Under robust conditions acids affect only sample introduction systems but not plasma conditions [56]. Running the instrument under robust conditions which means keeping the

nebulizer gas flow rate lower than 0.7 L/min. may be helpful to minimize but not to prevent acid effects. CeO^+/Ce^+ ratio is a well known plasma robustness criterion. This ratio indicates the efficiency of the plasma to break strong Ce-O bond. Plasma that is unable to decompose Ce-O bond is accepted to be insufficient to ionize elements with high first ionization potentials. Improving the decomposition of CeO which means reducing the CeO^+/Ce^+ ratio is known to decrease matrix interferences especially those of chloride and sulphate. For commercial ICP-MS instruments reported CeO^+/Ce^+ ratio is in between 0.3-3% [25].

To test the robustness of ICP-MS used in this study and to check if it is suitable to analyze acid containing matrices 200.0 ng/mL Ce standard was prepared and $^{140}Ce^+$ and $^{156}CeO^+$ signals were monitored. The signals obtained are shown in **Figure 3.5**. The two peaks overlapping above belong to Ce^+ duplicate signals whereas the other two at the bottom belong to two CeO^+ signals. When the mean areas of the peaks were compared, CeO/Ce ratio was found to be 3.1% indicating that the instrument operates under robust conditions.

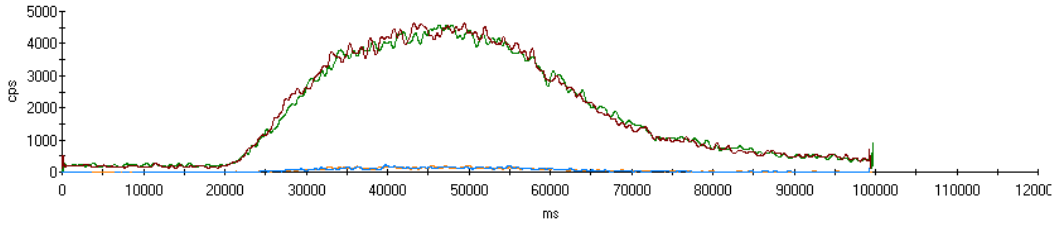

Figure 3.5 Comparison of 200.0 ng/mL $^{140}Ce^+$ (upper trace) and $^{156}CeO^+$ (lower trace) signals

There are some practical methods to obtain accurate results when analyzing acid matrices. These are matrix matching between the standards and samples, standard

addition method and mathematical corrections among which standard addition is the most practical method [59].

During the analysis of spice samples, standard addition method was applied for the determination of arsenic and selenium. SRM results were in good agreement with experimental values only for cadmium when direct calibration method was applied. For direct calibration method standards were prepared in 1.0 M HNO_3. This situation did not affect Cd signals since the suppression effect of HNO_3 on Cd signals are not as severe as that of on As and Se signals.

As seen in **Figure 3.6** the signals of 50.0 ng/mL Cd standards prepared in 1.0 M HNO_3 and 1.0 M H_2O_2 are compared with a 50.0 ng/mL Cd standard of which the matrix was matched with that of samples, do not differ according to t-test indicating no systematic error. However the situation is different for As and Se.

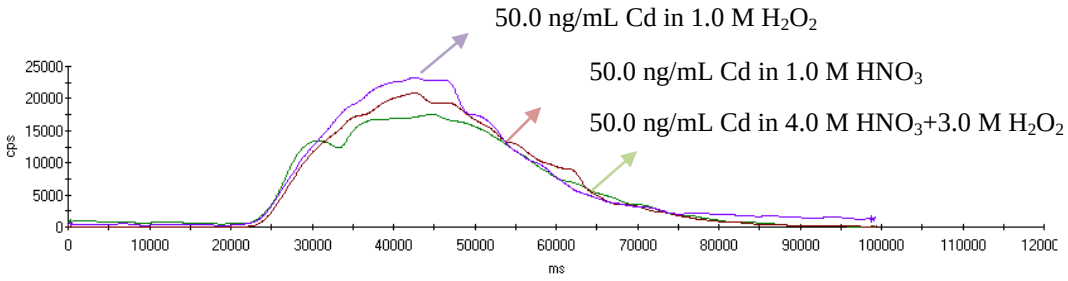

Figure 3.6 Comparison of the signals of 50.0 ng/mL Cd standards prepared in 1.0 M HNO_3 and 1.0 M H_2O_2 with a matrix matched standard containing 50.0 ng/mL Cd.

The strong suppression effect of HNO_3 on arsenic and selenium signals is seen in **Figure 3.7** and **Figure 3.8**. The signals of 50.0 ng/mL matrix match standards are much lower than those of prepared in 1.0 M HNO_3 and 1.0 M H_2O_2. This situation explains why direct calibration method is not applicable for arsenic and selenium. It

is possible to apply direct calibration method for the determination of arsenic and selenium only if the matrices of samples and standards are properly matched.

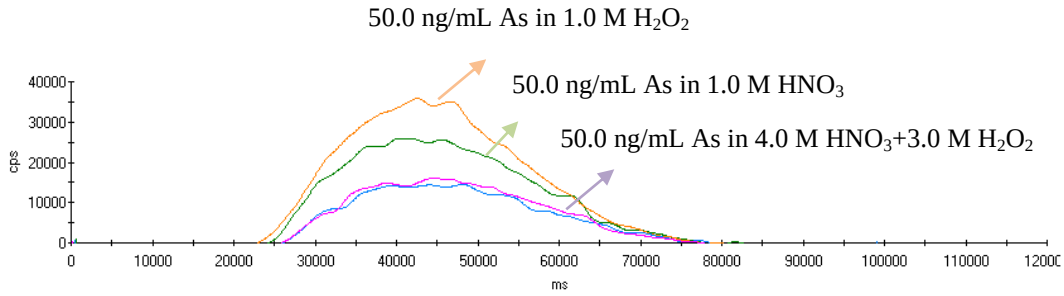

Figure 3.7 Comparison of the signals of 50.0 ng/mL As standards prepared in 1.0 M HNO_3 and 1.0 M H_2O_2 with a matrix matched standard containing 50.0 ng/mL As

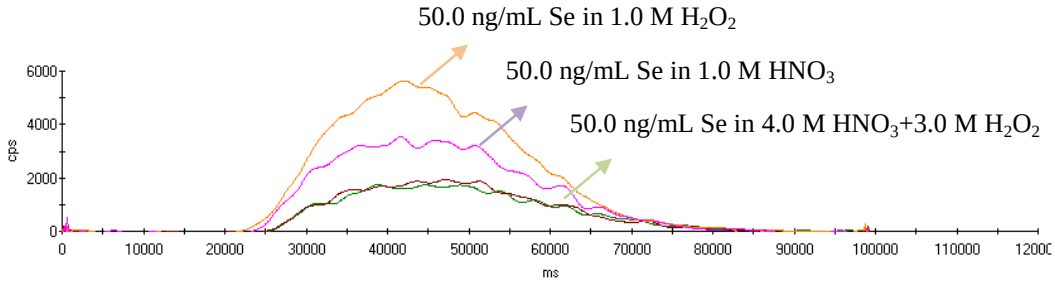

Figure 3.8 Comparison of the signals of 50.0 ng/mL Se standards prepared in 1.0 M HNO_3 and 1.0 M H_2O_2 with a matrix matched standard containing 50.0 ng/mL Se.

To study this situation arsenic and selenium standards prepared in 4.0 M HNO_3 and 3.0 M H_2O_2 were used for the analysis of 1566b Oyster Tissue SRM samples containing the same amount of nitric acid and hydrogen peroxide as a result of digestion and dilution procedures applied. When direct calibration method was used experimental values for As and Se were found to be 7.11±0.02 mg/kg and 2.04 ±

0.03 mg/kg, respectively. The certified value of arsenic in 1566b Oyster Tissue SRM is 7.65 ± 0.65 mg/kg whereas that of selenium is 2.06 ± 0.04 mg/kg.

3.3 Interference Studies for Arsenic

An important part of this study includes interference studies for arsenic. Although argon plasmas are powerful ion sources for mass spectrometry because of their relatively high atomization and ionization efficiencies, in many applications ICP-MS instruments suffer from both spectral and non-spectral interferences. Spectral interferences are caused by molecular or atomic ions having the same nominal mass to charge ratio with the analyte of interest. Especially with ICP-MS instruments equipped with quadruple mass analyzers providing limited resolution, the analyte signals are affected. Non-spectral interferences on the other hand may cause enhancement or suppression on analyte signal and are often referred as matrix effects. They may both be reversible or irreversible. The former is effective as long as the matrix is introduced into the plasma whereas the latter is caused by deposition of matrix salts or oxides at MS interface. This will suppress the signals gradually and may be effective although sample introduction has been terminated. Irreversible effects are well understood, reversible effects are not fully characterized. They probably result from a number of mechanisms including changes in (1) analyte ionization in ICP, (2) sampling processes, (3) interface region, and (4) ion transmission in the mass spectrometer [60].

During interference studies, effects of both spectral and non-spectral interferences on arsenic signals were investigated. Since ICP-MS is capable of monitoring different isotopes of an element at the same time, it is possible to recognize spectral interferences for elements having more than one naturally occurring isotopes.

Disagreement among the results of isotopes indicates a spectral interference problem [60]. **Table 3.4** represents % isotopic abundances of arsenic, cadmium and selenium.

Table 3.4 % Isotopic abundances of arsenic, cadmium and selenium [60]

Cd	Atom %	Se	Atom %	As	Atom %
^{106}Cd	1.25	^{74}Se	0.889	^{75}As	100
^{108}Cd	0.89	^{76}Se	9.366		
^{110}Cd	12.49	^{77}Se	7.635		
^{111}Cd	12.80	^{78}Se	23.772		
^{112}Cd	24.13	^{80}Se	49.607		
^{113}Cd	12.22	^{82}Se	8.731		
^{114}Cd	28.73				
^{116}Cd	7.49				

When **Table 3.4** is investigated it is seen that the isotopes of cadmium, selenium and arsenic having the highest abundances are ^{114}Cd, ^{80}Se and ^{75}As, respectively. During the analysis it is best to monitor the signals of the isotopes having the highest abundances to get the highest integrated count per seconds (ICPS) leading to more sensitive analysis. However this is not possible if the isotope of the analyte with the highest abundance such as ^{114}Cd and ^{80}Se suffers from spectral interferences. The most common spectral interference on $^{80}Se^{+}$ signals arises from the combination of $^{40}Ar^{+}$ ions to form $^{40}Ar^{40}Ar^{+}$ ions having the same m/z ratio with $^{80}Se^{+}$ ions leading to enhancement on signals. A similar situation is observed with $^{78}Se^{+}$ signals since this time $^{38}Ar^{40}Ar^{+}$ ions may be formed. However the abundance of ^{38}Ar is lower than the abundance of ^{40}Ar so the effect is less severe. For ^{112}Cd the possible spectral interferences are $^{96}Mo^{16}O^{+}$ and $^{96}Zr^{16}O^{+}$ ions whereas those for ^{114}Cd are $^{98}Mo^{16}O^{+}$ and $^{97}Mo^{16}O^{1}H^{+}$ ions [62].For selenium and cadmium determination $^{78}Se^{+}$ and $^{111}Cd^{+}$ signals were monitored, respectively since there are spectral interferences on $^{112}Cd^{+}$ signals too. However for As having only a single isotope, this method is not applicable. One of the most common spectral interference on $^{75}As^{+}$ signals arises

from the molecular ion formed by the combination of argon and chlorine ions of which the pre-cursers are carrier gases and sample matrix, respectively.

Relative isotopic abundances of argon and chlorine are listed in **Table 3.5**.

Table 3.5 Relative isotopic abundances of argon and chlorine [60]

Ar isotopes	Atom %	Cl isotopes	Atom %
^{36}Ar	0.34	^{35}Cl	75.5
^{38}Ar	0.06	^{37}Cl	24.5
^{40}Ar	99.6		

Under plasma conditions $^{40}Ar^{+}$ and $^{35}Cl^{+}$ ions may come together and form the molecular ion $^{40}Ar^{35}Cl^{+}$ which may overlap with the analytical signal of $^{75}As^{+}$ leading to enhancement on the signal. However ICP-MS is capable of correcting arsenic signals with respect to the following simple equations.$^{75}As^{+}$ denotes the signal intensity of this species; the other ion configurations are used in a similar meaning.

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

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

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

According to these equations first $^{82}Se^{+}$ signals are corrected with respect to $^{83}Kr^{+}$ signals. Then $^{40}Ar^{37}Cl^{+}$ signals are corrected with respect to the corrected $^{82}Se^{+}$ signals by using the ratio between the abundance ratios of ^{77}Se and ^{82}Se which is 0.860. Finally the corrected $^{40}Ar^{37}Cl^{+}$ signals are multiplied by the ratio between the relative abundances of ^{35}Cl and ^{37}Cl which is 3.12. The corrected $^{75}As^{+}$ signals are

obtained by subtracting this value from the enhanced experimental value. This simple correction which is automatically done by the instrument is optional. It is possible to set the instrument so that no correction is made.

It is possible to check the validity of the corrections made by the instrument software. To do this 20.0 ng/mL arsenic sample containing 200.0 ng/mL selenium and 0.2 M LiCl as chlorine source was prepared and analyzed both with and without correction.

Arsenic signals were corrected theoretically by using the peak areas of uncorrected signals in equations. Then the results were compared with arsenic signals corrected automatically by the instrument software. The results are seen in **Table 3.6** below.

Table 3.6 Corrected and uncorrected peak areas of $^{75}As^{+}$, $^{77}Se^{+}$ and $^{82}Se^{+}$ signals

	Peak Area (Uncorrected)	**Peak Areas (Corrected)**
$^{75}\mathbf{As}^{+}$	74931	52461
$^{77}\mathbf{Se}^{+}$	11836	10723
$^{82}\mathbf{Se}^{+}$	6649	6171

The peak areas given in the second column of **Table 3.6** belong to uncorrected signals and were obtained by taking the average of two results. The signals are corrected by calculations starting from $^{82}Se^{+}$ to $^{75}As^{+}$ as explained below.

$$^{77}ArCl^{+}_{(corrected)} = 11836 - 0.860 \times 6649$$

$$^{77}ArCl^{+}_{(corrected)} = 6118$$

$$^{75}As^{+}_{(corrected)} = 74931 - 3.12 \times 6118$$

$$^{75}As^{+}_{(corrected)} = 55843$$

When t-test was applied to the mean peak areas of mathematically and automatically corrected arsenic signals which are 55843 and 52461 no systematic error was found at 95% confidence interval indicating that the corrections done by the instrument are reliable.

In the first part of this study spectral interference of $^{40}Ar^{35}Cl^{+}$ on $^{75}As^{+}$ signals was investigated by monitoring $^{40}Ar^{37}Cl^{+}$ signals during arsenic determination with and without corrections. By comparing the results it is possible to observe how $^{75}As^{+}$ signals are affected by the presence of chlorine originating from sample matrix.

The second part of this study includes matrix induced signal intensity changes for arsenic. In general it is known that high concentrations of matrix elements such as high levels of salts and other dissolved solids cause suppression or enhancement on analyte signals because of three main reasons: (1) ionization effect, (2) space charge effect and (3) salt build-up at sampling or skimmer cones. It is stated that heavy matrix elements cause most severe matrix interferences and light analytes are more seriously affected than heavier ones. Furthermore effects of matrix elements having low ionization potentials are more pronounced [62].

With this knowledge, in order to investigate non-spectral interferences of matrix elements on arsenic signals, salts of various group 1A elements having low ionization potentials were prepared at various concentrations since it is known that

matrix effect depends more on the absolute concentration of the matrix element rather than the relative concentration of matrix element to analyte.

The types of salts used are LiCl, $LiNO_3$, NaCl, $NaNO_3$, CsCl and $CsNO_3.3H_2O$. The reason why group 1A elements were used as matrix elements is not only their low ionization potentials but also to investigate the trend among these elements and the nature of space charge effects which will be discussed later. In addition, the effect of HCl as a source of chlorine was also investigated.

3.3.1 Interference Effect of HCl

3.3.1.1 Effect of HCl with Software Correction

The aim of using HCl at various concentrations is to study the spectral interference of chlorine present in sample matrix on arsenic signals. Chloride salts of each matrix element were prepared because of similar reasons. By this way it is possible to investigate the non-spectral interference of the matrix element and the spectral interference of chlorine at the same time and to see their overall effect on arsenic signals. On the other hand since it is known that nitrate has no spectral or non-spectral interference on arsenic signals, nitrate salts allows one to investigate only non-spectral interferences of matrix elements.

Polyatomic ion interferences are one of the most important limiting features of ICP-MS especially for analytes having atomic masses below 80 amu. The polyatomic ion

$^{40}Ar^{35}Cl^{+}$ is a well known problem in mono-isotropic As determination since it overlaps with analyte the signal leading to incorrect results due to enhancement [63].

There are a number of approaches to overcome this problem when analyzing samples with high chloride content such as mathematical corrections based on $^{40}Ar^{37}Cl^{+}$, co-precipitation of chloride with silver, chromatographic separation of argon and chlorine or hydride generation ICP-MS [63]. In this study $^{40}Ar^{37}Cl^{+}$ signals were monitored in the presence of HCl in sample matrix and the effect of mathematical corrections on $^{75}As^{+}$ signals were investigated.

It was observed that $^{40}Ar^{37}Cl^{+}$ signals increase, as HCl concentration in sample matrix is increased. The results are shown in **Figure 3.9**. All the solutions tested contained 20.0 ng/mL As. HCl concentrations were increased from 0.025 to 0.4 M. The enhancement effect on $^{75}As^{+}$ signals is due to the overlapping of $^{40}Ar^{35}Cl^{+}$ signals which are 3.12 times greater than $^{40}Ar^{37}Cl^{+}$ signals, with $^{75}As^{+}$ signals.

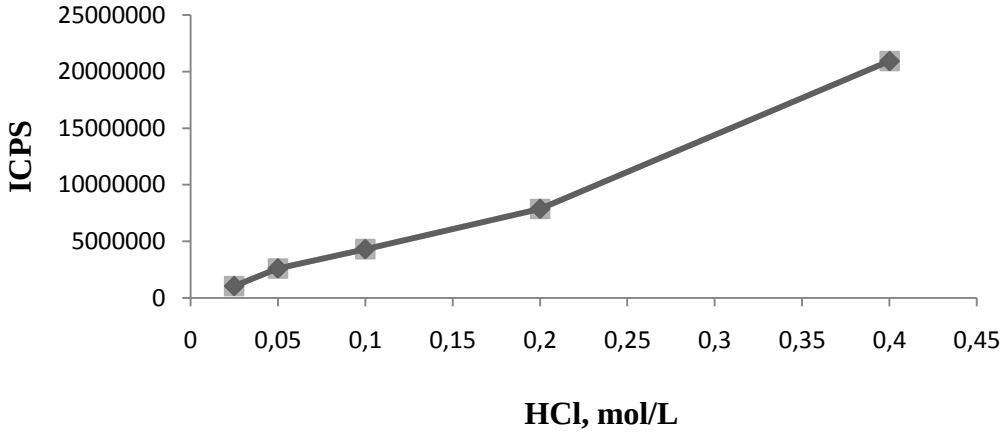

Figure 3.9 Effect of HCl concentration on $^{40}Ar^{37}Cl^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

$^{75}As^{+}$ signals at various HCl concentrations are shown in **Figure 3.10** when the instrument software correction was used. Although HCl concentration is increased up to 0.40 M arsenic signals are not affected by the presence of high chlorine content in the sample matrix. This indicates that mathematical corrections done by the instrument software are applicable for determination of arsenic in chlorine containing matrices. The first result at zero point belongs to 20.0 ng/mL As solution in H_2O.

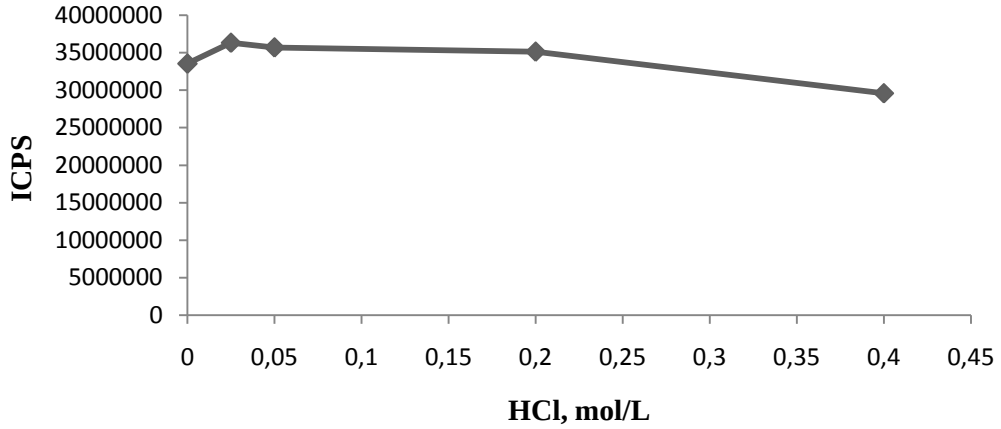

Figure 3.10 Effect of HCl concentration on $^{75}As^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

3.3.1.2 Effect of HCl without Software Correction

The effect of HCl on $^{77}ArCl^{+}$ signals is similar to that on corrected signals shown in **Figure 3.10.**

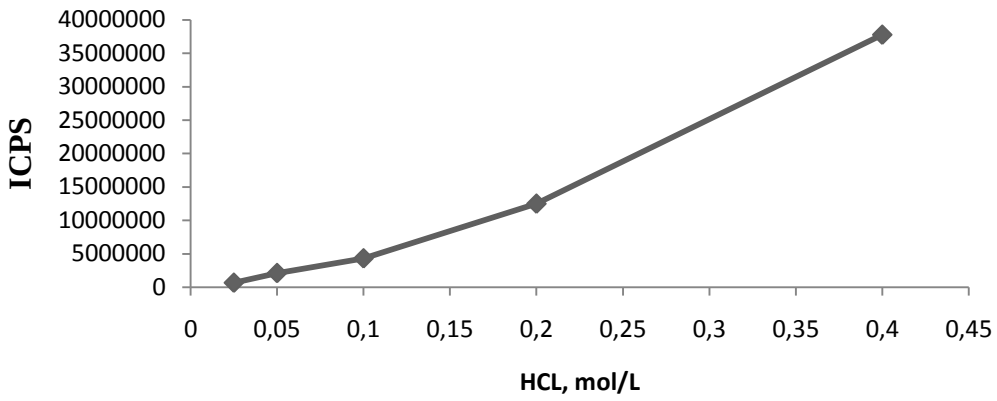

Figure 3.11 Effect of HCl concentration on $^{40}Ar^{37}Cl^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

However uncorrected arsenic signals are quite different than corrected arsenic signals. **Figure 3.12** illustrates clearly how spectral overlap of $^{75}ArCl^{+}$ signals causes positive error on $^{75}As^{+}$ signals in the presence of chlorine in sample matrix.

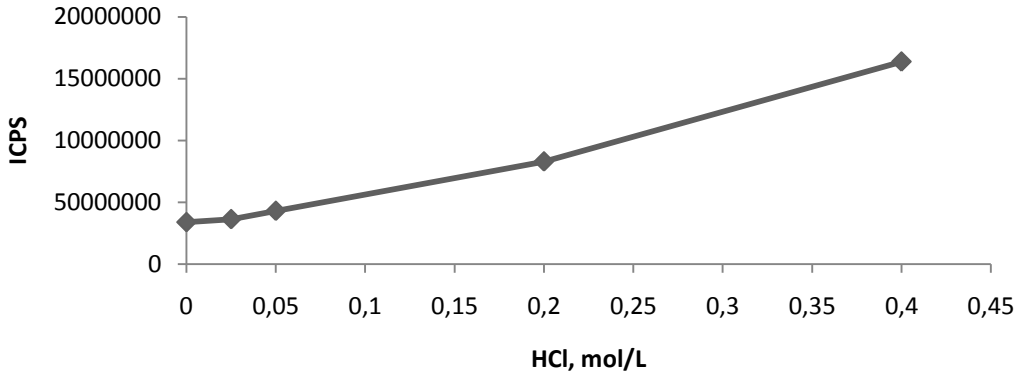

Figure 3.12 Effect of HCl concentration on $^{75}As^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

Analysis with HCl containing matrices showed that chlorine can cause positive error on $^{75}As^{+}$ signals due to the formation of $^{40}Ar^{35}Cl^{+}$ ion and the overlapping of this ion

with $^{75}As^{+}$ signals. Without software corrections even 0.025 M HCl in sample matrix causes positive error on $^{75}As^{+}$ signals.

3.3.2 Interference Effects of NaCl and $NaNO_3$

3.3.2.1 Effect of NaCl with Software Correction

Since the introduction of ICP-MS technique, both spectral and non-spectral interferences caused by NaCl matrix have been observed by many users. The most common spectral interferences arising from Na are $^{23}Na^{23}Na^{+}$, $^{23}Na^{23}Na^{1}H^{+}$, $^{23}Na^{23}Na^{16}O^{+}$, $^{40}Ar^{23}Na^{+}$ and $^{40}Ar^{23}Na^{16}O^{+}$ which cause positive error on $^{46}Ti^{+}$, $^{47}Ti^{+}$, $^{62}Ni^{+}$, $^{63}Cu^{+}$ and $^{79}Br^{+}$ signals respectively. For ^{75}As determination any spectral interference arising from sodium ion is not expected [64].

The change of $^{40}Ar^{37}Cl^{+}$ signals at different NaCl concentrations is shown in **Figure 3.13**. As NaCl concentration is increased $^{40}Ar^{37}Cl^{+}$ signals also increase as expected conforming the spectral interference caused by chlorine.

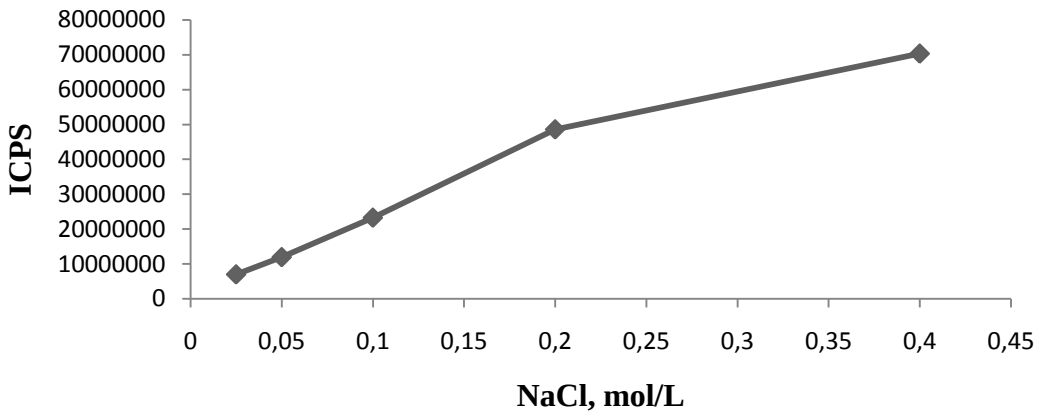

Figure 3.13 Effect of NaCl concentration on $^{40}Ar^{37}Cl^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

Corrected $^{75}As^{+}$ signals at various NaCl concentrations are seen in **Figure 3.14**. As seen in the figure, enhancement on $^{40}Ar^{37}Cl^{+}$ signals was observed due to the presence of chlorine. Since the abundance ratio of ^{35}Cl to ^{37}Cl is 3.12 the expected situation is almost three times higher for enhancement on $^{75}As^{+}$ signals. However it was observed that as concentration of NaCl increases $^{75}As^{+}$ signals decrease. The suppression can be attributed to non-spectral interference of sodium ions. Easily ionizable elements such as sodium significantly increase the electron density in the plasma medium. If plasma temperature is not increased, a decrease in the degree of ionization of the analyte occurs due to enhanced ion-electron re-combinations known as ionization effect. Elements having high ionization potentials are affected more severely. Furthermore increasing concentrations of NaCl change viscosity, density, surface tension, vapor pressure and evaporation rate of the sample solution which may cause local variations in plasma temperature and hence in analyte ionization during vaporization [65].

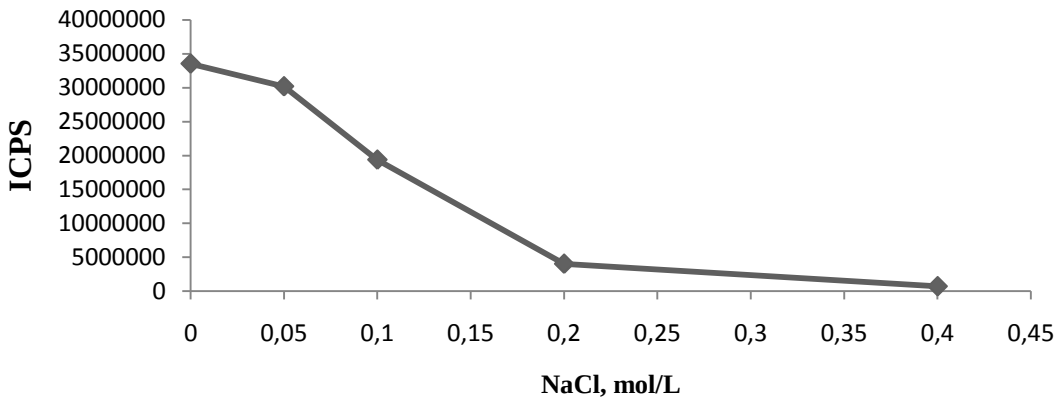

Figure 3.14 Effect of NaCl concentration on $^{75}As^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

It is known that the most easily ionized element in a salt dominates the matrix ion mass spectrum observed from ICP in the following order;

Na > Mg > I > Br > Cl

Under plasma conditions the ion population of Na is 98% whereas the ion population of As is 16%. In such an electron rich plasma medium, the degree of ionization of As decreases causing suppression on the signals [66].

3.3.2.2 Effect of NaCl without Software Correction

$^{77}ArCl^{+}$ signals when the instrument does no correction are seen in **Figure 3.15**. $^{77}ArCl^{+}$ signals increase as NaCl concentration is increased, regardless of correction.

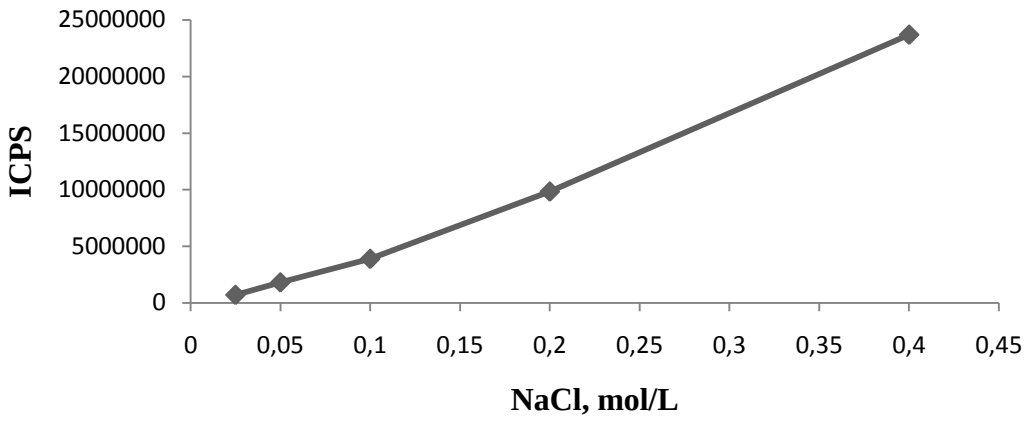

Figure 3.15 Effect of NaCl concentration on $^{40}Ar^{37}Cl^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

Although both corrected and uncorrected $^{40}Ar^{37}Cl^{+}$ signals are similar at various NaCl concentrations due to the spectral enhancement effect of chlorine when corrected and uncorrected, when $^{75}As^{+}$ signals were compared drastic differences were observed. As seen in **Figure 3.16** varying concentrations of NaCl have almost no effect on $^{75}As^{+}$ signals when the instrument software correction was not used. This is due to the fact that suppression effect of sodium is compensated by the enhancement effect of ^{35}Cl which combines with ^{40}Ar and later overlaps with $^{75}As^{+}$ signals. As a result no significant change was observed in arsenic signals except for 0.040 M NaCl solution of which the signals are relatively lower. This may be an indication of the limit of tolerance of ICP-MS to dissolved solids. The fluctuations in signals are due to the change of the stability of the plasma when exposed to dissolved solids. When **Figure 3.16** is compared with **Figure 3.14**, it is possible to observe the effect of instrument software correction and spectral interference of chlorine on arsenic signals.

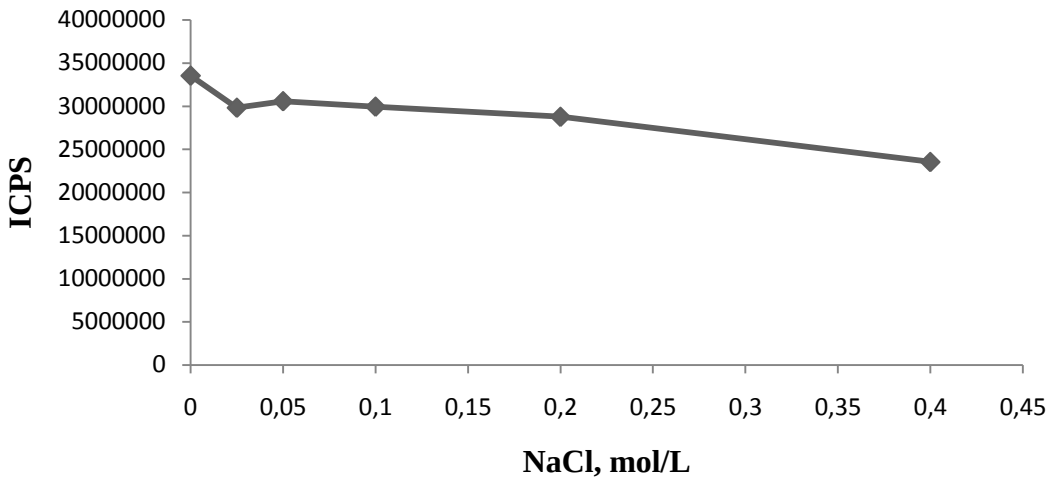

Figure 3.16 Effect of NaCl concentration on $^{75}As^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

According to the analysis with NaCl containing matrices, Na has suppression effect on $^{75}As^{+}$ signals even at low dissolved solid concentrations. Suppressions effect is less severe without instrument software correction. It is suggested that during analysis with ICP-MS dissolved solid levels must be controlled carefully and must not exceed typically 0.2% [25] Our findings in this study support this information.

3.3.2.3 Effect of $NaNO_3$ with Software Correction

As expected no $^{40}Ar^{37}Cl^{+}$ signal were observed in the presence of $NaNO_3$. When compared with increasing signals at various HCl concentrations this situation indicated that HCl in the sample matrix is the source of chlorine causing spectral overlap on $^{75}As^{+}$ signals.

Sodium is a well known problematic element which is commonly found in many sample matrices at high concentrations. As seen in **Figure 3.17,** sodium has suppression effect on $^{75}As^{+}$ signals. The effect depends on the absolute concentration of sodium in the sample matrix. The decrease in $^{75}As^{+}$ signals at increasing $NaNO_3$ concentrations indicates non-spectral interference of sodium on arsenic signals.

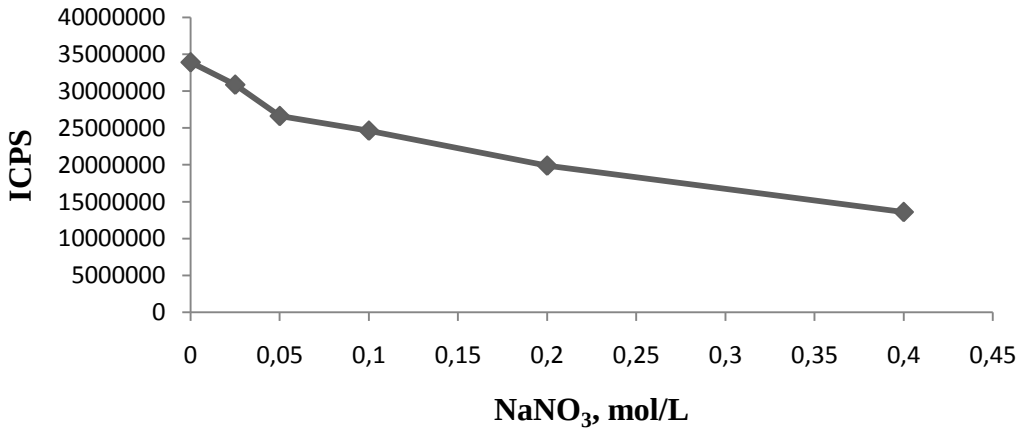

Figure 3.17 Effect of $NaNO_3$ concentration on $^{75}As^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

It is shown that in a matrix containing high concentrations of $NaNO_3$, software correction is not useful as the interference is not spectral.

3.3.2.4 Effect of $NaNO_3$ without Software Correction

The profile of uncorrected $^{40}Ar^{37}Cl^{+}$ signals is similar to that of corrected ones as expected since sample matrix including $NaNO_3$ is not a source of chlorine.

Similar to $^{40}Ar^{37}Cl^{+}$ signals corrected and uncorrected arsenic signals at various $NaNO_3$ concentrations are also similar to each other since the correction is done with

respect to $^{40}Ar^{37}Cl^{+}$ signals and there exists no $^{40}Ar^{37}Cl^{+}$ signals which may overlap with arsenic signals. As seen in **Figure 3.18** when no correction is done arsenic signals decrease at increasing $NaNO_3$ concentrations due the suppression effect of sodium.

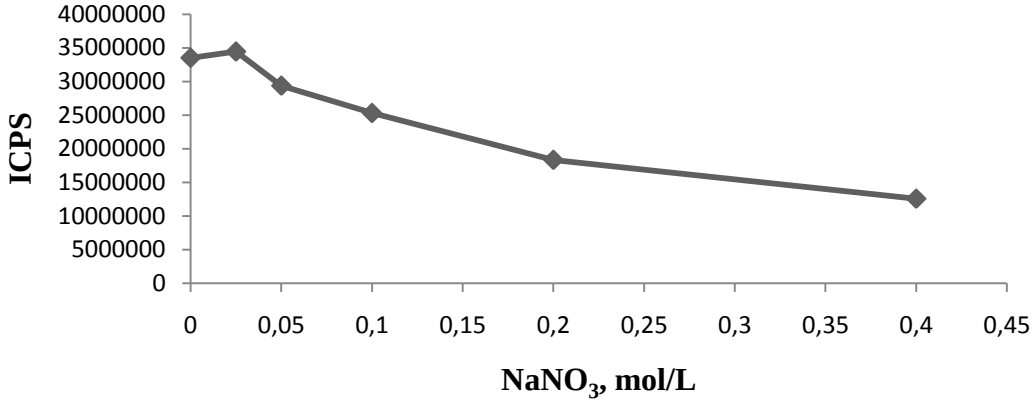

Figure 3.18 Effect of $NaNO_3$ concentration on $^{75}As^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

It is concluded that, as expected, software correction did not eliminate nonspectral interference by Na^{+} species.

3.3.3. Interference Effect of CsCl and $CsNO_3$

3.3.3.1 Effect of CsCl with Software Correction

The effect of sample matrices including CsCl on $^{75}As^{+}$ signals is expected to be similar to those containing NaCl at various concentrations since Cs and Na belong to the same group of elements in the periodic table. However $^{75}As^{+}$ signals are suppressed in the presence of CsCl in sample matrix and the suppression effect increases as CsCl concentration is increased and $^{40}Ar^{37}Cl^{+}$ signals obtained in the presence of CsCl are quite different than expected indicating some factors other than ionization effect. The signals are shown in **Figure 3.19**.

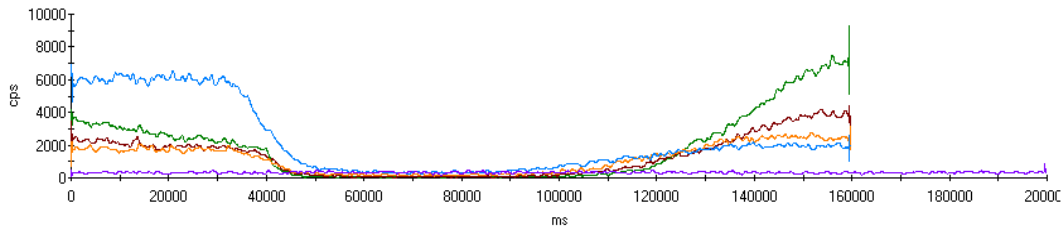

Figure 3.19 Effect of CsCl concentration on $^{40}Ar^{37}Cl^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

As seen in **Figure 3.19** $^{40}Ar^{37}Cl^{+}$ signals decrease and lose peak behavior in the presence of CsCl and as shown in **Figure 3.20** $^{75}As^{+}$ signals are suppressed at increasing CsCl concentrations.

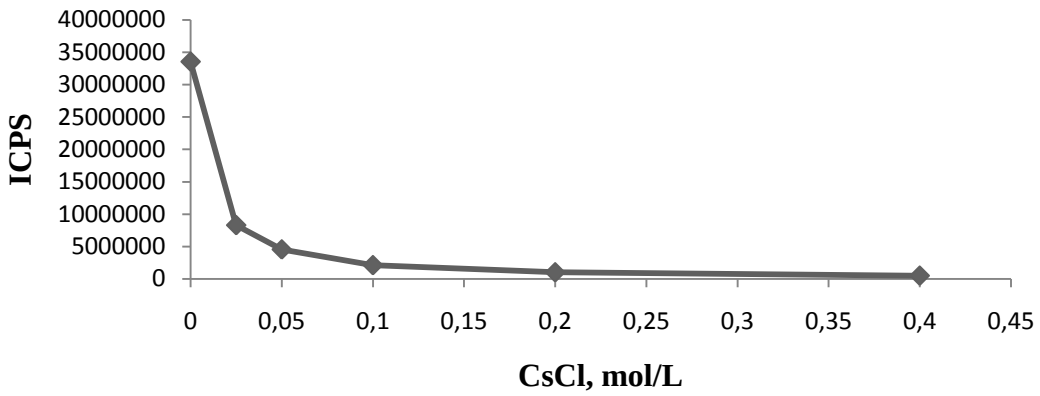

Figure 3.20 Effect of CsCl concentration on $^{75}As^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

The decrease in $^{40}Ar^{37}Cl^{+}$ signals does not indicate that this molecular ion is not formed although there is plenty of chlorine in the sample matrix. In fact there may be some factors similar to the ones causing suppression on $^{75}As^{+}$ signals which suppress also $^{40}Ar^{37}Cl^{+}$ signals. When $^{40}Ar^{37}Cl^{+}$ signals are investigated it is seen that the suppression is effective as long as the sample matrix is introduced into the plasma. In this study flow injection technique is used during sample introduction and it is possible to see the loop behavior in **Figure 3.19**. This shows that the suppression is caused by the sample matrix however Cs must have some other effects different than Na and Li causing such effective suppressions even on $^{40}Ar^{37}Cl^{+}$ signals.

The ionization potential of Cs (3.894 eV) is lower than that of Na (5.139 eV) and Li (5.392 eV) due to greater atomic radius. It can easily shift the ionization equilibrium of the analyte causing a decrease in the degree of ionization of As leading to greater suppression effect compared to Na and Li.

It is stated by some researchers that varying Na concentrations have the same effect on different analytes regardless of the atomic mass of the analyte whereas Cs matrix affects each analyte element differently depending on the atomic mass. This shows

that ionization effect is not the only reason of the drastic suppression on analyte signals [67].

There are two possible explanations for these results. The first one is ambipolar diffusion. The mechanism is such that the presence of a high mass easily ionizable element such as Cs in the plasma produces an electric field due to the diffusion of electrons at a greater rate than positively charged ions out of the central channel. Electric field results in the diffusion of the analyte ions, especially the ones with low atomic masses, towards the annular region of the plasma. As a result the number of ions sampled from the central region decreases leading to suppression on analyte signal [60].

Space charge effect also plays an important role in matrix induced suppressions.

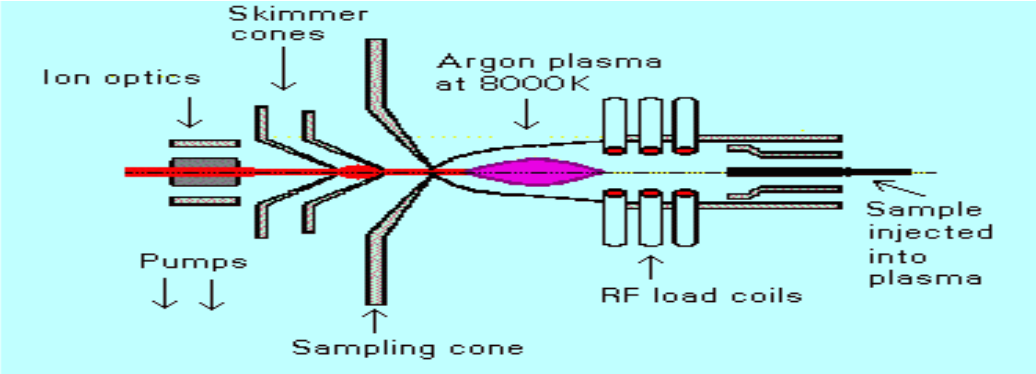

Figure 3.21 Schematic diagram of ICP-MS

Space charge is caused by excess of positively charged ions in the beam extracted from ICP. The repulsion among these ions spreads the beam radially leading to defocusing of the ion flow and loss in the ion transmission efficiency. Under continuum flows all ions travel at the speed of gas flow. The ion kinetic energy

depends on the ion mass (KE =m 1/2.v^2). That is why light analytes with low kinetic energies are defocused more easily than heavier analytes [67].

ICP is electrically neutral since positively charged ions are balanced by equivalent number of electrons so no space charge is developed between sampler and skimmer cones. However as the ion beam passes through the skimmer orifice pressure and density drop in the high vacuum stage leading to increase in the mobility of electrons which are much lighter than positively charged ions. As a result electrons diffuse leaving the positively charged ions behind in the flow which is not neutral anymore. Furthermore ion optics is designed to focus only positively charged ions and to repel electrons. The resulting beam rich in positively charged ions exhibit space charge repulsions. This indicates that the composition of the beam affects ion transmission efficiency and sensitivity for particular analytes [68, 69].

Space charge effect explains why Cs in sample matrix suppresses $^{40}Ar^{37}Cl^+$ signals and why the suppression effect on arsenic is much more effective than those of Li and Na. At low matrix concentrations ion trajectories are dominated by Ar^+ whereas at high matrix concentrations heavy matrix elements which exhibit space charge repulsions dominate the trajectories [67]. Cs having an atomic mass of 132.9054 amu which is greater than those of both $^{75}As^+$ and $^{40}Ar^{37}Cl^+$ affects the transmission efficiencies of these ions. Since the atomic weights of both Na (22.990 amu) and Li (6.939 amu) are lower than those of arsenic and ArCl they cannot exhibit space charge effects and that is why their suppression effects are much lower than that of cesium.

3.3.3.2 Effect of CsCl without Software Correction

The corrected and uncorrected $^{40}Ar^{37}Cl^{+}$ signals at various CsCl concentrations are similar. As a result the corrected and uncorrected $^{75}As^{+}$ signals are also similar since the correction is done with respect to $^{40}Ar^{37}Cl^{+}$ signals. Different from NaCl and LiCl containing matrices, in the presence of CsCl $^{40}Ar^{37}Cl^{+}$ signals are suppressed so much that the suppression effect on $^{75}As^{+}$ signals cannot be compensated by the enhancement effect of $^{40}Ar^{37}Cl^{+}$ so correction is meaningless in this situation as seen in **Figure 3.22.**

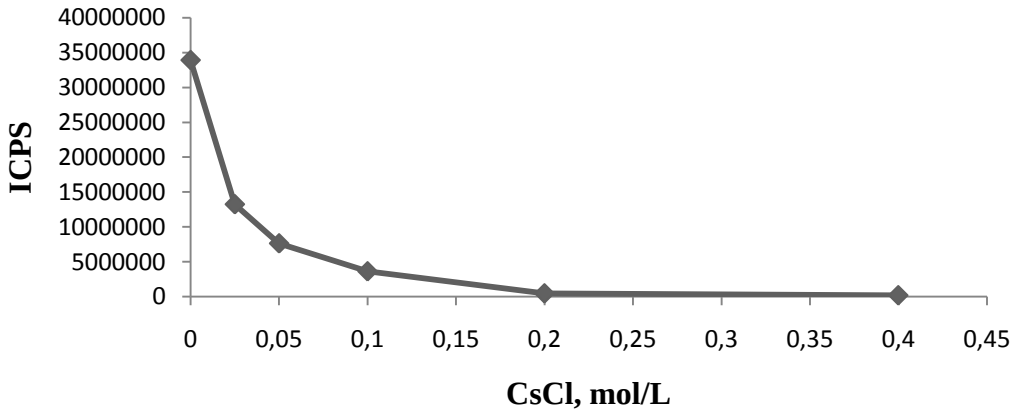

Figure 3.22 Effect of CsCl concentration on $^{75}As^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

3.3.3.3 Effect of $CsNO_3$ with Software Correction

To see the effect of non-spectral interference of Cs on arsenic signals, it is possible to use nitrate salts. So $CsNO_3.3H_2O$ was used at increasing concentrations into arsenic standards and both $^{40}Ar^{37}Cl^{+}$ and $^{75}As^{+}$ signals were monitored. However, no signals

were observed when the system does correction. This situation is observed when the system does overcorrection. Overcorrection is observed when the element used for isobaric correction forms monoxide or hydroxide ions [70]. However, in such a situation uncorrected arsenic signals observed in the presence of $CsNO_3$ is enough to investigate the effect of cesium when the system does correction since correction was proved to be meaningless when nitrate salts are used.

3.3.3.4 Effect of $CsNO_3$ without Software Correction

Uncorrected $^{40}Ar^{37}Cl^{+}$ and $^{75}As^{+}$ signals are seen in **Figure 3.23** and **Figure 3.24**, respectively. Similar to CsCl, in the presence of $CsNO_3$ $^{75}As^{+}$ signals are suppressed so much that they lose peak behavior as long as $CsNO_3$ is introduced into the plasma. The peak in **Figure 3.24** belongs to 20.0 ng/mL As standard containing no $CsNO_3$.

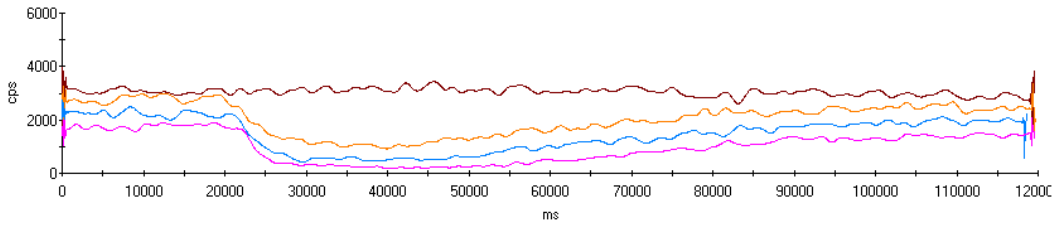

Figure 3.23 Effect of $CsNO_3$ concentration on $^{40}Ar^{37}Cl^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

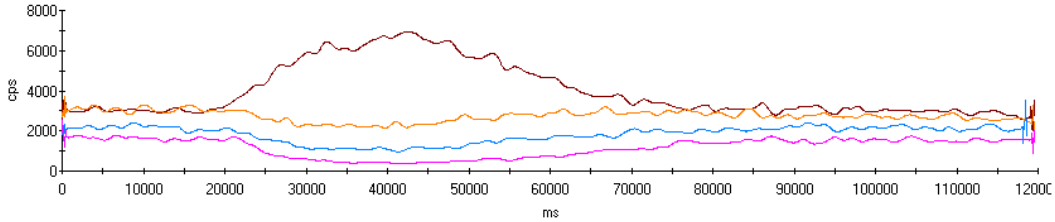

Figure 3.24 Effect of $CsNO_3$ concentration on $^{75}As^+$ signals without software correction; solutions contain 20.0 ng/mL As

3.3.4 Interference Effect of LiCl and $LiNO_3$

3.3.4.1 Effect of LiCl with Software Correction

As a matrix element Li is not as common as sodium. However since one of the aims of this study is to investigate how easily ionizable elements in the sample matrix can cause non-spectral interferences and if there exists a trend among the group 1A elements, effect of LiCl on arsenic signals should also be investigated.

$^{40}Ar^{37}Cl^+$ signals at increasing LiCl concentrations are shown in **Figure 3.25**. Similar to NaCl and CsCl, LiCl in sample matrix enhances $^{40}Ar^{37}Cl^+$ signals.

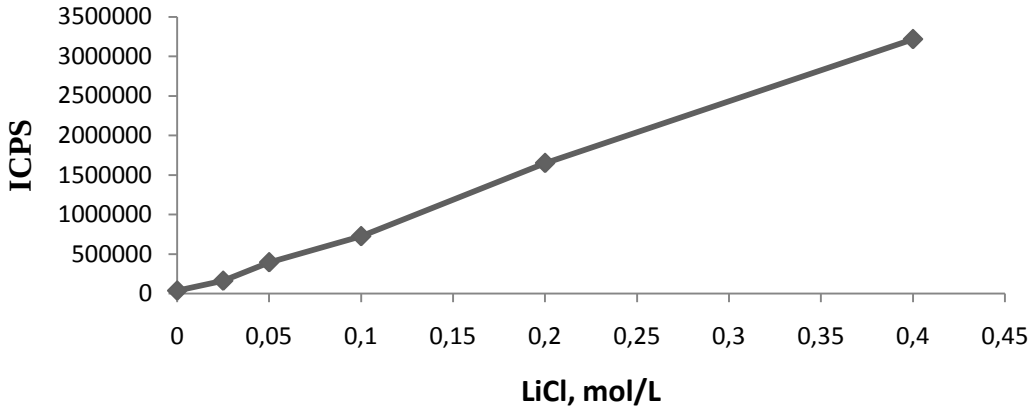

Figure 3.25 Effect of LiCl concentration on $^{40}Ar^{37}Cl^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

The effect of LiCl on $^{75}As^{+}$ signals is seen in **Figure. 3.26** The decreasing signals at increasing LiCl concentrations indicate the suppression effect of Li present in sample matrix due to ionization effects.

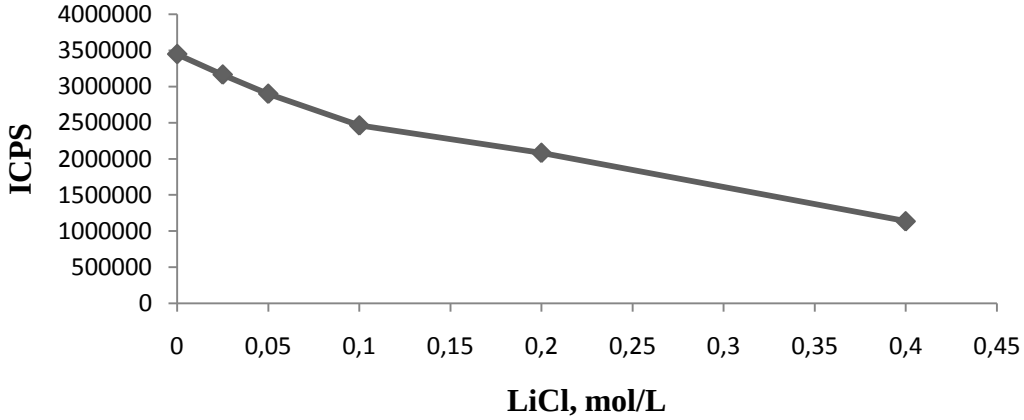

Figure 3.26 Effect of LiCl concentration on $^{75}As^{+}$ signals with software correction; solutions contain 20.0 ng/mL As

3.3.4.2 Effect of LiCl without Software Correction

$^{40}Ar^{37}Cl^{+}$ signals are enhanced due to the presence of chlorine in LiCl containing matrices when the instrument does no mathematical corrections. This is shown in **Figure 3.27**.

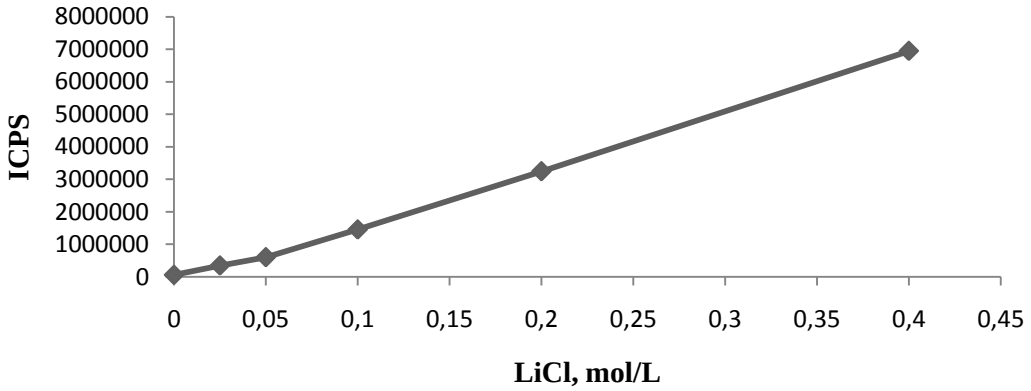

Figure 3.27 Effect of LiCl concentration on $^{40}Ar^{37}Cl^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

However $^{75}As^{+}$ signals obtained in the presence of LiCl in sample matrix are quite different than those obtained from NaCl containing matrices. As seen in **Figure 3.27** arsenic signals increase as LiCl concentration is increased when the instrument correction software was not used to correct arsenic signals to overcome the spectral overlap of $^{40}Ar^{35}Cl^{+}$ and therefore there is positive error. When compared with **Figure 3.17**, **Figure 3.28** .indicates that the enhancement effect of $^{40}Ar^{35}Cl^{+}$ cannot be compensated by the suppression effect of Li whereas at the same chloride concentrations Na suppresses arsenic signals so much that the enhancement effect of $^{75}ArCl^{+}$ is overcome. Suppression effect of Na is higher than that of Li; ionization potentials of Na and Li are 5.139 eV and 5.392 eV, respectively.

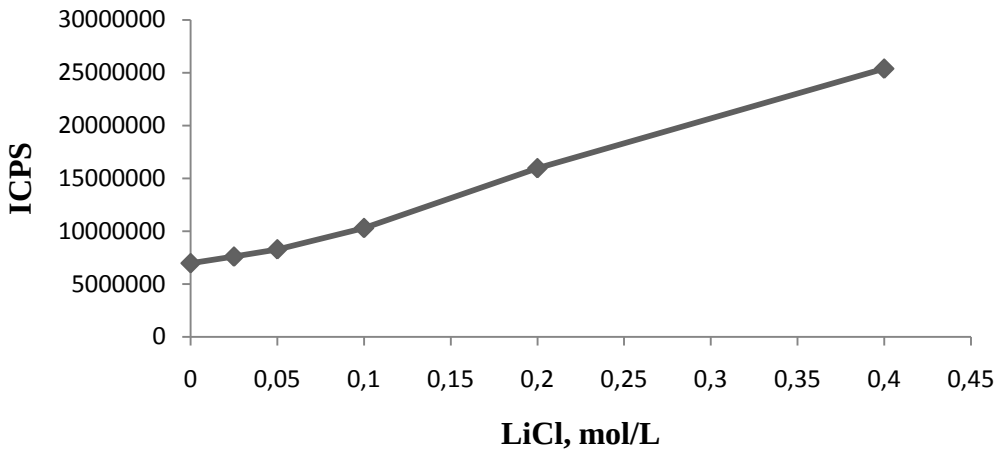

Figure 3.28 Effect of LiCl on concentration $^{75}As^{+}$ signals without software correction; solutions contain 20.0 ng/mL As

This is an expected result when ionization effect is taken into consideration. The ionization potentials and ion populations of Li, Na, Cs and As at several plasma temperatures are compared in the **Table 1.1** [66].

Among the four elements listed above arsenic has the first highest ionization potential so under plasma conditions ionization population of arsenic ions is low when compared to the other matrix elements. Since the ionization potential of Na is lower than that of Li it ionizes easily and forms greater number of electrons in the plasma medium. According to Le Chatelier's principle the ionization equilibrium is shifted more leading to a greater decrease in the degree of ionization of arsenic. This explains why the suppression effect of Na is more effective than that of Li. On the other hand Cs which has the lowest ionization population and so the highest ion population suppresses arsenic signals much more than Na. Furthermore Li and Na cannot exhibit space charge effects on As since their atomic masses are lower whereas Cs can do.

3.3.4.3 Effect of $LiNO_3$ with Software Correction

Similar to $NaNO_3$, in the presence of $LiNO_3$, no $^{40}Ar^{37}Cl^+$ signals were obtained. Corrected $^{75}As^+$ signals decrease as $LiNO_3$ concentration in the sample matrix is increased as seen in **Figure 3.29**. The only difference between **Figure 3.28** and **Figure 3.29** is that the suppression effect of $NaNO_3$ is more effective than that of $LiNO_3$ due to stronger ionization effects.

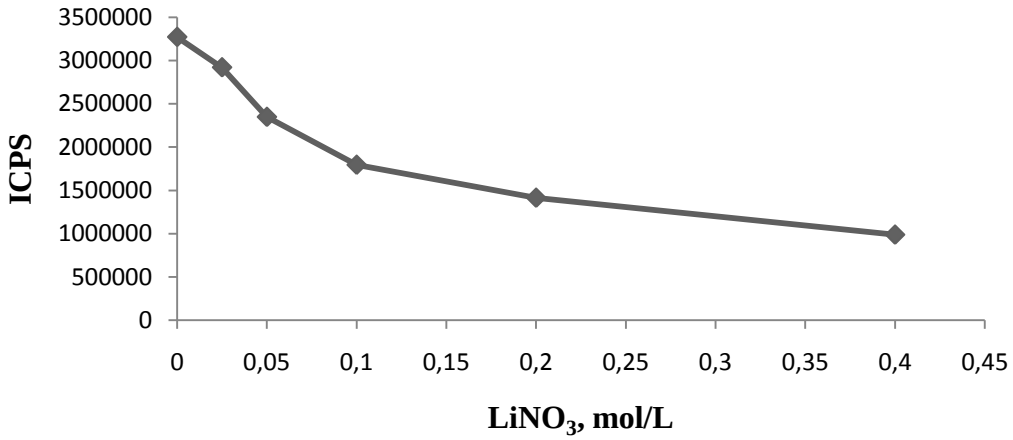

Figure 3.29 Effect of $LiNO_3$ concentration on $^{75}As^+$ signals with software correction; solutions contain 20.0 ng/mL As

3.3.4.4 Effect of $LiNO_3$ without Software Correction

Both corrected $^{40}Ar^{37}Cl^+$ and $^{75}As^+$ signals are similar to uncorrected $^{40}Ar^{37}Cl^+$ and $^{75}As^+$ signals. $^{40}Ar^{37}Cl^+$ signals are not affected by the presence of $LiNO_3$ in the sample matrix whereas $^{75}As^+$ signals are suppressed due to ionization effects.

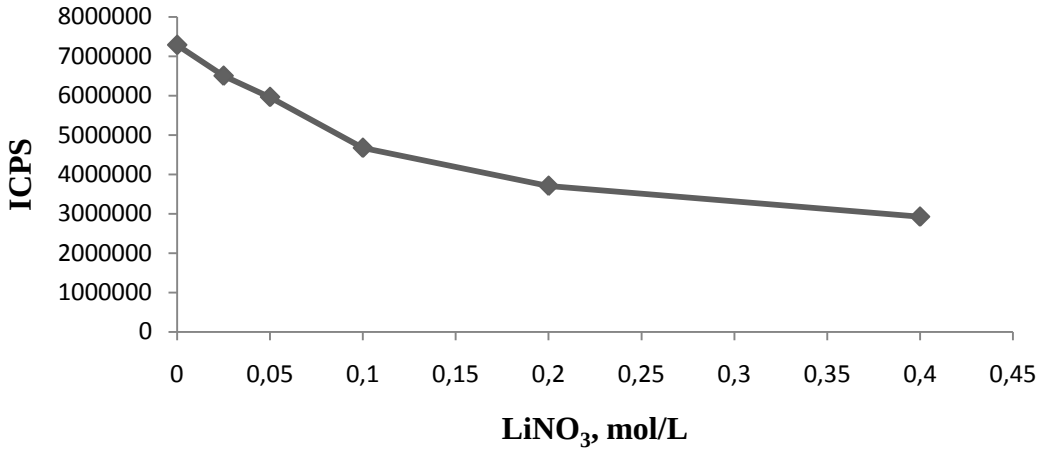

Figure 3.30 Effect of $LiNO_3$ on $^{75}As^+$ signals without software correction; solutions contain 20.0 ng/mL As

The effects of HCl, NaCl, $NaNO_3$, CsCl, $CsNO_3$, LiCl and $LiNO_3$ on $^{75}As^+$ and $^{40}Ar^{37}Cl^+$ signals are summarized in **Table 3.7**.

Table 3.7 The effects of HCl, NaCl, $NaNO_3$, CsCl, $CsNO_3$, LiCl and $LiNO_3$ on $^{75}As^+$ and $^{40}Ar^{37}Cl^+$ signals

	$^{40}Ar^{37}Cl^+$		$^{75}As^+$	
	With software correction	Without software correction	With software correction	Without software correction
HCl	Enhancement	Enhancement	No Effect	Enhancement
NaCl	Enhancement	Enhancement	Suppression	No Effect
$NaNO_3$	No Effect	No Effect	Suppression	Suppression
CsCl	Suppression	Suppression	Suppression	Suppression
$CsNO_3$	No Effect	No Effect	Suppression	Suppression
LiCl	Enhancement	Enhancement	Suppression	Enhancement
$LiNO_3$	No Effect	No Effect	Suppression	Suppression

During the analysis of spice samples not only $^{75}As^+$ signals but also $^{40}Ar^{37}Cl^+$ signals were monitored. For none of the samples which gives arsenic signals $^{40}Ar^{37}Cl^+$

signals were observed indicating that chlorine content of spices are sufficiently low to cause spectral overlaps. Furthermore the highest total concentration of Group 1A elements including Na, K and Li in arsenic containing spice samples was found to be less than $1.0x10^{-4}$ M. This value is quite lower than the lowest concentration of matrix elements investigated in this study. This shows that the analyte undertaken in this study is free of spectral and non-spectral interferences.

3.4 Analytical Figures of Merit for Cd, As and Se

In order to develop a suitable method for Cd determination by ICP-MS. Cd standards having concentrations of 0.50, 1.0, 5.0, 10.0, 20.0, 50.0, 100.0 ng/mL prepared in 1.0 M HNO_3 were analyzed by using a flow injection system for sample introduction. The linear calibration plot obtained is shown in **Figure 3.31**.

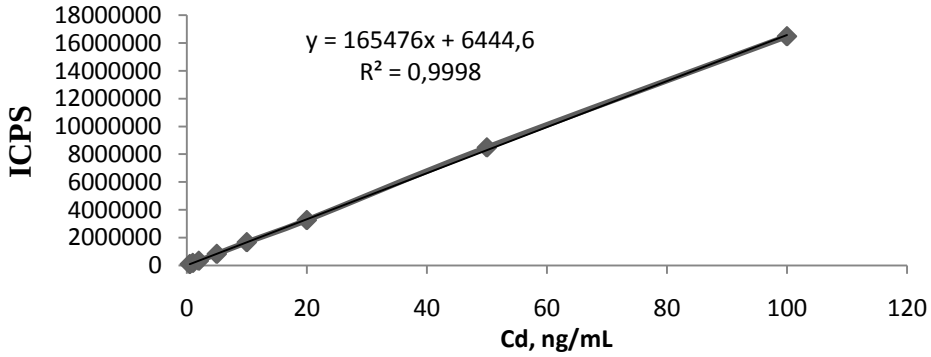

Figure 3.31 Linear calibration plot for Cd in 1.0 M HNO_3, using $^{111}Cd^{+}$ signal and an injection loop of 500 µL

During the analysis deionized water was used as the carrier solution. For sample introduction a loop having a volume of 0.5 mL was used. The ICP-MS signals obtained during the analysis of standards are given in **Figure 3.32**.

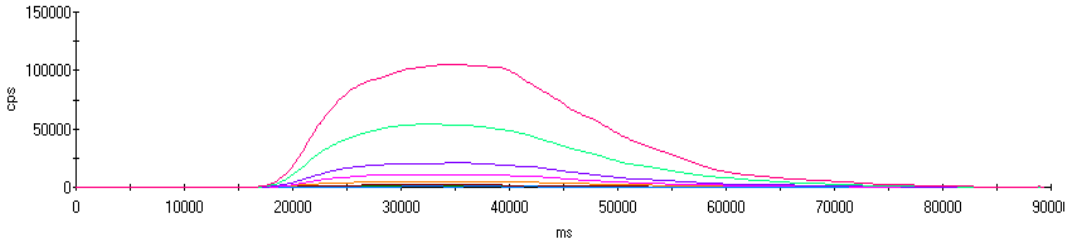

Figure 3.32 ICP-MS signals obtained from 0.5, 1.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/mL Cd standards in 1.0 M HNO_3, using $^{111}Cd^{+}$ and an injection loop of 500 µL

Analytical figures of merit for Cd are listed in **Table 3.8**. LOD (Limit of Detection) and LOQ (Limit of Quantification) values were obtained by using the signals of 0.1 ng/mL Cd standard solution which was measured six times.

Table 3.8 Analytical figures of merit for Cd, using $^{111}Cd^{+}$ signals and ICP-MS

LOD, ng/mL	0.04
LOQ, ng/mL	0.12
Linear Range, ng/mL	0.1-100.0

The accuracy of the method was checked by using 1566b Oyster Tissue SRM. 0.2 g SRM samples were digested in 4.0 mL of concentrated HNO_3 and 4.0 mL of concentrated H_2O_2 by using a MW digestion system. Digested solutions were diluted to 25.0 mL by using deonized water and analyzed by ICP-MS. The experimental and certified results are listed in **Table 3.9.**

Table 3.9 1566b Oyster Tissue SRM results for $^{111}Cd^{+}$ by using direct calibration

SRM, NIST	Certified Result ng/mL	Experimental Result ng/mL
Oyster Tissue, 1566b	2.48 ± 0.08	2.57 ± 0.07

The linear calibration plot for As is seen in **Figure 3.33**. To obtain this plot 1.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/mL As standard solutions prepared in 1 M HNO_3 were used.

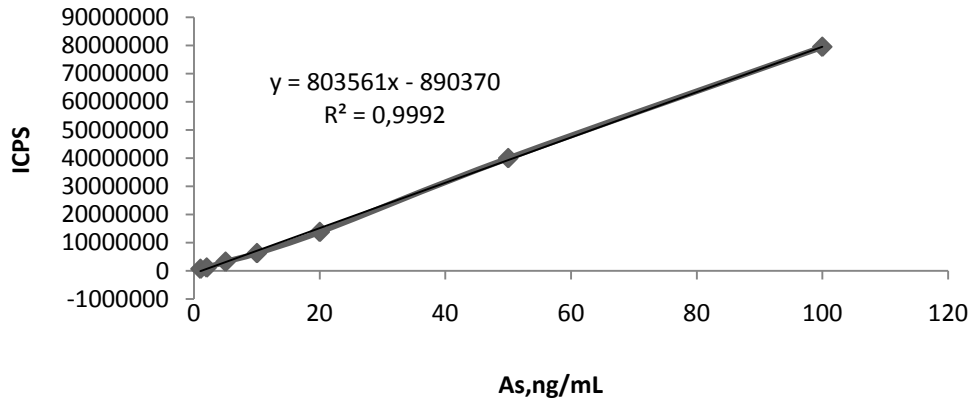

Figure 3.33 Linear calibration plot for As in 1.0 M HNO_3, using $^{75}As^{+}$ signal and an injection loop of 500 µL

ICP-MS signals obtained during the analysis of standard solutions are shown in **Figure 3.34.**

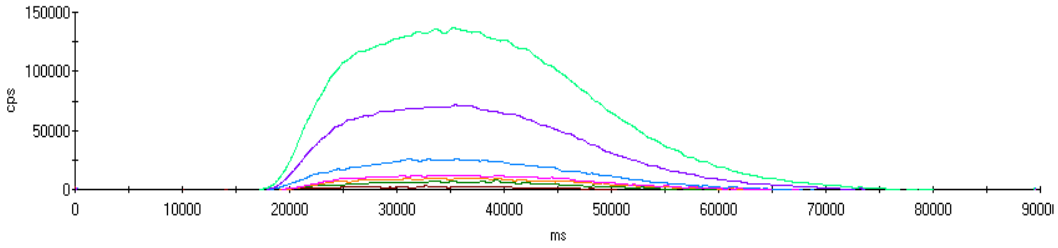

Figure 3.34 ICP-MS signals obtained from 1.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/mL As standards in 1.0 M HNO_3, using $^{75}As^{+}$ and an injection loop of 500 µL

LOD and LOQ values for As were obtained by using the signals of 0.2 ng/mL As standard solution which was measured six times. Analytical figures of merit for As are listed in **Table 3.10.**

Table 3.10 Analytical figures of merit for As, using $^{75}As^{+}$ signals and ICP-MS

LOD, ng/mL	0.15
LOQ, ng/mL	0.50
Linear Range, ng/mL	0.2-100.0

For Oyster Tissue, 1566b SRM experimental and certified results shown in **Table 3.11** were in good agreement when standard addition method was used for As determination. SRM samples were prepared and digested by using the same method explained for Cd. Direct calibration method was found to be not applicable.

Table 3.11 1566b Oyster Tissue SRM results for $^{75}As^{+}$ by using standard addition

SRM, NIST	**Certified Result ng/mL**	**Experimental Result ng/mL**
Oyster Tissue, 1566b	7.65 ± 0.65	7.47 ± 0.02

The linear calibration plot obtained by using 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/mL Se standard solutions prepred in 1.0 M HNO_3 is given in **Figure 3.35**.

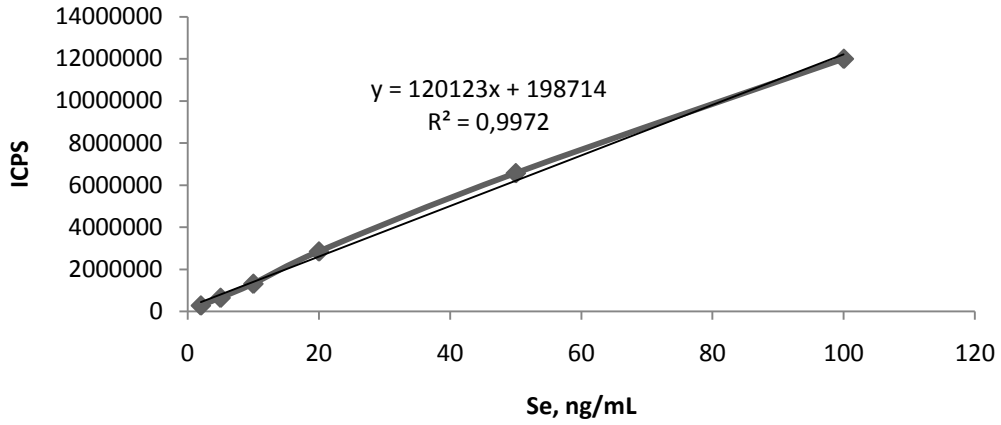

Figure 3.35 Linear calibration plot for Se in 1.0 M HNO_3, using $^{82}Se^{+}$ signal and an injection loop of 500 µL

Analytical figures of merit for Se are shown in **Table 3.12**. For LOD and LOQ determinations 2.0 ng/mL standard solution prepared in 1.0 M HNO_3 was measured six times.

Table 3.12 Analytical figures of merit for Se, using $^{82}Se^{+}$ signals and ICP-MS

LOD, ng/mL	0.35
LOQ, ng/mL	1.16
Linear Range, ng/mL	2.0-100.0

Similar to As, when BCR Human Hair, 397 SRM was analyzed for Se determination experimental and certified values were in good agreement when standard addition

method was applied. SRM results for Se in samples prepared and analyzed by using the same method applied for As for selenium determination are seen in **Table 3.13.**

Table 3.13 BCR Human Hair, 397 SRM results for $^{82}Se^{+}$ by using standard addition

SRM, NIST	Certified Result, ng/mL	Experimental Result, ng/mL
BCR Human Hair, 397	2.00 ± 0.08	2.00 ± 0.16

3.5 Determination of As, Cd and Se in Spice Samples

For this study 50 spice samples purchased from two different markets and three different bazaars were analyzed for As, Se and Cd contents. For each spice sample duplicate analyses were carried out. The average of the two results were calculated as final result.

The results of ICP-MS analyses for Cd, As and Se are given in **Table 3.14**, **Table 3.15** and **Table 3.16**. The results in each column named with capital letters A, B, C belong to spice samples purchased from three different markets whereas the numbers represent two different bazaars. N.D. (not detected) notation, refers to concentrations found to be near to LOD so that no meaningful signal was obtained. Results are the average values from duplicate spice samples.

Table 3.14 Cadmium concentrations in spice samples found by ICP-MS using $^{111}Cd^{+}$ signals. Results are average value from duplicate spice samples

^{111}Cd µg/kg	A	B	C	1	2
(1) Black Pepper	25.4 ± 1.0	23.3 ± 0.9	10.9 ± 0.4	12.7 ± 0.5	19.7 ± 0.8
(2) Mint	7.66 ± 0.53	20.9 ± 1.0	14.5 ± 0.6	6.48 ± 0.59	12.7 ± 0.5
(3) Thyme	28.9 ± 0.6	17.0 ± 1.2	9.96 ± 0.79	11.9 ± 0.6	14.2 ± 0.9
(4) Linden Flower	31.5 ± 2.4	40.8 ± 1.6	26.0 ± 1.0	57.5 ± 4.0	61.0 ± 4.3
(5) Daisy	30.3 ± 0.3	148 ± 3	659 ± 26	7.77 ± 0.54	48.7 ± 0.2
(6) Sumac	12.8 ±1.1	15.0 ± 1.2	13.3 ± 1.1	11.2 ± 0.9	14.3 ± 0.9
(7) Cumin	50.4 ± 1.7	70.9 ± 0.9	75.6 ± 0.5	44.7 ± 2.3	54.5 ± 0.8
(8) Sage	10.1 ± 0.3	40.0 ± 1.6	18.8 ± 1.3	10.6 ± 0.7	16.0 ± 1.4
(9) Bay Leaf	8.46 ±0.59	45.0 ± 2.3	39.8 ± 1.4	109 ± 6	31.3 ± 1.9
(10) Rosehip	7.40 ± 0.59	18.2 ± 2.3	12.6 ± 0.8	15.7 ± 0.9	7.53 ± 0.45

Table 3.15 Arsenic concentrations in spice samples found by ICP-MS using $^{75}As^{+}$ signals. Results are average value from duplicate spice samples

^{75}As µg/kg	A	B	C	1	2
(1) Black Pepper	73.1 ± 2.8	N.D.	125 ± 7	N.D.	440 ± 15
(2) Mint	*N.D.	N.D.	48.2 ±1.4	N.D.	54.1 ± 1.6
(3) Thyme	565 ± 2	174 ± 10	156 ± 3	134 ± 5	311 ± 13
(4) Linden Flower	N.D.	N.D.	8.97 ± 0.65	N.D.	N.D.
(5) Daisy	N.D	N.D.	1047±10	N.D.	N.D.
(6) Sumac	N.D.	N.D.	N.D.	N.D.	76.4 ± 2.0
(7) Cumin	24.5 ± 1.8	116 ± 2	380 ± 20	N.D.	72.3 ± 1.9
(8) Sage	N.D.	101 ± 1	98.8 ± 0.5	N.D.	N.D.
(9) Bay Leaf	N.D.	N.D.	N.D.	N.D.	N.D.
(10) Rosehip	N.D.	N.D.	N.D.	N.D.	N.D.

*N.D. = Not Detected

Table 3.16 Selenium concentrations in spice samples found by ICP-MS using $^{82}Se^{+}$ signals. Results are average value from duplicate spice samples

^{82}Se µg/kg	A	B	C	1	2
(1) Black Pepper	1288 ± 26	715 ± 45	397 ± 24	790 ± 50	1872 ± 119
(2) Mint	*N.D.	N.D.	464 ± 17	N.D.	N.D.
(3) Thyme	N.D.	N.D.	N.D.	N.D.	N.D.
(4) Linden Flower	N.D.	N.D.	138 ± 4	N.D.	N.D.
(5) Daisy	N.D.	58.5 ± 1.5	221 ± 6	N.D.	144 ± 4
(6) Sumac	N.D.	N.D.	N.D.	359 ± 9	451 ± 2
(7) Cumin	N.D.	327 ± 23	254 ± 4	144 ± 1	312 ± 22
(8) Sage	N.D.	N.D.	279 ± 4	N.D.	N.D.
(9) Bay Leaf	N.D.	N.D.	N.D.	N.D.	N.D.
(10) Rosehip	N.D.	N.D.	N.D.	N.D.	N.D.

*N.D. = Not Detected

The sample LOD values for As, Cd and Se are listed in **Table 3.17**. Sample LOD values are obtained by multiplying the LOD values in ng/mL units by dilution factor which is 50.0 for this study since 0.2 g samples were digested in 3.0 mL of HNO_3 and 3.0 mL of H_2O_2. After digestion they were taken in volumetric flasks and diluted to 10.0 mL by using deionized water. By this way the units are converted into µg/kg in order to make more accurate evaluations.

Table 3.17 Sample LOD values for As, Cd and Se

	LOD, µg/kg
^{111}Cd	3.5
^{75}As	7.5
^{82}Se	17.5

When **Table 3.14** is investigated it is seen that Cd concentrations ranges from around 10-100 µg/kg. The samples having the highest Cd concentrations are bay leaf and cumin. This may be attributed to the ability of these plants to accumulate cadmium of which the mobility in soil is very high [3].

The total concentrations Cd in bay leaf and cumin purchased from both bazaars and markets are on average 50.71 and 57.50 µg/kg, respectively. Based on the knowledge that the the weekly tolerable intake value of Cd is 15 µg/ kg body weight, to be exposed to Cd toxicity a 70 kg man should consume 2.96 kg of bay leaf and 2.6 kg of cumin in a day. These values are quite high indicating that exposure to Cd toxicity by consuming any of spice samples listed above is not realistic.

According to **Table 3.15,** As concentrations in the selected spices are in the range of 100-500 µg/kg. Spice samples with the highest arsenic concentrations are thyme, cumin and black pepper whereas arsenic is not detected in daisy, bay leaf, rosehip and linden flower. The weekly tolerable intake value of As is 7 µg/ kg body weight [1]. To exceed this limit the consumption of thyme, cumin and black pepper by a 70 kg man in a day should be 0.3 kg, 0.6 kg and 0.5 kg, respectively. On the other hand it is stated that 12-25 µg/ day As is enough to meet the requirements [2]. It is not possible to provide As by consuming only these spices since one should eat at minimum 44.8 g of thyme or 101.2 g of cumin or 94.0 g of black pepper in a day.

As seen in **Table 3.16** Se is not detected in most of the samples analyzed. However its concentration in black pepper is quite high followed by cumin and sumac. When RDA and tolerable intake values are taken in consideration one should consume minimum of 49.4 g of black pepper in a day to meet the requirements whereas to be exposed to Se toxicity the amount of black pepper that should be consumed in a day is 740.9 g.

These results indicate that the spices analyzed in this study are essential in daily diet since they contribute to As, and Se intake. Furthermore altough they contain Cd at relatively lower concentrations it is not possible to exceed toxicity levels for both Cd and As by spice consumption.

3.6 Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg, Pb Concentrations of Spice Samples

In this study the results of analyses including As, Cd and Se determination were combined with the results of the previous analysis for Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg, Pb performed in our laboratory [5].

For the determination of Na, K, Mg, Ca, Li, Zn, Fe, Cu, B and Mn 10 different spice samples were analyzed by using ICP-OES. For each spice sample two parallel samples were analyzed and the averages of the two results were taken as the final result. Hg determination in the same spice samples was performed by using cold vapor AAS. Pb determination was performed by using Hydride Generation AAS in six different spice samples each purchased from two different markets and one bazaar. For each spice sample two parallel samples were analyzed and the average is taken as the final result.

Some of the LOD values for the elements listed above are seen in **Table 3.18**.

Tablo 3.18 Sample LOD values for Zn, Fe, Cu, B, Mn, Hg and Pb determination

	LOD	Method
Zn	0.08 mg/kg	ICPOES
Fe	0.15 mg/kg	ICPOES
Cu	0.08 mg/kg	ICPOES
B	0.15 mg/kg	ICPOES
Mn	0.15 mg/kg	ICPOES
Hg	5.0 µg/kg	Cold Vapor AAS
Pb	0.3 µg/kg	HGAAS

The results obtained in these analyses were evaluated in three different ways and three different tables were obtained. Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg and Pb concentrations of spice samples purchased only from markets are listed in **Table 3.19**. In this table each result is the average of three markets except for Pb which was determined in samples taken from two different markets. Each sample was analyzed duplicate.

Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg and Pb concentrations in spice samples purchased only from different bazaars are listed in **Table 3.20**. Each concentration is the average of three results obtained from spice samples from three different bazaars. Each Pb concentration was obtained by taking the average of the results of two parallel spice samples purchased from one bazaar.

Finally the total Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg and Pb concentrations in each spice sample are listed in **Table 3.21**. In this table each result is obtained by taking the average of six results from the sources since there exist three different

bazaars and three different markets. Pb concentrations were obtained by taking the average of the results from two different markets and one bazaar.

Table 3.19 Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg and Pb concentrations of spice samples obtained from different markets.Number of markets, N, was three except for Pb, N=2

	Daisy	Bay Leaf	Mint	Rosehip	Sage	Thyme	Cumin	Sumac	Linden Flower	Paprika
Na	0.06±0.01	0.04±0.02	0.05±0.02	0.02±0.01	0.03±0.02	0.06±0.01	0.04±0.01	1.93±0.07	0.07±0.04	2.43±0.04
K	1.95±0.12	0.77±0.14	2.42±0.22	1.22±0.07	1.18±0.05	0.91±0.11	1.73±0.42	0.99±0.11	1.57±0.11	3.19±0.11
Mg	0.43±0.07	0.33±0.09	0.23±0.16	0.18±0.05	0.09±0.07	0.37±0.12	0.30±0.12	0.08±0.12	0.26±0.12	0.14±0.07
Ca	0.95±0.14	1.26±0.11	2.23±0.25	1.16±0.10	1.40±0.05	1.88±0.09	1.58±0.17	0.64±0.10	1.88±0.33	0.22±0.09
Li	0.71±0.07	*N.D.	0.92±0.08	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Zn	1.23±0.14	1.65±0.07	0.97±0.13	N.D.	1.26±0.38	1.53±0.27	1.93±0.23	0.89±0.27	1.42±0.20	0.84±0.38
Fe	12.1±0.5	13.2±0.5	18.5±0.5	0.92±0.51	14.1±0.3	17.7±0.7	17.8±1.0	17.6±0.6	5.95±0.40	14.3±0.89
Cu	0.53±0.09	0.33±0.06	0.60±0.06	N.D.	0.61±0.12	0.42±0.12	0.59±0.10	0.44±0.12	1.31±0.12	0.82±0.12
B	3.24±0.62	2.82±0.66	1.86±0.47	2.19±0.60	2.60±0.41	4.78±0.67	3.38±0.67	2.15±0.37	4.04±0.68	1.10±0.14
Mn	3.54±0.93	3.32±0.67	5.38±0.90	1.99±0.84	2.09±0.57	6.50±0.37	2.39±0.48	7.11±0.42	7.11±0.40	1.15±0.25
Hg	N.D.	N.D.	N.D.	N.D.	ND.	N.D.	N.D.	N.D.	N.D.	N.D.
Pb			0.35±0.02		0.90±0.04	0.44±0.12	0.37±0.02		0.66±0.03	0.32±0.01

*N.D. Not Detected

Table 3.20 Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg and Pb concentrations of spice samples obtained from different bazaars.Number of bazaars, N, was 3 except for Pb, N=1

	Daisy	Bay Leaf	Mint	Rosehip	Sage	Thyme	Cumin	Sumac	Linden Flower	Paprika
Na	0.07±0.06	0.03±0.01	0.06±0.02	0.03±0.01	0.05±0.01	0.04±0.01	0.05±0.03	2.13±0.03	0.03±0.01	3.27±0.05
K	1.95±0.09	1.10±0.07	2.30±0.39	2.35±0.09	1.81±0.06	1.08±0.07	1.78±0.23	0.68±0.07	1.28±0.07	3.07±0.07
Mg	0.22±0.06	0.10±0.07	0.27±0.22	0.22±0.07	0.13±0.09	0.28±0.10	0.37±0.06	0.06±0.01	0.15±0.10	0.09±0.06
Ca	1.38±0.11	1.34±0.06	2.35±0.11	0.95±0.08	1.41±0.06	1.94±0.05	1.34±0.09	0.64±0.08	1.55±0.04	0.21±0.07
Li	0.58±0.10	*N.D.	1.10±0.11	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Zn	1.52±0.12	1.17±0.06	1.36±0.08	N.D.	0.87±0.94	1.29±0.31	2.61±0.16	1.04±0.30	1.57±0.26	0.79±0.24
Fe	11.3±0.3	11.9±0.64	16.9±0.5	1.22±0.37	12.8±0.3	15.9±1.0	21.7±1.0	17.5±0.5	8.10±0.34	10.6±0.9
Cu	0.53±0.11	0.53±0.05	0.64±0.09	N.D.	0.54±0.13	0.70±0.07	1.15±0.05	0.53±0.01	0.98±0.07	0.69±0.11
B	2.52±1.1	4.47±0.83	1.94±0.80	1.85±1.07	1.00±0.67	5.57±0.15	4.78±0.15	2.12±0.25	2.96±0.20	1.57±0.13
Mn	2.41±1.24	5.29±0.66	6.17±1.14	1.19±0.30	2.14±0.81	4.68±0.56	2.65±0.57	5.52±0.56	5.86±0.50	0.87±0.08
Hg	N.D.	N.D.	N.D.	N.D.	ND.	N.D.	N.D.	N.D.	N.D.	N.D.
Pb			0.74±0.02		1.78±0.03	1.01±0.02	N.D.		0.93±0.02	0.80±0.05

*N.D. Not Detected

Table 3.21 Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg and Pb concentrations of spice samples obtained from different bazaars and markets.Number of sources, N, was 6 except for Pb, N=3

	Daisy	Bay Leaf	Mint	Rosehip	Sage	Thyme	Cumin	Sumac	Linden Flower	Paprika
Na	0.07±0.06	0.03±0.01	0.06±0.02	0.03±0.01	0.05±0.01	0.04±0.01	0.05±0.03	2.13±0.03	0.03±0.01	3.27±0.05
K	1.95±0.09	1.10±0.07	2.30±0.39	2.35±0.09	1.81±0.06	1.08±0.07	1.78±0.23	0.68±0.07	1.28±0.07	3.07±0.07
Mg	0.22±0.06	0.10±0.07	0.27±0.22	0.22±0.07	0.13±0.09	0.28±0.10	0.37±0.06	0.06±0.01	0.15±0.10	0.09±0.06
Ca	1.38±0.11	1.34±0.06	2.35±0.11	0.95±0.08	1.41±0.06	1.94±0.05	1.34±0.09	0.64±0.08	1.55±0.04	0.21±0.07
Li	0.58±0.10	N.D.	1.10±0.11	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Zn	1.52±0.12	1.17±0.06	1.36±0.08	N.D.	0.87±0.94	1.29±0.31	2.61±0.16	1.04±0.30	1.57±0.26	0.79±0.24
Fe	11.3±0.3	11.9±0.64	16.9±0.5	1.22±0.37	12.8±0.3	15.9±1.0	21.7±1.0	17.5±0.5	8.10±0.34	10.6±0.9
Cu	0.53±0.11	0.53±0.05	0.64±0.09	N.D.	0.54±0.13	0.70±0.07	1.15±0.05	0.53±0.01	0.98±0.07	0.69±0.11
B	2.52±1.1	4.47±0.83	1.94±0.80	1.85±1.07	1.00±0.67	5.57±0.15	4.78±0.15	2.12±0.25	2.96±0.20	1.57±0.13
Mn	2.41±1.24	5.29±0.66	6.17±1.14	1.19±0.30	2.14±0.81	4.68±0.56	2.65±0.57	5.52±0.56	5.86±0.50	0.87±0.08
Hg	N.D.	N.D.	N.D.	N.D.	ND.	N.D.	N.D.	N.D.	N.D.	N.D.
Pb			0.74±0.02		1.78±0.03	1.01±0.02	N.D.		0.93±0.02	0.80±0.05

*N.D. Not Detected

When **Table 3.21** is investigated it is seen that the type of spices having the highest concentrations of Na and K is paprika. Na and K concentrations are also relatively high in sumac and mint samples, respectively. Fe concentrations are high in cumin, sumac, thyme and mint samples. Li was detected only in daisy and mint samples. Mg and Ca concentrtions are highest in daisy and mint samples, respectively. Mn concentration is high in sumac, thyme and linden flower whereas Cu concentration in cumin and linden flower is higher than the other spice samples. The highest boron concentrations were obtained with thyme samples whereas sage was found to be rich in Pb. Hg was not detected in any of the spice samples analyzed.

3.7 Statistical Evaluation of Results

In this section the analytical results will be evaluated using statistical methods. By using **Table 3.19** and **Table 3.20** it is possible to investigate whether there exist a siginificant difference in concentrations of the analyte elements between the spice samples obtained from bazaars and from markets. In order to investigate any possible difference between these two groups of sample source, Student's t-test was applied to all the results listed in **Table 3.19** and **Table 3.20** and the results were evaluated at 95% confidence level. For the Student's t-test the following formula was used.

$$T = \frac{\overline{X} - \overline{Y}}{\sqrt{\frac{S_X^2}{n_1} + \frac{S_Y^2}{n_2}}}$$

The t values obtained for each sample are listed in **Table 3.22-Table 3.32**. According to the tabulated t-tables for Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn and Hg the stated t-value is 2.776 when the degrees of freedom is 4 since each value in each

table is the average of three results. Degrees of freedom is calculated by summing up the number of independent scores which is 6 for these analyses and subtracting 2 from this value. This value is 3.182 for As, Cd and Se since samples from three different markets and two different bazaars were analyzed for their determination. For Hg the tabulated t-value is 12.706 [71]. In case of greater value than these tabulated values, there is a significant difference in the mean scores of two groups. In each table N.D. indicates that the analyte is not detected in both groups.

Table 3.22 Calculated and tabulated t values for daisy samples at 95% confidence level

DAISY	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	0.222	2.776	**Zn**	**16.283**	**2.776**	As	N.D.	3.182
K	0.071	2.776	Fe	13.101	**2.776**	**Cd**	**17.565**	**3.182**
Mg	**3.857**	**2.776**	Cu	1.012	2.776	Se	17.078	3.182
Ca	**4.240**	**2.776**	B	1.026	2.776	Hg	N.D.	2.776
Li	1.764	2.776	Mn	1.406	2.776			

Table 3.23 Calculated and tabulated t values for bay leaf samples at 95% confidence level

BAY LEAF	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	0.630	2.776	**Zn**	**9.133**	**2.776**	As	N.D.	3.182
K	**3.716**	**2.776**	**Fe**	**3.326**	**2.776**	**Cd**	**10.321**	**3.182**
Mg	**3.641**	**2.776**	Cu	0.638	2.776	Se	N.D.	3.182
Ca	1.189	2.776	B	2.705	2.776	Hg	N.D.	2.776
Li	N.D.	2.776	**Mn**	**3.628**	**2.776**			

Table 3.24 Calculated and tabulated t values for mint samples at 95% confidence level

MINT	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	5.56	2.776	**Zn**	**4.45**	**2.776**	**As**	**11.362**	**3.182**
K	0.486	2.776	**Fe**	**3.932**	**2.776**	**Cd**	**5.313**	**3.182**
Mg	0.239	2.776	Cu	0.152	2.776	**Se**	**16.198**	**3.182**
Ca	0.764	2.776	B	0.144	2.776	Hg	N.D.	2.776
Li	1.825	2.776	Mn	0.934	2.776	**Pb**	**17.611**	**12.706**

Table 3.25 Calculated and tabulated t values for rosehip samples at 95% confidence level

ROSEHIP	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	0.979	2.776	Zn	N.D.	2.776	As	N.D.	3.182
K	**18.156**	**2.776**	Fe	0.818	2.776	Cd	0.734	3.182
Mg	0.769	2.776	Cu	N.D.	2.776	Se	N.D.	3.182
Ca	0.506	2.776	B	0.482	2.776	Hg	N.D.	2.776
Li	N.D.	2.776	Mn	0.892	2.776			

Table 3.26 Calculated and tabulated t values for sage samples at 95% confidence level

SAGE	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	1.750	2.776	Zn	0.667	2.776	**As**	**99.61**	**3.182**
K	**14.650**	**2.776**	**Fe**	**4.905**	**2.776**	**Cd**	**6.478**	**3.182**
Mg	0.523	2.776	Cu	0.615	2.776	**Se**	**35.85**	**3.182**
Ca	0.372	2.776	**B**	**3.526**	**2.776**	Hg	N.D.	2.776
Li	N.D.	2.776	Mn	0.093	2.776	**Pb**	**21.343**	**12.706**

Table 3.27 Calculated and tabulated t values for thyme samples at 95% confidence level

THYME	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	**4.672**	**2.776**	Zn	1.007	2.776	**As**	**7.408**	**3.182**
K	2.089	2.776	Fe	2.589	2.776	**Cd**	**5.103**	**3.182**
Mg	1.076	2.776	Cu	0.841	2.776	Se	N.D.	3.182
Ca	1.063	2.776	B	2.019	2.776	Hg	N.D.	2.776
Li	N.D.	2.776	**Mn**	**4.716**	**2.776**	**Pb**	**24.599**	**12.706**

Table 3.28 Calculated and tabulated t values for cumin samples at 95% confidence level

CUMIN	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	0.121	2.776	**Zn**	**4.250**	**2.776**	**As**	**11.761**	**3.182**
K	0.156	2.776	**Fe**	**4.839**	**2.776**	**Cd**	**8.893**	**3.182**
Mg	0.924	2.776	Cu	1.617	2.776	Se	1.849	3.182
Ca	2.551	2.776	**B**	**3.581**	**2.776**	Hg	N.D.	2.776
Li	N.D.	2.776	Mn	0.602	2.776	**Pb**	**21.802**	**12.706**

Table 3.29 Calculated and tabulated t values for sumac samples at 95% confidence level

SUMAC	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	**4.925**	**2.776**	Zn	0.631	2.776	**As**	**45.142**	**3.182**
K	**3.987**	**2.776**	Fe	0.169	2.776	Cd	0.724	3.182
Mg	0.333	2.776	Cu	1.857	2.776	**Se**	**73.472**	**3.182**
Ca	0.110	2.776	B	0.117	2.776	Hg	N.D.	2.776
Li	N.D.	2.776	**Mn**	**3.909**	**2.776**			

Table 3.30 Calculated and tabulated t values for linden flower samples at 95% confidence level

LINDEN FLOWER	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	1.917	2.776	Zn	0.776	2.776	**As**	**23.90**	**3.182**
K	**3.932**	**2.776**	**Fe**	**7.191**	**2.776**	**Cd**	**6.906**	**3.182**
Mg	1.202	2.776	Cu	0.779	2.776	**Se**	**22.314**	**3.182**
Ca	1.679	2.776	B	2.662	2.776	Hg	N.D.	2.776
Li	N.D.	2.776	Mn	3.378	2.776	Pb	10.502	12.706

Table 3.31 Calculated and tabulated t values for paprika samples at 95% confidence level

PAPRIKA	t-value	Tabulated t-value		t-value	Tabulated t-value		t-value	Tabulated t-value
Na	**16.865**	**2.776**	Zn	0.209	2.776	Hg	N.D.	2.776
K	1.553	2.776	**Fe**	**5.130**	**2.776**	Pb	8.833	12.706
Mg	0.988	2.776	Cu	1.433	2.776			
Ca	0.059	2.776	**B**	**4.207**	**2.776**			
Li	N.D.	2.776	Mn	0.531	2.776			

Table 3.32 Calculated and tabulated t values for black pepper samples at 95% confidence level

BLACK PEPPER	t-value	Tabulated t-value
As	**13.44**	**3.182**
Cd	**3.596**	**3.182**
Se	**9.549**	**3.182**

The elements whose concentrations in two groups are significantly different at 95% confidence level are written in bold in statistics **Tables 3.21** to **3.31**. When these tables are investigated it is seen that the number of elements having different

concentrations in the two groups is high. However it is not easy to get favorable results. The most significant difference is obtained with Fe. Fe concentrations in 7 out of 10 spice samples are significantly different. For most of the samples Fe concentrations in spices from markets is higher than that of purchased from bazaars. A similar situation is observed for As and Cd. As and Cd concentrations in market groups are higher in most of the samples. Zn concentrations are significantly different in daisy, bay leaf, mint and cumin samples. However Zn concentrations are higher in market group for daisy and bay leaf whereas concentrations of bazaar group are higher for mint and cumin samples. For Pb determination six kinds of spice samples were analyzed including mint, paprika, linden flower, cumin, sage and thyme. Except for paprika, Pb concentrations in two groups are significantly different with higher concentrations belonging to the bazaar group. There is no significant difference between B, Se and Mn concentrations with a few exceptions. K and Na concentrations are also significantly different in bay leaf, mint, paprika, daisy, sage, thyme, sumac and linden flower, however there is not a trend among the groups having higher concentrations.

CONCLUSIONS

In this sudy the analysis of a variety of spices including *daisy (Chamomillae Vulgaris),* bay leaf *(Folium Lauri),* mint *(Folium Menthane)* rosehip *(Rosae Caninae),* sage *(Folium Salviae Officinalis),* thyme *(Herba Thymi),* cumin *(Fructus Cummuni),* sumac *(Folium Rhois Coriariae),* linden flower *(Flos Tilliae*) and black pepper *(Piper Nigrum)* were performed by using inductively coupled plasma mass spectrometry (ICP-MS) for As, Cd and Se determinatin.

50 spice samples each purchased from 3 different markets and 2 different bazaars were analyzed. For each of 50 spice samples two parallel samples were analyzed and the final results were expressed by taking the average of the results of two measurements.

Spice samples were digested by using MW digestion method. HNO_3 and H_2O_2 were used as digestion reagents. The concentrations of these reagents were optimized to minimize possible matrix interferences. Four different MW temperature programs were investigated to obtain maximum digestion efficiency with minimum digestion reagent consumption.

Effects of HNO_3 and H_2O_2 concentrations on As, Cd and Se signals were investigated to see the effect of matrix matching on ICP-MS signals and it was found that increasing concentrations of HNO_3 has suppression effect on As and Se signals whereas the effect on Cd signals were not that severe allowing one to perform Cd determination by using direct calibration method without the need for matrix matching or standard addition method by ICP-MS.

Based on this knowledge direct calibration method was used for Cd determination whereas for As and Se determination standard addition method was applied. Direct calibration method was also found to be applicable for As and Se determination by ICP-MS when matrix matching is used.

For arsenic, interference studies were performed by using a variety of salts of Group IA elements. To investigate both spectral and nonspectral interferences seperately HCl, $NaNO_3$, $LiNO_3$ and $CsNO_3$ solutions including 20.0 ng/mL As were prepared at various concentrations ranging from 0.025 M to 0.40 M. The overall effect of spectral and nonspectral interferences were investigated by using chloride salts of Na, Li and Cs at the given concentration range.

As a result of interference studies it was found that easily ionizable elements including Li, Na and Cs suppress As signals due to ionization and space charge effects. However salt concentrations of the spice samples analyzed were found too low to exhibit any kind of suppression effect. So, the samples were analyzed by just applying mathematical correction methods which are done automatically by the instrument to prevent possible spectral interferences arising from chlorine present in sample matrix. The validity of these correction methods were also checked by running the instrument both with and without corrections and comparing ICP-MS signals.

As a result of the analysis Cd concentrations were found to be in the range of 10-100 µg/kg where as As and Se concentrations were ranging from 100 to 500 µg/kg with a few exceptions.

Spice samples with the highest Cd concentrations were found to be bay leaf and cumin. Thyme, cumin and black pepper contain the highest As concentrations whereas As was not detected in daisy, bay leaf, rosehip and linden flower. Although

Se was not detected in most of the analyzed samples it has the highest concentartions in black pepper, cumin and sumac.

After the determination of As, Cd and Se concentrations, the amounts of spice samples that should be consumed to meet the daily requirements for these elements and to be exposed to toxicity were calculated by taking RDA values and daily or weekly tolerable intake values stated by WHO in consideration. The calculations indicated that the minimum amounts of any of spice samples analyzed to exceed toxicity levels or to meet daily or weekly requirements are too high to be possibly consumed.

In the last part of the study the results of previous analyses for Na, K, Mg, Ca, Li, Zn, Fe, Cu, B, Mn, Hg and Pb determination in spice samples were evaluated together with the results of this study for statistical analysis. For each spice sample the results of all elements obtained from different markets and bazaars were compared by using Student's t-test. At 95% confidence interval mainly Fe, As, Zn, Cd and Pb concentrations of market and bazaar groups were found to be significantly different in most of the samples analyzed.

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