T.C.
GAZİ UNIVERSITY
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SPECIATION STUDIES BY CAPILLARY ELECTROPHORESIS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-HYDRIDE GENERATION-ATOMIC FLUORESCENCE SPECTROMETRY

Ph.D. THESIS

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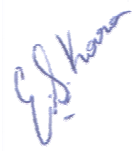

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Symbols and Abbreviations

Abbreviation	Definition
AAS	atomic absorption spectrometry
AB	arsenobetaine
AC	arsenocholine
ACN	acetonitrile
AFS	atomic fluorescence spectrometry
AU	absorbance units
BGE	background electrolyte
CE	capillary electrophoresis
CE–UV	capillary electrophoresis–ultraviolet
CRM	certified reference material
CTAB	cetyltrimethylammonium bromide
CVG	chemical vapor generation
CZE	capillary zone electrophoresis
DI	deionized
DLLME	dispersive liquid-liquid microextraction
DMA	dimethylarsinic acid
DNA	deoxyribonucleic acid
EOF	electroosmotic flow
EPA	Environmental Protection Agency
ES	electrospray
ESI–MS	electrospray ioinization-mass spectrometry
EU	European Union
FASI	field-amplified sample injection
FASS	field-amplified sample stacking
GC	gas chromatography
GF-AAS	graphite furnace-atomic absorption spectroscopy
HF–LPME	hollow fiber-liquid phase microextraction

Abbreviation	Definition
HG	hydride generation
HG–AAS	hydride generation-atomic absorption spectroscopy
HG–AFS	hydride generation-atomic fluorescence spectroscopy
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HSDC	high-sensitivity detection cell
IC	ion chromatography
IC–MS	ion chromatography-mass spectrometry
ICP	inductively coupled plasma
ICP–AES	inductively coupled plasma-atomic emission spectrometry
ICP–MS	inductively coupled plasma-mass spectrometry
ICP–OES	inductively coupled plasma-optical emission spectrometry
IEC	ion-exchange chromatography
IF	improvement factor
INAA	instrumental neutron activation analysis
IUPAC	International Union of Pure and Applied Chemistry
LC	liquid chromatography
LIF	laser induced fluorescence
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
LPME	liquid-phase microextraction
LVSS	large-volume sample stacking
MEKC	micellar electrokinetic chromatography
MMA	monomethylarsonic acid
MS	mass spectrometry
NIST	National Institute of Standards and Technology
NSAID	non-steroidal anti-inflammatory drug
NSM	normal-stacking mode

Abbreviation	Definition
RSD	relative standard deviation
S/N	signal to noise ratio
SBME	solvent bar microextraction
SD	standard deviation
SDME	single-drop microextraction
SDS	sodium dodecyl sulfate
SEC	size exclusion chromatography
SFOD–ME	solidification of floating organic drop microextraction
SPE	solid phase extraction
SPME	solid-phase microextraction
SRM	standard reference material
TBAH	tetrabutylammonium hydroxide
TMAO	trimethylamine oxide
TTAB	tetradecyl trimethyl ammonium bromide
TÜBİTAK	Scientific and Technological Research Council of Turkey
USAEME	ultrasound-assisted emulsification microextraction
UV	ultraviolet
WHO	World Health Organization

TO MY BELOVED FAMILY

1 OVERVIEW

Arsenic and antimony play detrimental roles in the environment and human health and therefore their speciation is of increasing concern[1,2]. Among arsenic species, inorganic arsenic compounds are highly toxic, whereas methylation is the most important pathway to transform inorganic arsenic into organic arsenic species of lower toxicity. Consequently, arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are among the most important arsenic species[3]. Antimony is a non-essential element for human and its toxicity depends on its oxidation state. Antimonite [Sb(III)] is more toxic than antimonate [Sb(V)]. The importance of antimony determination is reflected by the fact that the Environmental Protection Agency (EPA) considers this element as a priority pollutant[2].

Simultaneous speciation of arsenic and antimony has been done using high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC–ICP–MS)[2,4], graphite furnace-atomic absorption spectrometry (GF–AAS)[5], hydride generation-atomic absorption spectrometry (HG–AAS)[6], hydride generation-atomic fluorescence spectrometry (HG–AFS)[7], hydride generation-inductively coupled plasma-atomic emission spectrometry (HG–ICP–AES)[8], and high performance liquid chromatography-hydride generation-atomic absorption spectrometry (HPLC–HG–AAS)[4].

In the past, liquid chromatography (LC) and gas chromatography (GC) have been the predominant separation techniques for elemental speciation in general[1]. However, in recent years, there has been an

increasing interest in the application of capillary electrophoresis (CE) for speciation of arsenic[9,10] and antimony[11-13]. CE is a powerful alternative technique to LC and GC and has rapidly spread into a wide array of analytical areas as it is considered a green analytical technique, due to its low consumption of samples and reagents, extremely high separation efficiency, short analysis time, high versatility in terms of multiple separation modes and excellent biocompatibility[14]. Nevertheless, one of the drawbacks of CE equipped with direct ultraviolet (UV) detection is the poor concentration sensitivity resulting from minute injection volumes needed to maintain high separation efficiency and a short optical pathlength equal to the capillary diameter[15]. Solutions to this problem include the use of capillaries designed for extended detection pathlength (e.g. multi-reflection and bubble cell), the use of highly sensitive detectors (e.g. laser-induced fluorescence (LIF), MS and electrochemical), and offline sample preconcentration techniques, e.g., liquid-liquid extraction (LLE) and solid-phase extraction (SPE). However, all these techniques require rather expensive and somewhat complex hardware and/or time-consuming procedures[15].

On the other hand, a high-sensitivity detection cell (HSDC) which provides a detection pathlength more than an order of magnitude longer than conventional on-capillary detection path[1] and/or online preconcentration techniques such as sweeping[16] and sample stacking[17] have been used to overcome this problem. Stacking techniques include (i) field-amplified sample stacking (FASS)[18], (ii) large volume sample stacking (LVSS) with polarity switching[19], and (iii) the less often applied field-amplified sample injection (FASI) with electroosmotic flow (EOF) reversal (FASI/EOF reversal)[10].

In the present study, sensitivity enhancement as compared to conventional capillary zone electrophoresis (CZE) has been achieved using sweeping, the above-mentioned three sample stacking techniques, and through the use of HSDC. A UV detector at 192 nm was used. The lowest limits of detection (LOD) were obtained by combining FASI/EOF reversal and HSDC. LODs for the target analytes were 2.1, 16, 14, 1.9, 1.1 and 13 μg L^{-1} for As(III), As(V), MMA, DMA, Sb(III) and Sb(V), respectively. Compared to conventional CZE, this method gave improvement factors (IF) in the range of 0.4×10^3–30×10^3. The developed CE method was successfully applied for speciation of arsenic and antimony in spiked tap water and recoveries in the range of 97.7–102.5% were obtained.

Speciation of arsenic in urine is indispensible because it is a good marker for exposure to arsenic. It has been demonstrated to correlate well for a number of chronic effects related to arsenic levels in drinking water. However confounding factors must be taken into account to avoid misinterpretation and this may require speciation[20]. AFS is an ideal detection technique for speciation studies concerning chemical vapor generating (CVG) elements which include arsenic. The analytical features of AFS, such as high selectivity, high sensitivity, i.e., LODs below μg L^{-1}, and the wide linear dynamic range, up to mg L^{-1}, allow its application to a great variety of environmental, biological and food samples. AFS represents a suitable alternative to other atomic spectrometers commonly employed in speciation studies such as AAS and ICP–MS[21].

The other part of this study, which was a part of a research project for investigating carcinogenic risk in residents chronically exposed to inorganic arsenic via drinking water in Nevşehir area, Turkey, represents a methodology for arsenic speciation in urine by HPLC–HG–AFS.

Inorganic [As(III), As(V)] and organic (DMA, MMA) arsenic species were separated on a strong anion exchange column under optimized separation conditions (mobile phase, phosphate buffer at pH 5.8; flow rate, 1.0 mL min^{-1}; column at ambient room temperature; and by using a C18 guard column). In order to assess accuracy of the speciation studies, "SRM NIST 2669 arsenic species in frozen human urine" was analyzed for arsenic species by the developed method and the results were statistically in a good agreement with the certified values. Total arsenic concentration was also determined by HG–AFS after acid digestion and pre-reduction with potassium iodide and L-ascorbic acid. The same procedure was applied to SRM 2669 at two levels (i.e., low and high) and the results were also in a good agreement. LODs with HPLC–HG–AFS, for a 100-µL injection loop, were in the range of 2.2–2.9 µg L^{-1} (at gain 10), 0.3–0.7 µg L^{-1} (at gain 100) and that with HG–AFS was 3 ng L^{-1}. The developed method was applied for the speciation of arsenic in urine samples collected from the study area. The results were classified into three groups according to the arsenic level in drinking water people consumed. The first group with arsenic concentration below 10 µg L^{-1} (Low-Level), the second with arsenic in the range of 10–50 µg L^{-1} (Middle-Level), and the third with arsenic above 50 µg L^{-1} (High-Level).

2 INTRODUCTION

2.1 Arsenic and antimony

Arsenic and antimony are metalloids belonging to Group 15 of the periodic table. They both occur naturally in the environment at trace levels. Because of their identical s^2p^3 outer orbital electron configuration, antimony and arsenic display the same range of oxidation states in environmental systems (-3, 0, +3, +5). Both most commonly occur as oxides, hydroxides or oxoanions either in the +5 state in relatively oxic environments (antimonates and arsenates) or in the +3 state in anoxic environments (antimonites and arsenites).

2.2 Applications

Arsenic is used industrially in the manufacture of numerous products including glass, ceramics, electronics, cosmetics, and fireworks[22]. In the latter half of the 20th century, arsenic was also widely used in pesticide and herbicide formulations and in wood preservation, but such use is now declining[23].

Antimony has a wide range of uses including the manufacture of semiconductors, diodes, flame-proof retardants, lead hardeners, batteries, small arms, tracer bullets, automobile brake linings, and pigments[24]. Antimony also remains the treatment of choice for several tropical protozoan diseases, such as leishmaniasis and human immunodeficiency virus (HIV)[22,25].

2.3 Production and occurrence

World production of antimony is considerably larger than arsenic. In 2008, the estimated antimony mine output was 165,000 tonnes compared to 40,500 tonnes of arsenic[22]. Environmental enrichment of both metalloids occurs naturally in areas of geological mineralization, but also anthropogenically[24,26-29] although the majority of antimony contamination appears to originate from mining and industrial emission sources, often smelting, co-occurring frequently with arsenic[29].

2.4 Toxicity and health effects

Arsenic has long been recognized as a potentially harmful element, evidenced through its historic use as a popular poison[22,23]. Less is known about antimony effects. The understanding of its toxicity and environmental behavior is much more limited[30]. Often, antimony is considered to behave similarly to arsenic, not always with justification[31]. Chemical similarities between the two metalloids have prompted concerns over the enrichment of this metalloid in many environments[24,32].

The toxicity of antimony and arsenic in the environment strongly depends upon speciation[24,33]. The general order of toxicity for antimony species is given as: organoantimonials (e.g. methylated species) < antimonates [Sb(V)] < antimonites [Sb (III)][24,32,33]. This is similar to arsenic: organoarsenicals (e.g. methylated species) < arsenates [As(V)] < arsenites [As (III)][34]. Oral route in humans is the most important way of arsenic intake, while the dermal route into the body is minimal. It is known that methylation is the most important metabolic pathway of arsenic. Upon

intake of inorganic arsenic, it is methylated to monomethylarsonic acid [MMA(V)] which is then reduced to monomethylarsonous acid [MMA(III)]. Next, the latter is further methylated to dimethylarsinic acid [DMA(V)], which is then reduced to dimethylarsinous acid [DMA(III)]. It has been shown that typical profile of arsenic in human urine is inorganic arsenic (10-30%), MMA (10-20%) and DMA (60-80%). It was thought that biotransformation of inorganic arsenic into organic arsenic through methylation is a detoxification route. However, it has been recently shown that methylated trivalent arsenic is more genotoxic than inorganic arsenic. Thus, methylation of arsenic may play an important role in understanding its toxicity[35,36].

It is debatable whether arsenic is essential to human health, but there is no known human requirement for antimony[22]. Both metalloids are clastogenic (i.e., capable of causing damage to chromosomes) in the trivalent state, and have carcinogenic potential[33]. Both have a strong affinity for thiol groups and may substitute for phosphorus in biological reactions, which explains their inhibitory role in deoxyribonucleic acid (DNA) replication and metabolic processes. There is evidence that arsenic is detoxified via methylation in biological systems, but less evidence for the same process occurring for antimony[33].

2.5 Occurrence of arsenic and antimony in Turkey's water

Col et al.[37] determined arsenic concentrations in surface, well and tap (drinking) water in Hisarcık (Kütahya). The authors used ICP–MS for the analysis and reported arsenic concentrations ranging from non-detectable to 760 µg L^{-1} (n = 74) in tap water, 300 µg L^{-1} (n = 34) in well water and up to 3000 µg L^{-1} (n = 257) in surface water. This was attributed to the closeness of the town to a large open pit boron mine.

Gemici et al.[38] evaluated the hydrogeochemical properties of the Heybeli spa (Afyon) to determine the quality of thermal water. Using inductively coupled plasma-optical emission spectrometry (ICP–OES), arsenic concentrations in the range of 30–1417 μg L^{-1} for well water and 30–1123 μg L^{-1} for spring water were reported and it was noted that arsenic had a high positive correlation with concentrations of boron, zinc, copper and lithium, which suggested possible rock leaching of arsenic. They concluded that thermal waters from the Heybeli spa are suitable for space heating, greenhouses, bathing, swimming and balneotherapy but are not suitable as mineral water.

Dogan et al.[39] studied arsenic mineralization, source, distribution, and abundance in Kütahya region through analyzing different types of rocks and minerals by ICP–OES and water samples for their arsenic content by means of GF–AAS. Arsenic concentration varied greatly with location. The authors reported arsenic concentrations of up to 950 μg L^{-1} (geothermal water), 1070 μg L^{-1} (groundwater) and 10 μg L^{-1} (surface water).

Gemici et al.[40] investigated the level of pollutants in waters around untreated abandoned Türkönü mercury mine (İzmir). Three water samples were collected from the mine area and were analyzed using ICP–MS for their contents of 19 elements including arsenic and antimony. It was observed that there was a remarkable enrichment of some elements especially in waters collected from puddles on the mine wastes. Arsenic concentrations ranged from 77 μg L^{-1} to 53.3 mg L^{-1} while for antimony they were found to vary from 0.58 to 418.6 μg L^{-1}.

In another study, Gemici et al.[41] related the high arsenic concentrations in groundwater of Bigadiç district determined using ICP–OES (33–911 μg L^{-1}), to borate mining activity in the region which has the largest colemanite and ulexite deposits in the world. Arsenic minerals or arsenic-bearing pyrite were not found in the deposits. However, results of the chemical analyses of the colemanite and ulexite minerals showed that arsenic concentration was around 70 mg kg^{-1}, coinciding with the high arsenic concentrations in groundwater.

Guler et al.[42] investigated the mineral content of 70 bottled water brands sold on the Turkish market. Brands consisting of ‘‘natural spring’’ and ‘‘processed drinking’’ types, collected from markets and food stores in eight provinces of Turkey, were analyzed for their major and trace element constituents to ascertain their suitability for human consumption. Analyses using ICP–MS revealed concentrations in the ranges of 0.29–1.23 μg L^{-1} and 0.12–30.63 μg L^{-1} for antimony and arsenic, respectively. While values for antimony were well below the parametric values set by the European Community Council Directive 98/83/EC of 5 μg L^{-1} and the World Health Organization (WHO) guideline values of 20 μg L^{-1}, arsenic concentrations in one sample was 30.63 μg L^{-1} which was more than three times the standard value set by the recommended value of 10 μg L^{-1} in drinking water. Additionally, in five other samples, arsenic concentrations were higher than 5 μg L^{-1}.

Aksoy et al.[43] comprehensively elucidated groundwater contamination mechanism in a geothermal field in Balçova (İzmir). Due to natural and anthropogenic activities including uncontrolled discharge of waste geothermal fluid to the natural drainage network, cold groundwater reserves were found to be contaminated in such a way that various toxic elements including arsenic and antimony were introduced to the heavily

used surficial aquifer waters hindering their use for human consumption and agricultural irrigation. Water samples collected from 39 stations were analyzed using ICP–MS. Antimony concentrations ranged between 26 and 689 μg L^{-1} in hot geothermal waters; between undetected and 26 μg L^{-1} in groundwaters; and between undetected and 24 μg L^{-1} in surface waters. Arsenic concentrations in geothermal waters were between 164 μg L^{-1} and 1420 μg L^{-1}. High correlations were observed between arsenic, boron and lithium, which indicated that arsenic was introduced into the environment via geothermal fluid. Arsenic concentrations in groundwater samples were between 0.7 and 170.1 μg L^{-1}. Surface water samples contained up to 182.4 μg L^{-1} arsenic.

Gunduz et al.[44] conducted a study to assess groundwater quality of Simav, Kütahya, by collecting water from 27 wells drilled in the surficial aquifer. Using ICP–MS, the authors reported an average arsenic concentration of 99.1 μg L^{-1} and a maximum concentration of 561.5 μg L^{-1}. It was concluded that high arsenic levels in groundwater primarily originated from local geological formations. The aquifer material containing metamorphic rocks was considered to be a general mechanism for arsenic enrichment in the aqueous phase. Arsenic levels in metamorphic rocks of the study area ranged between 10.4 mg kg^{-1} and 660.4 mg kg^{-1}, where the highest value was obtained in a sample collected from a former Cu-Pb-Zn mine site situated next to the Simav district center.

Altas et al.[45] determined arsenic levels in 62 stations utilized as drinking and potable water resources by local community in Aksaray Province. Sampling was implemented every two months for one year. Using HG–ICP–OES, maximum arsenic concentrations were found as

52.4 µg L^{-1} (well-drinking), 201 µg L^{-1} (well-irrigation), 13.8 µg L^{-1} (spring-drinking), and 54.6 µg L^{-1} (river).

In another study, Guler et al.[46] analyzed a total of 100 samples from 25 brands of carbonated natural mineral waters produced in Turkey for a total of 36 water quality constituents, including arsenic and antimony, to determine their suitability for human consumption. Using ICP–MS, concentration ranges of 0.03–16.93 µg L^{-1} for arsenic and 0.03–3.04 µg L^{-1} for antimony were reported. The authors stated that substantial amounts of antimony can be released from commercially available glass and PET containers used for drinking waters. PET plastic is usually made with antimony-based catalysts. Therefore, antimony concentrations in PET bottles increase over time during storage, especially when they are exposed to high temperatures. The authors concluded that high concentrations of arsenic in some of the studied brands could pose a risk to individuals who consume those carbonated natural mineral waters on a regular basis.

Caylak[47] investigated the levels of arsenic in water sources of Çankırı Province. The samples, analyzed using AAS, had arsenic concentrations of up to 64.5 µg L^{-1} (reservoir), 71.9 µg L^{-1} (well-drinking) and 51.2 µg L^{-1} (spring-drinking). Studies performed to determine the concentrations of arsenic and antimony in Turkey's water are summarized in Table 1.

Table 1. Determination of total arsenic and antimony concentrations in Turkey's waters.

Study location	Analytical technique	Sample	Maximum arsenic concentration ($\mu g\ L^{-1}$)	Maximum antimony concentration ($\mu g\ L^{-1}$)	Ref. (year)
Hisarcık, Kütahya	ICP–MS	Tap	760	–	37 (2004)
		Well	300		
		Surface	3000		
Heybeli, Afyon	ICP–OES	Well	1417	–	38 (2004)
		Spring	1123		
Kütahya	GF–AAS	Geothermal	950	–	39 (2007)
		Ground	1070		
		Surface	10		
Türkönü mine, İzmir	ICP–MS	Surface	53.3	418.6	40 (2007)
Bigadiç, Balıkesir	ICP–OES	Ground	911	–	41 (2008)
–	ICP–MS	Bottled waters	30.63	1.23	42 (2009)
Balçova, İzmir	ICP–MS	Geothermal	1420	689	43 (2009)
		Ground	170.1	26	
		Surface	182.4	24	
Simav, Kütahya	ICP–MS	Ground	561.5	–	44 (2010)
Aksaray	HG–ICP–OES	Well-drinking	52.4	–	45 (2011)
		Well-irrigation	201		
		Spring-drinking	13.8		
		River	54.6		
–	ICP–MS	Carbonated natural mineral waters	16.93	3.04	46 (2011)
Çankırı	AAS	Reservoir	64.5	–	47 (2012)
		Well-drinking	71.9		
		Spring-drinking	51.2		

2.6 Capillary electrophoresis

A CE system is comparatively simple. The basic components (Fig. 1) include a power supply, which provides the high voltage necessary for the separation, a capillary in which the separation takes place, a detector that monitors the analytes, and a data acquisition system that records the electropherogram.

A background electrolyte (BGE) in CE is a continuous electrolyte system that provides an electrically conducting and buffering medium along the migration path. If its composition is constant, the electric field and the effective mobilities of the analytes remain constant, and they migrate with mutually different but constant velocities.

To make an injection, the BGE inlet vial is replaced with the sample vial. A small volume of the sample is introduced into the capillary and then the BGE vial is returned to its original position. The power supply is switched on, and the analytes migrate toward the detector. As the analytes pass by the detector, the signal is recorded on the data acquisition device. The majority of the literature using CE describes the application of UV or fluorescence detection since these were the only detectors offered by the early commercial instrument manufacturers. However, nowadays, CE instrument manufacturers offer conductivity and MS detectors hyphenated to CE.

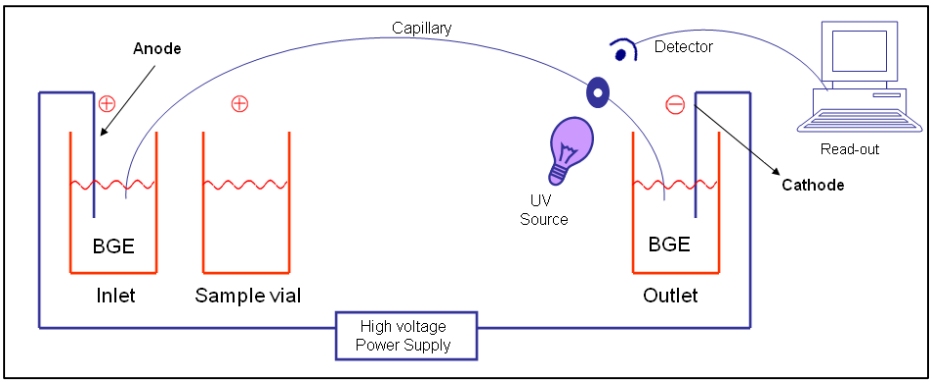


Fig. 1. Schematic diagram of a CE instrument

A high-voltage DC power supply provides the electrical field necessary to establish the EOF of the bulk solution as well as electrophoretic mobility of the charged analytes. Most power supplies provide voltages in the range of –30 kV to +30 kV with current levels of about 100 to 300 µA. As it is necessary to make the detector end either anodic or cathodic depending on the application, it must be possible to switch polarity. Field programming is the ability to run voltage (sometimes current or power) gradients, which is another feature that is often useful in CE instruments.

Only small volumes of the sample can be introduced into the capillary if the high separation efficiencies characteristic of the technique are to be maintained. In general, the sample length should be less than 2% of the total capillary length. Although this can be an advantage for applications with limited sample volumes, it can be a problem from the point of view of detection sensitivity. The sample is introduced into the capillary either by hydrostatic injection (gravity, pressure, or vacuum) or electrokinetic injection. Hydrostatic injection, which is a more common approach, can be achieved by (1) raising the sample vial a given distance above the level of the destination vial for a predetermined time (gravity), (2) applying pressure to the sample vial (pressure), or (3) applying a

vacuum to the destination end of the capillary (vacuum). With hydrostatic injection mechanisms, injection reproducibility (expressed as relative standard deviation, %RSD) can be better than 1–2%. The volume of sample loaded is a function of the capillary dimensions, viscosity of the buffer, the applied pressure, and time. Electrokinetic injection is performed by replacing the BGE vial with the sample vial and applying voltage. The advantage of this approach over the other is that more sample can be introduced, thereby improving LOD. The disadvantage is that the sample plug that enters the capillary is not representative of the original sample, because ions with higher mobility will enter at a greater rate than ones with lower mobility.

Separation in CE is based on the principle that, under the influence of an applied potential, different species in a BGE solution migrate at different velocities from one another. Each ion moves toward the electrode of opposite charge. The velocities of the migrating species depend not only on the electric field, but also on the size of the species and their environment. Historically, electrophoresis has been performed on a support medium such as a semisolid slab gel or in non-gel support media such as paper or cellulose acetate. The support media provided the physical support and mechanical stability for the fluidic buffer system. CE has emerged as an alternative form of electrophoresis, where the capillary walls provide the mechanical stability for the carrier electrolyte.

In electrophoresis, there are two main contributions to the movement of analyte ions: first, the electrophoretic mobility (μ) of the analyte ion itself and, second, the speed and direction of the EOF. The latter is the term used to describe the movement of the bulk solution in contact with a solid surface, when an electric field is applied. It is used to improve separations of inorganic species in CE, but is often suppressed

during the analysis of larger molecules in order to improve resolution. The EOF may be induced to travel in the same direction as the analyte ions or in the opposite direction from the analyte ions, or it can be suppressed so that the flow is negligible.

If both the analytes and the EOF move in the same direction, the system is classified as co-electroosmosis, and the analytes reach the detector faster than they would as a result of their electrophoretic mobilities. If the analytes move in the opposite direction to the EOF, the system is classified as counter-electroosmosis, and the analytes reach the detector later than they would as a result of their electrophoretic mobilities. If the EOF is suppressed by eliminating the effective charge on the capillary walls, then the analytes reach the detector solely as a result of their own electrophoretic mobilities.

Resolution, R_s, depends on both the efficiency of the system and the differences in migration times of the analytes of interest. Resolution is affected by the speed and direction of the EOF, the mobility of the analytes, and, to a lesser degree, on the applied voltage. R_s is defined as:

$$R_s = \frac{2\,(t_B - t_A)}{w_A + w_B}$$

where t_A and t_B are the migration times, w_A and w_B are the baseline width in time for the peaks of species A and B, respectively.

CE is similar to chromatography in many aspects, and most of the terms used in chromatography are found in CE. For example, resolution and efficiency of separation are common to both techniques and are

defined in a similar way. However, some of the terminology is different, as illustrated in Table 2. For example, in chromatography, a column is used to separate the analytes; in CE, a capillary is used. In chromatography a pump is used to propel the sample through the column; in CE, there is no external pumping system, and the sample constituents move as a result of their mobilities in an applied potential and the EOF, if present.

Table 2. Comparison of electrophoretic and chromatographic terms.

CE	Chromatography
Electropherogram	Chromatogram
Applied potential	Flow rate
BGE	Eluent or mobile phase
Injection mode (hydrostatic or electrokinetic)	Injector
Migration time	Retention time
Electrophoretic mobility	Column capacity factor
EOF	–
High-voltage power supply	Pump
Capillary	Column

2.6.1 Online preconcentration methods in CE

CE with online UV detection has been widely applied for the speciation of arsenic and antimony. Its popularity may be attributed to its extremely high efficiency, short analysis time, and wide application range. However, this technique suffers from low concentration sensitivity[10]. This arises from the small dimensions of the CE capillary, typically 25–75 µm inner diameter (i.d.) and 30–100 cm in length, which limits the volume of sample that can be injected and restricts the optical path length when optical detection is used. To address this problem, several techniques

have been developed. These include the use of high sensitivity detectors such as LIF[48], MS[49], chemiluminescence[50] and electrochemical detectors[51]. Nevertheless, these detectors are rather expensive, may require derivatization, long procedures, interface problems and/or complicated software. Alternatively, the extended path length capillary with bubble cell or Z-type cell are available to increase sensitivity up to 10-fold[10]. Other techniques include offline sample preconcentration techniques e.g., LLE and SPE. Shortcomings associated with LLE such as emulsion formation, use of large sample volumes and toxic organic solvents make it labor-intensive, expensive, time-consuming and environmentally unfriendly. Although SPE uses much less solvent than LLE, it can still be considered significant, and normally an extra step is needed to preconcentrate the analytes further into smaller volumes. SPE is also time-consuming and relatively expensive[52].

The drive for “green” methods to overcome these inherent problems of conventional LLE and SPE has led to the development of solventless and solvent-minimized microextraction techniques such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME). Despite the advantages provided by SPME, most commercial fibers used are relatively expensive, fragile and have limited lifetime. Moreover, sample carry-over is a possible problem[53]. LPME with its various modes such as dispersive liquid-liquid microextraction (DLLME)[54], single drop microextraction (SDME)[55], hollow fiber-liquid phase microextraction (HF–LPME)[56] and solvent-bar microextraction (SBME)[57], among others, has emerged to overcome such problems of SPME.

Recently, we have introduced the use of DLLME, ultrasound-assisted emulsification microextraction (USAEME) and solidification of floating organic drop microextraction (SFODME) along with online

preconcentration techniques in CE as efficient techniques for the analysis of ephedrines in human urine[58], non-steroidal anti-inflammatory drugs (NSAIDs) in milk and dairy products[59], bisphenol A in water and human urine[60] and beta(2)-agonists in bovine urine[61].

Online sample preconcentration can be performed just by injecting a large volume of the sample solution without modification of the instrument and the analyte can be focused (through stacking or sweeping) into a minimum volume inside the capillary. Therefore, online sample preconcentration is a useful technique to improve the concentration sensitivity taking advantage of the small sample volume requirement in CE[62]. These include sweeping and stacking, the latter can be performed through several modes such as (i) FASS[18], (ii) LVSS with polarity switching[19], and (iii) FASI/EOF reversal[10].

2.6.1.1 Sweeping-micellar electrokinetic chromatography

Micellar electrokinetic chromatography (MEKC), a separation mode of CE, has enabled the separation of charged as well as neutral analytes. MEKC can be performed by adding an ionic micelle to the BGE without modifying the instrument. Its separation principle is based on the differential migration of the ionic micelles and the BGE under electrophoretic conditions and on the interaction between the analyte and the micelle. Hence, separation principle of MEKC combines those of chromatography and CE. MEKC is a useful technique particularly for the separation of small molecules, both neutral and charged, and yields high-efficiency separations in a short time with minimum amounts of sample and reagents. To improve the concentration sensitivity of detection,

several online sample preconcentration techniques in MEKC including sweeping have been developed[63].

Sweeping is defined as the picking and accumulating of the analytes by charged micelles that fill or penetrate through the sample zone upon application of a voltage[64]. Both anionic and cationic surfactants can be used for this purpose. A schematic diagram of sweeping-MEKC with sodium dodecyl sulfate (SDS) as an anionic surfactant is shown in Fig. 2. In this method, the capillary is conditioned with a non-micellar (NM) acidic BGE. The magnitude of the EOF is suppressed almost to zero by the use of an acidic buffer[65]. The sample, generally prepared in a low-conductivity buffer is injected and the inlet vial is replaced with a high-conductivity buffer containing SDS (micellar, M-BGE) keeping the outlet vial the same. When a negative polarity is applied, the SDS micelles enter the capillary from the inlet and penetrate through the sample zone. Then, they sweep the anionic, neutral and cationic analytes by varying interaction strength. Since they are negatively charged micelles, they interact with cationic analytes the most and the anionic ones the least. Accordingly, the SDS-analyte moieties are separated by the MEKC mode. Small cations reach the detector first and large anions reach last (Fig. 2).

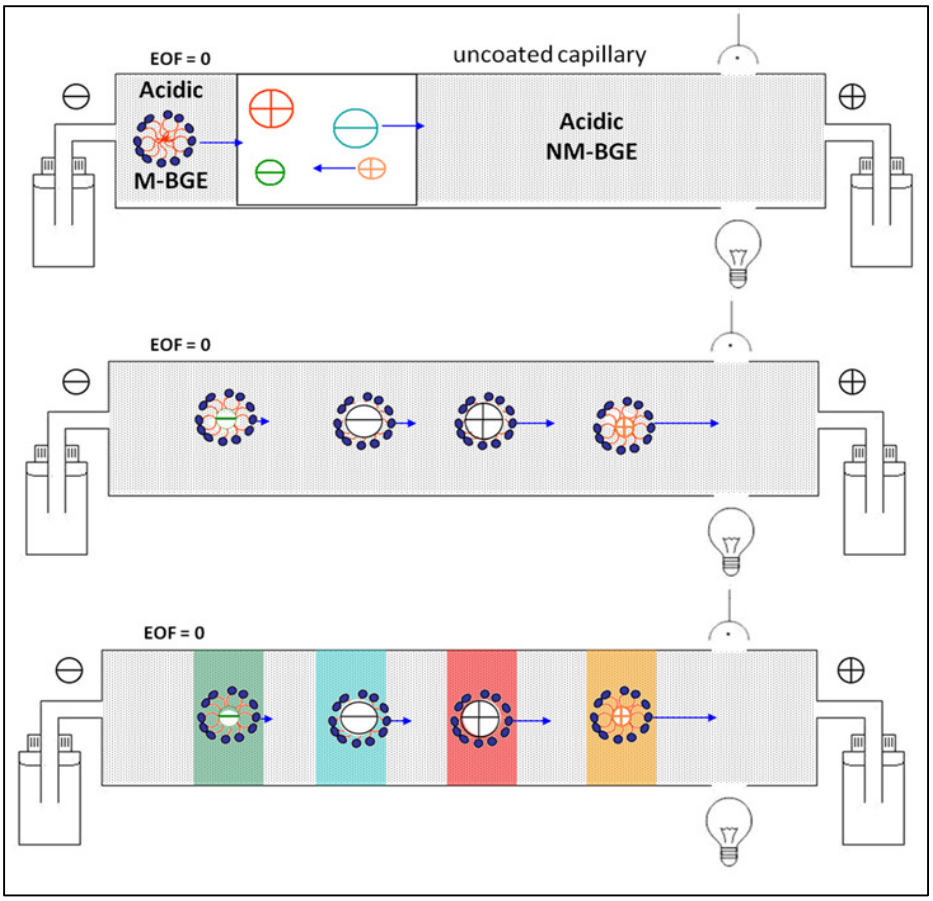


Fig. 2. Schematic diagram of sweeping-MEKC model with SDS

2.6.1.2 Large volume sample stacking

As described by Chien and Burgi[19], LVSS can be performed by preparing the sample in a low-conductivity matrix, which is different from the BGE (a high-conductivity buffer). A convenient sample preparation method uses a 1/100–1/1000 diluted BGE, resulting in a low-conductivity solution, in which the sample is dissolved. A schematic diagram of the LVSS method is illustrated in Fig. 3. After filling the capillary with the BGE, the sample solution is injected into the capillary to a certain length and a negative polarity is applied. At this moment, the direction of the EOF is toward the inlet. Owing to their negative charge, anionic analytes tend to move toward the detection end (outlet) and thus stack at one side of the

boundary between the sample zone and the BGE. Meanwhile, the cations and neutral species move and exit the capillary at the injection end (inlet). The electrophoretic current should be monitored carefully until it reaches approximately 90–95% of its original value, and the polarity is then quickly returned to positive, leading to a reversed EOF. If there is a delay, the anionic analytes may be lost. The following separation occurs by the CZE mode. For acquiring a good reproducibility, monitoring the current requires some care. Furthermore, it should be noted that this method cannot be used for the simultaneous separation of anions and cations and is also limited to analytes with low mobilities.

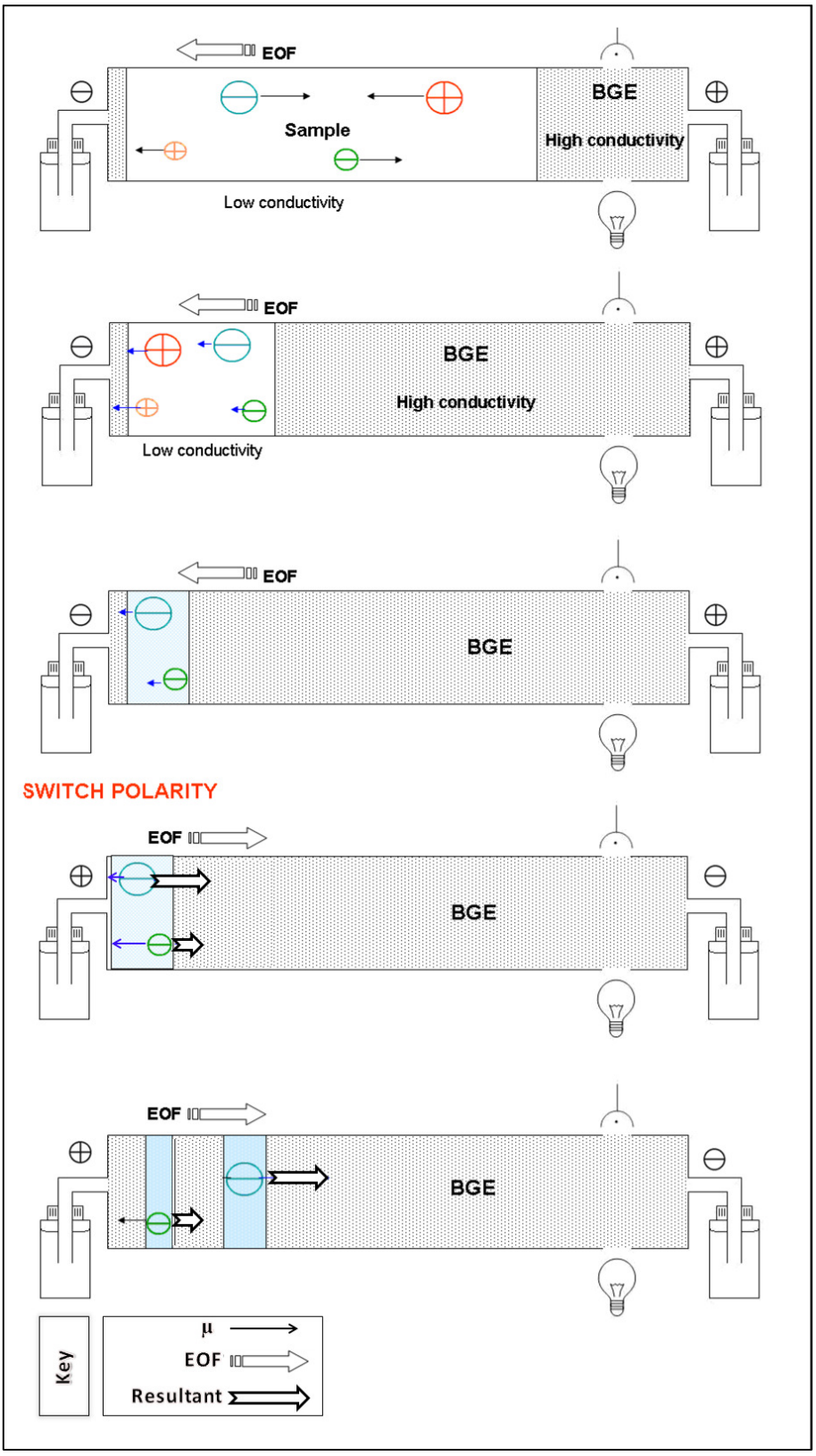


Fig. 3. Schematic diagram of the LVSS model

2.6.1.3 Field-amplified sample stacking

The simplest and most commonly used sample stacking technique is FASS[65]. It is based on the concept that ions electrophoretically migrating through a low-conductivity solution (sample plug) into a high-conductivity solution (i.e., BGE) slow down dramatically at the boundary between the two solutions. A schematic diagram of FASS is shown in Fig. 4.

In this method, the sample is prepared in a low-conductivity matrix, which is different from the BGE (a high-conductivity buffer). As in LVSS, a convenient sample preparation method uses a 1/100–1/1000 diluted BGE, resulting in a low-conductivity solution, in which the sample is dissolved. In the initial step, the capillary is filled with the BGE and the sample solution is then injected into the capillary to a certain length. Upon application of a high positive voltage, a proportionally greater electric field develops across the sample zone causing the ions to migrate more rapidly. Once the ionic analytes reach the boundary between the sample zone and the BGE, the electric field strength suddenly decreases and migration becomes slower, causing the analytes to be focused at the boundary. If the EOF is greater than electrophoretic mobility of the analytes, they will move toward the detection window (the cations migrating faster than the anions). Then, they will be separated by the CZE mode. In this method, the sample injection volume must be optimized, however, because the mismatch in EOF between the sample solution and the BGE can cause peak broadening and even a total loss of resolution if a long sample plug is injected. Consequently, the best results are obtained when a proper compromise is found, which may cause the sensitivity enhancement of this method to be limited.

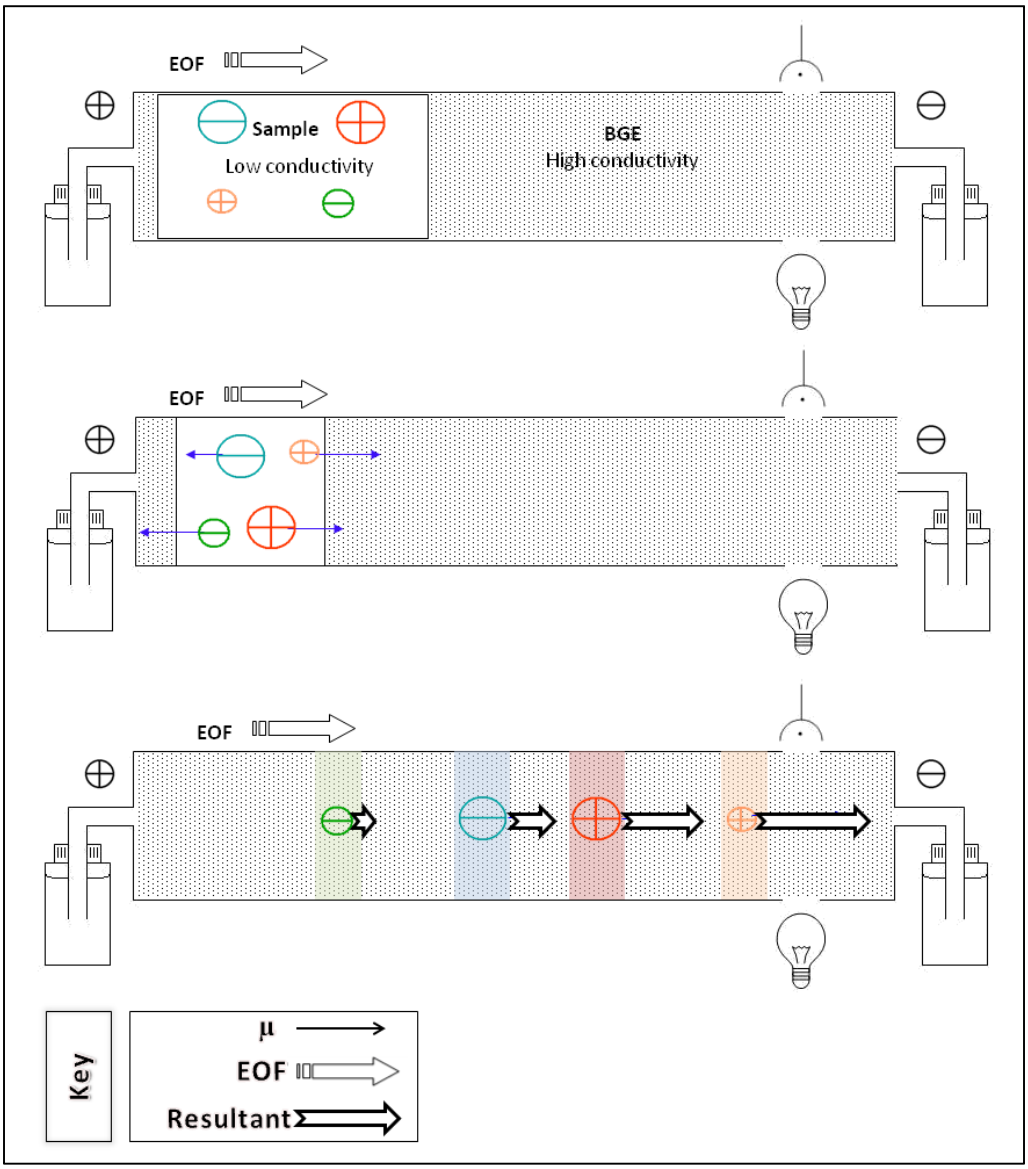


Fig. 4. Schematic diagram of the FASS model

2.6.1.4 Field-amplified sample injection with EOF reversal

One of the most commonly used sample stacking techniques is FASI, the principle of which is similar to FASS except that the analytes are introduced into the capillary by electrokinetic injection. To enhance the efficiency of this method, a short deionized water plug is generally

injected hydrodynamically into the capillary prior to the introduction of the analytes. Ions enter the capillary by their own electrophoretic mobilities as well as by the EOF upon electrokinetic injection of the sample[66]. More ions will be injected if the electrophoretic mobility of the ion is in the same direction as the EOF. In some instances, such as in the separation of inorganic ions, complete elimination of the EOF is undesirable. Changing the velocity of the EOF or reversing the direction may be more preferable. To take advantage of the EOF, the charge on the capillary can be reversed by the addition of cationic surfactants such as cetyltrimethylammonium bromide (CTAB) to the BGE. The surfactant coats the inner capillary walls, as illustrated in Fig. 5, B, resulting in a capillary wall with a positive charge. The positively charged hydrophilic ends of the quaternary amines are attached to the capillary wall through ionic interactions with the silanoate groups on the wall. The hydrophobic hydrocarbon ends of these amines associate with the other hydrocarbon ends of quaternary amines in the solution through hydrophobic interactions. The hydrophilic, positively charged ends of the associated amines attract anions from the buffer. The solvated anions migrate toward the anode, and drag the bulk solution with them, which results in the reversal of the direction of the EOF. A schematic diagram of FASI/EOF reversal is shown in Fig. 6.

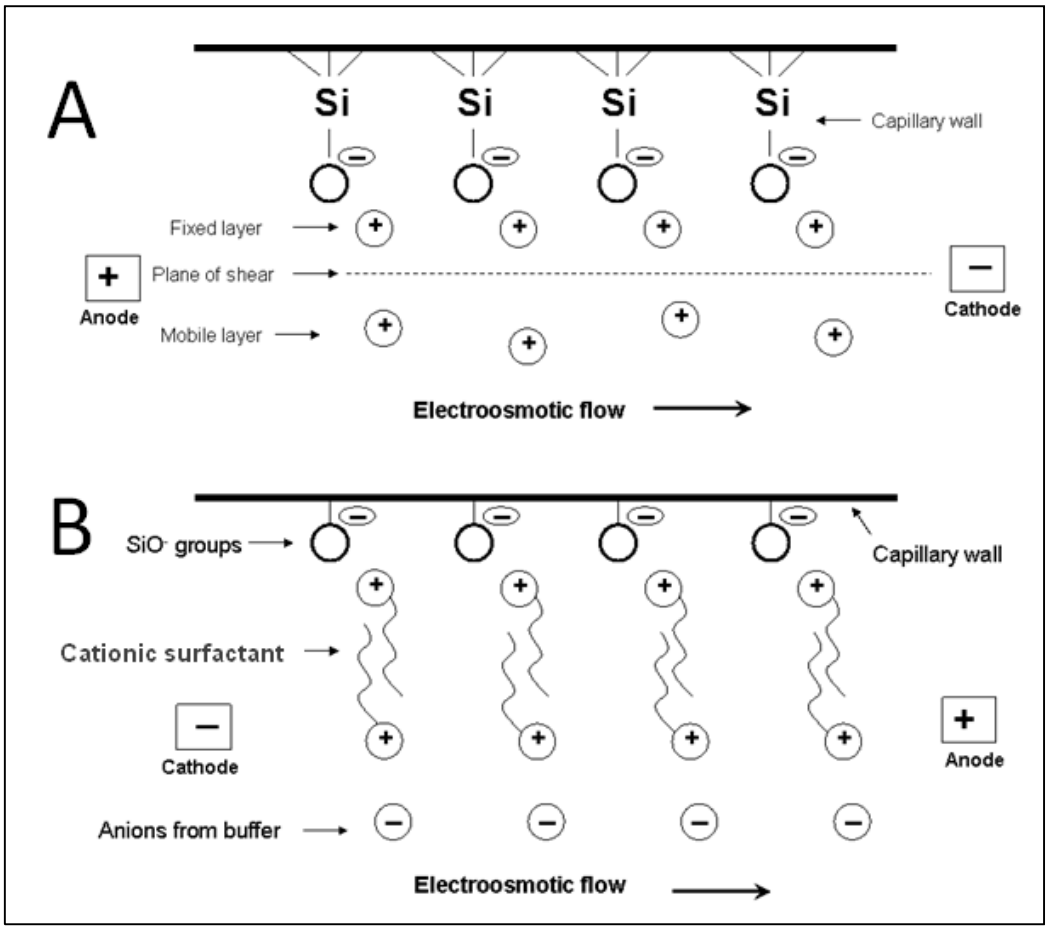


Fig. 5. Schematic illustration of the capillary wall. (A) no surfactant added and (B) cationic surfactant added to reverse the EOF

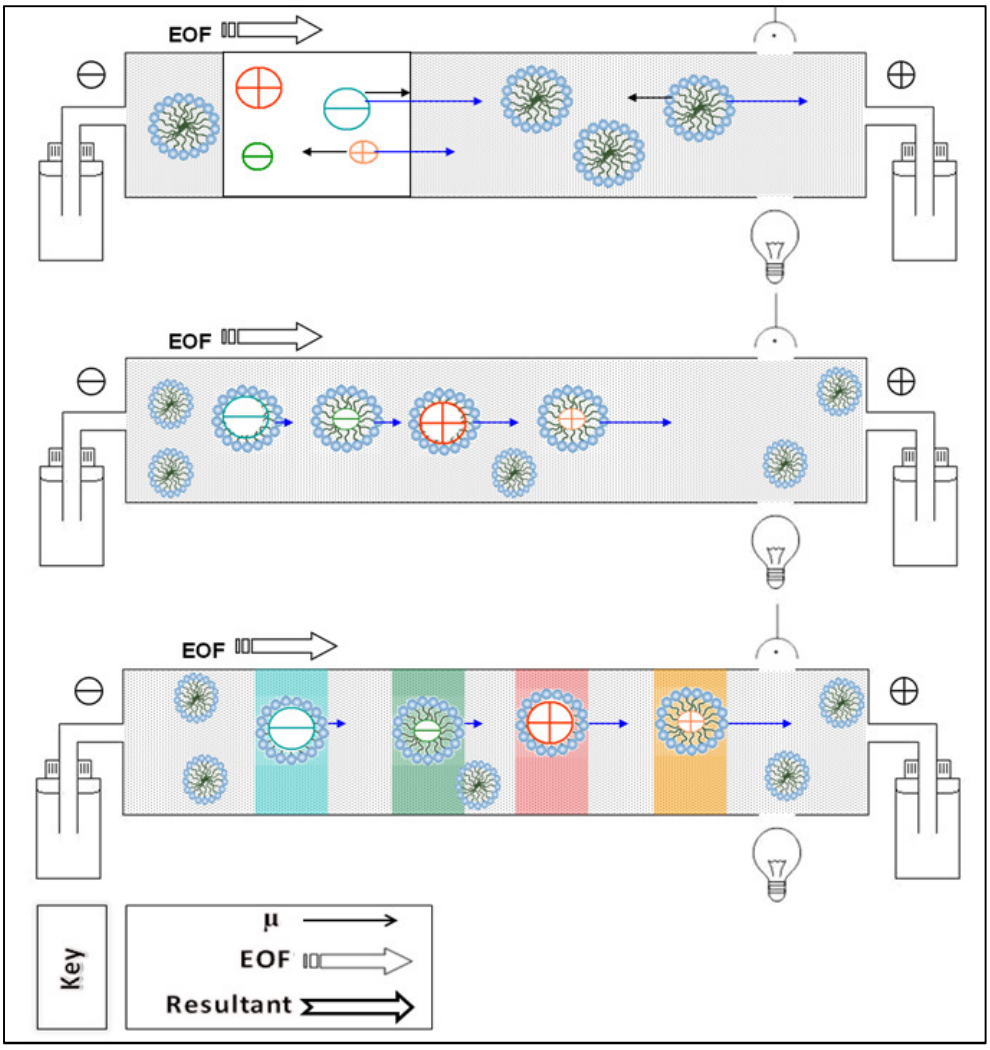


Fig. 6. Schematic diagram of the FASI/EOF reversal model

2.6.2 High-sensitivity detection cell

In CE with on-capillary optical detectors, sensitivity is defined by the diameter of the capillary. Because sensitivity is proportional to the path length, sensitivity of photometric methods is limited. Thus, different capillary geometries have been developed to overcome this problem. In the Z-cell also known as HSDC (Fig. 7), two pieces of the capillary are attached through the cell to create a Z-shaped path, resulting into a longer

longitudinal optical path length and sensitivity enhancements of up to 10 times over standard 75 µm id capillaries.

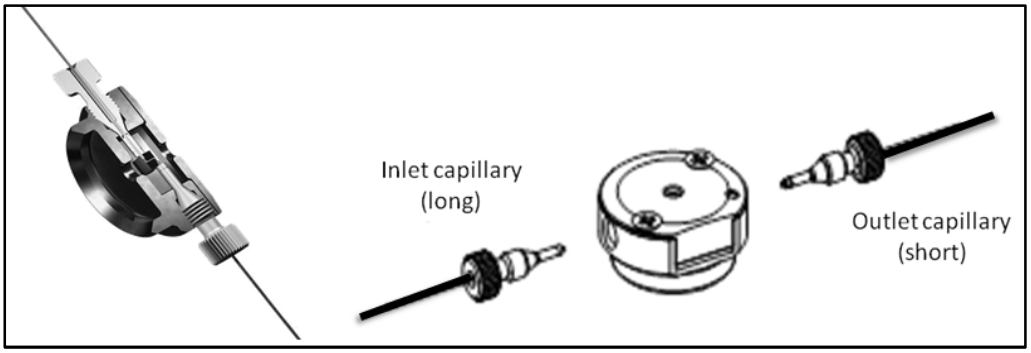


Fig. 7. A schematic diagram of the HSDC

2.7 Speciation analysis

A chemical species is a specific form of a chemical element, defined as its molecular or complex structure, or oxidation state. While, the term speciation analysis refers to the analytical activity of identifying and measuring species[67]. It must be stressed that identifying and measuring includes strictly a clear identification of the species (elements and possibly binding partners) as well as an exact quantification in a representative sample[68,69].

Analytical chemists have increasingly realized within the last two decades that determining total concentrations of the elements cannot provide the required information about mobility, bioavailability, and finally the impact of elements on ecological systems or biological organisms. Only knowledge about the chemical species of the elements can lead to understanding of chemical and biochemical reactions involving these species, thus providing information about toxicity or essentiality. Elements usually interact as parts of macromolecules (proteins, enzymes,

hormones, etc.) or according to their oxidation states. Thus, problem-related speciation analysis promises to become an essential tool for useful risk assessment of elements in the environment, leading to more effective diagnosis and therapy of trace elements depletion or toxicity and better health. This type of research will lead to legislations based on maximal element species concentrations rather than total element concentrations. Element speciation analysis should allow a speciation of the different elements in a particular sample, reflecting the actual interrelationships. However, since this aim is not easily achieved, new challenges arise concerning sampling, sample storage, preparation, as well as separation and detection technologies[70].

2.8 CE for speciation of arsenic and antimony

Separation principles in CE and MEKC are quite different, providing completely different characterization and identification mechanisms for element species. This is important, because species identification is rarely done by one single method, but requires multidimensional strategies. Besides, this flexibility offers the advantage of facilitating the development of separations for nearly every separation problem. Whereas CE supplements conventional LC methods, it shows unique promise for speciation purposes by exerting only minor disturbance on the existing equilibrium between different species. The lack of a stationary phase in CE avoids the problems that arise as a result of the large surface area and various possibilities for undesired interactions. Therefore, species integrity is not as likely to be compromised as with LC. However, the use of CE for speciation does entail several limitations, among them is the small sample volume, typically only a few nanoliters, that can be analyzed. This causes problems with representativity and demands extreme homogeneity of the sample. Also, the concentration detection

limits are generally one to two orders of magnitude higher than those with LC separations. The high voltage itself can sometimes alter the integrity of elemental species. Similarly, the BGE composition and nature of additives can have detrimental effects on species stability[71].

The mobility of analytes is dependent on the actual strength of the electric field and the (pH-dependent) EOF. Unfortunately, the EOF may be influenced by the sample itself. Therefore, migration time of a specific compound may be shifted from standard to sample and from sample to sample. This phenomenon has been widely reported in the literature and is known as ‘‘migration-time shifts’’[72]. Species identification according to comparison with standard electropherograms is thus most questionable. Among the solutions proposed was the addition of an internal standard, preferably a compound not occurring in the native sample. Alterations in its known migration time permit correction for species migration times according to standard electropherograms. Another possible solution is the ‘‘standard addition method.’’ If co-migration of other analytes can be excluded this clearly identifies the species. Alternatively, peaks can be identified by the spike method, through the addition of a single species to a mixture of all species and the recognition of the peak with increased height and/or area.

There exists extensive literature on speciation of arsenic with a variety of CE separation modes and detection techniques. This can be attributed, on the one hand, to the wide occurrence of various forms of arsenic in the environment, industrial products, and living organisms and, on the other, to the pronounced influence of the chemical form on arsenic toxicity, where e.g. As(III) is several orders of magnitude more toxic than organoarsenic species[73]. Hence, if the toxicity risk to arsenic exposure is

based on the total arsenic concentration, this can be significantly underestimated or overestimated.

For speciation of arsenic, three main modes of separation have been employed (Table 3), either with the anode or with the cathode at the detection end of the capillary. In the first case, when a suppressed EOF was used, anions with higher electrophoretic mobility than the EOF are separated. In the second case, the EOF was reversed towards the anode by the addition of positively charged surfactants, the separation of analytes was based on co-electroosmotic migration. Finally, in the system with cathodic polarization at the detector end, the analytes migrated together against the EOF and separation had counter-electroosmotic character because electrophoretic mobilities were less than the EOF[73].

Direct UV detection in CE has been the main technique for speciation purposes of arsenic and antimony. However, indirect UV and LIF have also been used. Element-specific detectors have been integrated with CE and applied for the same purpose, which included ICP–MS and HG–AFS. Recent studies using CE for speciation of arsenic and antimony in different matrices are summarized in Table 3 with emphasis on the offline and/or online preconcentration techniques used as well as the CE conditions under which optimum separation was obtained.

Table 3. Recent studies using CE for speciation of arsenic and antimony.

Matrix/ sample	Species	Detector	Pretreatment/ preconcentration method	CE conditions	Ref.
Arsenic speciation					
Coal fly ash, dogfish liver SRMs	As(III), DMA, MMA, As(V), AB	ICP–MS	MW[a]-assisted extraction	Capillary: 60 cm, 75 µm, uncoated; BGE: 25 mM 3-(cyclohexylamino)-1-propanes-ulfonic acid, 0.5 mM SDS (pH 9.5); Separation voltage: 25 kV; Injection: pressure, 50 s.	74
Standards	As(III), DMA, MMA, As(V)	UV (indirect)	FASI	Capillary: 72 cm, 50 µm, coated; BGE: 10 mM 2,6-pyridinedicarboxylic acid (pH 10.3); Separation voltage: –25 kV; Injection: electrokinetic, 10 s, –20 kV.	9
Mya arenaria Linnaeus, *Shrimp*	As(III), DMA, MMA, As(V)	ICP–MS	MW-assisted extraction	Capillary: 60 cm, 75 µm, uncoated; BGE: 20 mM NaH_2PO_4-5.00 mM $Na_2B_4O_7$ (pH 6.50); Separation voltage: 12 kV; Injection: pressure, 10 s.	75
Dried shrimps	As(III), DMA, MMA, As(V), p-aminophenyl arsenic acid	UV	MW-assisted extraction	Capillary: 60 cm, 75 µm, uncoated; BGE: 20 mM NaH_2PO_4-5.00 mM $Na_2B_4O_7$ (pH 6.25); Separation voltage: 20 kV; Injection: pressure, 10 s, 3.0 kPa.	76
Natural and contamin-ated waters	As(III), DMA, MMA, As(V)	UV	–	Capillary: 60 cm, 75 µm, uncoated; BGE: 10 mM Na_2MoO_4, 10 mM $NaClO_4$ (pH 3.0); Separation voltage: −13 kV; Injection: pressure, 100 s, 30 mbar.	77
Fish	As(III), DMA, As(V), AB	ICP–MS	US[b]-assisted extraction	Capillary: 34.5 cm, 75 µm, uncoated; BGE: 25 mM borate, 10% methanol (pH 9.2); Separation voltage: 30 kV; Injection: pressure, 1 s, 50 mbar.	78
Aqueous soil extracts	As(III), DMA, MMA, As(V), phenylarsonic acid, p-aminophenyl-arsonic acid, o-amino-phenylarsonic acid, roxarsone, phenylarsine oxide	UV	US-assisted extraction and LVSS with polarity switching	Capillary: 56 cm, 50 µm, uncoated; BGE: 50 mM phosphate (pH 8.0); Stacking: pressure, 700 s, 50 mbar, for 120 s at –30 kV, then polarity switched to 30 kV.	79
Chicken litter and soil	As(III), MMA, As(V)	UV	US-assisted extraction and dynamic pH junction	Capillary: 66.5 cm, 50 µm, uncoated; BGE: 15 mM phosphate (pH 10.6); Separation voltage: 25 kV; Injection: pressure, 150 s, 50 mbar.	80
Standards	As(III), DMA, MMA, As(V), trimethylarsine oxide, tetramethyl-arsonium ion, AB, arsenocholine	UV (indirect), MS	–	Capillary: 40–70 cm, 50 µm, uncoated; BGE: 5 mM lactic acid, 10 mM 3,4-diamino-benzoic acid (pH 3.2); Separation voltage: 25 kV (cationic analytes) and –30 kV (anionic analytes); Injection: pressure, 6 s, 50 mbar.	81
Ground-water	As(III), DMA, As(V),	UV	–	Capillary: 42 cm, 50 µm, uncoated; BGE: 10 mM phosphate, 0.35 mM TTAB (pH 9); Separation voltage: –20 kV; Injection: electrokinetic, 10 s, –10 kV.	82

Matrix/ sample	Species	Detector	Pretreatment/ preconcentration method	CE conditions	Ref.
Sediment	As(III), DMA, MMA, As(V)	UV	Extraction; co-EOF-NSM[c]; HSDC	Capillary: 56 cm, 50 µm, coated; BGE: 15 mM phosphate (pH 10.6), Separation voltage: −25 kV; Injection: pressure, 40 s, 50 mbar.	1
Lake and river water	As(III), DMA, MMA, As(V)	HG–AFS	pH-mediated stacking	Capillary: 50 cm, 75 µm, uncoated; BGE: 25 mM NaH_2PO_4 (pH 6.5); Separation voltage: 20 kV; Injection: pressure.	48
Tap and lake water	As(III), DMA, MMA, As(V), roxarsone, p-amino-phenylarsonic acid, 4-nitrophenyl-arsonic acid, phenylarsonic acid	UV	LVSS with polarity switching; NSM; co-EOF NSM with EOF reversal; HSDC	Capillary: 40 cm, 75 µm, uncoated; 51.5 cm, 50 µm, coated; BGE: 20 mM carbonate (pH 10); Separation voltage: 18 kV (normal EOF), −25 kV (reversed EOF); Injection: e.g., pressure, 160 s, 50 mbar (LVSS).	10
Animal feed	As(III), DMA, MMA, As(V), p-aminophenylarsonic acid, 4-hydroxy-3-nitro-benzenearsonic acid, 4-nitrophenylarsonic acid, phenylarsonic acid, phenylarsine oxide	UV	Extraction; co-EOF; counter-EOF	Capillary: 40 cm, 75 µm, uncoated; 51.5 cm, 50 µm, coated; BGE: 20 mM $NaHCO_3$-Na_2CO_3 (pH 10.0); Separation voltage: 18 kV (normal EOF), −25 kV (reversed EOF); Injection: pressure, 2 s, 50 mbar (counter-EOF), pressure, 7 s, 50 mbar (co-EOF).	3
Tap and mineral water	As(III), DMA, MMA, As(V)	LIF (indirect)	–	Capillary: 40 cm, 50 µm, uncoated; BGE: 2.0 mM $NaHCO_3$, 10^{-7} M fluorescein (pH 9.28); Separation voltage: 25 kV; Injection: pressure, 5 s, 0.5 psi.	83
Antimony speciation					
Standards	Sb(III), Sb(V)	LIF (indirect)	–	Capillary: 40 cm, 50 µm, uncoated; BGE: 10 mM borate, 1.0 mM fluorescein (pH 9.5); Separation voltage: 25 kV; Injection: pressure, 10 s.	11
Sewage sludge	Sb(III), Sb(V), $(CH_3)_3SbCl_2$	ICP–MS	Extraction, isoelectric stacking	Capillary: 150 cm, 50 µm, uncoated; BGE: 20 mM Na_2HPO_4/NaH_2 PO_4 (pH 5.6); Separation voltage: −18 kV; Injection: pressure, 7 s, 8 bar.	13
Simultaneous arsenic and antimony speciation					
Aqueous soil extracts	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	ICP–MS	Extraction	Capillary: 80 cm, 75 µm, uncoated; BGE: 0.5 mM sodium chromate, 0.5 mM TTAB (pH 11.2); Separation voltage: −20 kV; Injection: electrokinetic, 10 s, −20 kV.	84
Aqueous soil extracts	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	UV (indirect)	Extraction	Capillary: 52 cm, 75 µm, uncoated; BGE: 5 mM sodium chromate, 0.5 mM TTAOH[d] (pH 11.2); Separation voltage: −20 kV; Injection: electrokinetic, 20 s, −20 kV.	12

[a] Microwave

[b] Ultrasound

[c] Co-electroosmotic flow normal stacking mode

[d] Trimethyltetradecylammonium hydroxide (cationic surfactant)

2.9 HPLC–HG–AFS

AFS is an ideal detection technique for speciation studies concerning chemical-vapor-generating elements. The analytical features of AFS, such as LODs below the μg L^{-1} and the wide linear dynamic range, up to the mg L^{-1}, allow its application to a great variety of environmental, biological and food samples. AFS represents a suitable alternative to other atomic spectrometers commonly employed in speciation studies such as AAS and ICP–MS[21].

Since atomic fluorescence can be quenched by many molecules, an effective separation of the analytes from the matrix and/or a very efficient atomization is required. The popularity of HG arises from its significant advantages, including high analyte-transport efficiency, high sensitivity, low cost, separation of analyte from the matrix (hence high selectivity), preconcentration potential, simplicity, automation, good sampling frequencies, and well-established methodology.

AFS has been described to be superior to AAS, especially with respect to its greater sensitivity because the fluorescence signal has a very low background. Compared to ICP–MS, AFS has very similar sensitivity and linear range, but with a relatively more simple set-up (Fig. 8) and has lower investment and maintenance costs[21,85]. In addition, adoption of the chemical vapor generation (CVG) system to the AFS detector is easy and yields good results. Unlike ICP–MS, the AFS detector is more tolerant to the external hydrogen formed during the HG process[85]. Hence, it is compatible with the online vapor-generation system, which makes it most suitable for measuring vapor-forming species[86].

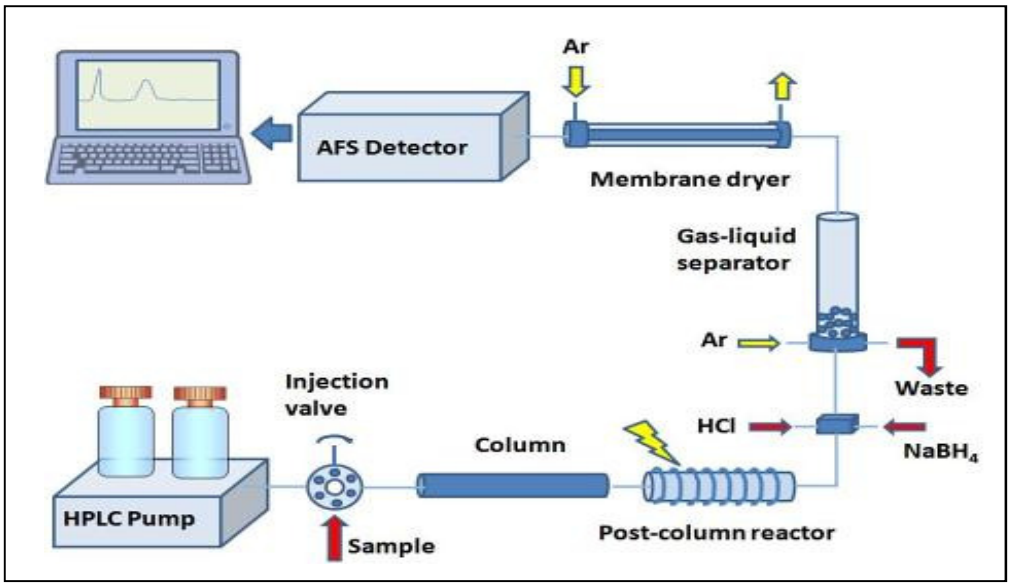


Fig. 8. Schematic diagram of an HPLC–AFS system

AFS instruments are commonly based on the use of non-dispersive instruments, equipped with a boosted discharge hollow cathode lamp (BDHCL) or a mercury vapor discharge lamp as the excitation radiation source for mercury determination. Historically, other more powerful atomizers such as ICP have also been investigated. However, no commercial instrument is nowadays available.

Upon chromatographic separation of the species, online HG transforms them into volatile species, which are carried with an argon flow to a gas-liquid separator, followed by atomization and excitation in an argon-hydrogen diffusion flame (Fig. 9). For those chemical species that do not readily form volatile species, additional online derivatization steps are needed before HG.

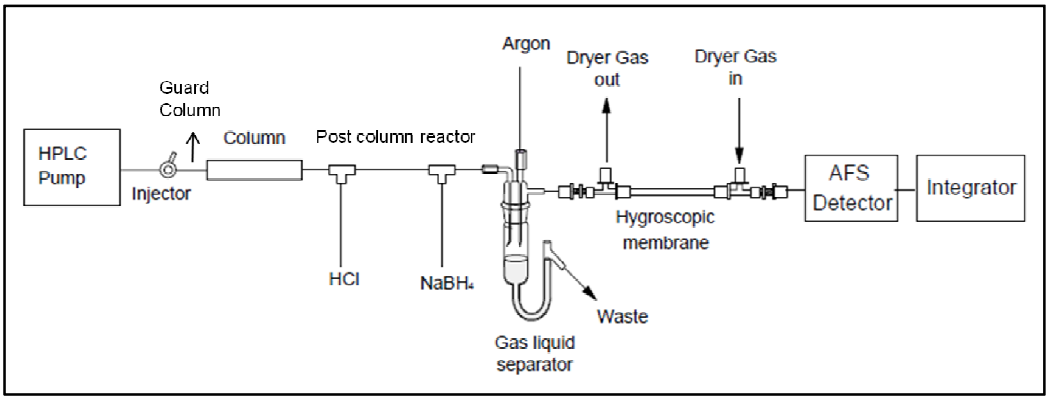


Fig. 9. Schematic diagram of reducible arsenic speciation system

Although HPLC–HG–AFS is one of the most popular techniques used for speciation of hydride generating elements, unfortunately, the development of this technique has not been as fast as one would like and also is supported by only very few instrument manufacturers. The key reasons for this situation are: (i) currently, the technique is limited to chemical-vapor-generating elements; (ii) commercially available AFS instruments are non-dispersive and thus are not suitable for multi-element determination; and (iii) the post-column treatment generally required by the nature of the technique in order to assure that all species eluting from the column are transformed to volatile species. Such additional derivatization in general is performed online with post-column reactors aiming at the destruction of the species to only free elemental cations accessible for CVG. Online oxidation with strong oxidants, photo-oxidation, or microwave digestion have been used for such purpose.

2.10 HPLC–HG–AFS for speciation of arsenic

Speciation of arsenic using HG involves several chemical species, both inorganic and organic ones. As(III), As(V), MMA and DMA generate volatile arsines when reduced with sodium borohydride ($NaBH_4$) in acidic

media. Other organic arsenic species, such as the trimethyl arsenic oxide (TMAO), tetramethyl arsenic ion (TETRA), arsenobetaine, arsenocholine and arsenosugars do not readily form volatile species. In this case, the destruction of the organic part of the molecules is required prior to the HG step, which can be achieved by means of either photo-oxidation (in the presence of a strong oxidant such as $K_2S_2O_8$ in basic media and UV radiation), thermooxidation (in the presence of a strong oxidant such as $K_2S_2O_8$ in basic media and heating), or a MW-assisted digestion[21].

HPLC based on ion exchange is the preferred separation technique for arsenic speciation combined with AFS detection. As most of the arsenic species exist in solution as negative or neutral species at mild pH values, they are commonly separated by HPLC using either strong anion-exchange columns[87] or in some cases with reversed-phase columns with ion-paring reagents[88]. The use of anion exchange columns has been widely employed in most speciation studies, as the separation process is more reproducible and less prone to sample matrix interferences than with reversed-phase columns with ion-paring reagents[21]. Up to twelve arsenic species have been determined in a single run on an anion-exchange column[89].

HPLC–HG–AFS has been widely applied for speciation of arsenic in water and human urine as summarized in Table 4. In addition, the technique has also been applied for a variety of other matrices such as leachates from chromate-copper-treated wood[90], wastewater[91], acid mine drainage[92] and beer[93].

For urine and water samples (Table 4), both isocratic and gradient modes have been used. Tetrabutylammonium hydroxide (TBAH) and phosphate were the main buffers used in the reversed-phase and ion

exchange modes, respectively. With a strong anion-exchange column, phosphate buffer alone with concentrations varying between 5.0 and 60 mM, and pH in the range of 4.8–8.8 was applied to achieve separation.

2.11 Other techniques used for speciation of arsenic and antimony

It is estimated that approximately 50% of all publications on the subject of speciation analysis involve only five elements, i.e., arsenic, selenium, mercury, chromium, and tin[94]. Further 30% of papers are devoted to copper, zinc, lead, and iron[95]. Less information on antimony speciation is available. Among the most popular hyphenated techniques used for speciation of arsenic and antimony, there are couplings of various liquid chromatography types. These include HPLC[96], ion chromatography (IC)[97,98], ion-exclusion chromatography (IEC)[99], or size exclusion chromatography (SEC)[100] with ICP–MS or electrospray ionization-mass spectrometry (ESI–MS). The most popular hyphenated techniques utilizing IC are IC–ICP–MS, IC–ICP–OES, and IC–MS[101,102]. The application of MS allows not only to obtain information on the qualitative and quantitative content of samples but also to determine the structure and molar masses of the analytes.

Other coupled analytical techniques can also be found in the literature for speciation of arsenic and antimony, which include HPLC–HG–AAS[103,104], HPLC–HG–ICP–OES[105] and HPLC coupled to neutron activation analysis (HPLC–NAA)[106].

Table 4. HPLC–HG–AFS for the determination of arsenic species in human urine and water.

Matrix/ sample	Species	Column	Eluent/type (I: isocratic; G: gradient)	Ref.
Urine	As(III), As(V), MMA, DMA, AB	Hamilton PRP-X100	10 mM phosphate buffer; pH 5.75 (G)	107
Urine	As(III), As(V), MMA, DMA	Phenomenex ODS C18	5 mM TBAH, 3 mM malonic acid, 5% (v/v) MeOH; pH 5.9 (I)	108
Urine	As(III), As(V), MMA, DMA	Phenomenex ODS-3 C18	5 mM TBAH, 2–5 mM malonic acid, 5% (v/v) MeOH; pH 5.9 (I)	109
Urine	As(III), As(V), MMA, DMA	Phenomenex ODS C18	5 mM TBAH, 2–5 mM malonic acid, 5% (v/v) MeOH; pH 5.9 (I)	110
Urine	As(III), As(V), MMA, DMA	Phenomenex ODS-3 C18	4.7% TBAH, 2 mM malonic acid, 4% (v/v) MeOH (I)	111
Water and urine	As(III), As(V), MMA, DMA	Phenomenex ODS-3	5 mM TBAH, 1 mM malonic acid, 5% (v/v) MeOH; pH 5.5 (I)	112
Urine	As(III), As(V), MMA, DMA, DMPS-MMA	Phenosphere	20 mM phosphate buffer, 5% MeOH; pH 6.0 (I)	113
Urine	As(III), As(V), MMA, DMA	Phenomenex ODS-3 C18	5 mM TBAH, 3 mM malonic acid, 5% (v/v) MeOH; pH 5.9 (I)	114
	TMAO	Hamilton PRP-X100	5 mM phosphate (I)	
Urine	As(III), As(V), MMA, DMA	Hamilton PRP-X100	10–60 mM phosphate buffer; pH 5.8 (G)	115
Natural freshwater	As(III), As(V), MMA	Prodigy ODS	(a) 5% (w/v) MeOH, 5 mM 2-mercapto ethanol, 20 mM TBAH; pH 4.0; (b) MeOH (G)	116
Bottled mineral waters	As(III), As(V)	Hamilton PRP-X100	20 mM phosphate buffer; pH 6.1 (I)	117
Water (mine site)	As(III), As(V)	Hamilton PRP-X100	Phosphate buffer (I)	118
Mineral, tap and river waters	As(III), As(V), MMA, DMA	Hamilton PRP-X100	(a) 5 mM phosphate buffer; pH 4.8; (b) 30 mM phosphate buffer; pH 8.0 (G)	119
Groundwater and urine	As(III), As(V), MMA, DMA	Hamilton PRP-X100	15 mM phosphate buffer; pH 6.0 (I)	120
River and estuarine waters	As(III), As(V), MMA, DMA	Hamilton PRP-X100	20 mM phosphate buffer; pH 5.8 (I)	121
River water	AsIII), As(V)	Hamilton PRP-X100	Phosphate buffer (I)	122

2.12 Objectives of this work

This study may be divided into two main parts: (i) simultaneous speciation of arsenic and antimony in water by CE, and (ii) speciation of arsenic in water and urine by HPLC–HG–AFS. The first part aimed at the development of a CE method for the simultaneous separation of As(III), As(V), MMA, DMA, Sb(III) and Sb(V) within the same run. In order to improve the detection sensitivity of CE, different online sample preconcentration techniques were investigated. These include (1) sweeping-MEKC with SDS, (2) LVSS with polarity switching, (3) FASS, and (4) FASI with reversal of EOF. The results obtained with each method were compared with conventional CZE in terms of IFs. The last technique, giving the highest IFs thus lowest LODs, was coupled to HSDC to lower LODs further to the levels of the target analytes in real-world samples. Tap water from our laboratory was analyzed by the proposed method.

The second part of the study, which was a component of a terminating research project (The Scientific and Technological Research Council of Turkey (TÜBİTAK), Project No. 109S419) for investigating carcinogenic risk in residents chronically exposed to inorganic arsenic via drinking water in Nevşehir area, aimed at developing an HPLC methodology for separation of As(III), As(V), MMA and DMA before their quantitation by HG–AFS. The most influential separation conditions (e.g., mobile phase composition and pH, sample pH, flow rate, column temperature, and the use of guard column) were optimized. To evaluate accuracy of the developed methodology, SRM 2669 "arsenic species in frozen human urine" was analyzed at two concentration levels, i.e., low and high. Urine samples collected from exposed people were analyzed with the developed method. Total arsenic concentration was also

determined in all samples by HG–AFS after acid digestion and pre-reduction with potassium iodide and L-ascorbic acid.

3 EXPERIMENTAL

3.1 Instrumentation

The CE instrument used was an HP^{3D} CE (Agilent Technologies, Germany). Conventional CZE and stacking modes were performed using uncoated fused-silica capillaries (Agilent Technologies, USA) of 75 µm i.d. and 64.5 cm length; effective length to the detector was 56 cm. Online UV diode-array detector (DAD) operated at a wavelength of 192 nm was used. Optimum wavelengths for the target analytes were determined using 'Isoabsorbance' and '3D' plots in the instrument's 'Data Analysis' software. Pressure or electrokinetic injections were employed throughout the experiments as indicated in the figure captions. An Orion, 720A pH meter equipped with a glass electrode was used for measuring the adjusted pH of all samples and aqueous solutions throughout the experiments. Deionized (DI) water (18.2 MΩ.cm) treated with Millipore (Simplicity, 185) Milli-Q water purification system was used for all aqueous solutions.

The chromatographic separation of arsenic species was performed with a Hewlett Packard HPLC system, 1050 model, equipped with quaternary pumps, an injector with a 100-µL loop, a Hamilton PRP-X100 anion-exchange column (250 mm×4.1 mm) with particle size of 10 µm, and a Hewlett Packard Hypersil guard column (20 mm×2.1 mm) with particle size of 5 µm. The signal output of the AFS system was recorded with a computer having chromatographic software (Agilent ChemStation for LC systems software, Rev. B. 03.01) interfaced to the AFS instrument via an analogue-to-digital converter "Agilent Interface 35900 E".

Hydride generation of volatile arsines was carried out by the online addition of 1.5 M HCl (4 mL min^{-1}) and 1.4% (w/v) $NaBH_4$ (4 mL min^{-1}) solutions at the outlet of the HPLC column by means of the two peristaltic pumps of the HG–AFS system (PS Analytical Excalibur Millennium System, PS Analytical, UK) (Fig. 10). These pumps could be operated at two speed levels only, i.e., half and full. At half speed, the flow rate was measured to be 4 mL min^{-1} with the type of tubing used, 1.8 mm i.d. An argon flow fixed at 250 mL min^{-1} was used to carry the volatile arsines generated to the detector. The gas was dried through a hygroscopic membrane drying tube (Perma Pure) using nitrogen gas at a flow rate of 2.5 L min^{-1}. An arsenic boosted discharge arsenic hollow cathode lamp (As BDHCL Super lamp, Photon, Victoria, Australia) was used as the radiation source of the AFS detector.

Fig. 10. HPLC–HG–AFS system

3.2 Reagents and solutions

Stock solutions (1000 mg L^{-1} arsenic and antimony, concentration expressed as species of arsenic and antimony) were prepared in DI water from the following materials: As(III) from AAS As(III) standard (Fisher

Scientific, UK); As(V) from arsenic pentoxide, As_2O_5 (Supelco, USA); MMA from disodium methyl arsonate hexahydrate, $CH_3AsNa_2O_3.6H_2O$ (Supelco, USA); DMA from sodium dimethylarsinate trihydrate, $(CH_3)_2AsO_2Na.3H_2O$ (Supelco, USA), Sb(III) from potassium antimonyl tartrate trihydrate, $K_2[Sb_2(C_4H_2O_6)_2].3H_2O$ (Sigma-Aldrich, Germany) and Sb(V) from potassium pyroantimonate tetrahydrate, $K_2H_2Sb_2O_7.4H_2O$ (Fisher Scientific, UK). Analytes used in this study, names, abbreviations, formulas and pK_a values are given in Table 5. Borate buffer was prepared from sodium borate decahydrate, $Na_2B_4H_7.10H_2O$ (Riedel De Haen, Germany). Solutions of pluronic F-127 (Sigma-Aldrich, Germany), SDS (J.T.Baker, Germany) and CTAB (Sigma-Aldrich, Germany) were prepared in DI water.

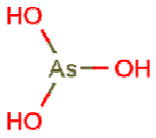
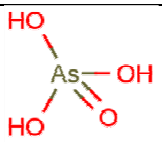
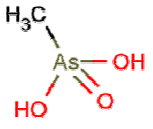
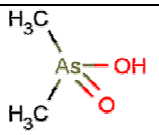
All BGE and standard solutions were prepared in DI water and were stored in the dark at 4°C. Standard and working solutions were prepared daily by appropriate dilutions of the stock solutions. pH of the solutions was adjusted with 0.1 M NaOH (Merck, Germany) or 0.1 M HCl (Merck, Germany) as requested. Standard and electrolyte solutions were degassed using a sonicator (Sonorex Bandelin Electronic, Germany) for 10 min and were filtered through 0.20 µm filters (Econofilters, Agilent Technologies, Germany) before use. A 1.0 M sodium hydroxide solution was used to condition the capillary at the beginning of each working session. Tap water was obtained from our laboratory, filtered through 0.20 µm filters, and spiked with the target analytes to give final concentrations of 20.0 µg L^{-1} for Sb(III), As(III) and DMA (each species) and 80.0 µg L^{-1} for As(V), MMA and Sb(V) (each species) after being diluted to 10.0 mL with DI water.

Standard solutions of arsenic species for AFS studies were prepared in blank urine (for HPLC-HG-AFS calibrations) and As(III) in 1.5

M HCl solution (for HG-AFS calibrations) from the above-mentioned respective materials. Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), and $NaBH_4$ (p.a. 98%) were purchased from Merck (Darmstadt, Germany). The mobile phase consisting of 8.0 mM KH_2PO_4/K_2HPO_4 adjusted to pH 5.8 with HCl was filtered through 0.45 µm membrane filters (Sartolon Stedim, Goettingen, Germany) and degassed using a sonicator for 10 min. Urine samples were filtered through 0.20 µm filters (Econofilters, Agilent Technologies, Germany) prior to injection. Potassium iodide (KI) and L-ascorbic acid ($C_6H_8O_6$) were obtained from Sigma-Aldrich (Steinheim, Germany).

Standard Reference Material 2669 “Arsenic Species in Frozen Human Urine” was purchased from the National Institute of Standards and Technology (NIST), MD, USA.

Table 5. Names, abbreviations, formulas and pK_a values of the studied analytes.

Species	Formula	pK_a	Ref.
Arsenous acid, As(III)		9.22, 12.13, 13.4	22
Arsenic acid, As(V)		2.20, 6.97, 11.53	22
Monomethylarsonic acid, MMA		3.6, 8.2	1
Dimethylarsinic acid, DMA		1.3, 6.2	1
Antimonite, Sb(III)	$HSbO_2$	11.9	22
Antimonate, Sb(V)	Sb_2O_5	2.72	22

3.3 Procedures

3.3.1 CE

For conventional CZE, sweeping-MEKC with SDS, LVSS, FASS, and FASI/EOF reversal, new capillaries were successively flushed with DI water (10 min), 1 M NaOH (20 min), DI water (20 min) and finally with the BGE (20 min). To assure a good reproducibility, capillaries were successively flushed with DI water (1 min), 1 M NaOH (0.5 min), DI water (2 min) and finally with the BGE (2 min) at the end of each run. At the end of each working session, capillaries were flushed with the BGE (3 min) and DI water (3 min).

In conventional CZE, the capillary was conditioned with a BGE (25 mM borate buffer, pH 9.3); the sample, prepared in this BGE, was injected for 10 s at 50 mbar and a positive voltage of 25 kV was applied. The analytes migrated in a homogeneous conductivity medium and were separated by the CZE mode.

In sweeping-MEKC with SDS, the capillary was conditioned with a non-micellar acidic BGE (25 mM phosphate containing 20% (v/v) ACN at pH 1.9). The sample prepared in DI water (pH adjusted to 1.9) was injected for 40 s at 50 mbar and the inlet vial was replaced with a micellar BGE (25 mM phosphate buffer containing 35 mM SDS and 20% (v/v) ACN at pH 1.9). Under these acidic conditions, the EOF was suppressed to almost zero. Upon the application of a negative polarity, the micelles entered the capillary from the inlet vial and penetrated through the sample zone, where they swept the analytes by varying interaction strength depending on their size, charge and the distribution coefficient between

the bulk of the solution and the micelles. Then, the SDS-analyte moieties were separated by the MEKC mode.

In LVSS, the capillary was first conditioned with a 25-mM borate buffer. The sample, prepared in water, was then injected for 120 s at 50 mbar, and then a negative voltage of –25 kV was applied. The electrophoretic current was carefully monitored until it reached exactly 91% of its original value (the current was originally recorded with the BGE alone without the sample being injected), and the polarity was then quickly switched to positive. The following separation occurred at 25 kV by the CZE mode.

In FASS, the capillary was conditioned with a BGE of 25 mM borate buffer (a high-conductivity medium); the sample, prepared in DI water (a low-conductivity medium), was then injected for 50 s at 50 mbar and a positive voltage of 25 kV was applied. This resulted in stacking of the analytes at the boundary between the sample zone and the BGE. Then, the stacked analytes migrated and were separated by the CZE mode.

In FASI/EOF reversal, the capillary was conditioned with 25 mM borate buffer containing 0.1 mM CTAB to dynamically coat the negative walls of the capillary and reverse the EOF; the sample dissolved in 0.1 mM CTAB was then electrokinetically injected for 50 s at –20 kV after the introduction of a water plug hydrodynamically injected for 5 s at 50 mbar. The following separation occurred at –25 kV by the CZE mode.

3.3.2 HPLC–HG–AFS

Standard solutions in urine were prepared by the matrix-matched method. Blank urine collected from a male volunteer (34 years old) was spiked with varying amounts of arsenic species to give final concentrations of 2.5, 5.0, 10.0 and 20.0 μg L^{-1} with gain 100, and 10.0, 30.0, 50.0, 70.0 and 100.0 μg L^{-1} with gain 10. Urine samples were allowed to thaw at room temperature. They were shaken and allowed to rest for 5 min. Afterwards, few milliliters of the supernatant solution were filtered through 0.20 μm filters, 900 μL of which were collected in a test tube and were buffered with 100 μL of 50.0 mM KH_2PO_4/K_2HPO_4 buffer at pH 6.9. Peak areas of the different arsenic species were plotted against added arsenic species concentrations and calibration graphs were used to obtain linear regression equations for As(III), DMA, MMA and As(V).

The same procedure was followed for real urine samples and, when necessary, dilutions of the samples were carried out with DI water. SRM 2669 was thawed at room temperature and used within 4 hours after being thawed. Once the pouches were cut open, urine was homogenized by gently inverting the vial several times. As recommended by the SRM supplier, the sample was filtered through 0.20 μm filters before analysis. An aliquot of 900 μL was transferred into a test tube and buffered with 100 μL of 50.0 mM KH_2PO_4/K_2HPO_4 buffer at pH 6.9. The solution was homogenized by being vortex-mixed for 10 s and 100 μL were injected into the system.

3.3.3 HG–AFS

One milliliter of urine was transferred into a long-neck digestion flask and 2 mL of concentrated nitric acid were added. The mixture was

heated to 80°C and retained at this temperature for 10 min. Then, the temperature was increased to 130°C and retained for 15 min. The flask was cooled down to room temperature and 0.5 mL of concentrated perchloric acid ($HClO_4$) was added. The mixture was heated to 130°C (10 min) and then to 170°C (15 min). After cooling down to room temperature, 0.5 mL of sulfuric acid was added. Next, the temperature was successively increased to 140°C (10 min), 170°C (10 min) and 220°C (15 min). The temperature was then increased to 280°C and maintained at this temperature until acid fuming stopped completely. When cooled, the solution was transferred into a volumetric flask, the digestion flask was rinsed twice with DI water and the volume was completed to the mark (10 mL) with DI water. Two parallel digestions were done for each urine sample. SRM 2669 was digested by the same procedure.

For total arsenic determination, 1.0 mL of each parallel was transferred into a 10-mL volumetric flask and 200 µL mixture of 50% (m/v) potassium iodide and 10% (m/v) L-ascorbic acid were added. To this solution, an appropriate amount of concentrated hydrochloric acid was added to have a final concentration of 1.5 M of the acid after dilution to the mark with DI. Two parallel solutions were prepared for each digested sample. Thus, for each urine sample, four parallel solutions were prepared. Total arsenic concentration in each parallel solution was measured with HG–AFS.

The instrument was always run for at least 20 min to reach a steady state signal before analysis. Calibration graphs were constructed at the beginning of each working session with As(III) standards at the concentrations of 0.10, 0.25, 0.50, 1.0, 2.5 and 5.0 µg L^{-1} (gain 10) and 0.025, 0.100, 0.250 and 0.500 µg L^{-1} (gain 100). Any signal drift was

checked with a standard at the middle of the calibration graph after measuring five samples.

4 RESULTS AND DISCUSSION

4.1 CE

4.1.1 Conventional CZE

Separation parameters were optimized to obtain optimum separation and efficiency with this method. These parameters included capillary dimensions, identity of the BGE and its pH, buffer concentration in the BGE and the sample, pH of the sample, temperature of the capillary, injection mode and time, and separation voltage.

Identification of the peaks in this method and all the other preconcentration methods described below was carried out in two ways: (i) by comparing the migration time of known species in a standard of a single species with those in a mixture of all species; and (ii) by the spike method using the addition of a single species to a mixture of all species and the recognition of the peak with increased height and/or area. A typical electropherogram obtained under optimized conditions with this method is shown in Fig. 11. Under these conditions, LODs obtained ranged from 2.3 mg L^{-1} for As(III) to 23 mg L^{-1} for Sb(III) as shown in Table 6.

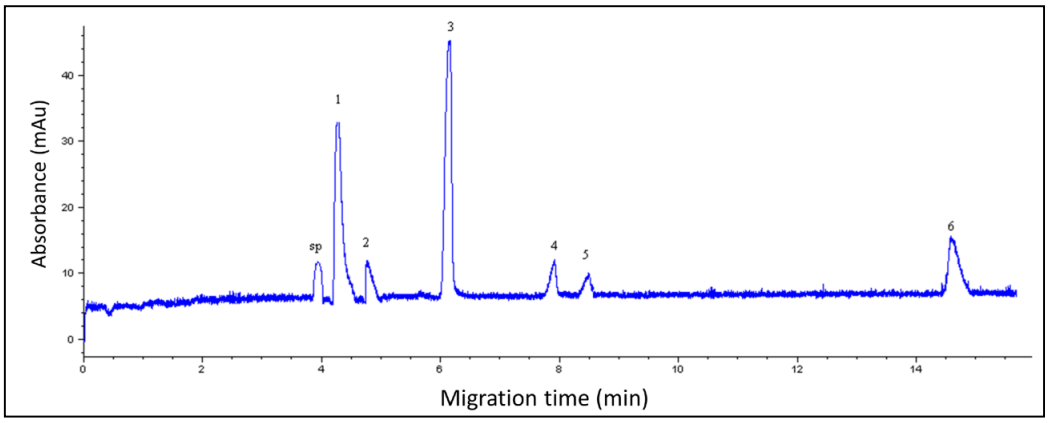


Fig. 11. Electropherogram with conventional CZE. Conditions: λ=192 nm; separation voltage: 25 kV; BGE: 25 mM borate buffer (pH 9.3); temperature: 25℃; sample injection: pressure, 50 mbar, 10 s; sample: arsenic and antimony (50.0 mg L^{-1}, each) in 25 mM borate buffer; peaks: sp, system peak; 1, DMA; 2, Sb(V); 3, As(III); 4, MMA; 5, Sb(III); 6, As(V)

Table 6. Analytical performance parameters for conventional CZE, LVSS and FASS.

Species	LOD with conventional CZE[a] (mg L^{-1})	LOD with LVSS (mg L^{-1})	IF[b]	LOD with FASS (mg L^{-1})	IF[c]
DMA	3.0	0.9	3.3	0.9	3.3
Sb(V)	15	2.7	5.6	2.5	6.1
As(III)	2.3	0.1	23	0.2	12
MMA	14	2.8	4.9	1.9	7.3
Sb(III)	23	0.7	33	2.2	10
As(V)	8.8	0.5	18	1.3	6.8

[a] Conditions: same as in Fig. 11

[b] Improvement factor (Ratio of LOD in conventional CZE to that with LVSS)

[c] Improvement factor (Ratio of LOD in conventional CZE to that with FASS)

4.1.2 Sweeping-MEKC with SDS

The basic concept behind sweeping and separation using an anionic surfactant (i.e., SDS) was shown in Fig. 2. Influential parameters affecting sweeping efficiency and thus IFs were systematically studied and optimized. These parameters included: type and pH of the buffer in both the micellar and non-micellar BGEs, type and concentration of surfactant (anionic, cationic and neutral) in the micellar BGE, concentration of ACN in both BGEs, capillary temperature, injection mode and time, and separation voltage. Under optimum sweeping conditions (shown in Fig. 12), IFs ranged from 123.0 to 4713 times as compared to conventional CZE (Table 7). High preconcentration efficiency of this method resulted into LODs in the range of 4.88 μg L^{-1} for Sb(III) to 18.7 μg L^{-1} for As(III) (Table 7).

Despite the high IFs obtained, a major limitation of this method was that upon introduction of the micellar BGE into the capillary to sweep the analytes, the walls of the capillary were dynamically coated with SDS, the removal of which necessitated long equilibration times with the non-micellar BGE for the next run, which resulted into a poor reproducibility of the method.

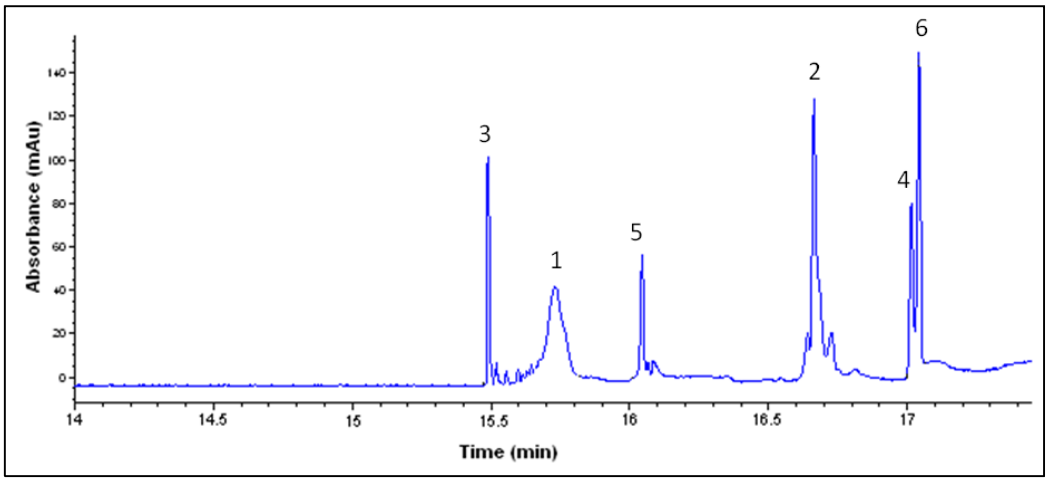


Fig. 12. Electropherogram with sweeping-MEKC with SDS. Temperature: 25℃; Injection: pressure at 50 mbar, 40 s; Separation voltage: –20 kV; Non-micellar BGE: 25 mM phosphate, 20 % (v:v) ACN (pH 1.9); Sample: 0.1 mg L^{-1} As, 0.1 mg L^{-1} Sb (each) in DI water (pH 1.9); Micellar BGE: 25 mM phosphate buffer, 35 mM SDS, 20 % (v:v) ACN (pH 1.9). Peaks: as in Fig. 11

Table 7. Analytical performance parameters for sweeping-MEKC with SDS[a].

Species	DMA	Sb(V)	As(III)	MMA	Sb(III)	As(V)
LOD with sweeping ($\mu g\ L^{-1}$)	11.6	5.92	18.7	12.9	4.88	6.48
IF[b]	259.0	2534	123.0	1085	4713	1358

[a] Conditions: same as in Fig. 12

[b] Improvement factor (Ratio of LOD in conventional CZE to that with sweeping-MEKC)

4.1.3 LVSS with polarity switching

One of the technical challenges encountered in LVSS was the ability to switch the applied polarity when the current exactly reached 91% of its original value. Our experiments showed that even a delay of 3 seconds beyond this value resulted in a significant loss of some of the analytes especially those with high electrophoretic mobilities such as

As(III), DMA and Sb(III); less number of peaks was obtained in the electropherograms or a decrease in peak areas of those peaks was observed. On the other hand, early switching of the polarity resulted in loss of resolution between some of the peaks, especially the two peak pairs of DMA/Sb(III) (peaks 1, 5) and Sb(V)/MMA (peaks 2, 4) (Fig. 13) and in a decrease of peak areas due to reduced stacking efficiency that resulted from larger sample volume remaining in the capillary. Experimental parameters affecting separation and stacking efficiency were thus investigated to obtain the optimum performance.

Out of these parameters, pH of the BGE was the most critical; it significantly influenced the migration time of the analytes and potentially the separation efficiency as well. The effect of pH can be attributed to its influence on the bulk EOF and on the electrophoretic mobilities of arsenic and antimony species. Deprotonation of the analytes increases their ionic charge, consequently increasing their electrophoretic mobilities. Hence, it was observed that some species were lost during the stacking step at pH values lower than 12.0.

Injection time, studied in the range of 60–120 s, was also of great importance. It was observed that resolution between DMA and Sb(III) (peaks 1, 5) as well as Sb(V) and MMA (peaks 2, 4) was improved as injection time increased. Peak areas also increased linearly with the injection volume. However, at injection times higher than 120 s, these two pairs overlapped. Although injection volumes up to 100% of the capillary have been reported in the literature with LVSS[123], loss of the target analytes in the back-out step and poor resolution and reproducibility occurred when the injection time was too large for the studied analytes. Therefore, injection time of 120 s (50% of the total capillary length) was set optimum. In LVSS, IFs were in the range of 3.3–33 and LODs

obtained with this method ranged from 0.1 mg L^{-1} for As(III) to 2.8 mg L^{-1} for MMA (Table 6).

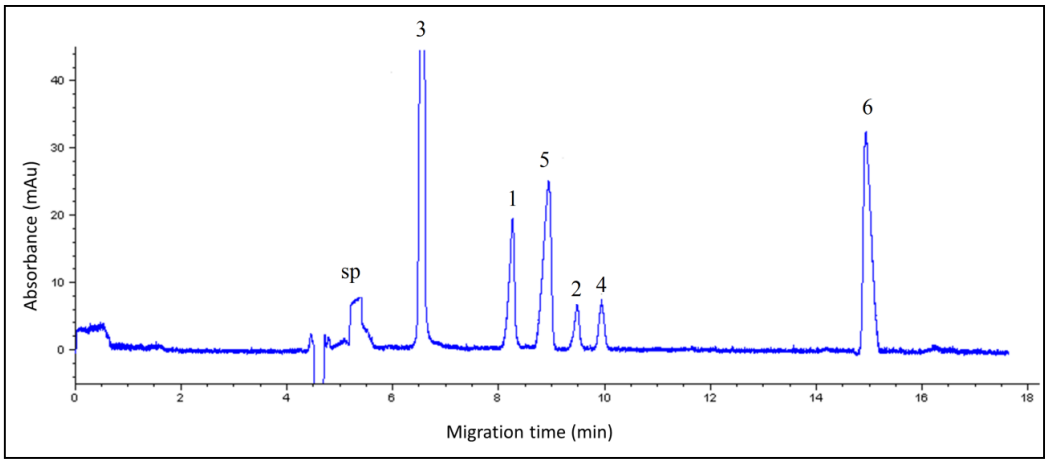


Fig. 13. LVSS with polarity switching. Temperature: 15°C; Injection: pressure, 50 mbar, 120 s; Stacking voltage: –25 kV till 91% of initial current; Separation voltage: 25 kV; BGE: 25 mM borate buffer; Sample: arsenic and antimony (10.0 mg L^{-1}, each) in water (pH 12.0); Peaks: same as in Fig. 11

4.1.4 FASS

In this method, the maximum field enhancement factor was adopted by dissolving the analytes in DI water. It was found that the injected sample volume could not exceed 21% (50 s) of the total capillary length. The maximum volume of the sample that could be introduced into the capillary was calculated by increasing the injection time until a sudden change in the baseline was observed; this corresponded to 240 s with pressure injection (325 s with electrokinetic injection). At injection times higher than 50 s, separation efficiency became progressively poor and the peaks of DMA, Sb(III) and Sb(V) (peaks 1, 5, 2) (Fig. 14) could not be baseline-separated. Under optimum conditions: temperature, 15 °C; separation voltage, 25 kV; BGE: 25 mM borate buffer (pH 9.3); Injection,

pressure, 50 mbar, 50 s, IFs were in the range of 3.3–12 and LOD values ranged from 0.2 mg L^{-1} for As(III) to 2.5 mg L^{-1} for Sb(V) (Table 6).

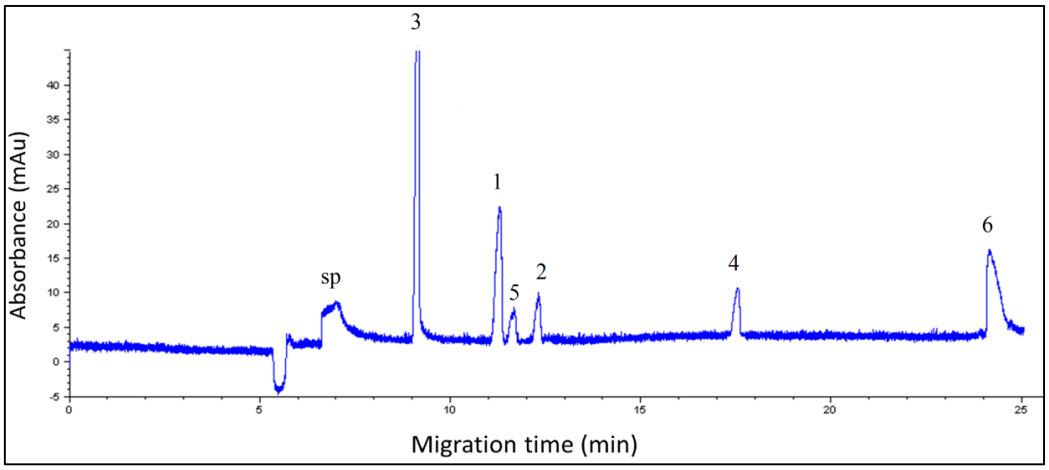


Fig. 14. Electropherogram with FASS. Temperature: 15°C; Separation voltage: 25 kV; BGE: 25 mM borate buffer (pH 9.3); Injection: pressure, 50 mbar, 50 s; Sample: arsenic and antimony (5.0 mg L^{-1}, each) in water (pH 12.0); Peaks: same as in Fig. 11

4.1.5 FASI with EOF reversal

In order to reach at the optimum analytical performance of the FASI with EOF reversal stacking method, several influential parameters were studied and optimized. The effect of borate buffer concentration in the BGE was studied within the range of 25–40 mM. Although a baseline resolution was not achieved at this point, stable baseline was obtained at 25 mM and 30 mM concentrations. Increasing the buffer concentration not only decreased the stability of the baseline but also resulted in longer migration times (Fig. 15) and reduced peak areas for all analytes with 25 mM being optimum.

It is well known that the addition of cationic surfactants into the BGE causes the reversal of the EOF owing to the positively charged capillary wall on account of the adsorption of cationic surfactants[64]. Because of the reversed EOF, the polarity of the electrodes had to be reversed in order to direct the analytes toward the detector. Hence, a negative separation voltage was used. Although increasing the separation voltage from –10 to –30 kV (Fig. 16) decreased the retention times steadily (from 16.2 to 3.7 min for the last peak), it had no effect on peak areas. Therefore, –20 kV was set optimum to avoid Joule heating.

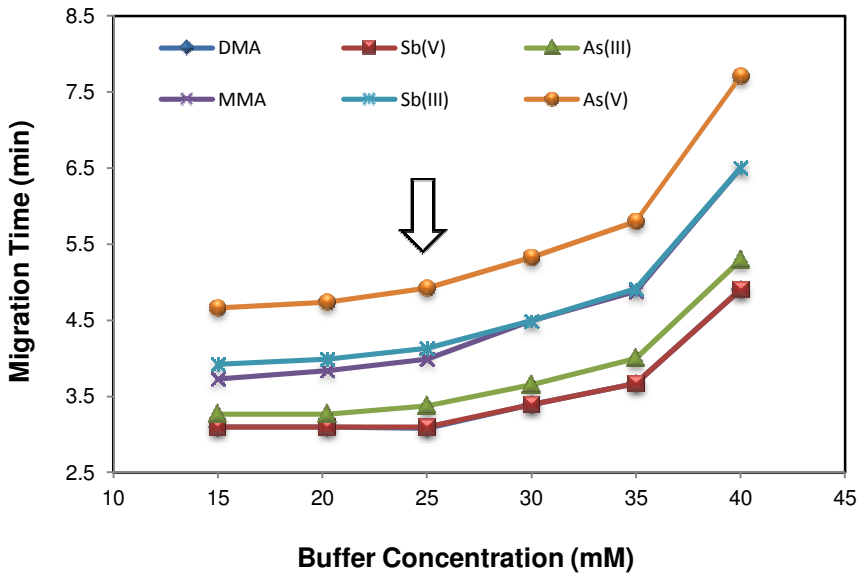


Fig. 15. Effect of buffer concentration on migration time. Temperature: 30℃; Injection: pressure, 50 mbar, 40 s; Separation voltage: −25 kV; Sample: arsenic and antimony (10 mg L^{-1}, each) in water (pH 10.0)

The maximum concentration of CTAB in the BGE that resulted in a baseline resolution of all peaks was 0.1 mM. At higher concentrations, migration times decreased (Fig. 17) and resolution became progressively poor. Effect of injection time (pressure) was studied in the range of 5 to 30 s. Migration times (Fig. 17) and peak areas increased linearly for all peaks

as injection time increased. However, the maximum injection time that resulted in a baseline resolution among four peaks was 25 s beyond which critical peaks overlapped. Hence, 25 s was chosen for further experiments.

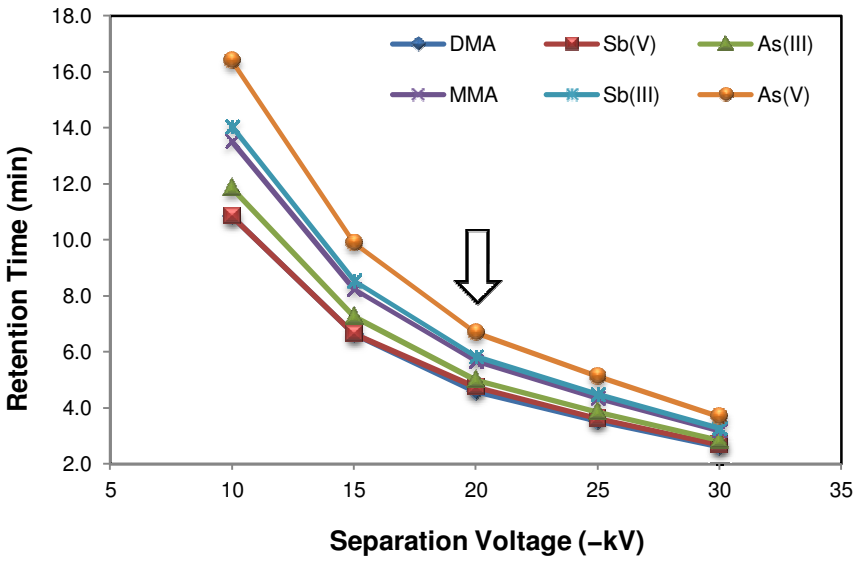


Fig. 16. Effect of separation voltage on migration time. Temperature: 15℃; Injection: pressure, 50 mbar, 25 s; BGE: 25 mM borate buffer, 10^{-4} M CTAB; Sample: arsenic and antimony (10 mg L^{-1}, each) in water (pH 7.0)

Increasing the temperature of the capillary from 10–30℃ increased peak areas of all peaks only slightly but resulted in a decrease in retention times (Fig. 19); 15℃ was optimum because at higher temperatures resolution between some peaks decreased below 1.5.

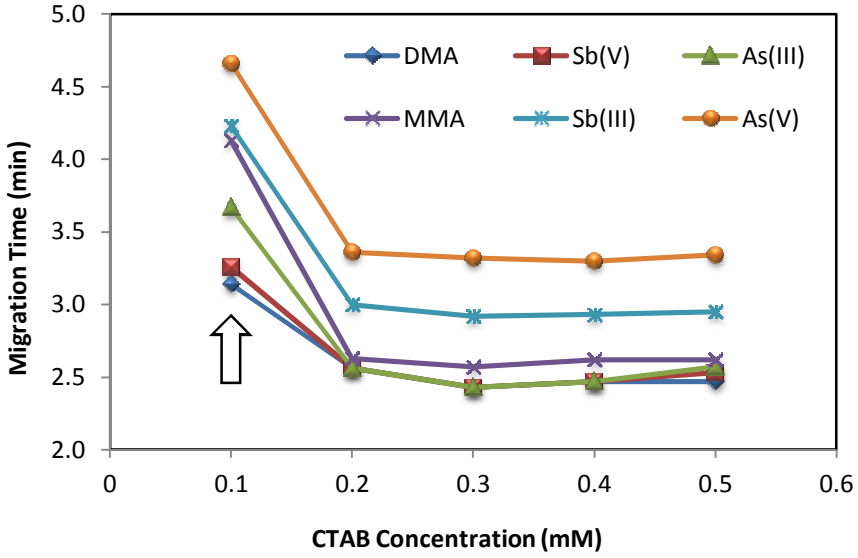


Fig. 17. Effect of CTAB concentration on migration time. Temperature: 30℃; Injection: pressure, 50 mbar, 40 s; Separation voltage: −25 kV; BGE: 25 mM borate buffer, CTAB Sample: arsenic and antimony (10 mg L^{-1}, each) in water (pH 10.0)

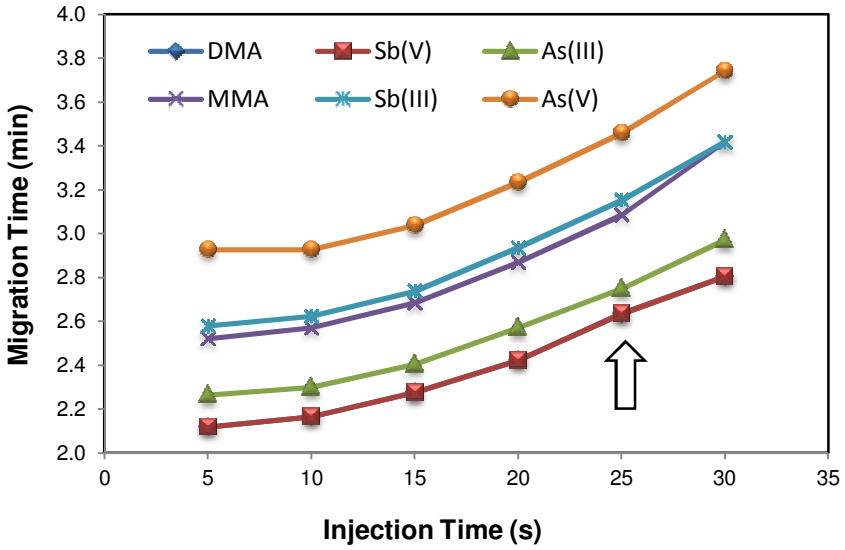


Fig. 18. Effect of injection time on migration time. Temperature: 30℃; Injection: pressure, 50 mbar; Separation voltage: −25 kV; BGE: 25 mM borate buffer, 10^{-4} M CTAB; Sample: arsenic and antimony (10 mg L^{-1}, each) in water (pH 7.0)

Effect of injection mode was critical in this method. Pressure and electrokinetic injection (FASI) with and without water plug were investigated. It can be inferred from Fig. 20 (a, b) that stacking efficiency of the method with electrokinetic injection without water plug was almost the same as that with pressure injection. The injection of a water plug prior to electrokinetic injection of the sample [Fig. 20 (c)] resulted in an IF of at least ten as compared to pressure injection (considering 10 times dilution of the sample, see the figure caption for concentrations) but electrokinetic injection resulted in the overlap of Sb(III) and As(V) (peaks 5, 6). Electrokinetic injection of the sample with a water plug was set optimum at that point but an effort was needed to resolve these two peaks.

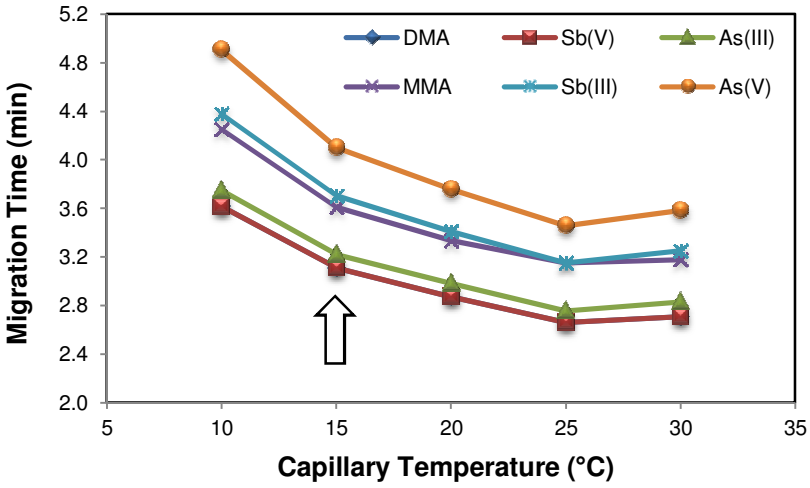


Fig. 19. Effect of capillary temperature on migration time. Injection: pressure, 50 mbar, 25 s; Separation voltage: −25 kV; BGE: 25 mM borate buffer, 10^{-4} M CTAB; Sample: arsenic and antimony (10 mg L^{-1}, each) in water (pH 7)

In order to achieve this goal, the effect of adding a surfactant (CTAB) to the sample was investigated. The resulting electropherogram is shown in Fig. 20 (d). The addition of CTAB to the sample improved peak

shapes and separation efficiency greatly; all analytes were baseline resolved upon addition of 0.1 mM of the surfactant to the sample, which was equal to the concentration of CTAB in the BGE. This was thought to be due to decreasing the mismatch between the sample and the BGE, which minimized de-stacking of the analytes from the stacked bands. The addition of SDS (anionic surfactant) and pluronic F-127 (neutral surfactant) to the sample had adverse effects on resolution and thus were not adopted.

Under optimized conditions, IFs in terms of sensitivity, calculated as the ratio of LOD in conventional CZE to that with FASI/EOF reversal, were in the range of 25–767 with LODs ranging from 23 μg L^{-1} for As(III) to 350 μg L^{-1} for As(V) (Table 8).

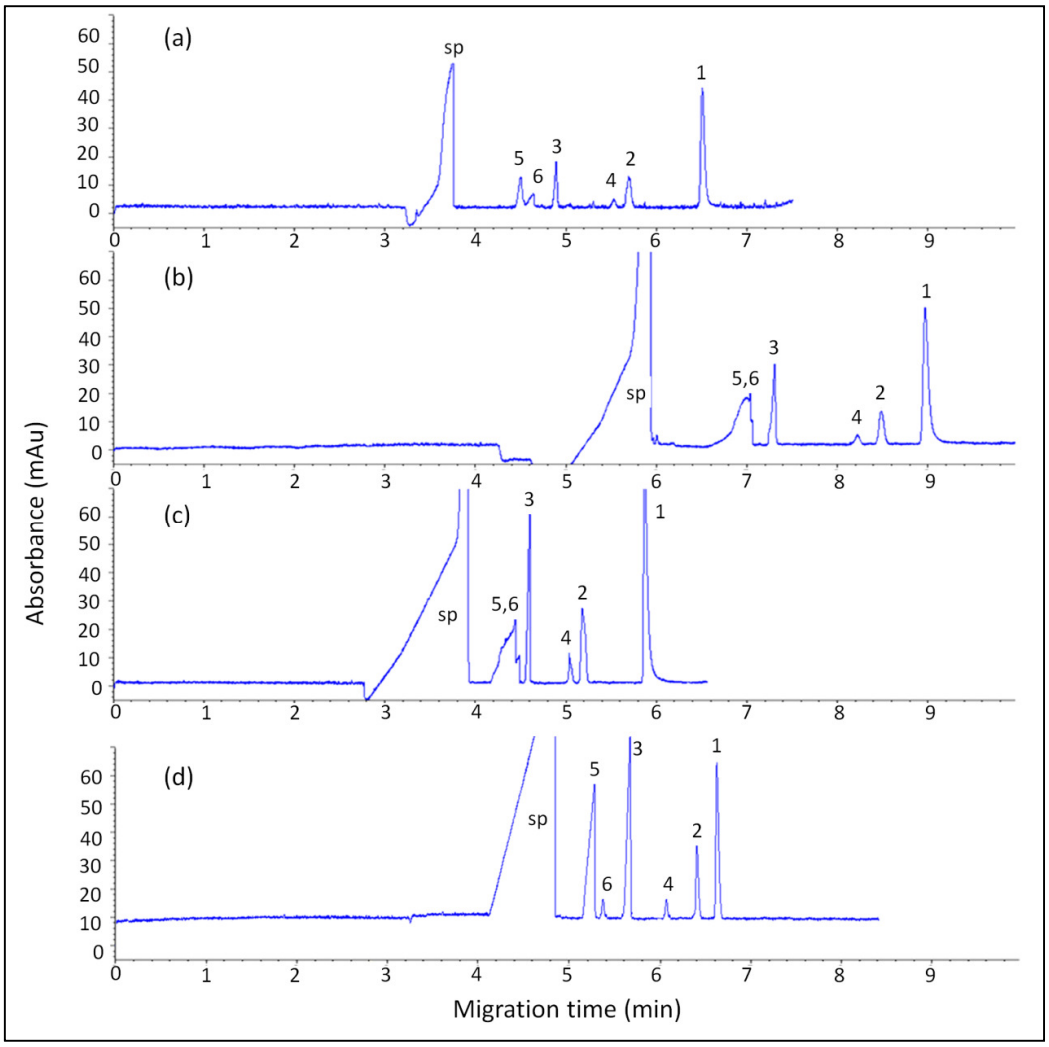


Fig. 20. Effect of injection mode and addition of CTAB to the sample in FASI/EOF reversal. Temperature: 15℃; Separation voltage: −20 kV; BGE: 25 mM borate buffer, 0.1 mM CTAB (pH 9.3); pH of sample: 7.0 (a) Pressure injection. 50 mbar, 25 s; Sample: arsenic and antimony (10.0 mg L^{-1}, each) in water. (b) Electrokinetic injection. −20 kV, 25 s; BGE: 25 mM borate buffer, 0.1 mM CTAB (pH 9.3); Sample: arsenic and antimony (10.0 mg L^{-1}, each) in water. (c) Electrokinetic injection with water plug. Water plug: 50 mbar; 5 s; Sample injection: electrokinetic, –20 kV, 50 s; Sample: arsenic and antimony (1.0 mg L^{-1}, each) in water. (d) Optimum FASI/EOF reversal conditions. Water plug: 50 mbar, 5 s; Sample injection: electrokinetic, −20 kV, 50 s; Sample: arsenic and antimony (1.0 mg L^{-1}, each) in 0.1 mM CTAB; peaks: same as in Fig. 14

Table 8. Analytical performance parameters for FASI/EOF reversal.

Species	DMA	Sb(V)	As(III)	MMA	Sb(III)	As(V)
LOD with FASI/EOF reversal (μg L^{-1})	28	57	23	340	30	350
IF[a]	107	263	100	41	767	25

[a] Improvement factor (Ratio of LOD in conventional CZE to that with FASI/EOF reversal)

4.1.6 FASI/EOF reversal combined with HSDC

Although high IFs were obtained with FASI/EOF reversal, LODs were still above the guideline values recommended by WHO in drinking water, i.e., 10 μg L^{-1} for total arsenic[124] and 20 μg L^{-1} for total antimony[125]. The use of a HSDC combined with FASI/EOF reversal was therefore examined to reduce LODs further to an appropriate level.

Analytical figures of merit for FASI/EOF reversal combined with HSDC including precision, linearity, LOD, limit of quantitation (LOQ), IF and precision were evaluated (Table 9). These results indicated that a linear relationship was attainable over wide concentration ranges with coefficients of determination (R^2) in the range of 0.9810–0.9986. LOD, calculated for a S/N ratio of 3, ranged from 1.1 μg L^{-1} for Sb(III) to 16 μg L^{-1} for As(V) due to IFs in the range of 0.6×10^3–21×10^3. These results indicate high sensitivity of the method. Precision of migration time, peak area and height was calculated in terms of percentage relative standard deviation (RSD%); for n = 5, RSD values were in the range of 0.5–1.2%, 3.4–6.0% and 1.2–4.4%, respectively. Therefore, this method was of good precision for all analytes. Since RSD of peak height was lower than that of peak area, the former was used for calibration of the method.

Selectivity and accuracy were investigated by applying the method to blank and spiked tap water samples. Neutral compounds and cations that may be present in tap water could be eliminated by this anion-selective electrokinetic injection of the sample, resulting into interference-free electropherograms. Accuracy was checked by spiking the tap water with 20.0 µg L^{-1} for Sb(III), As(III) and DMA and 80.0 µg L^{-1} for As(V), MMA and Sb(V) and calculated recoveries were in the range of 97.7–102.5% (Table 9). Electropherograms of tap water analyzed under optimum conditions before and after being spiked with arsenic and antimony species are shown in Fig. 21.

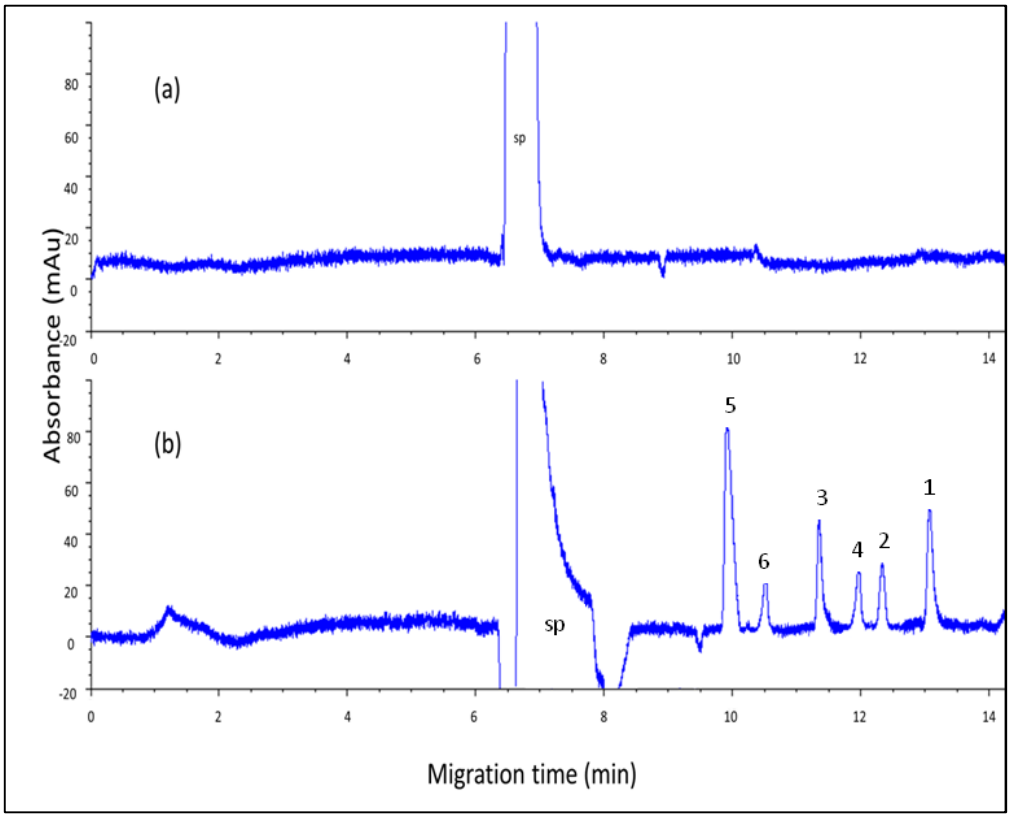


Fig. 21. Electropherograms of tap water. (a) Before and (b) after being spiked with arsenic and antimony species. Conditions as given in Fig. 20(d); Spiked concentrations: 20.0 µg L^{-1} for Sb(III), As(III) and DMA (each); 80.0 µg L^{-1} for As(V), MMA and Sb(V) (each); Peaks: same as in Fig. 11

Table 9. Figures of merit of FASI/EOF reversal with HSDC[a].

		DMA	Sb(V)	As(III)	MMA	Sb(III)	As(V)
Linear regression equation[b]		y = 2.0416x + 7.9213	y = 0.3004x + 3.5432	y = 0.8185x + 28.525	y = 0.0792x + 21.816	y = 3.8205x + 3.4573	y = 0.0483x + 17.248
Coefficient of determination (R^2)		0.9966	0.9810	0.9986	0.9902	0.9980	0.9980
Linear range ($\mu g\ L^{-1}$)		6.3–400	43–500	7.0–400	47–500	3.7–500	53–500
LOD (S/N = 3, $\mu g\ L^{-1}$)		1.9	13	2.1	14	1.1	16
IF ($\times 10^3$)		1.6	1.2	1.1	1.0	21	0.6
RSD (%, n = 5)	Migration time	0.9	0.5	1.2	0.9	0.6	1.0
	Peak area	6.0	3.4	4.7	4.7	5.7	4.9
	Peak height	3.4	1.2	4.4	2.2	2.0	3.9
Recovery (%)[c]		102.1±4.9	97.9±2.0	101.3±3.3	98.5±2.2	102.5±5.0	97.7±3.0

[a] Conditions: same as in Fig. 20 (d)

[b] Peak area = slope × concentration ($\mu g\ L^{-1}$) + intercept

[c] Spiked concentrations: same as in Fig. 21; recovery and standard deviation values were based on three parallel measurements (n = 5)

4.2 HPLC–HG–AFS and HG–AFS

4.2.1 Effect of guard column

Preliminary experiments with a strong anion exchange column with arsenic standards in aqueous samples without using a guard column resulted in well-resolved peaks of the four arsenic species (Fig. 22). Attempt to transfer the same methodology to urine samples was disappointing, however, in terms of peak shapes, resolution and retention time (Fig. 23, A). It was observed that backpressure and retention times gradually increased over time and thus it seemed necessary to use a

guard column both for extending the life of the analytical column by removing impurities from the mobile phase and/or samples as well as for improving peak characteristics. The use of a reversed phase guard column (Hewlett Packard Hypersil C18, 20 mm×2.1 mm, particle size of 5 µm) resulted in a better chromatogram (Fig. 23, B). This was thought to be due to elimination of large molecules present in urine, which would otherwise interact with the analytes/column and distort their peak shapes.

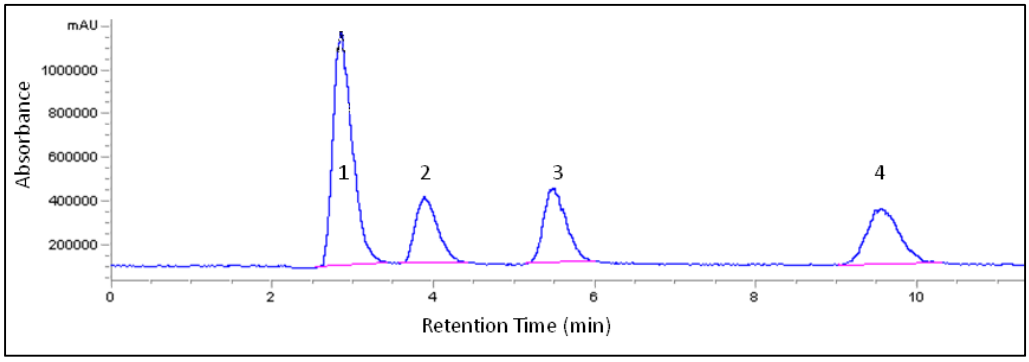


Fig. 22. Separation of arsenic species in standard aqueous solution. Separation conditions: mobile phase (MP): 8.0 mM phosphate buffer (pH 5.8); Flow rate (FR): 1.0 mL min^{-1}; Data acquisition rate 100 Hz; Gain: 10; No guard column; Sample: arsenic (100 µg L^{-1}, each) in DI water. Peaks: 1, As(III); 2, DMA; 3, MMA; 4, As(V)

4.2.2 Effect of buffer concentration

In order to check the distribution of arsenic species with pH, software (MarvinSketch, version 5.3.8, ChemAxon) was used. As can be seen in Fig. 24, As(III), DMA, MMA and As(V) exist either in neutral or anionic forms in water (at pH 5.8, optimized later). Hence, chromatographic separation of the four arsenic species was carried out on an anion-exchange column using phosphate buffer in the mobile phase. In the literature, anion-exchange has been widely employed for arsenic speciation studies, as the separation process is more reproducible and

less prone to sample matrix interferences than with reversed-phase columns with ion-paring reagents. In addition, organic solvents added to the mobile phase in reversed-phase may affect stability of the flame when AFS is used for detection together with signal quenching effect caused by CO and CO_2.

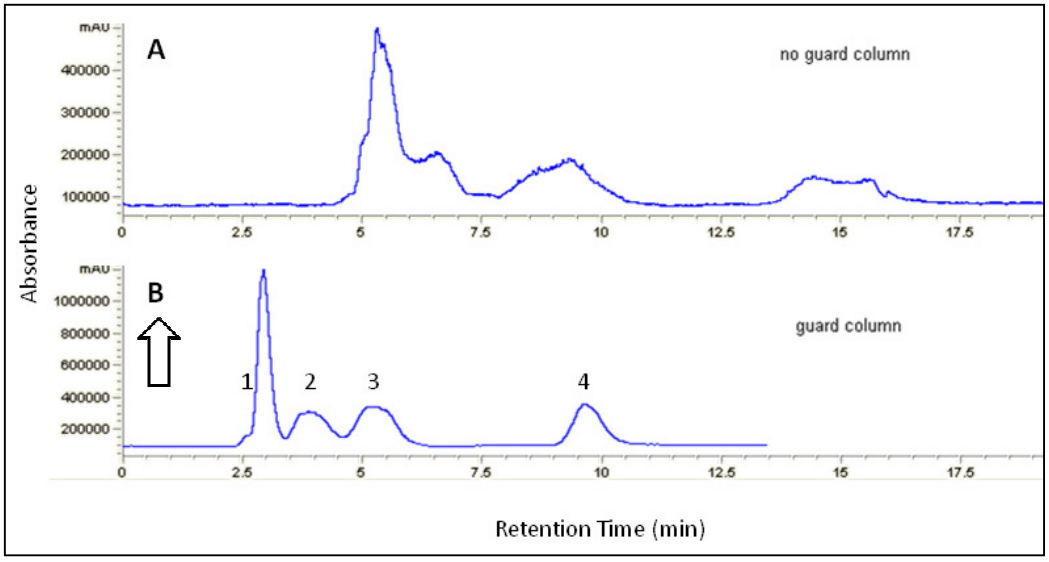


Fig. 23. Effect of guard column. Separation conditions: MP: 8.0 mM phosphate buffer; pH 5.8; FR: 1.0 mL min^{-1}; Data acquisition rate 100 Hz; Gain: 10; (A) Without guard column, (B) With guard column (Hewlett Packard Hypersil C18, 20 mm×2.1 mm, particle size of 5 µm); Sample: arsenic (100 µg L^{-1}, each) in urine. Peaks: same as in Fig. 22

The concentration of phosphate buffer (at pH 5.6) in the mobile phase was investigated, as it is important to use the lowest possible concentration while still effectively control the pH, so as not to cause any increase in the background signal by contamination of the column or detector. A concentration of 5.0–20.0 mM was considered adequate relying on the assumption that the volume of injected sample is small and/or the sample is not buffered at very different pH to the mobile phase. The lowest concentration found to give reproducible results was 5.0 mM.

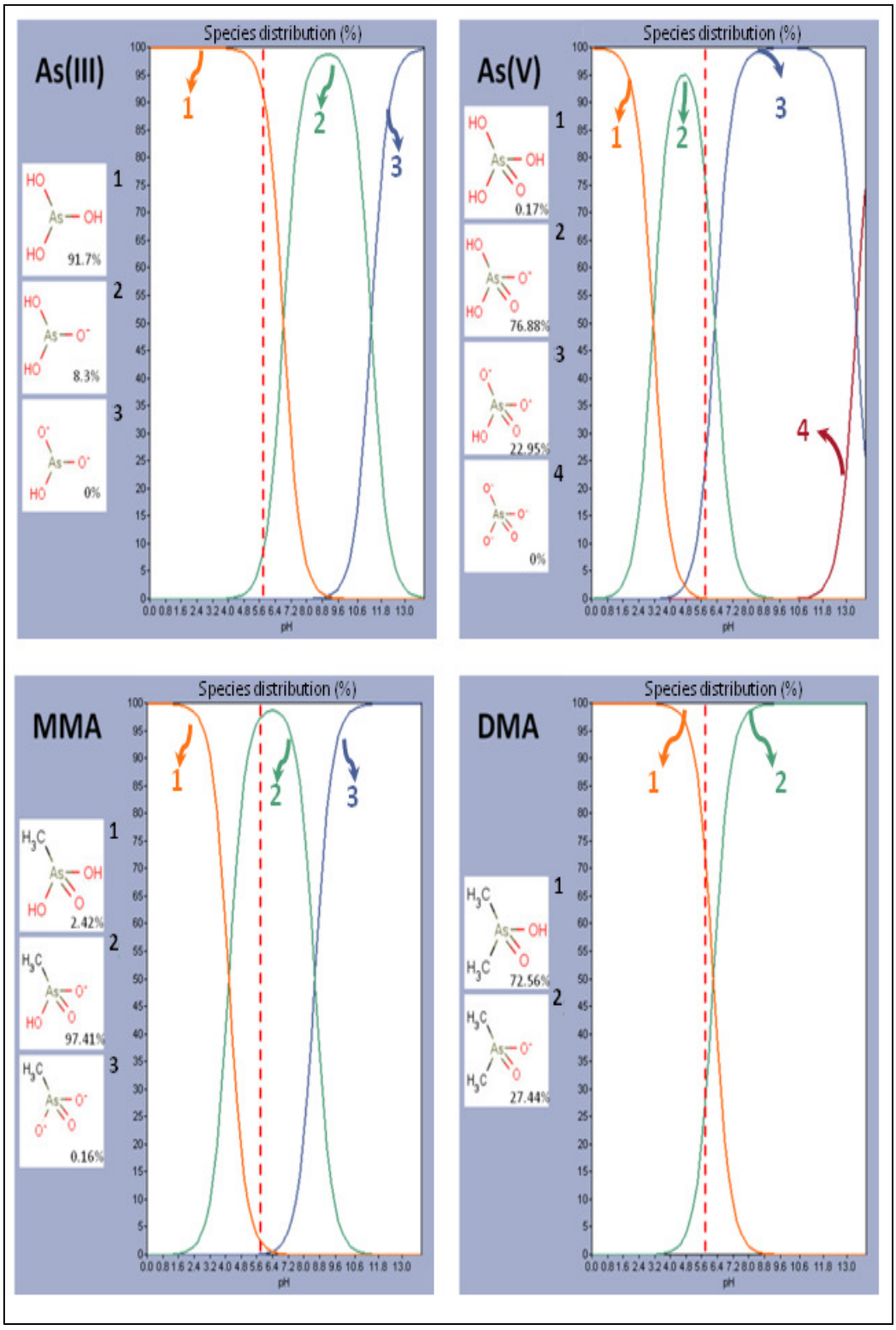


Fig. 24. Species distribution (%) versus pH curves of the target analytes

In ion-exchange chromatographic systems, an increase of the buffer concentration in the eluent results in a decrease in retention times[120] due to increase of the strength of the mobile phase. In this experiment, it was observed that while the use of low buffer concentration resulted into peak broadening, at high concentrations, co-elution of the early peaks occurred (Fig. 25). Based on these observations and considering a baseline resolution higher than 1.5 among all peaks, a concentration of 8.0 mM was selected.

4.2.3 Effect of mobile phase pH

pH of the mobile phase containing 10.0 mM phosphate buffer was varied across the pH range of 5.0–6.9 (Fig. 26), avoiding extreme values which would degrade the stationary phase. It was suggested by the manufacturer, PS Analytical, that a 10.0-mM phosphate buffer at pH 5.6 with the same column used in our study was optimum for separation. However, with this composition and pH, a baseline resolution between As(III) and DMA (peaks 1,2) was not achieved (Fig. 26, B), probably due to small variations among column batches. Therefore, the effect of the mobile phase pH on the retention of arsenic species on the chromatography column was studied. It was observed that variations in pH drastically influenced the retention times of the species (Fig. 26). Generally, for the separation of multivalent ions it is necessary to alter the pH of the mobile phase with an appropriate acid or a base since retention pattern changes with the degree of dissociation of the multivalent ion, which can be controlled by the pH[126]. In this study, hydrochloric acid (1.5 M) was used to lower the pH of the buffer solution while sodium hydroxide (1.0 M) was used to raise it. A pH value of 5.8 was found to give the best peak resolution (Fig. 26, C). Therefore, this value was used in subsequent experiments.

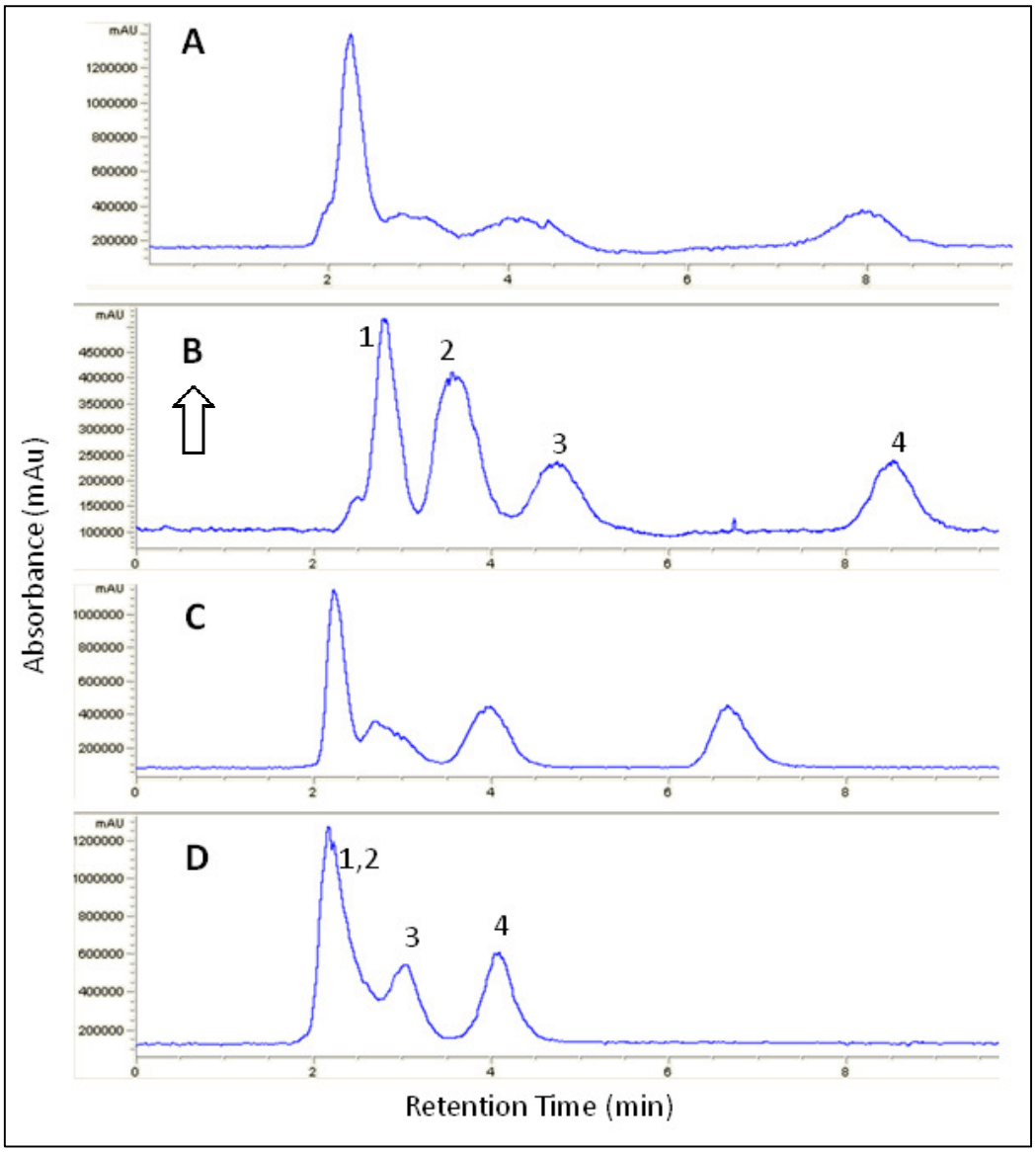


Fig. 25. Effect of buffer concentration. Separation conditions: MP: phosphate buffer; pH 5.6; FR: 1.2 mL min^{-1}; Data acquisition rate 100 Hz; Gain: 10; With guard column; Buffer concentration: (A) 5.0, (B) 8.0, (C) 10.0, (D) 20.0 mM; Sample: arsenic (100 µg L^{-1}, each) in urine. Peaks: same as in Fig. 22

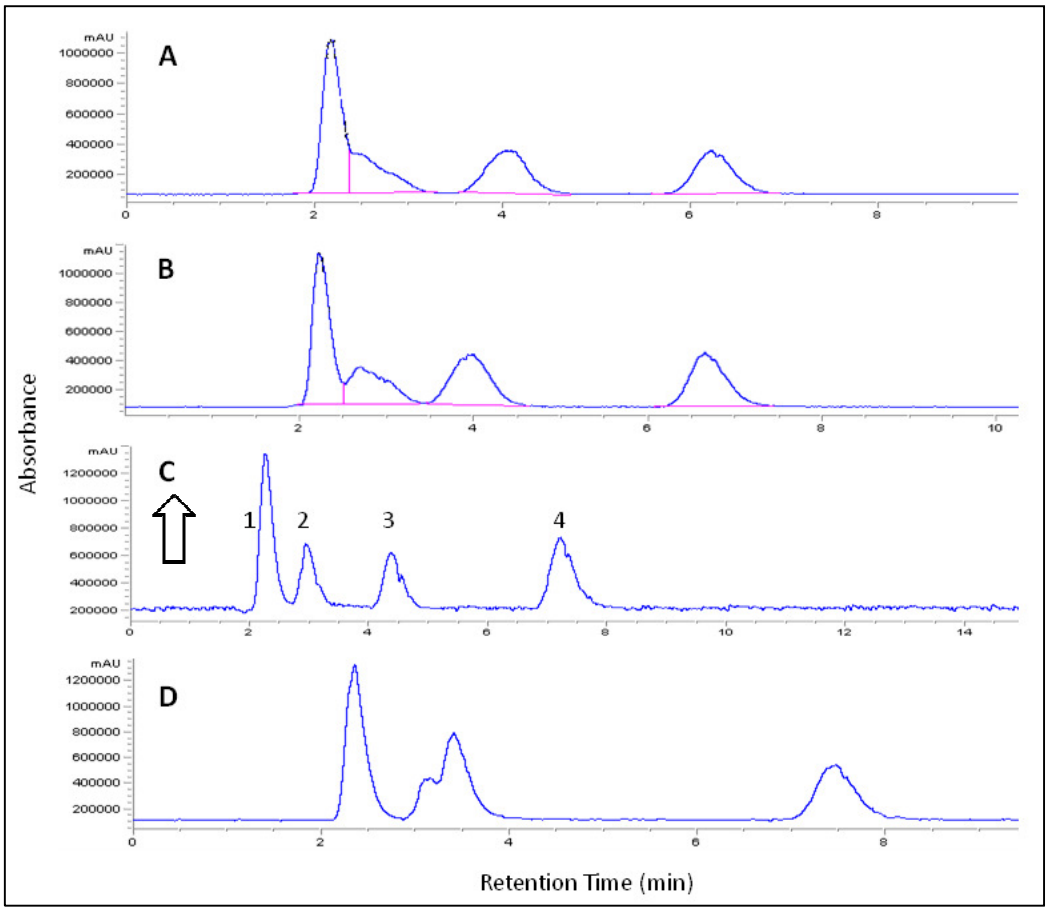


Fig. 26. Effect of buffer pH. Separation conditions: MP: 10.0 mM phosphate buffer; FR: 1.2 mL min^{-1}; Data acquisition rate 100 Hz; Gain: 10; With guard column; Buffer pH: (A) 5.0, (B) 5.6, (C) 5.8, (D) 6.9; Sample: 100 µg L^{-1} arsenic species (each) in urine. Peaks: same as in Fig. 22

4.2.4 Effect of flow rate

The effect of mobile phase flow rate on separation of the four species was investigated within the range of 1.0–1.5 mL min^{-1} (Fig. 27). Optimum separation was obtained at a flow rate of 1.0 mL min^{-1} but slightly at the expense of the analysis time. As so, a flow rate of 1.0 mL min^{-1} was set optimum in further experiments.

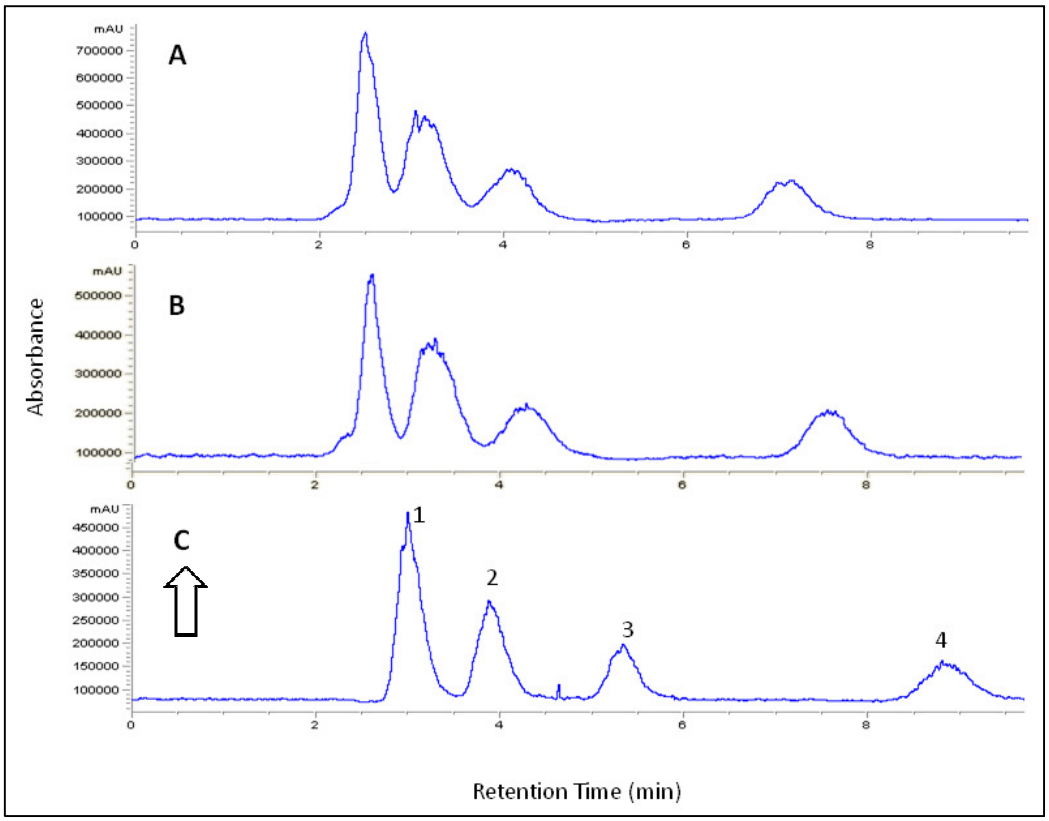


Fig. 27. Effect of flow rate. Separation conditions: MP: 8.0 mM phosphate buffer, pH 5.8; Data acquisition rate 100 Hz; Gain: 10; with guard column; FR: (A) 1.5, (B) 1.2, (C) 1.0 mL min^{-1}; Sample: arsenic (50 µg L^{-1}, each) in urine. Peaks: same as in Fig. 22

4.2.5 Effect of column temperature

The effect of column temperature was studied at two levels, i.e., 5 and 24ºC (ambient temperature of the laboratory) (Fig. 28). It was observed that separation was independent of the column temperature, except for As(V) (peak 4) which was retained more at low temperature. Therefore, the column was used at laboratory temperature in further experiments. Optimum conditions for HPLC–HG–AFS are summarized in Table 10.

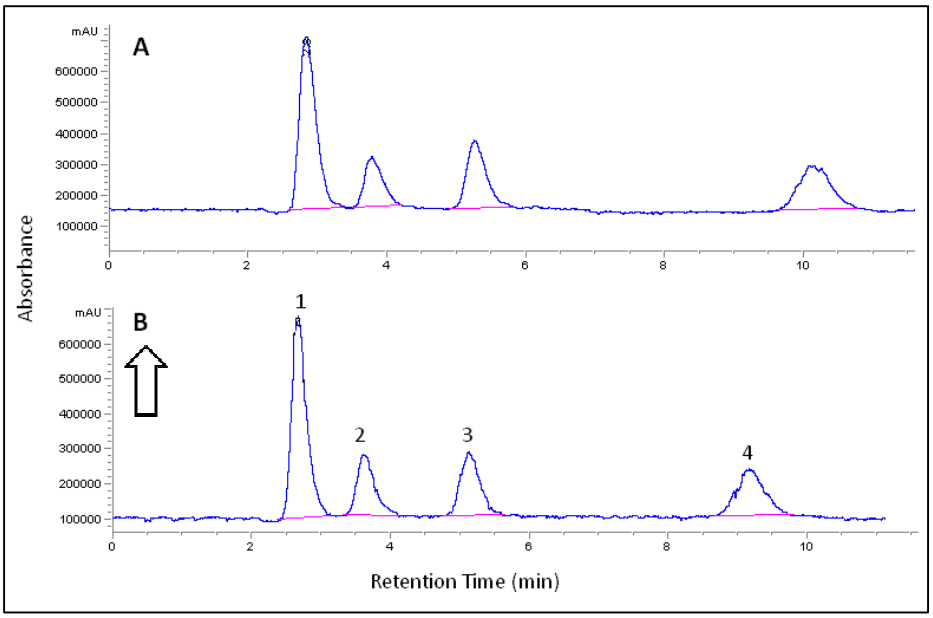


Fig. 28. Effect of column temperature. Separation conditions: MP: 8.0 mM phosphate buffer, pH 5.8; FR: 1.0 mL min^{-1}; Data acquisition rate 100 Hz; Gain: 10; with guard column; column temperature: (A) 5℃, (B) 24℃; Sample: arsenic (50 µg L^{-1}, each) in urine. Peaks: same as in Fig. 22

Table 10. Optimum conditions for HPLC–HG–AFS.

HPLC (Hewlett Packard HPLC system, 1050 model)	
Column	Hamilton PRP-X100 anion-exchange column (250 mm×4.1 mm) with particle size of 10 µm
Guard column	Hewlett Packard Hypersil C18, 20 mm×2.1 mm, particle size of 5 µm
Column temperature	ambient
Mobile phase	isocratic, 8.0 mM KH_2PO_4/K_2HPO_4 (at pH 5.80)
Flow Rate	1.0 mL min^{-1}
Injection volume	100 µL
HG–AFS (PS Analytical Ltd, Millennium Excalibur system)	
As BDHCL	Photron Super Lamp
Primary current	27.5 mA
Boosted	35.0 mA
HCl	1.5 M; 4 ml min^{-1}
$NaBH_4$	1.4% (w/v) in 0.4% (w/v) NaOH; 4 ml min^{-1}
Argon flow rate	250 mL min^{-1}
Signal process and data handling	Agilent interface 39500 E (AD converter) Agilent ChemStation software

4.2.6 Analytical figures of merit of HPLC–HG–AFS and HG–AFS

To evaluate the performance of the developed HPLC–HG–AFS method for separation and speciation of the four arsenic species and that of HG–AFS for determination of the total arsenic concentration, calibration graphs were constructed.

In each method, the instrument was operated at high detector sensitivity level (gain 100) and at low level (gain 10). For samples with high arsenic concentration, gain 10 was used while gain 100 was used for samples with low concentration level. Calibration graphs with HPLC–HG–AFS at gain 10 were plotted by spiking blank urine with the four arsenic species in the range of 10–100 µg L^{-1} (Fig. 29), while at gain 100, concentrations in the range of 2.5–20.0 µg L^{-1} were used (Fig. 30). For total arsenic determination, calibration graphs of HG–AFS were constructed in the ranges of 0.1–5.0 µg L^{-1} for gain 10 and 2.5–500 ng L^{-1} for gain 100 using aqueous standard solutions of As(III).

Linear regression equations, coefficients of determination (R^2), linear dynamic ranges, LODs, LOQs, and %RSDs are summarized in Table 11 for gain 10 and in Table 12 for gain 100. The response was linear over the concentration ranges mentioned above with R^2 ranging from 0.9971 to 0.9999. LOD was calculated based on a signal-to-noise (S/N) ratio of 3; N was the noise of the baseline calculated for eleven noise peaks chosen at different places of the baseline void of analytical peaks. LODs at gain 10 ranged from 2.2 to 0.9 µg L^{-1} for the species and 30 ng L^{-1} for total arsenic (Table 11). For gain 100, they were in the range of 0.3 to 0.7 µg L^{-1} for the species and 3 ng L^{-1} for the total (Table 12). Representative chromatograms obtained under optimum HPLC–HG–AFS conditions for urine spiked with 5.0 and 20.0 µg L^{-1} are shown in Fig. 31.

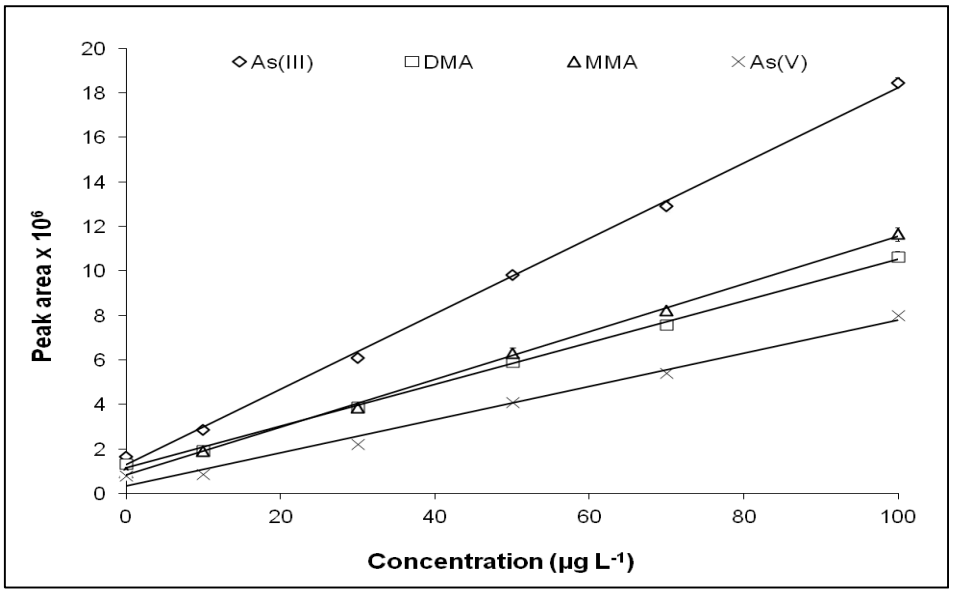


Fig. 29. Calibration graphs of HPLC–HG–AFS at gain 10. Conditions: mobile phase: 8.0 mM phosphate buffer at pH 5.8; flow rate: 1.0 ml min^{-1}; Data acquisition rate 100 Hz

Table 11. Figures of merit of HPLC–HG–AFS and HG–AFS (Gain 10).

		HPLC–HG–AFS[a]				HG–AFS[b]
		As(III)	DMA	MMA	As(V)	Total As
Linear regression equation[c]		$y = 1.7\times10^5$ x + 1.3×10^6	$y = 9.4\times10^4$ x + 1.1×10^6	$y = 1.1\times10^5$ x + 8.3×10^5	$y = 7.9\times10^4$ x + 4.8×10^4	$y = 3.3\times10^3$ x + 80
Coefficient of determination (R^2)		0.9983	0.9983	0.9989	0.9971	0.9999
Linear dynamic range (µg L^{-1})		7.3–100	7.3–100	9.6–100	9.6–100	0.1–5.0 (µg L^{-1})
LOQ ($10s_b/m$, µg L^{-1})		7.3	7.3	9.6	9.6	0.1 (µg L^{-1})
LOD ($3s_b/m$, µg L^{-1})		2.2	2.2	2.9	2.9	30 (ng L^{-1})
RSD (%, n = 5)	Retention time	0.66	0.44	1.54	1.41	–
	Peak area	0.92	1.47	1.75	1.76	–
	Peak height	0.42	2.31	0.11	1.41	–

[a] Conditions: mobile phase: 8.0 mM phosphate buffer at pH 5.8; flow rate: 1.0 ml min^{-1}; Data acquisition rate 100 Hz; Gain: 100; Sample: 100 µL urine (buffered to pH 6.9 with 5.0 mM phosphate buffer)

[b] Conditions: as given in Fig. 31

[c] Peak area = slope × concentration (µg L^{-1}) + intercept

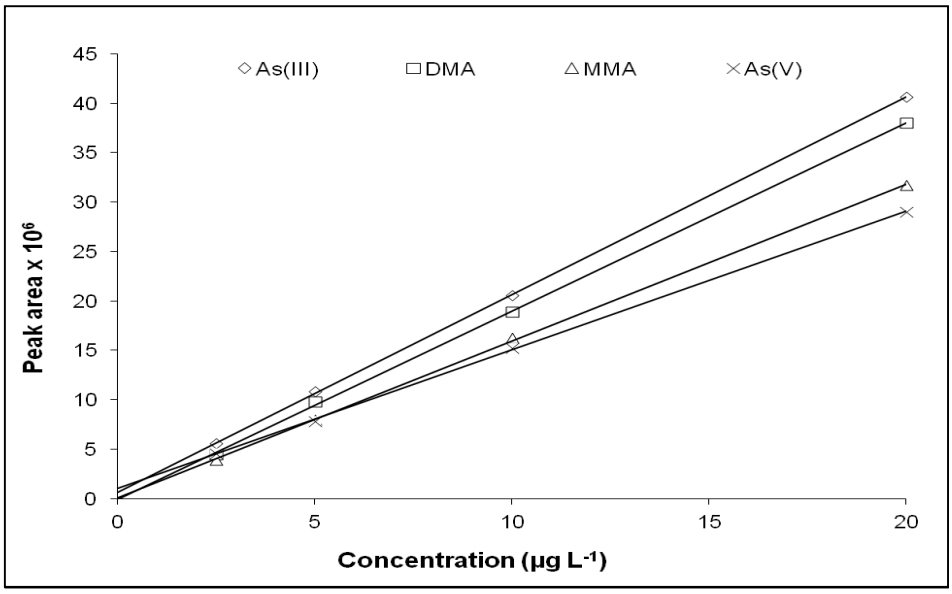


Fig. 30. Calibration graphs of HPLC–HG–AFS at gain 100. Conditions: mobile phase: 8.0 mM phosphate buffer at pH 5.8; flow rate: 1.0 ml min^{-1}; Data acquisition rate 100 Hz

Table 12. Figures of merit of HPLC–HG–AFS and HG–AFS (Gain 100).

	HPLC–HG–AFS[a]				HG–AFS[b]
	As(III)	DMA	MMA	As(V)	Total As
Linear regression equation[c]	$y = 2.1\times10^6 x + 8.2\times10^4$	$y = 2.0\times10^6 x - 2.0\times10^5$	$y = 1.6.1\times10^6 x - 5.6\times10^4$	$y = 1.4\times10^6 x + 5.8\times10^5$	$y = 9674.4x + 75.6$
Coefficient of determination (R^2)	0.9995	0.9995	0.9999	0.9998	0.9998
Linear dynamic range (µg L^{-1})	1.0–20	1.0–20	2.3–20	2.3–20	25–500 (ng L^{-1})
LOQ ($10s_b/m$, µg L^{-1})	1.0	1.0	2.3	2.3	10 (ng L^{-1})
LOD ($3s_b/m$, µg L^{-1})	0.3	0.3	0.7	0.7	3 (ng L^{-1})

[a] Conditions: mobile phase: 8.0 mM phosphate buffer at pH 5.8; flow rate: 1.0 ml min^{-1}; Data acquisition rate 100 Hz; Gain: 100; Sample: 100 µL urine (buffered to pH 6.9 with 5.0 mM phosphate buffer)

[b] Conditions: as given in Fig. 31

[c] Peak area = slope × concentration (µg L^{-1}) + intercept

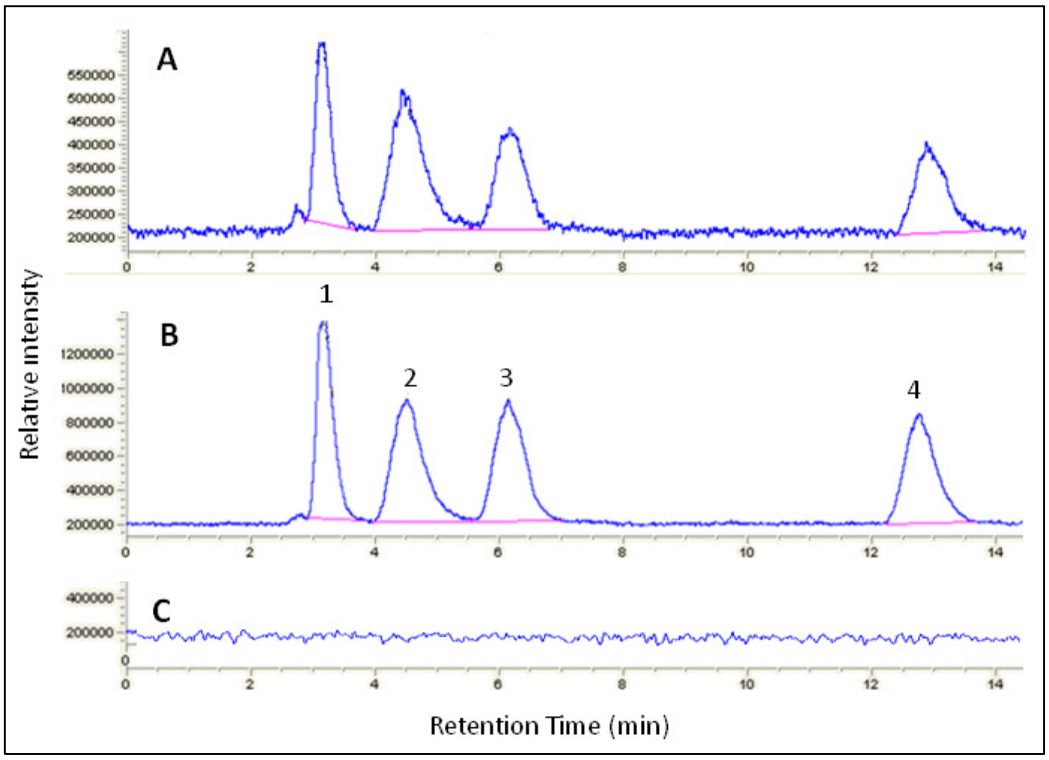


Fig. 31. Chromatograms under optimized separation conditions. MP: 8.0 mM phosphate buffer, pH 5.8; FR: 1.0 ml min^{-1}; Data acquisition rate 100 Hz; Gain: 100; Standard urine solution spiked with: (A) 5.0 µg L^{-1}; (B) 20.0 µg L^{-1} of each arsenic species; (C) Blank urine. Peaks: same as in Fig. 22

A representative signal profile, at gain 10, obtained with continuous flow HG–AFS for blank and standard solutions at concentrations of 1.0, 2.5 and 5.0 µg L^{-1} is shown in Fig. 32**.**

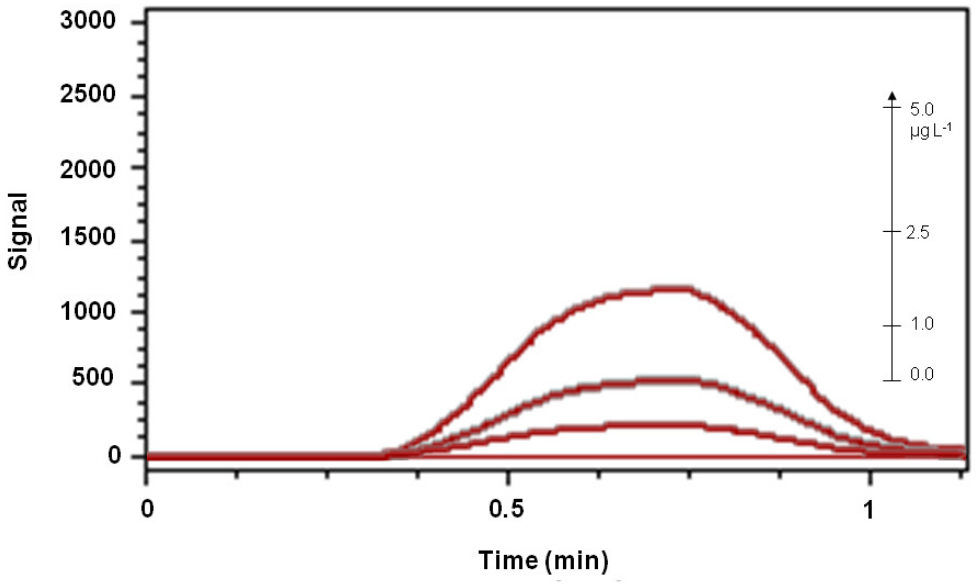


Fig. 32. Signal profile of HG–AFS at gain 10. Arsenic concentration: blank, 1.0, 2.5 and 5.0 µg L^{-1} bottom to top, respectively.

4.2.7 Accuracy check

Accuracy of HPLC–HG–AFS was assessed by analyzing the standard reference material "SRM NIST 2669 arsenic species in frozen human urine" (level II, High-Level). The results obtained (Table 13) were statistically not different from the certified values at 95% confidence level. Low-Level of the SRM was not analyzed because the certified values were in the range of 1.47–3.47 µg L^{-1} that would fall below the linear dynamic range after diluting 900 µL of the urine with 100-µL buffer. Accuracy of HG–AFS was checked by determining total arsenic concentration in level I and II of the same urine SRM (Table 14). The results obtained were also statistically in a good agreement with the certified values.

Table 13. Arsenic speciation in certified reference material (CRM) by HPLC–HG–AFS.

SRM NIST 2669 "arsenic species in frozen human urine" Level II (μg L^{-1})		
Arsenic species	Certified value	Found[a]
As(III)	5.03±0.31	5.12±0.45
As(V)	6.16±0.49	6.64±0.49
DMA	25.3±0.7	26.5±1.0
MMA	7.18±0.56	7.21±1.04

[a] The results are the mean values of 5 measurements

Table 14. Total arsenic concentration in CRM by HG–AFS.

"SRM NIST 2669 arsenic species in frozen human urine" Total arsenic concentration (μg L^{-1})			
Level I		Level II	
Certified value	Found[a]	Certified value	Found[a]
22.2±4.8	22.3±0.4	50.7±6.3	50.7±0.6

[a] The results are the mean values of 5 measurements

4.2.8 Speciation of arsenic in urine from Nevşehir

The developed HPLC–HG–AFS method was applied for the speciation of arsenic in urine samples collected from the study area from people chronically exposed to arsenic through drinking water. The analysis results were classified into three groups according to the arsenic level in drinking water they consumed. The first group was for water with arsenic concentration below 10 μg L^{-1} (Low-Level), the second with arsenic in the range of 10–50 μg L^{-1} (Middle-Level), and the third with arsenic above 50 μg L^{-1} (High-Level). The results of the analysis are given in the APPENDIX section. Representative chromatograms from High-Level (with 1:2, v/v dilution) and Low-Level samples (no dilution) are given

in Fig. 33 A and B, respectively. Total arsenic concentration in all samples was also determined using the HG–AFS method described in Section 3.3.3.

Table 15. Concentration (µg L^{-1}) of arsenic species and total concentration in sample groups.

As in water (µg L^{-1})	Arsenic species in urine (mean±SD)				Sum of species (mean±SD)*	Total arsenic, (mean±SD)§
	As(III)	As(V)	MMA	DMA		
As>50 (n = 174)	9.0±8.45	10.9±13.6	19.9±23.9	75.0±90.8	102.3±110.6	157.2±121.8
10<As<50 (n = 256)	4.9±3.6	8.7±5.8	6.5±6.1	34.6±53.2	40.5±53.9	71.0±54.2
As<10 (n = 146)	0.9±0.3	<LOD	1.1±0.4	2.3±1.1	3.0±1.5	17.3±26.5

* Mean values are statistically different (Student t-Test, $p<0.05$)

§ Mean values are statistically different (Student t-Test, $p<0.05$)

Mean values for arsenic species and the total concentration (Table 15) of Middle-Level were compared with those of both Low- and High-Levels using the t-Test. The results showed that the three groups are statistically different from each other at 95% confidence level. It can also be observed from Table 15 that in average DMA was the predominant species with the highest concentration in the three groups, which can be preliminarily attributed to methylation. It was also observed that the sum of concentrations of organic arsenic species were always much higher than those of inorganic species.

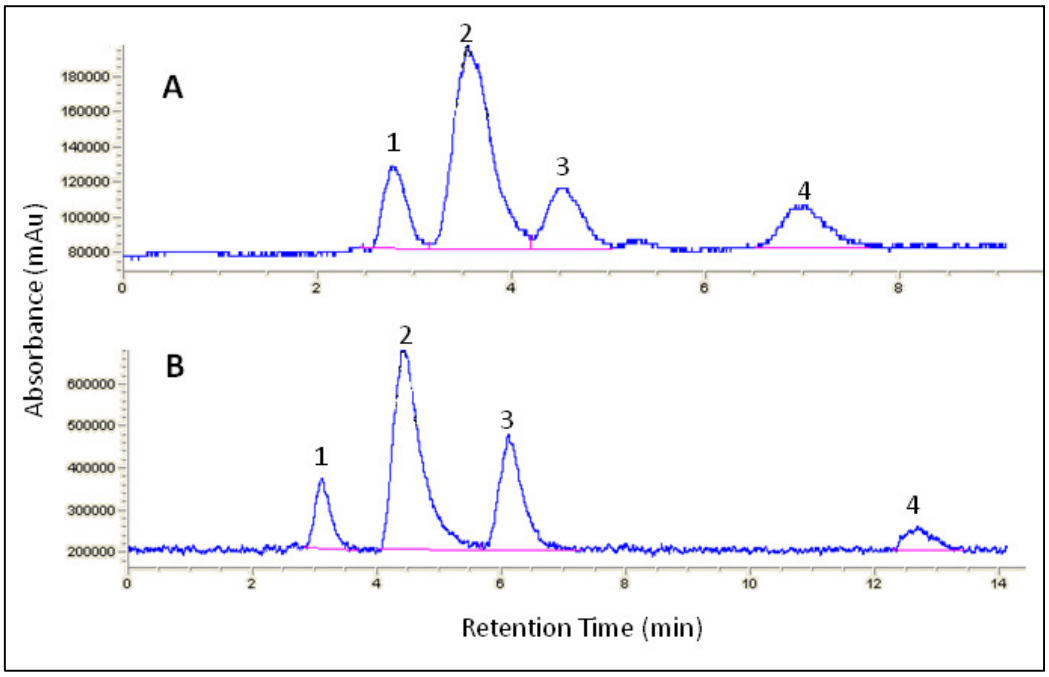


Fig. 33. Chromatograms of real urine samples. MP: 8.0 mM phosphate buffer, pH 5.8; FR: 1.0 ml min^{-1}. (A) Urine sample, Code No. 35 (High-Level, 1:2 dilution, Gain: 100). Peaks: 1: As(III) [16.9±1.2]; 2: DMA [116.8±3.5]; 3: MMA [16.6±0.4]; 4: As(V) [1.6±0.1], Sum: 151.9±3.7; (B) Urine sample, Code No. 419 (Low-Level, no dilution, Gain: 100). Peaks: 1: As(III) [1.15±0.04]; 2: DMA [7.68±0.12]; 3: MMA [3.76±0.01]; 4: As(V) [1.04±0.18], Sum: 13.63±0.22, concentrations in µg L^{-1}

5 CONCLUSION

In the first part of this study, simultaneous preconcentration of As(III), As(V), MMA, DMA, Sb(III) and Sb(V) by CE was achieved by using: (i) sweeping-MEKC with SDS, (ii) LVSS with polarity switching, (iii) FASS, and (iv) FASI/EOF reversal. The lowest LODs were obtained by combining FASI/EOF reversal with HSDC. LODs for the target analytes were 2.1, 16, 14, 1.9, 1.1 and 13 μg L^{-1} for the six species, respectively. Compared to conventional CZE, this method gave IFs in the range of 0.6×10^3–21×10^3. The developed CE method was successfully applied for the speciation of arsenic and antimony in spiked tap water and recovery values in the range of 97.7–102.5% were obtained.

The developed FASI/EOF reversal combined with HSDC was compared with other studies in the literature using CE with different detection systems for speciation of arsenic and/or antimony in waters. The results shown in Table 16 indicate that the developed method provided lower LODs than all other methods using UV, LIF and HG–AFS. When ICP–MS was used, LODs were comparable with those obtained with hydrodynamic injection, but electrokinetic injection gave better results. However, UV detectors are both cheaper and more compatible with CE than ICP–MS.

Table 16. Comparison of the developed CE method with others in the literature.

Matrix/sample	Species	Detector	Pretreatment/ preconcentration method	LOD ($\mu g\ L^{-1}$)	Ref.
Standards	As(III), DMA, MMA, As(V)	UV (indirect)	FASI	20–70	9
Tap and lake water	As(III), DMA, MMA, As(V), roxarsone, p-aminophenylarsonic acid, 4-nitrophenylarsonic acid, phenylarsonic acid	UV	LVSS with polarity switching; NSM; co-EOF NSM with EOF reversal; HSDC	5.61–17.1	10
Aqueous soil extracts	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	UV (indirect)	Extraction	84–304 (hydrodynamic injection), 44–147 (electrokinetic injection),	12
Lake and river water	As(III), DMA, MMA, As(V)	HG–AFS	pH-mediated stacking	5.0–9.3	48
Natural and contaminated waters	As(III), DMA, MMA, As(V)	UV	–	5.0–20	77
Ground-water	As(III), DMA, As(V),	UV	–	100–500	82
Tap and mineral water	As(III), DMA, MMA, As(V)	LIF (indirect)	–	120–540	83
Aqueous soil extracts	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	ICP–MS	Extraction	6–17 (hydrodynamic injection), 0.08–0.31 (electrokinetic injection),	84
Tap water	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	UV	FASI/EOF reversal with HSDC	1.9–16	This study

In the second part, an HPLC–HG–AFS methodology was developed and applied for speciation of As(III), As(V), MMA and DMA in residents chronically exposed to inorganic arsenic via drinking water in Nevşehir area. The four arsenic species were separated on an anion exchange column under optimized chromatographic conditions (i.e., mobile phase 8.0 mM phosphate buffer at pH 5.8, flow rate 1.0 mL min^{-1}, column at ambient room temperature, with C18 guard column). Accuracy of the speciation studies was checked by analyzing, “SRM NIST 2669

arsenic species in frozen human urine" and the results were not different from the certified values at 95% confidence level. Total arsenic concentration in the samples was also determined by HG–AFS after acid digestion and pre-reduction with potassium iodide. The same procedure was applied to SRM 2669 at two levels (i.e., low and high) and the results were also in a good agreement. LODs with HPLC–HG–AFS, for a 100-µL injection loop, were in the range of 0.3–0.7 µg L^{-1} and that with HG–AFS was 3 ng L^{-1} (gain 100). The results were classified into three groups as low, middle and high according to the arsenic level in drinking water people consumed. The mean values for the sum of arsenic species concentration and total arsenic of the three groups were compared. The results showed that they were statistically different from each other. It was also observed that in average DMA was the species with the highest concentration in the three groups, which was preliminarily attributed to methylation. In addition, the sum of concentrations of organic arsenic species was always much higher than those of inorganic species.

6 ÖZET

UV dedektörlü CE arsenit [As(III)], arsenat [As(V)], monometiylarsonik asit (MMA), dimetilarsinik asit (DMA), antimonit [Sb(III)] ve antimonat'ın [Sb(V)] aynı anda zenginleştirilmesi ve tayini için dört kapileriçi zenginleştirme tekniği kullanılmıştır. Bunlar (i) sodyum dodesil sülfat ile süpürmeli- misel elektrokinetik kromatografi (MEKC), (ii) polarite değiştirmeli yüksek hacimli numune istifleme (LVSS), (iii)Yükseltilmiş alan numune istifleme (FASS) ve (iv) setiltrimetilamonyum bromür (CTAB) ile ters elektroozmotik akışlı yükseltilmiş alan numune istifleme (ters EOF-FASI) teknikleridir. En düşük teşhis sınırı (LOD), ters EOF-FASI ile, elektrokinetik numune enjeksiyonu öncesinde kapilere kısa süreli su tabakası enjekte edildiğinde gözlenmiştir. Bu teknik, yüksek duyarlıklı hücre ile birlikte kullanıldığında hedef analitlerin LOD değerleri sırasıyla 2.1, 16, 14, 1.9, 1.1 and 13 μg L^{-1} dır. LOD değerlerinde konvansiyonel kapiler elektroforeze kıyasla 0.6×10^3–21×10^3 kez artış sağlanmıştır. Geliştirilen yöntem arsenik ve antimon standardı eklenmiş çeşme suyuna başarı ile uygulanmış ve geri kazanım değerleri %97.7-102.5 arasında bulunmuştur.

Türkiye'nin Nevşehir bölgesinde içme suyu yolu ile arseniğe maruz kalan insanlarda kanser riskinin araştırıldığı projenin bir kısmı olarak bölgeden toplanan idrar numunelerinde yüksek performanslı sıvı kromatografisi-hidrür oluşturmalı-atomik floresans spektrometrisi (HPLC–HG–AFS) ile arsenik türleme analizi yapılmıştır. İdrarda arsenik türlemesi için bir HPLC–HG–AFS yöntemi geliştirilmiştir. Arsenik türleri, As(III), As(V), DMA ve MMA, optimize edilmiş kromatografik koşullarda (pH'sı 5.8'e ayarlanmış, 8 mM fosfat tampon ve 1.0 mL min^{-1} akış hızındaki hareketli faz, numune pH'sı 5.8, C18 koruyucu kolon) kuvvetli anyon değiştirici kolonda ayrılmıştır. Geliştirilen yöntemin doğruluğunu

test etmek için SRM NIST 2669 (dondurulmuş insan idrarında arsenik türleri) sertifikalı referans maddesinde arsenik türlerinin tayini yapılmış ve sonuçların %95 güven aralığında sertifikalı değerler ile uyumlu olduğu görülmüştür. İdrar numuneleri asit ile yıkılanmış ve potasyum iyodür ile ön-indirgeme sonrası HG-AFS ile toplam arsenik tayini yapılmıştır. Aynı şekilde yıkılanan SRM 2669'un yüksek ve düşük olmak üzere iki faklı seviyesinde toplam arsenik tayini yapılmış ve sertifikalı değerler ile uyumlu değerler bulunmuştur. Arsenik türleri için HPLC-HG-AFS ile 100 µL enjeksiyon hacmi kullanılarak hesaplanan LOD değerleri 0.3-0.7 µg L^{-1} arasında, HG-AFS ile toplam arsenik tayininde ise 2.5 ng L^{-1}'dir (gain 100). Bölgeden toplanan idrar numunelerinde arsenik türleri ve toplam arsenik tayini yapılmıştır. Sonuçlar, bölgede yaşayan insanların tükettikleri içme suyundaki arsenik derişimine göre düşük, orta ve yüksek olarak gruplandırıldığında, hesaplanan toplam arsenik ortalamaları ve türlerin toplamlarının ortalamaları arasındaki fark %95 güven aralığında anlamlı bulunmuştur. Üç grupta da türleme analizlerinde derişimi en yüksek olan tür DMA dır. Sonuçlar HPLC-HG-AFS ve HG-AFS yönteminin idrar ve su numunelerinde arsenik türlemesi ve toplam arsenik tayini için duyarlı, tekrarlanabilir ve doğru olduğunu göstermektedir.

ANAHTAR KELİMELER: antimon, arsenik, atomik floresans spektrometri, kapiler elektroforez, hidrür oluşturma, türleme, istifleme, süpürme

7 SUMMARY

Capillary electrophoresis (CE) with direct on-capillary UV detection was applied along with four online sample preconcentration techniques for the simultaneous preconcentration and determination of arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), antimonite [Sb(III)] and antimonate [Sb(V)]. These techniques were (i) sweeping-micellar electrokinetic chromatography (MEKC) with sodium dodecyl sulphate (SDS), (ii) large volume sample stacking (LVSS) with polarity switching, (iii) field-amplified sample stacking (FASS), and (iv) field-amplified sample injection (FASI) with electroosmotic flow (EOF) reversal using cetyltrimethylammonium bromide (CTAB). The lowest limits of detection (LOD) were obtained with FASI/EOF reversal with electrokinetic injection of the sample after a water plug. By combining this technique with high-sensitivity detection cell (HSDC), LODs for the target analytes were 2.1, 16, 14, 1.9, 1.1 and 13 μg L^{-1} for the six species, respectively. Compared to conventional capillary zone electrophoresis (CZE), this method gave improvement factors in LODs in the range of 0.6×10^3–21×10^3. The developed method was successfully applied for the speciation of arsenic and antimony in spiked tap water and recovery values in the range of 97.7–102.5% were obtained.

Arsenic speciation in residents chronically exposed to inorganic arsenic via drinking water in Nevşehir area, Turkey, by HPLC–HG–AFS was a part of a research project for investigating carcinogenic risks of arsenic in those people. A methodology for arsenic speciation in urine by HPLC–HG–AFS was developed and presented. Inorganic [As(III), As(V)] and organic (DMA, MMA) arsenic species were separated on a strong anion exchange column under optimized chromatographic conditions (i.e., mobile phase 8.0 mM phosphate buffer at pH 5.8, flow rate 1.0 mL min^{-1},

column at ambient room temperature, and using a C18 guard column). In order to assess accuracy of the speciation studies, “SRM NIST 2669 arsenic species in frozen human urine” was analyzed for arsenic species by the developed method and the results were not different from the certified values at 95% confidence level. Total arsenic concentration was also determined by HG–AFS after acid digestion and pre-reduction with potassium iodide. The same procedure was applied to SRM 2669 at two levels (i.e., low and high) and the results were also in a good agreement. LODs with HPLC–HG–AFS, for a 100-µL injection loop, were in the range of 0.3–0.7 µg L^{-1} and that with HG–AFS was 3 ng L^{-1} (at gain 100). Urine samples collected from the study area were analyzed. The results were classified into three groups as low, middle and high according to the arsenic level in drinking water people consumed. The mean values for the sum of arsenic species concentration and total arsenic were compared and were found to be statistically different from each other. It was also observed that in average DMA was the species with the highest concentration in the three groups, which was preliminarily attributed to methylation.

KEYWORDS: antimony, arsenic, atomic fluorescence spectrometry, capillary electrophoresis, hydride generation, speciation, stacking, sweeping

8 TÜRKÇE GENİŞ ÖZET

8.1 GİRİŞ

Arsenik ve antimonun çevre ve insan sağlığında önemli rolü olduğundan bu elementlerin türleme analizi de ilgi çekmektedir[1,2]. Arsenik türleri arasında inorganik arsenik bileşikleri daha toksiktir ve metilasyon, inorganik arsenik türlerinin daha az toksik olan organik arsenik türlerine dönüştürüldüğü en önemli yolaktır. Arsenit [As(III)], arsenat [As(V)], monometilarsonik asit (MMA) ve dimetilarsinik asit (DMA) en önemli arsenik türleridir[3]. Antimon insan sağlığı için esansiyel olmayan elementlerdendir ve toksisitesi yükseltgenme basamağına bağlıdır. Antimonit [Sb(III)], antimonat'dan [Sb(V)] daha toksiktir. Çevre Koruma Ajansının (EPA) bu elementi öncelikli kirletici olarak listelemiş olması antimon tayinini önemli kılmaktadır[2].

Arsenik ve antimon türlerinin aynı anda tayini: yüksek performanslı sıvı kromatografi-endüktif eşleşmiş plazma-kütle spektrometri (HPLC–ICP–MS)[2,4], grafit fırın atomik absorbsiyon spektrometri (GF–AAS)[5], hidrür oluşturmalı-atomik absorbsiyon spektrometri[6], hidrür oluşturmalı-atomik floresans spektrometri[7], hidrür oluşturmalı-endüktif eşleşmiş plazma-atomik emisyon spektrometri (HG–ICP–AES)[8], ve yüksek performanslı sıvı kromatografi-hidrür oluşturmalı-atomik absorbsiyon spektrometri (HPLC–HG–AAS)[4] kullanılarak yapılmıştır.

Yüksek performanslı-sıvı kromatografisi (HPLC) ve gaz kromatografi (GC) elemental türleme analizlerinde en sık kullanılan ayırma tekniklerdendir[1]. Ancak, son zamanlarda arsenik[9,10] ve antimon[11-13] türleme analizlerinde kapiler elektroforez (CE) uygulamalarına ilgi

artmaktadır. Bunun nedenleri arasında yüksek ayırma verimliliği, kısa analiz süreleri, düşük işlem maliyeti ve uygulama alanının geniş olması gösterilebilir[10,12]. Bununla beraber, CE'nin dezavantajları: ayırma verimliliğinin korunabilmesi için numune enjeksiyon hacminin çok küçük tutulması ve optik yolun çok kısa olması sonucu düşük konsantrasyon duyarlılığıdır[15]. Duyarlılığın artırılması için balon tipi, Z-tipi hücre veya çok yansıtmalı kapiler tasarımları, yüksek duyarlılığa sahip dedektörler (lazer indükleyici floresans, elektrokimyasal, kütle spektrometri) veya offline zenginleştirme teknikleri (sıvı-sıvı veya katı-faz ekstraksiyon) kullanılmıştır. Ancak, bu tekniklerin göreceli olarak pahallı olmaları veya CE ile eşleştirmede yaşanan sorunlar mevcuttur. Offline zenginleştirme yöntemleri ise uzun süren ön işlemler gerektirmektedir. Diğer taraftan, standart kapiler ışık yoluna kıyasla 10 kattan fazla ışık yolu sağlayan yüksek duyarlıklı hücre (HSDC)[1] veya süpürme[16] ve numune istifleme[17] gibi kapiler içi zenginleştirme yöntemleri bu probleme çözüm olarak kullanılmıştır. İstifleme teknikleri (i) yükseltilmiş alan numune istiflemesi (FASS)[18], (ii) yüksek hacimli numune istifleme (LVSS)[19], (iii) yükseltilmiş alan numune enjeksiyonu (FASI)[10] tekniklerini içermektedir.

Bu çalışmada süpürme ve üç ayrı numune istifleme yöntemi denenmiş ve ters EOF–FASI istifleme yöntemi HSDC ile birlikte kullanılarak konvansiyonel kapiler elektroforeze (CZE) kıyasla duyarlılık artışı sağlanmıştır. As(III), As(V), MMA, DMA, Sb(III) ve Sb(V) için teşhis sınırları (LOD) sırasıyla, 2.1, 16, 14, 1.9, 1.1 ve 13 $\mu g\ L^{-1}$, duyarlılık artışı $0.6x10^3$–$21x10^3$ kezdir. Geliştirilen CE yöntemi arsenik ve antimon ilave edilmiş içme suyunda türleme analizine uygulanmış ve % 97.7–102.5 geri kazanım değerleri elde edilmiştir.

Arsenik maruziyetinin izlenmesinde idrar numunesinde arsenik türleme analizi önemli bir belirteçtir. İçme suyundaki arsenik düzeyi ile

bazı kronik etkiler arasında korelasyon olduğu gösterilmiştir. Ancak, bu korelasyonun doğru yorumlanabilmesi için maruz kalınan arsenik türleri ile birlikte çeşitli ortak etkiler de göz önünde bulundurulmalıdır[20]. AFS, arsenik gibi hidrür oluşturabilen elementlerin türleme çalışmalarında ideal bir tayin yöntemidir. AFS yüksek seçilik ve duyarlılık, geniş dinamik aralık gibi özellikleri ile türleme çalışmalarında sıklıkla kullanılan AAS, ICP–MS gibi atomik tekniklere alternatif oluşturmaktadır.

Bu çalışmanın ikinci kısmında, Nevşehir bölgesinde içme suyu yoluyla kronik arsenik maruziyeti sonucu insanlarda kanser riskinin araştırıldığı projenin bir parçası olarak HPLC–HG–AFS ile idrar numunelerinde arsenik türlerinin tayini için yöntem geliştirilmiştir (Türkiye Bilimsel ve Teknolojik Araştırma Kurumu, TÜBİTAK, Proje No 109S419). Arsenik türleri kuvvetli anyon değiştirici kolonda ayırılmıştır. Türleme çalışmalarının doğruluğunu test etmek amacıyla sertifikalı referans madde (NIST SRM 2669) HPLC–HG–AFS ile analiz edilmiştir. Aynı SRM'de yıkılama işlemi sonrası toplam arsenik tayini de HG–AFS ile yapılmıştır.

8.2 GENEL BİLGİLER

8.2.1 Arsenik ve Antimon

Arsenik ve antimon peryodik cetvelde 15. grupta bulunan metaloid grubundandır. Her ikisi de s^2p^3 dış orbital konfigürasyona sahip olmaları nedeniyle aynı yükseltgenme basamaklarına (-3, 0, +3, +5) sahiptir. Genel olarak oksit, hidroksit veya oksoanyonlar halinde bulunurlar. Oksitleyici ortamlarda +5 yükseltgenme basamağında (arsenat ve antimonat halinde)

veya oksitleyici olmayan ortamlarda +3 yükseltgenme basamağında (arsenit ve antimonit halinde) bulunurlar.

8.2.2 Toksisite ve Sağlık Etkileri

Tarihte popüler bir zehir olarak kullanılması nedeniyle uzun süreden beri arseniğin zararlı bir element olduğu bilinmektedir[22,23]. Antimonun toksik ve çevresel etkileri daha az bilinmektedir. Antimonun, arsenik ile benzer etkileri olduğu değerlendirilse de henüz doğrulanmamıştır[30]. Bu iki metaloid arasındaki kimyasal benzerlikler ve bunların çeşitli ortamlarda derişiminin artıyor olması endişeye yol açmaktadır[30].

Antimon ve arseniğin toksisitesi türlerine bağlıdır[24,33]. Antimon türlerinin genel toksisite sıralaması: Organoantimonlar (metillenmiş türler) < antimonatlar [Sb(V)] < antimonitler [Sb (III)] şeklinde verilmektedir[24,32,33]. Bu sıralama arsenik için de benzerdir: Organoarsenikler (metillenmiş türler) < arsenatlar [As(V)] < arsenitler [As (III)][34]. Arseniğin insanlarda en önemli absorpsiyon yolu oral yol olup, dermal yol ile vücuda girişi minimumdur. İnsanlar tarafından vücuda alınan arseniğin vücutta en önemli metabolik yolağının metilasyon olduğu bilinmektedir. Ağız yolu ile vücuda alındıktan sonra arsenik, monometilarsonik aside [MMA(V)] metillenir, bu da monometil arsinöz aside redüklenir [MMA(III)]. MMA(III) daha sonra dimetilarsinik aside [DMA(V)], bu da dimetil arsinöz aside [DMA(III)] redüklenir. İdrarda arsenik metabolitlerinin tipik profili inorganik arsenik (%10-30), MMA (%10-20) ve DMA (%60-80) olduğu gösterilmiştir. Geçmişte bu biyotransformasyon işleminin arseniğin detoksifasyon yolağı olabileceği düşünülmüştü, fakat arsenik (III) içeren metillenmiş metabolitlerinin inorganik formundan daha genotoksik olduğu

gösterildiğinden bu yana bu metabolik yolak tartışmalıdır. Bu nedenle arsenik metilasyonu, arseniğin toksik etkisini anlamada anahtar bir rol oynayabilir[35,36].

Arseniğin insan sağlığı için essansiyel olup olmadığı konusunda şüpheler bulunmakla birlikte antimonun insan için gerekli olabileceği hiç bir durum bilinmemektedir[22]. Her iki metalloid de trivalent formunda klastojeniktir ve kanser oluşturma potansiyeline sahiptir[33]. Her ikisinin de tiyol gruplarına afinitesi yüksektir ve biyolojik reaksiyonlarda fosfor ile yer değiştirebilir. Arseniğin biyolojik sistemlerde metilasyon yolu ile detoksifike edildiğine dair veriler mevcut ise de antimon için aynı işlemin gerçekleştiğine dair yeterli veri yoktur[33].

8.2.3 Türkiye'deki sularda arsenik ve antimon tayin çalışmaları

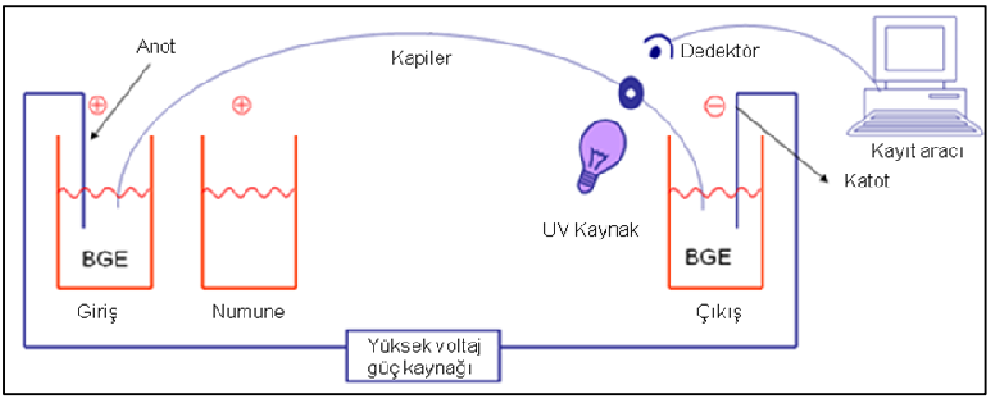
Türkiyenin çeşitli bölgelerindeki içme suyu, termal ve kuyu sularında arsenik ve antimon tayinine yönelik çalışmalar Tablo 1'de özetlenmiştir.

Tablo 1. Türkiye sularında arsenik ve antimon tayini çalışmaları.

Bölge	Analitik teknik	Numune	Maksimum arsenik derişimi (μg L^{-1})	Maksimum antimon derişimi (μg L^{-1})	Kaynak (yıl)
Hisarcık, Kütahya	ICP–MS	İçme suyu	760	–	37 (2004)
		Kuyu	300		
		Yüzey	3000		
Heybeli, Afyon	ICP–OES	Kuyu	1417	–	38 (2004)
		Kaynak	1123		
Kütahya	GF–AAS	Jeotermal	950	–	39 (2007)
		Yeraltı	1070		
		Yüzey	10		
Türkönü madeni, İzmir	ICP–MS	Yüzey	53.3	418.6	40 (2007)
Bigadiç, Balıkesir	ICP–OES	Yüzey	911	–	41 (2008)
–	ICP–MS	Şişelenmiş	30.63	1.23	42 (2009)
Balçova, İzmir	ICP–MS	Jeotermal	1420	689	43 (2009)
		Yeraltı	170.1	26	
		Yüzey	182.4	24	
Simav, Kütahya	ICP–MS	Yeraltı	561.5	–	44 (2010)
Aksaray	HG–ICP–OES	Kuyu-içme	52.4	–	45 (2011)
		Kuyu-sulama	201		
		Kaynak-içme	13.8		
		Nehir	54.6		
–	ICP–MS	Maden suyu	16.93	3.04	46 (2011)
Çankırı	AAS	Rezervuar	64.5	–	47 (2012)
		Kuyu-içme	71.9		
		Kaynak-içme	51.2		

8.2.4 Kapiler elektroforez

CE sistemi göreceli olarak basittir. Temel bileşenleri, ayırma için gerekli yüksek voltajı sağlayan güç kaynağı, ayırma işleminin gerçekleştirildiği kapiler, dedektör ve verileri işlemek, saklamak için sinyal işlemciden oluşmaktadır (Şekil 1).



Şekil 1. Kapiler elektroforez cihazının şematik gösterimi

Kapiler elektroforezde genellikle eritilmiş silika kapiler kullanılır. Eritilmiş silika kapilerin iç yüzeyi silanol grupları (SiOH) ile kaplıdır ve bu grupların pK_a'sı 2 ila 9 arasında değişmektedir. Kapiler kolon, çalışma tamponu ile doldurulduğunda eksi yüklü kapiler duvarı tampondaki katyonları çeker. Böylece kapiler duvarının yakınında elektriksel bir çift tabaka oluşur. Çepere çok yakın artı yüklü iyonlar hareketsizken çeperden uzaklaştıkça çeperle artı yüklü tanecikler arasındaki elektrostatik çekim kuvveti zayıfladığından kapilere uygulanan elektriksel alanın iyonlar üzerindeki kuvveti baskın hale gelir ve bu artı yüklü iyonlar topluca katoda doğru göç ederler. Bu toplu göç kapiler içindeki tamponun katoda doğru akmasına neden olur. Bu akışa elektroosmotik akış (EOF) denir. Konvansiyonel CZE çalışmalarında iyonların göç hareketinde EOF'un baskın olması için silanol gruplarının iyonize olduğu pH ≥ 3 tamponlar önerilir.

CE'de ayırma, uygulanan potansiyel etkisi ile BGE çözeltisindeki türlerin göç hızlarının birbirlerinden farklı olması temeline dayanmaktadır. Her iyon sahip olduğu yüke zıt yüklü elektroda doğru hareket eder. Bu göç hareketi elektroforetik hareket olarak isimlendirilir. Türlerin elektroforetik göç hızları uygulanan potansiyele, türlerin boyutuna ve bulundukları

ortama bağlıdır. Elektroforezde analit iyonlarının toplam hareketi ise analitin elektroforetik hareketi ve elektrozmotik akışın (EOF) hızı ve yönüne bağlıdır. Standart CZE yönteminde EOF her zaman baskın olduğundan iyonların hareket yönünü tayin eder. Eğer elektroforetik hareket ve EOF aynı yönde ise, sistem co-elektroozmosis olarak sınıflandırılır ve analitlerin dedektöre ulaşmaları daha kısa sürede gerçekleşir. Eğer elektroforetik hareket ile EOF zıt yönlerde ise sistem counter-elektroozmosis olarak sınıflandırılır ve dedektöre daha uzun sürede ulaşırlar. EOF'nin bastırıldığı durumlarda ise analitlerin hareket yönlerine ve hızlarına uygulanan potansiyelin polaritesi ve iyonik türlerin yüküne bağlı olarak elektroforetik hareketleri karar verir.

CE bir çok açıdan kromatografiye benzerdir ve kromatografide kullanılan bir çok terim CE'de de kullanılır. Örneğin, çözünürlük ve ayırma verimliliği her iki teknikte de kullanılır ve tanımları aynıdır. Ancak, terminolojide mevcut bazı farklılıklar Tablo 2'de özetlenmiştir.

Tablo 2. Bazı kromatografik terimlerin elektroforezdeki karşılıkları.

CE	**Kromatografi**
Elektroferogram	Kromatogram
Potansiyel	Akış hızı
Elektrolit çözelti	Elüent veya hareketli faz
Enjeksiyon modu (hidrostatik veya elektrokinetik)	Enjektör
Göç zamanı	Alıkonma zamanı
Elektroforetik hareketlilik	Kolon kapasite faktörü
Elektroozmotik akış	–
Yüksek voltaj güç kaynağı	Pompa
Kapiler	Kolon

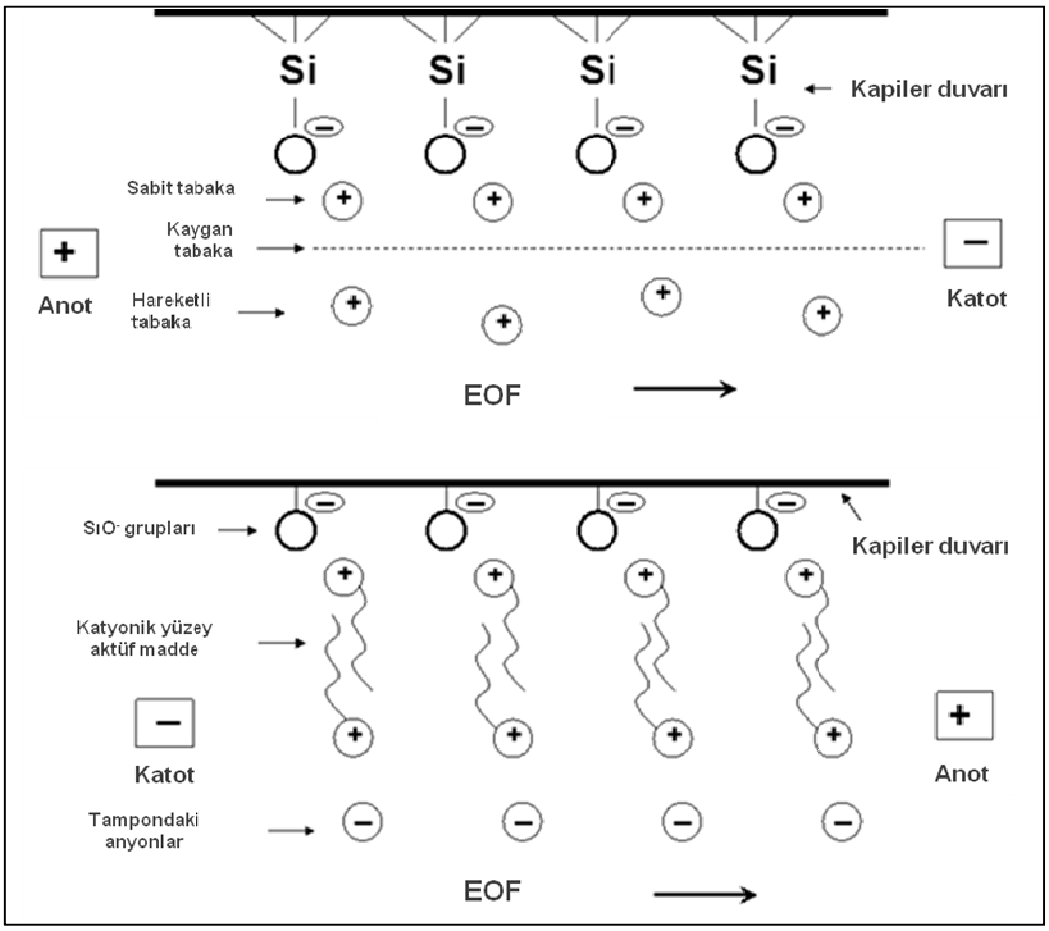
8.2.4.1 CE'de kapiler içi zenginleştirme yöntemleri

Kapiler içi zenginleştirme basit olarak daha yüksek hacimlerde numunenin kapilere enjekte edilmesi ve daha sonra analitin istifleme veya süpürme yöntemleri ile küçük bir hacme sıkıştırılması ile sağlanır. Bunun için ek bir aparata veya cihaz üzerinde modifikasyona gerek yoktur[62]. Kapiler içi zenginleştirme yöntemleri genel olarak istifleme ve süpürme başlıkları altında incelenebilir. Her bir başlık altında uygulamada küçük farklılıklar içeren çok sayıda metodoloji mevcuttur. Bu çalışmada kullanılan metodolojiler ana metinde açıklandığından bu kısımda sadece en duyarlı sonuçların elde edildiği ters çevrilmiş EOF ile yükseltilmiş alan numune injeksiyonu (FASI) modu ele alınmıştır.

8.2.4.1.1 Ters çevrilmiş EOF ile FASI

İstifleme yöntemlerinden biri yükseltilmiş alan numune enjeksiyonudur (FASI). Temel prensibi FASS ile aynı olmakla birlikte enjeksiyon elektrokinetik olarak gerçekleştirilir. Bu yöntemde elektrokinetik enjeksiyon süresince iyonlar kapilere elektroforetik hareketlerinin yanısıra ve EOF ile sürüklenerek girerler[66]. Duyarlılığı artırmak amacıyla elektrokinetik enjeksiyon öncesi hidrodinamik olarak kısa süreli deiyonize su enjeksiyonu yapılır. Bu sayede kapilerin içine transfer edilen analit iyonları su ile zemin elektrolit çözeltisi arasında istiflenmektedir. Analit iyonunun elektroforetik hareketi ve EOF aynı yönde olduğunda daha yüksek miktarda analit enjekte edilmektedir. Elektrokinetik enjeksiyon sırasında EOF'un kapilerin dışına doğru olduğu durumlarda daha fazla analit enjeksiyonunu sağlamak için elektrozmotik akış minimize edilebilir veya tercihen katyonik bir yüzey aktif maddesi içeren elektrolit çözeltisi kullanılarak akış yönü ters çevrilir. Katyonik yüzey aktif madde Şekil 2'de gösterildiği gibi kapilerin iç yüzeyini pozitif yükle kaplar ve tampon

çözeltisindeki anyonları çekerek bir çift tabaka oluşturur. Çift tabakadan uzaklaştıkça anyonlar üzerindeki baskın güç anot potansiyeli olacağından, anyonların anoda doğru elektroforetik hareketleri çözeltinin yığın halinde anoda doğru sürüklenmesine yani ters EOF'a neden olur.



Şekil 2. EOF'un şematik gösterimi A) çıplak ve B) EOF'u ters çevirmek üzere katyonik yüzey aktif madde ile kaplanmış kapiler duvarı

8.2.5 CE ile arsenik ve antimon türlemesi

CE en güçlü ayırma tekniklerinden biridir ve çoğunlukla LC ayırma tekniklerinden üstündür. Ayrıca, tek bir CE cihazı ile CZE, MEKC gibi

farklı ayırma modlarında çalışılabilir. Bu modlarının hepsinde ayırma işlemi yüksek voltaj varlığında gerçekleşmekle birlikte ayırma mekanizmaları farklıdır. Türleme çalışmalarında farklı türler arasındaki dengeye en az etki eden ayırma yöntemi olması CE'nin eşsiz bir özelliğidir. Sabit fazın olmaması geniş yüzey alanı ile olası istenmeyen etkileşimlerden kaynaklanan problemleri ortadan kaldırmaktadır. Bu nedenle HPLC'de olduğu gibi türlerin değişime uğrama riski daha azdır. Ancak, türleme için CE kullanımı da bazı şartları gerektirir. Bunlardan biri numune hacminin birkaç nanolitre düzeyinde olması nedeniyle numunenin homojenliğinin garanti edilmesinin gerekliliğidir. Ayrıca LC'ye kıyasla derişim duyarlılığı 1–2 mertebe daha kötüdür. Bazı durumlarda ise uygulanan yüksek voltaj, türlerin dağılımındaki dengeyi bozabilmektedir. Benzer şekilde BGE'nin kompozisyonu ve BGE'ye katılan kimyasallar, türlerin kararalılığını azaltabilmektedir[71].

CE ile yapılan türleme analizlerinde en çok çalışılan elementlerden biri arseniktir. Bu çalışmalarda çeşitli CE modları ve dedektörler kullanılmıştır. Bunun sebebi, çevre, endüstriyel ürün ve canlı organizmalarda arsenik türlerine sık rastlanması ve arseniğin farklı türlerinin toksisitelerinin farklı olmasının sık sık vurgulanmasına bağlanabilir[73]. CE ile arsenik türleme çalışmalarında üç temel CE modu kullanılmıştır. Birincisi, minimize edilmiş ters yöndeki EOF varlığında analitlerin elektroforetik hareketleri sayesinde dedektöre doğru göçleri, ikincisi, yüzey aktif madde ilavesi ile EOF ters çevrilerek anyonların elektroforetik hareketleri sayesinde birlikte göç ettirilmesidir (co-electroosmotic migration). Son olarak, kapilere dedektör ucu negatif olacak şekilde potansiyel uygulandığında analitler elektroforetik olarak ters yöne hareket ederlerken daha güçlü EOF tarafından dedektöre doğru sürüklenirler (counter-electroosmotic mod)[73].

Arsenik ve antimon türleme çalışmalarında CE–UV en sık kullanılan tekniktir. Bunun yanında, indirekt UV ve lazer-indükleyici floresans (LIF), ICP–MS ve HG–AFS gibi element spesifik dedektörlerin kullanıldığı çalışmalar da mevcuttur. Arsenik ve antimon türleme analizlerine yönelik son CE çalışmaları, kullanılan zenginleştirme yöntemleri ve optimum ayırma koşulları Tablo 3'te özetlenmiştir.

Tablo 3. CE ile gerçekleştirilen arsenik ve antimon türleme çalışmaları.

Matriks/ numune	Türler	Dedektör	Önişlem/ Zenginleştirme yöntemi	CE koşulları	Kaynak
Arsenik türleme analizi					
Kömür külü, köpek balığı karaciğeri SRMs	As(III), DMA, MMA, As(V), arsenobetain	ICP–MS	MW[a]-destekli ekstraksiyon	Kapiler: 60 cm, 75 µm, BGE: 25 mM 3–(siklohegzilamino)–1–propan-sülfonik asit, 0.5 mM SDS (pH 9.5); Ayırma voltajı: 25 kV; Enjeksiyon: basınç, 50 s.	74
Standart	As(III), DMA, MMA, As(V)	UV (indirek)	FASI	Kapiler: 72 cm, 50 µm, BGE: 10 mM 2,6-piridindikarboksilik asit (pH 10.3); Ayırma voltajı: −25 kV; Enjeksiyon: elektrokinetik, 10 s, −20 kV.	9
Mya arenaria Linnaeus, K*arides*	As(III), DMA, MMA, As(V)	ICP–MS	MW-destekli ekstraksiyon	Kapiler: 60 cm, 75 µm, BGE: 20 mM NaH_2PO_4-5.00 mM $Na_2B_4O_7$ (pH 6.50); Ayırma voltajı: 12 kV; Enjeksiyon: basınç, 10 s.	75
Kurutulmuş K*arides*	As(III), DMA, MMA, As(V), p–aminofenil arsenik asit	UV	MW-destekli ekstraksiyon	Kapiler: 60 cm, 75 µm, BGE: 20 mM NaH_2PO_4-5.00 mM $Na_2B_4O_7$ (pH 6.25); Ayırma voltajı: 20 kV; Enjeksiyon: basınç, 3.0 kPa.	76
Doğal ve Kirletilmiş sular	As(III), DMA, MMA, As(V)	UV	–	Kapiler: 60 cm, 75 µm, BGE: 10 mM Na_2MoO_4, 10 mM $NaClO_4$ (pH 3.0); Ayırma voltajı: −13 kV; Enjeksiyon: basınç, 100 s, 30 mbar.	77
Balık	As(III), DMA, As(V), arsenobetain	ICP–MS	US[b]-destekli ekstraksiyon	Kapiler: 34.5 cm, 75 µm; BGE: 25 mM borat, 10% metanol (pH 9.2); Ayırma voltajı: 30 kV; Enjeksiyon: basınç, 1 s, 50 mbar.	78
Toprak ekstraktı	As(III), DMA, MMA, As(V), fenilarsonik asit, p-aminofenil-arsonik asit, o-aminofenilarsonik asit, roksarson, fenilarsin oksit	UV	US-destekli ekstraksiyon ve LVSS polarite değiştirmeli	Kapiler: 56 cm, 50 µm, BGE: 50 mM fosfat (pH 8.0); istifleme: basınç, 700 s, 50 mbar, 120 s süreyle –30 kV, daha sonra +30 kV.	79
Tavuk altlığı ve toprak	As(III), MMA, As(V)	UV	US-destekli ekstraksiyon ve dinamik pH sınırı	Kapiler: 66.5 cm, 50 µm, BGE: 15 mM fosfat (pH 10.6); voltage: 25kV; Enjeksiyon: basınç, 150 s, 50 mbar.	80

Matriks/ numune	Türler	Dedektör	Önişlem/ Zenginleştirme yöntemi	CE koşulları	Kaynak
Standart	As(III), DMA, MMA, As(V), trimetilarsin oksit, tetrametil-arsonyum iyonu, arsenobetain, arsenokolin	UV MS	–	Kapiler: 40–70 cm, 50 µm, BGE: 5 mM laktik asit, 10 mM 3,4−diamino−benzoik asit (pH 3.2); Voltaj: 25 kV (katyonik analitler) ve −30 kV (anyonik analitler); Enjeksiyon: basınç, 6 s, 50 mbar.	81
Yeraltı suyu	As(III), DMA, As(V),	UV	–	Kapiler: 42 cm, 50 µm, BGE: 10 mM fosfat, 0.35 mM TTAB (pH 9); Voltaj: −20 kV; Enjeksiyon: Elektrokinetik, 10 s, −10 kV.	82
Çökelti	As(III), DMA, MMA, As(V)	UV	Ekstraksiyon; co−EOF−NSM[c]; HSDC	Kapiler: 56 cm, 50 µm, kaplanmış; BGE: 15 mM fosfat (pH 10.6), Voltaj: −25 kV; Enjeksiyon: basınç, 40 s, 50 mbar.	1
Göl ve nehir suyu	As(III), DMA, MMA, As(V)	HG−AFS	pH-vasıtasıyla istifleme	Kapiler: 50 cm, 75 µm, kaplanmış; BGE: 25 mM NaH_2PO_4 (pH 6.5); Voltaj: 20 kV; Enjeksiyon: basınç.	48
Çeşme ve göl suyu	As(III), DMA, MMA, As(V), roksarson, p−aminofenilarsonik asit, 4−nitrofenilarsonik asit, fenilarsonik asit	UV	LVSS polarite değiştirmeli; NSM; co−EOF NSM ters EOF; HSDC	Kapiler: 40 cm, 75 µm, 51.5 cm, 50 µm, kaplanmış; BGE: 20 mM karbonat (pH 10); Voltaj: 18 kV (normal EOF), −25 kV (ters EOF); Enjeksiyon: basınç, 160 s, 50 mbar (LVSS).	10
Hayvan yemi	As(III), DMA, MMA, As(V), p−aminofenil arsonik asit, 4−hidroksi −3−nitro benzenarsonik asit, 4−nitrofenillarsonik asit, fenilarsonik asit, fenilarsin oksit	UV	Ekstraksiyon; co−EOF; counter−EOF	Kapiler: 40 cm, 75 µm, 51.5 cm, 50 µm, kaplanmış; BGE: 20 mM $NaHCO_3$-Na_2CO_3 (pH 10.0); Voltaj: 18 kV (normal EOF), −25 kV (ters EOF); enjeksiyon: basınç, 2 s, 50 mbar (counter−EOF), basınç, 7 s, 50 mbar (co−EOF).	3
Çeşme suyu ve maden suyu	As(III), DMA, MMA, As(V)	LIF (indirek)	–	Kapiler: 40 cm, 50 µm, BGE: 2.0 mM $NaHCO_3$, 10^{-7} M fluoressein (pH 9.28); Voltaj: 25 kV; Enjeksiyon: basınç, 5 s, 0.5 psi.	83
Antimon türlemesi					
Standart	Sb(III), Sb(V)	LIF (indirek)	–	Kapiler: 40 cm, 50 µm; BGE: 10 mM borat, 1.0 mM fluoressein (pH 9.5); Voltaj: 25 kV; Enjeksiyon: basınç, 10 s.	11
Lağım	Sb(III), Sb(V), $(CH_3)_3SbCl_2$	ICP−MS	Ekstraksiyon, izoelektrik istifleme	Kapiler: 150 cm, 50 µm; BGE: 20 mM Na_2HPO_4/NaH_2 PO_4 (pH 5.6); Voltaj: −18 kV; Enjeksiyon: basınç, 7 s, 8 bar.	13
Arsenik ve antimonun aynı anda türlemesi					
Toprağın sulu ekstraktı	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	ICP−MS	Ekstraksiyon	Kapiler: 80 cm, 75 µm; BGE: 0.5 mM sodyum kromat, 0.5 mM TTAB (pH 11.2); Voltaj: −20 kV; Enjeksiyon: elektrokinetik, 10 s, −20 kV.	84

Matriks/ numune	Türler	Dedektör	Önişlem/ Zenginleştirme yöntemi	CE koşulları	Kaynak
Toprağın sulu ekstraktı	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	UV (indirek)	Ekstraksiyon	Kapiler: 52 cm, 75 µm; BGE: 5 mM sodyum kromat, 0.5 mM TTAOH (pH 11.2); Voltaj: −20 kV; Enjeksiyon: elektrokinetik, 20 s, −20 kV.	12

[a] Mikrodalga

[b] Ultrasonik

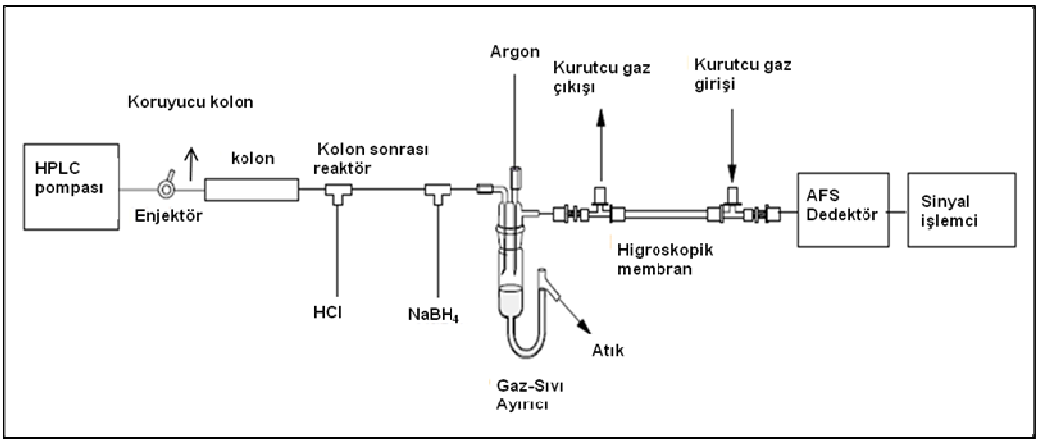
[c] Co-elektroozmotik akış normal istifleme modu

8.2.6 HPLC–HG–AFS

Hidrür oluşturan elementler için AFS ideal bir dedektördür. AFS, düşük teşhis sınırı, geniş dinamik aralık, basit ve ucuz olması, işletim maliyetinin düşük olması gibi özellikleri ile türleme analizlerinde AAS ve ICP–MS gibi diğer atomik tekniklere alternatif oluşturmaktadır[21]. Atomik floresans girişimlere açık bir yöntem olduğundan, analitin mattriksten etkili bir şekilde ayrılmasını gerektirir. HG düzenekleri ile analit transfer verimliliğinin yüksek olması, yüksek duyarlılık, düşük maliyet, analitin matriksten seçici olarak ayrılarak girişimlerin azaltılması, zenginleştirmeye ve otomasyona uygunluğu, hızlı, basit ve oturmuş bir metodolojiye sahip olması bu iki tekniğin birlikte kullanılmasına yol açmıştır.

AFS, zemin sinyallerinin düşük olması nedeniyle AAS'den daha duyarlıdır. ICP–MS ile kıyaslandığında ise benzer duyarlılık ve doğrusal aralığa sahiptir, fakat AFS çok daha basit ve ekonomik bir cihazdır (Şekil 3). Ayrıca, hidrür oluşturma düzeneklerinin AFS ile eşleştirilmeleri daha kolaydır ve reaksiyon ürünü olan hidrojen gazına toleransı ICP–MS'e kıyasla daha yüksektir.

AFS enstrumentasyonunda çoğunlukla artırılmış deşarjlı oyuk katot lambaları (BDHCL) ile donatılmış dispersif olmayan düzenekler kullanılır. Önceleri daha güçlü atomlaştırıcı olarak ICP kullanılmışsa da günümüzde bu tarz ticari bir cihaz mevcut değildir. HPLC–AFS hidrür oluşturabilen elementlerin türleme çalışmalarında göreceli olarak sık kullanılmakla birlikte genel olarak diğer atomik teknikler kadar yaygınlaşamamıştır. Bunun temel nedenleri: (i) soğuk buhar ve hidrür oluşturabilen elementler ile sınırlı kalması; (ii) ticari olarak mevcut AFS cihazları nondispersif tasarıma sahip olduklarından multielement atomik tekniklerle rekabet edememeleri; (iii) kolondan yıkanan tüm analit türlerinin uçucu hale getirilmesi için kolon sonrası işlem gerektirmesi olarak özetlenebilir. Kolon sonrası işlemlerin gerçekleştirildiği reaktörler analit türünün yıkılanarak uçucu buhar oluşturabileceği forma dönüştürülmesi amacına yöneliktir. Online kimyasal oksidasyon, fotooksidasyon veya mikrodalga yıkılama bu amaçla kullanılan yöntemlerdir.



Şekil 3. Arsenik türlemesi için kullanılan HPLC-HG-AFS'nin şeması

8.2.7 Arsenik türlemesi için HPLC–HG–AFS

Arsenik türlemesi hem inorganik, hem organik arsenik türlerini kapsamaktadır. As(III), As(V), MMA ve DMA, $NaBH_4$ ile asidik ortamda indirgendiklerinde uçucu arsin oluştururlar. Trimetil arsenik oksit (TMAO), tetrametil arsenik (TETRA), arsenobetain (AB), arsenokolin (AC) ve arsenoşeker gibi diğer arsenik bileşikleri hidrür oluşturmazlar. Bu durumda HG basamağından önce molekül foto-oksidasyon (bazik ortamda $K_2S_2O_8$ ve UV radyasyonu), termal oksidasyon (bazik ortamda $K_2S_2O_8$ ile ısıtılarak) veya mikrodalga düzenekleri ile yıkılanır.

AFS ile arsenik türleme analizlerinde nötral pH'larda birçok arsenik türü anyon veya yüksüz olduğundan iyon değiştirici kolon ve HPLC tercih edilen ayırma yöntemidir. Kuvvetli anyon değiştirici kolonlar sık kullanılmakla birlikte, iyon çifti kullanarak ters faz kromatografi ile yapılmış çalışmalar da mevcuttur. Anyon değiştirici kolon ile 12 arsenik türünün birlikte tayin edilebildiği rapor edilmiştir[87]. HPLC–HG–AFS, arsenik türlerinin su ve insan idrarı gibi numunelerde tayininin yanısıra kromat-bakır ile işlenmiş ahşap malzemelere[90], atık sulara[91], asidik maden drenajlarına[92] ve bira[93] gibi çeşitli matrikslere de uygulanmıştır. Tablo 4'te özetlendiği gibi, idrar ve su numunelerindeki türleme çalışmalarında hem izokratik hem gradient HPLC modları kullanılmıştır. Arsenik türleme analizlerinde tetrabütilamonyum hidroksit (TBAH) ve fosfat sırasıyla ters faz ve iyon değiştirici kolonlar ile sıklıkla kullanılan tampon çözeltileridir.

8.2.8 Çalışmanın amacı

Bu çalışma su numunelerinde arsenik ve antimonun CE ile aynı anda türlemesi ve insan idrarında HPLC–HG–AFS ile arsenik türlemesi

olarak iki kısma ayrılmıştır. İlk kısımda As(III), As(V), MMA, DMA, Sb(III) ve Sb(V)'in CE ile aynı anda ayrılmaları ve duyarlılığın artırılması için farklı kapiler içi zenginleştirme yöntemleri çalışılmıştır. Bunlar (1) SDS ile süpürme–MEKC, (2) polarite değiştirmeli LVSS, (3) FASS ve (4) ters EOF ile FASI yöntemleridir. Her bir yöntem ile elde edilen sonuçlar konvansiyonel CZE ile kıyaslanmıştır. En yüksek zenginleştirme faktörü, HSDC hücresinin ters EOF–FASI yöntemi ile birlikte kullanıldığında gözlenmiştir.

Çalışmanın ikinci kısmında Nevşehir bölgesinde içme suyu kaynaklı kronik arsenik maruziyeti olan insanlarda kanser riskinin araştırıldığı projenin bir parçası olarak As(III), As(V), MMA ve DMA'nın HPLC–HG–AFS ile tayinine yönelik yöntem geliştirilmiştir. Ayırmayı etkileyen parametreler (hareketli faz kompozisyonu, pH, akış hızı, kolon sıcaklığı ve koruyucu kolon kullanımı gibi) çalışılarak deneysel koşullar optimize edilmiştir. İdrar numunelerinde arsenik türlerinin yansıra, asit ortamında yıkılanmış numunelerde HG–AFS ile toplam arsenik tayini yapılmıştır. Yöntemin doğruluğunu test etmek amacıyla NIST–SRM 2669'da (dondurulmuş insan idrarında arsenik türleri) As(III), As(V), MMA ve DMA ve toplam arsenik tayin edilmiştir.

Tablo 4. Su ve insan idrarı numunelerinde HPLC–HG–AFS ile arsenik türlerinin tayini.

Matriks/ numune	Türler	Kolon	Eluent/tipi (I: isokratik; G: gradient)	Kaynak
İdrar	As(III), As(V), MMA, DMA, AB	Hamilton PRP–X100	10 mM fosfat tamponu; pH 5.75 (G)	107
İdrar	As(III), As(V), MMA, DMA	Phenomenex ODS C18	5 mM TBAH, 3 mM malonik asit, 5% (v/v) MeOH; pH 5.9 (I)	108
İdrar	As(III), As(V), MMA, DMA	Phenomenex ODS–3 C18	5 mM TBAH, 2–5 mM malonik asit, 5% (v/v) MeOH; pH 5.9 (I)	109
İdrar	As(III), As(V), MMA, DMA	Phenomenex ODS C18	5 mM TBAH, 2–5 mM malonik asit, 5% (v/v) MeOH; pH 5.9 (I)	110
İdrar	As(III), As(V), MMA, DMA	Phenomenex ODS–3 C18	4.7% TBAH, 2 mM malonik asit, 4% (v/v) MeOH (I)	111
Su ve İdrar	As(III), As(V), MMA, DMA	Phenomenex ODS–3	5 mM TBAH, 1 mM malonik asit, 5% (v/v) MeOH; pH 5.5 (I)	112
İdrar	As(III), As(V), MMA, DMA, DMPS–MMA	Phenosphere	20 mM fosfat tamponu, 5% MeOH; pH 6.0 (I)	113
İdrar	As(III), As(V), MMA, DMA	Phenomenex ODS–3 C18	5 mM TBAH, 3 mM malonik asit, 5% (v/v) MeOH; pH 5.9 (I)	114
	TMAO	Hamilton PRP–X100	5 mM fosfat (I)	
İdrar	As(III), As(V), MMA, DMA	Hamilton PRP–X100	10–60 mM fosfat; pH 5.8 (G)	115
Doğal tatlı su	As(III), As(V), MMA	Prodigy ODS	(a) 5% (w/v) MeOH, 5 mM 2–merkapto etanol, 20 mM TBAH; pH 4.0; (b) MeOH (G)	116
Şişelenmiş maden suyu	As(III), As(V)	Hamilton PRP–X100	20 mM fosfat tamponu; pH 6.1 (I)	117
Su (maden ocağı)	As(III), As(V)	Hamilton PRP–X100	Fosfat tamponu (I)	118
Maden suyu çeşme suyu Nehir suyu	As(III), As(V), MMA, DMA	Hamilton PRP–X100	(a) 5 mM fosfat tamponu; pH 4.8; (b) 30 mM fosfat tamponu; pH 8.0 (G)	119
Yer altı suyu ve idrar	As(III), As(V), MMA, DMA	Hamilton PRP–X100	15 mM fosfat tamponu; pH 6.0 (I)	120

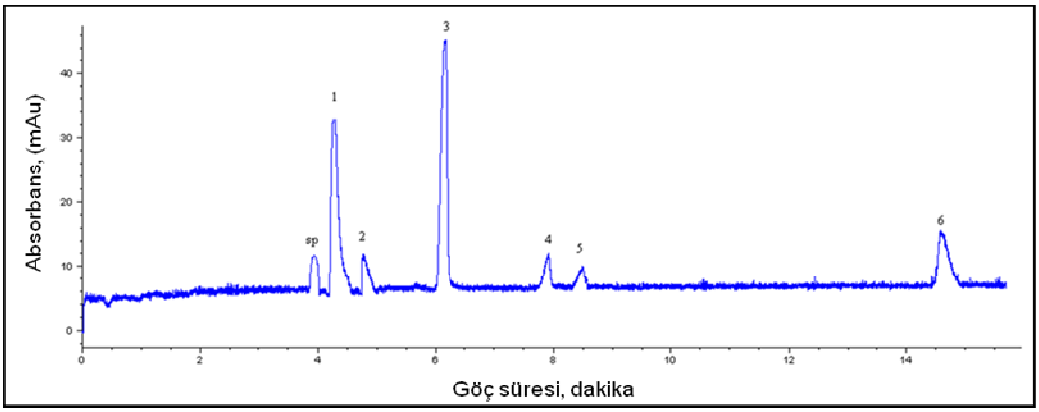
Matriks/ numune	Türler	Kolon	Eluent/tipi (I: isokratik; G: gradient)	Kaynak
Nehir ve Nehir ağzı suyu	As(III), As(V), MMA, DMA	Hamilton PRP–X100	20 mM fosfat tamponu; pH 5.8 (I)	121
Nehir suyu	AsIII), As(V)	Hamilton PRP–X100	Fosfat tamponu (I)	122

8.3 BULGULAR VE TARTIŞMA

8.3.1 Kapiler Elektroforez

8.3.1.1 Konvansiyonel CZE

Konvansiyonel CZE ile en iyi ayırma ve duyarlılık sağlayan koşulların tespiti için kapiler uzunluğu, BGE tipi ve pH'sı, BGE'deki ve numunedeki tampon derişimi, kapiler sıcaklığı, enjeksiyon modu ve süresi ve ayırma voltajı taranarak optimize edilmiştir. Optimum koşullarda elde edilen elektroferogram Şekil 4'te verilmiştir. Kapiler içi zenginleştirme yöntemlerinden LVSS ve FASS için ana metinde açıklanan deneysel koşullar optimize edilmiş ve optimum koşullar için UV dedektör ile hesaplanan LOD değerleri Tablo 5'te verilmiştir.



Şekil 4. Konvansiyonel CZE ile optimum koşullarda elde edilen elektroferogram. Koşullar: ayırma voltajı: 25 kV; BGE: 25 mM borat tamponu (pH 9.3); sıcaklık: 25 ºC; enjeksiyon: basınç, 50 mbar, 10 s; numune kompozisyonu: 50.0 mg L^{-1} arsenik ve antimon türleri 25 mM borat tampon içinde; pikler: sp, sistem piki; 1, DMA; 2, Sb(V); 3, As(III); 4, MMA; 5, Sb(III); 6, As(V)

Tablo 5. Konvansiyonel CZE, LVSS, ve FASS yöntemleri için analitik performans verileri.

Türler	Konvansiyonel CZE ile LOD[a] (mg L^{-1})	LVSS ile LOD (mg L^{-1})	ZF[b]	FASS ile LOD (mg L^{-1})	ZF[c]
DMA	3.0	0.9	3.3	0.9	3.3
Sb(V)	15	2.7	5.6	2.5	6.1
As(III)	2.3	0.1	23	0.2	12
MMA	14	2.8	4.9	1.9	7.3
Sb(III)	23	0.7	33	2.2	10
As(V)	8.8	0.5	18	1.3	6.8

[a] Koşullar:Şekil 4'te verildiği gibidir

[b] Zenginleştirme faktörü (CZE yönteminin LOD değerinin LVSS'in LOD değerine oranı)

[c] Zenginleştirme faktörü (CZE yönteminin LOD değerinin FASS'in LOD değerine oranı)

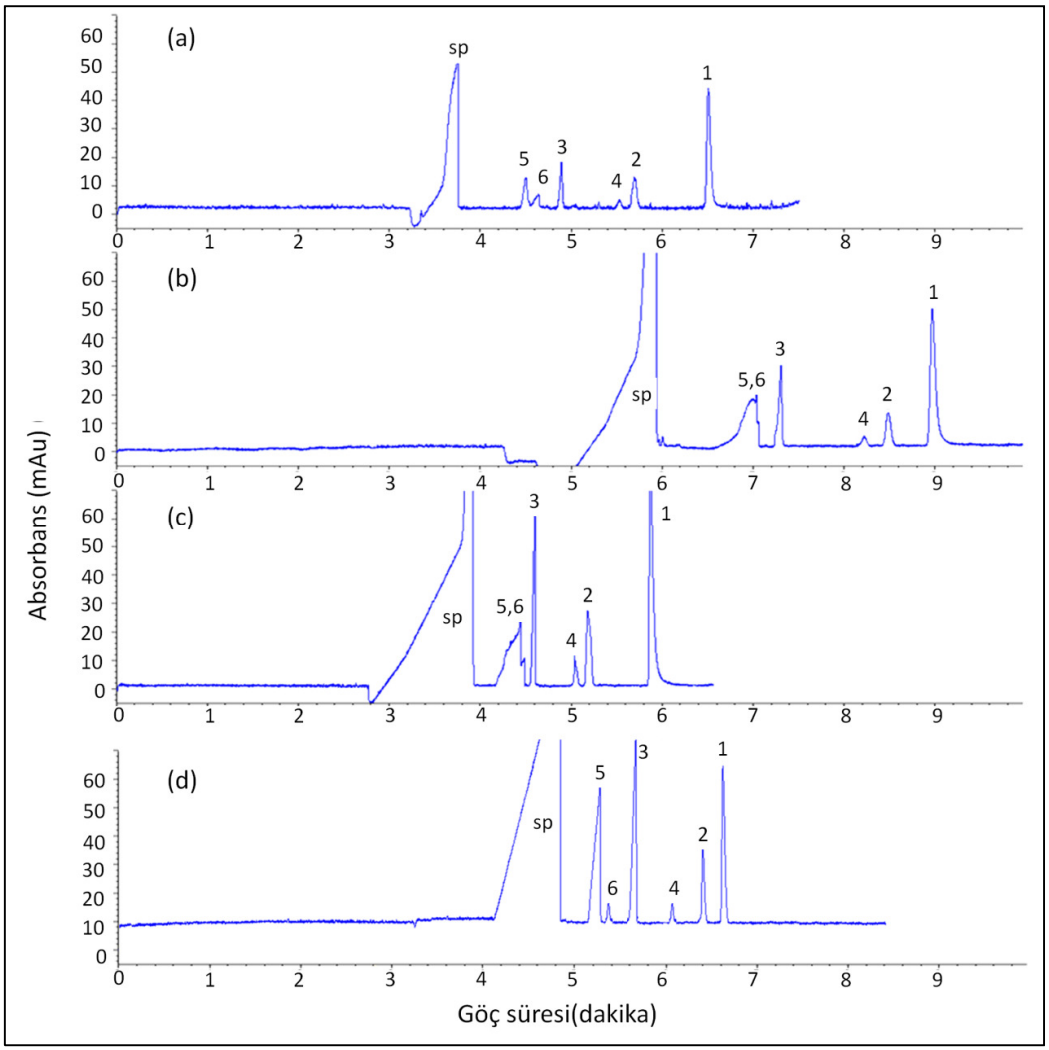
8.3.1.2 Ters EOF ile FASI

Ters EOF–FASI için BGE'deki borat tampon derişiminin, BGE ve numune çözeltisine yüzey aktif madde (setiltrimetilamonyum bromür, CTAB) ilavesinin pik ayırımına ve duyarlılığa etkisi incelenmiş (Şekil 5) ve optimize edilmiştir. Numuneye 0.1 mM CTAB eklenmesinin pik şekillerini ve kromatografik çözünürlüğü önemli derecede iyileştirdiği gözlenmiştir (Şekil 5 d). Optimum koşullarda zenginleştirme faktörü, konvansiyonel CZE için hesaplanan LOD değerinin ters EOF–FASI'nin LOD değerine oranından hesaplanmıştır. LOD değerleri ve zenginleştirme faktörleri Tablo 6'da verilmiştir.

Tablo 6. Ters FASI–EOF analitik performans verileri.

Türler	DMA	Sb(V)	As(III)	MMA	Sb(III)	As(V)
Ters EOF–FASI ile LOD ($\mu g\ L^{-1}$)	28	57	23	340	30	350
ZF[a]	107	263	100	41	766	25

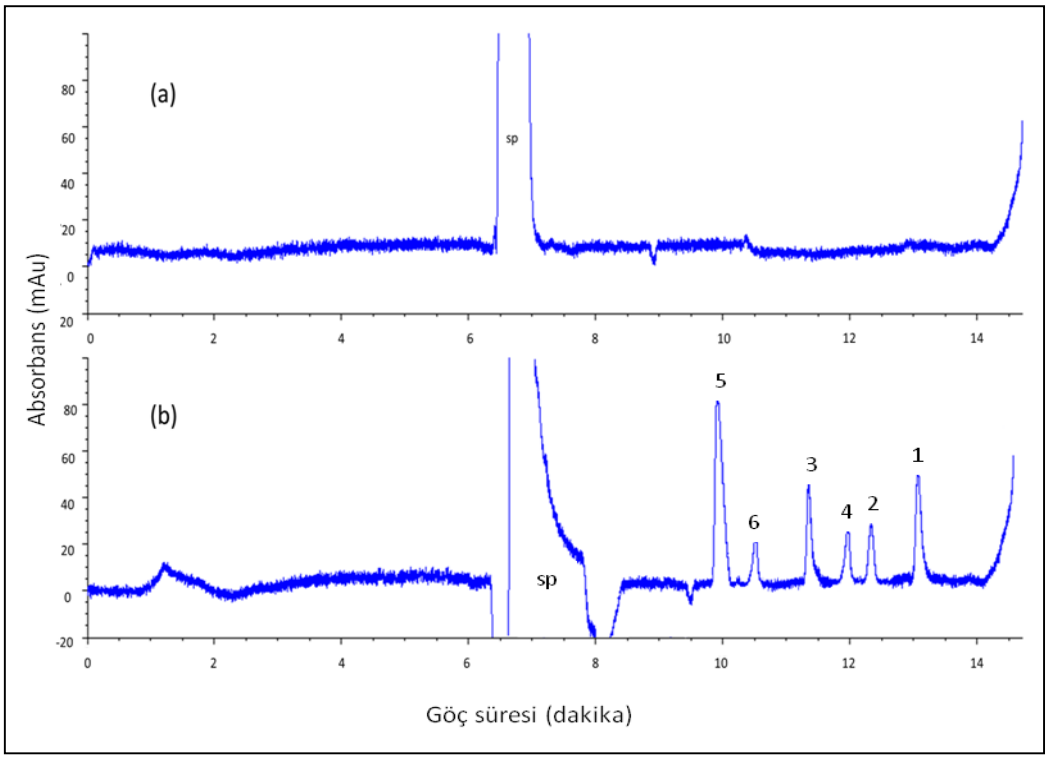
[a] Zenginleştirme faktörü (CZE yönteminin LOD değerinin ters FASI/EOF'in LOD değerine oranı)



Şekil 5. Enjeksiyon modunun ve numuneye CTAB ilavesinin ters EOF-FASI elektrofrogramına etkisi. Sıcaklık: 15 °C; Ayırma voltajı: −20 kV; BGE: 25 mM borat tamponu, 0.1 mM CTAB (pH 9.3); numune pH'sı: 7.0 (a) Enjeksiyon: 50 mbar, 25 s; Numune: arsenik ve antimon (her biri 10.0 mg L^{-1}) suda. (b) Enjeksiyon: elektrokinetik, −20 kV, 25 s; BGE: 25 mM borat tamponu, 0.1 mM CTAB; Numune: arsenik ve antimon (her biri 10.0 mg L^{-1}) suda. (c) Su tabakalı elektrokinetik enjeksiyon. Su tabakası: 50 mbar; 5 s; Numune enjeksiyonu: –20 kV, 50 s; Numune: arsenik ve antimon (her biri 1.0 mg L^{-1}) suda. (d) Optimum ters EOF–FASI koşulları. Su tabakası: 50 mbar, 5 s; Numune enjeksiyonu: elektrokinetik, −20 kV, 50 s; Numune: arsenik ve antimon (her biri 1.0 mg L^{-1}) 0.1 mM CTAB içinde; pikler: 1, DMA; 2, Sb(V); 3, As(III); 4, MMA; 5, Sb(III); 6, As(V). sp, sistem piki

Ters EOF–FASI ile yüksek zenginleştirme faktörleri elde edilmişse de yöntemin duyarlılığı içme sularında WHO tarafından önerilen, toplam arsenik, 10 μg L^{-1},[124] ve toplam antimon, 20 μg L^{-1},[125] değerlerinin üstündedir. Bu nedenle HSDC'nin ters EOF–FASI ile birleştirilerek daha düşük LOD değerlerine inilmesi hedeflenmiştir. Tablo 7'de gösterildiği gibi zenginleştirme faktörleri 0.6×10^3–21×10^3 ve LOD değerleri Sb(III) için 1.1 μg L^{-1} As(V) için 16 μg L^{-1} olarak hesaplanmıştır. Ters EOF–FASI için kesinlik, doğrusallık, LOD ve zenginleştirme faktörü gibi analitik performans verileri Tablo 7'de değerlendirilmiştir. Geniş bir derişim aralığında, 0.9810–0.9986 arasında değişen korelasyon katsayıları ile doğrusal davranan kalibrasyon grafikleri elde edilebilmiştir. Göç sürelerinin, pik alanı ve pik yüksekliklerinin kesinliği 5 ardışık ölçüm için %RSD cinsinden Tablo 7'de verilmiştir. Pik yüksekliklerinin %RSD değeri daha düşük olduğundan kalibrasyonlarda pik yüksekliği kullanılmıştır.

Yöntemin seçiciliği ve doğruluğunu test etmek amacıyla standart ilave edilmiş çeşme suyu numunesi ile çalışılmıştır. Numune içindeki girişim yapabilecek nötral ve katyonik türler anyon seçici elektrokinetik enjeksiyon ile elimine edildiğinden oldukça temiz zemine sahip elektroferogramlar elde edilmiştir (Şekil 6 a). Çeşme suyuna 20.0 μg L^{-1} for Sb(III), As(III), DMA ve 80.0 μg L^{-1} As(V), MMA ve Sb(V) ilave edilmiş ve geri kazanım değerleri %97.7–102.5 arasında bulunmuştur (Tablo 7). Analiz edilen boş ve standart ilave edilmiş çeşme suyuna ait elektroferogramlar Şekil 6'da gösterilmiştir.



Şekil 6. Optimum ters EOF–FASI koşullarında (a) boş, (b) standart ilave edilmiş çeşme suyuna ait elektroferogramlar. Koşullar: Sıcaklık: 15 °C; voltaj: −20 kV; BGE: 25 mM borat tampon, 0.1 mM CTAB (pH 9.3); numune pH'sı: 7.0, su tabakası: 50 mbar, 5 s; Enjeksiyon: elektrokinetik, −20 kV, 50 s; Analit derişimleri: Sb(III), As(III) ve DMA 20.0 µg L^{-1}; As(V), MMA ve Sb(V) 80.0 µg L^{-1}; 1, DMA; 2, Sb(V); 3, As(III); 4, MMA; 5, Sb(III); 6, As(V)

Tablo 7. Ters EOF–FASI ve HSDC[a] ile analitik performans verileri.

		DMA	Sb(V)	As(III)	MMA	Sb(III)	As(V)
Regresyon denklemi[b]		y = 2.0416x + 7.9213	y = 0.3004x + 3.5432	y = 0.8185x + 28.525	y = 0.0792x + 21.816	y = 3.8205x + 3.4573	y = 0.0483x + 17.248
Tamamlayıcılık katsayısı (R^2)		0.9966	0.9810	0.9986	0.9902	0.9980	0.9980
Doğrusal aralığı ($\mu g\ L^{-1}$)		6.3–400	43–500	7.0–400	47–500	3.7–500	53–500
LOD (S/N = 3, $\mu g\ L^{-1}$)		1.9	13	2.1	14	1.1	16
ZF ($\times 10^3$)		1.6	1.2	1.1	1.0	21	0.6
RSD (%, n = 5)	Göç süresi	0.9	0.5	1.2	0.9	0.6	1.0
	Pik alanı	6.0	3.4	4.7	4.7	5.7	4.9
	Pik yüksekliği	3.4	1.2	4.4	2.2	2.0	3.9
Geri kazanım (%)[c]		102.1±4.9	97.9±2.0	101.3±3.3	98.5±2.2	102.5±5.0	97.7±3.0

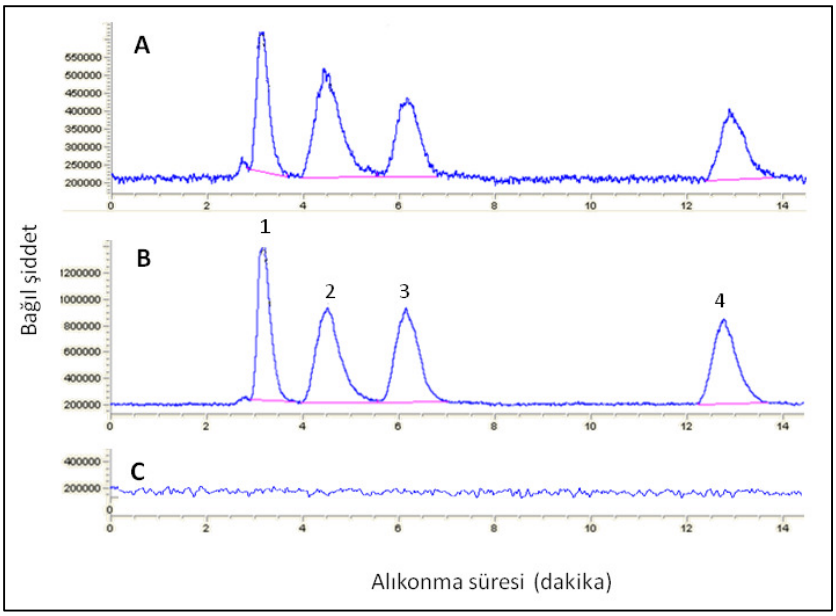
[a] Koşullar: Fig. 20 d'deki gibi

[b] Pik alanı = eğim $\times$ derişim ($\mu g\ L^{-1}$) + kesim noktası

[c] Eklenen derişimler: Fig. 21'deki gibi; geri kazanım ve standart sapma değerlerinin hesaplanmasında üç paralel ölçümleri esas alındı (n = 5)

8.3.2 HPLC–HG–AFS ve HG–AFS

HPLC–HG–AFS düzeneği ile yapılan optimizasyon çalışmalarında fosfat tamponu derişiminin, koruyucu kolonun, hareketli fazın pH'sının ve akış hızının arsenik türlerinin sinyal şekline ve kromatografik çözünürlüklerine etkisi incelenmiş ve optimum koşullar Tablo 8'de özetlenmiştir. Optimize edilmiş koşullarda elde edilen örnek kromatogramlar Şekil 7'de verilmiştir.



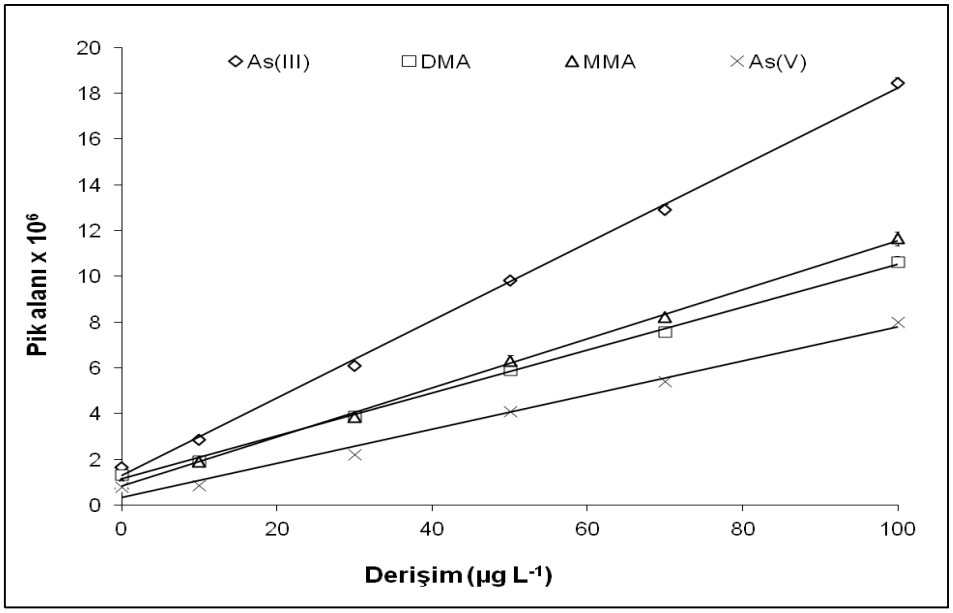
Şekil 7. Optimize edilmiş koşullarda elde edilen kromatogramlar. Hareketli faz: pH 5.8, 8.0 mM fosfat tamponu; akış hızı: 1.0 ml min^{-1}; Gain: 100; (A) 5.0 µg L^{-1}; (B) 20.0 µg L^{-1} arsenik türlerin katılmış idrar; (C) Kör idrar. Pikler: 1. As(III), 2. DMA, 3. MMA, 4 As(V)

Tablo 8. HPLC–HG–AFS için koşullar.

HPLC (Hewlett Packard HPLC sistemi, 1050 model)	
Kolon	Hamilton PRP-X100 anyon-değiştirici kolon (250 mm×4.1 mm), parçacık boyutu 10 µm
Koruyucu kolon	Hewlett Packard Hypersil C18, 20 mm×2.1 mm, parçacık boyutu 5 µm
Kolon sıcaklığı	oda sıcaklığı
Hareketli faz	izokratik, 8.0 mM KH_2PO_4/K_2HPO_4 (pH 5.80)
Akış hızı	1.0 mL min^{-1}
Enjeksiyon hacmi	100 µL
HG–AFS (PS Analytical Ltd, Millennium Excalibur sistemi)	
As BDHCL	Photron Super Lamba
Primer akım	27.5 mA
Güçlendirilmiş	35.0 mA
HCl	1.5 M; 4 ml min^{-1}
$NaBH_4$	1.4% (w/v) in 0.4% (w/v) NaOH; 4 ml min^{-1}
Argon akış hızı	250 mL min^{-1}
Sinyal işleme ve veri depolama	Agilent 39500 E (AD dönüştürücü) Agilent ChemStation yazılımı

8.3.2.1 HPLC–HG–AFS ve HG–AFS için analitik performans verileri

Geliştirilen HPLC–HG–AFS ve HG–AFS yöntemlerinin arsenik türlerinin ve toplam arsenik tayinindeki analitik performans verilerini belirlemek için her iki yöntemle de kalibrasyon grafikleri hazırlandı. HPLC–HG–AFS ile kalibrasyon grafikleri iki farklı dedektör duyarlılığında çalışıldı. Arsenik derişimleri yüksek olan numuneler için 10.0, 20,0, 30.0, 50.0, 70.0 ve 100.0 µg L^{-1} aralığında (gain 10), arsenik derişimi düşük numuneler için ise 2.5, 5.0, 10.0 ve 20.0 µg L^{-1} (gain 100) arsenik standardı ilave edilmiş boş idrar numunesi kullanıldı. Benzer şekilde toplam arsenik tayininde 100.0, 250, 500, 1000, 2500 ve 5000 ng L^{-1} (gain 10) veya 25.0, 50.0, 100.0, 250, 500 ng L^{-1} (gain 100) derişimlerinde sulu ortamda hazırlanmış standart çözeltiler kullanıldı. Yüksek derişimde arsenik içeren numunelerde arsenik türlerinin tayininde kullanılan kalibrasyon grafiğine örnek Şekil 8'de, analitik performans verileri ise Tablo 9'da özetlenmiştir.



Şekil 8. HPLC–HG–AFS ile çizilen kalibrasyon grafiği. Koşullar: hareketli faz: pH 5.8, 8.0 mM fosfat tamponu; akış hızı: 1.0 ml min^{-1}; Gain: 10

Düşük derişimlerde arsenik içeren numuneler için arsenik türlerinin tayininde kullanılan kalibrasyon grafiğine örnek Şekil 9'da, analitik performans verileri ise Tablo 10'da özetlenmiştir.

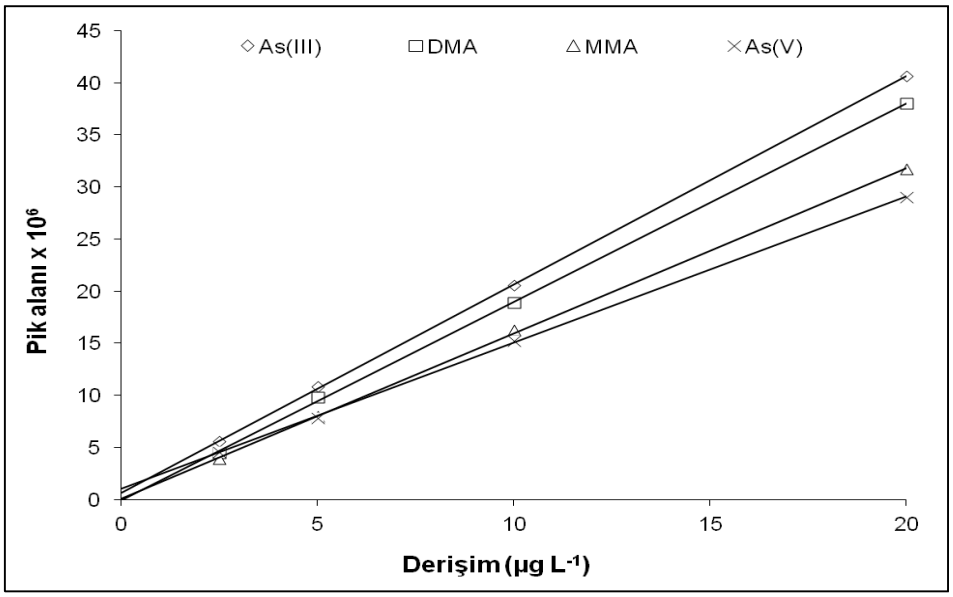
Tablo 9. HPLC–HG–AFS ve HG–AFS için anlamlı figürler (Gain 10).

		HPLC–HG–AFS[a]				HG–AFS[b]
		As(III)	DMA	MMA	As(V)	Toplam As
Regresyon denklemi[c]		$y = 1.7\times10^5 x + 1.3\times10^6$	$y = 9.4\times10^4 x + 1.1\times10^6$	$y = 1.1\times10^5 x + 8.3\times10^5$	$y = 7.9\times10^4 x + 4.8\times10^4$	$y = 3.3\times10^3 x + 80$
Tamamlayıcılık katsayısı (R^2)		0.9983	0.9983	0.9989	0.9971	0.9999
Doğrusal aralık (μg L^{-1})		7.3–100	7.3–100	9.6–100	9.6–100	0.1–5.0 (μg L^{-1})
LOQ ($10s_b/m$, μg L^{-1})		7.3	7.3	9.6	9.6	0.1 (μg L^{-1})
LOD ($3s_b/m$, μg L^{-1})		2.2	2.2	2.9	2.9	30 (ng L^{-1})
RSD (%, n = 5)	Alıkonma zamanı	0.66	0.44	1.54	1.41	–
	Pik alanı	0.92	1.47	1.75	1.76	–
	Pik yüksekliği	0.42	2.31	0.11	1.41	–

[a] Koşullar: hareketli faz: 8.0 mM fosfat tampon, pH 5.8; akış hızı: 1.0 ml min^{-1}; Gain: 10; Numune: 100 μL idrar (5.0 mM fosfat tampon ile pH 6.9'a tamponlanmış)

[b] Koşullar Şekil 7'da verilmiştir

[c] Pik alanı = eğim × derişim (μg L^{-1}) + kesişim



Şekil 9. HPLC–HG–AFS ile çizilen kalibrasyon grafiği. Koşullar: hareketli faz: pH 5.8, 8.0 mM fosfat tamponu; akış hızı: 1.0 ml min^{-1}; Gain: 100

Tablo 10. HPLC–HG–AFS ve HG–AFS için anlamlı figürler (Gain 100).

	HPLC–HG–AFS[a]				HG–AFS[b]
	As(III)	DMA	MMA	As(V)	Toplam As
Regresyon denklemi[c]	$y = 2.1\times10^6 x + 8.2\times10^4$	$y = 2.0\times10^6 x - 2.0\times10^5$	$y = 1.6.1\times10^6 x – 5.6\times10^4$	$y = 1.4\times10^6 x + 5.8\times10^5$	$y = 9674.4x + 75.6$
Tamamlayıcılık katsayısı (R^2)	0.9995	0.9995	0.9999	0.9998	0.9998
Doğrusal aralık (µg L^{-1})	1.0–20	1.0–20	2.3–20	2.3–20	25–500 (ng L^{-1})
LOQ ($10s_b/m$, µg L^{-1})	1.0	1.0	2.3	2.3	10 (ng L^{-1})
LOD ($3s_b/m$, µg L^{-1})	0.3	0.3	0.7	0.7	3 (ng L^{-1})

[a] Koşullar: hareketli faz: 8.0 mM fosfat tampon, pH 5.8; akış hızı: 1.0 ml min^{-1}; Gain: 100; Numune: 100 µL idrar (5.0 mM fosfat tampon ile pH 6.9'a tamponlanmış)

[b] Koşullar Şekil 7'da verilmiştir

[c] Pik alanı = eğim × derişim (µg L^{-1}) + kesişim

8.3.2.1 Doğruluk Çalışması

HPLC–HG–AFS yönteminin doğruluğu standart referans maddede "SRM NIST 2669 dondurulmuş insan idrarında arsenik türleri" hidrür oluşturabilen arsenik türlerinin tayini ile gerçekleştirilmiştir. Bulunan değerler ile sertifika değerleri arasında %95 güven seviyesinde anlamlı bir fark yoktur (Tablo 11). Toplam arsenik tayininde kullanılan HG–AFS yönteminin doğruluğu da aynı SRM kullanılarak test edilmiştir (Tablo 12). Bulunan sonuçlar ile sertifika değerleri arasında %95 güven seviyesinde anlamlı bir fark yoktur.

Tablo 11. Sertifikalı referans maddede HPLC–HG–AFS ile arsenik türlerinin tayini

"SRM NIST 2669 İnsan idrarında arsenik türleri" Level II (μg L^{-1})		
Arsenik türleri	Sertifikalı değerleri[a]	Bulunan[b]
As(III)	5.03±0.31	5.12±0.45
As(V)	6.16±0.49	6.64±0.49
DMA	25.3±0.7	26.5±1.0
MMA	7.18±0.56	7.21±1.04

[a] Derişimler arsenik cinsinden verilmiştir

[b] Sonuçlar 5 ölçümün ortalamasıdır

Tablo 12. Sertifikalı referans maddede HG-AFS ile toplam arsenik tayini

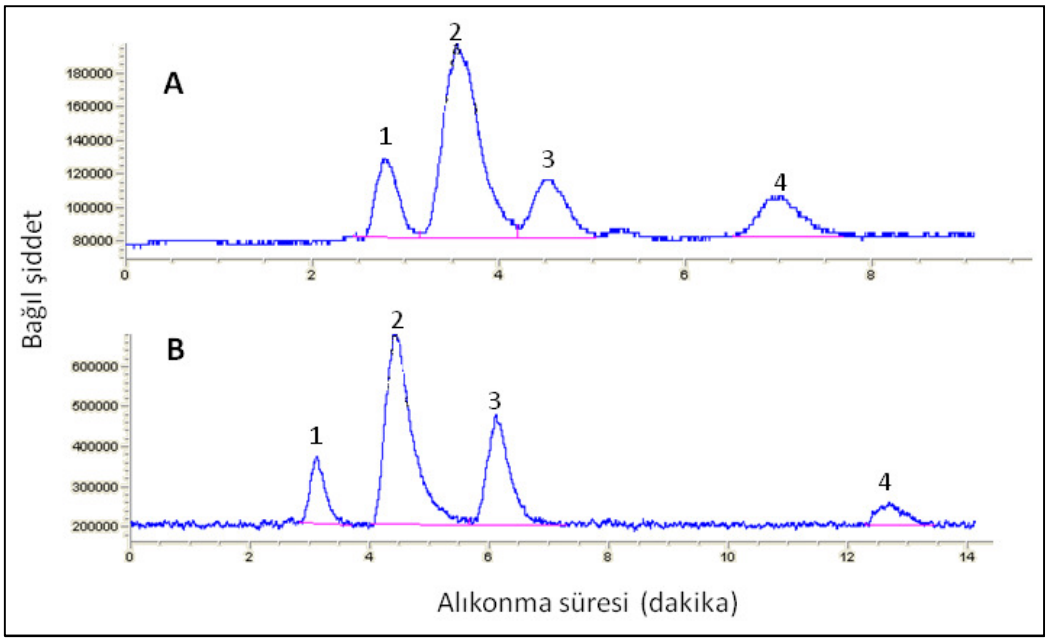
"SRM NIST 2669 İnsan idrarında arsenik türleri" Toplam arsenik derişimi (μg L^{-1})			
Level I		Level II	
Sertifikalı değer	Bulunan[a]	Sertifikalı değer	Bulunan[a]
22.2±4.8	22.3±0.4	50.7±6.3	50.7±0.6

[a] Sonuçlar iki parallel çözeltinin beşer kez alınan ölçümlerinin ortalamasıdır

8.4 İdrar numunelerinde türleme analizi

Geliştirilen HPLC–HG–AFS yöntemi Nevşehir bölgesinde yaşayan insanlardan toplanan idrar numunelerinde arsenik türlerinin tayinine uygulandı. Yüksek derişimde (1:1 seyreltilmiş) ve düşük derişimde (seyreltilmemiş) arsenik içeren numunelere ait örnek kromatogramlar sırasıyla Şekil 10 A ve B'de verilmiştir.

Sonuçlar idrar numunelerinin toplandığı bölgelerdeki içme suyunda arsenik düzeyine göre, arsenik derişimi 10 µg L^{-1}'den düşük, 10–50 µg L^{-1} arasında ve 50 µg L^{-1}'den yüksek olmak üzere gruplandırıldı. As(III), As(V), DMA ve MMA'nın derişimleri, türlerin derişimlerinin toplamı ve HG-AFS ile yapılan toplam arsenik tayin sonuçları Apendiks'te tablo halinde sunulmuştur. Her bir grup için arsenik türlerinin, türlerin toplamının ve toplam arsenik tayininin ortalaması standart sapmaları ile birlikte Tablo 13'te özetlenmiştir. Türlerin toplamları ve toplam arsenik için ortalama değerler kendi içinde karşılaştırıldığında %95 güven seviyesinde farkın istatiksel olarak anlamlı olduğu görülmüştür. Ayrıca DMA'nın tüm gruplarda en yüksek derişime sahip tür olduğu organik arsenik türlerinin toplamının da inorganik arsenik türlerinin toplamından büyük olduğu da Tablo 13'te görülmektedir.



Şekil 10. Gerçek numune ile elde edilen kromatogramlar. MP: 8.0 mM fosfat tamponu, pH 5.8; Akış hızı: 1.0 ml min^{-1}. (A) İdrar numunesi, Kod No. 35 (Yüksek derişim, 1:2 seyreltme, Gain: 10). Pikler: 1: As(III) [16.9±1.2]; 2: DMA [116.8±3.5]; 3: MMA [16.6±0.4]; 4: As(V) [1.6±0.1], Türlerin toplamı: 151.9±3.7; (B) İdrar numunesi, Kod No. 419 (düşük derişim, Gain: 100). Pikler: 1: As(III) [1.15±0.04]; 2: DMA [7.68±0.12]; 3: MMA [3.76±0.01]; 4: As(V) [1.04±0.18], Türlerin toplamı: 13.63±0.22, derişimler µg L^{-1} cinsinden

Tablo 13. Gruplandırılmış numunelerde arsenik türlerinin ve toplam arsenik derişimleri (µg L^{-1}).

Suda arsenik (µg L^{-1})	İdrar numunelerinde arsenik türleri (ortalama±SD)				Türlerin toplamı (ortalama±SD)*	Toplam arsenik (ortalma±SD)§
	As(III)	As(V)	MMA	DMA		
As>50 (n = 174)	9.0±8.45	10.9±13.6	19.9±23.9	75.0±90.8	102.3±110.6	157.2±121.8
10<As<50 (n = 256)	4.9±3.6	8.7±5.8	6.5±6.1	34.6±53.2	40.5±53.9	71.0±54.2
As<10 (n = 146)	0.9±0.3	<LOD	1.1±0.4	2.3±1.1	3.0±1.5	17.3±26.5

* Ortalamalar karşılaştırıldığında (Student t-Test, $p<0.05$) fark anlamlıdır

§ Ortalamalar karşılaştırıldığında (Student t-Test, $p<0.05$) fark anlamlıdır

8.5 SONUÇ

Bu çalışmanın ilk kısmında As(III), As(V), MMA, DMA, Sb(III) ve Sb(V)'in kapiler içi zenginleştirilmesi ve CE'de tayini için: (i) SDS ile süpürmeli–MEKC, (ii) polarite değiştirmeli LVSS, (iii) FASS ve (iv) ters EOF–FASI yöntemleri ile çalışılmıştır. En düşük teşhis sınırı HSDC ile birleştirilmiş ters EOF–FASI ile elde edilmiştir. Çalışılan analitler için hesaplanan LOD değerleri sırasıyla 2.1, 16, 14, 1.9, 1.1 ve 13 μg L^{-1} dir. Konvansiyonel CZE'ye kıyasla 0.6×10^3–21×10^3 kez duyarlılık artışı sağlanmıştır. Geliştirilen CE yöntemi arsenik ve antimon türleri ilave edilmiş çeşme suyuna uygulanmış ve geri kazanım değerleri % 97.7–102.5 arasında bulunmuştur.

Geliştirilen ters EOF–FASI yöntemi literatürde su numunelerinde arsenik ve/veya antimon türleme analizi için çeşitli dedektör tiplerinin kullanıldığı CE çalışmalarıyla kıyaslanmış ve Tablo 14'de özetlenmiştir. Tablo 14'de görüldüğü gibi geliştirilen yöntem UV, LIF ve HG–AFS dedektörü kullanılan diğer CE çalışmalarının bir çoğundan daha düşük LOD değerlerine sahiptir. Dedektör olarak ICP–MS'in elektrokinetik enjeksiyonla birlikte kullanıldığında daha düşük LOD değerlerine ulaşılmıştır. Fakat, UV dedektörler ICP–MS'e kıyasla daha ucuz ve CE ile uyumludur.

İkinci kısımda insan idrarında As(III), As(V), MMA ve DMA türlerinin tayini için HPLC–HG–AFS yöntemi geliştirilmiş ve Nevşehir ilinde içme sularında arsenik derişimi 50 μg L^{-1} den yüksek, arsenik derişimi 10-50 μg L^{-1} arasında ve arsenik derişimi 10 μg L^{-1} den düşük olan bölgelerde yaşayan insanlardan toplanan idrar numunelerine uygulanmıştır. Arsenik türlerinin ayırılmasında kuvvetli anyon değiştirici kolon kullanılmıştır. Ayırma sırasında pH'sı 5.8'e ayarlanmış 8.0 mM fosfat tamponundan

oluşan hareketli faz ve 1 mL min^{-1} akış hızında çalışılmıştır. İdrar numuneleri ile çalışıldığında koruyucu kolon kullanımının zorunlu olduğu tespit edilmiştir. Türleme çalışmalarının doğruluğu, sertifikalı referans madde "SRM NIST 2669 dondurulmuş insan idrarında arsenik türleri" ile test edilmiş ve As(III), As(V), MMA ve DMA için bulunan değerler ile

Tablo 14. Geliştirilen CE yönteminin literatürdeki benzer yöntemler ile kıyaslanması.

Matriks/ numune	Türler	Detektör	Önişlem/ Zenginleştirme yöntemi	LOD ($\mu g\ L^{-1}$)	Kaynak
Standart	As(III), DMA, MMA, As(V)	UV (indirek)	FASI	20–70	9
Çeşme ve göl suyu	As(III), DMA, MMA, As(V), roksarson, p-aminofenilarsonik asit, 4-nitrofenilarsonik asit, fenilarsonik asit	UV	LVSS polarite değiştirmeli; NSM; ters çevrilmiş EOF ile co-EOF NSM; HSDC	5.61–17.1	10
Toprağın sulu ekstraktı	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	UV (indirek)	Ekstraksiyon	84–304 (hidrodinamik enjeksiyon), 44–147 (elektrokinetik enjeksiyon),	12
Göl ve nehir suyu	As(III), DMA, MMA, As(V)	HG–AFS	pH-vasıtasıyla istifleme	5.0–9.3	48
Doğal ve kirletilmiş sular	As(III), DMA, MMA, As(V)	UV	–	5.0–20	77
Yeraltı suyu	As(III), DMA, As(V),	UV	–	100–500	82
Çeşme ve maden suyu	As(III), DMA, MMA, As(V)	LIF (indirek)	–	120–540	83
Toprağın sulu ekstraktı	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	ICP–MS	Ekstraksiyon	6–17 (hidrodinamik enjeksiyon), 0.08–0.31 (elektrokinetik enjeksiyon),	84
Çeşme suyu	As(III), DMA, MMA, As(V), Sb(III), Sb(V)	UV	HSDC ve ters EOF-FASI	1.9–16	Bu çalışma

sertifika değerleri arasında %95 güven seviyesinde istatiksel olarak anlamlı bir fark olmadığı görülmüştür. Aynı idrar numunelerinde yıkılama sonrası toplam arsenik tayini de yapılmış ve sertifikalı değerlerle uyumlu olduğu görülmüştür. HPLC–HG–AFS ile 100 µL enjeksiyon hacmi için LOD değerleri 0.3-0.7 µg L^{-1} (Gain 100) olarak hesaplanmıştır. HG-AFS için ise LOD değeri 3 ng L^{-1} dir (Gain 100). İdrar numuneleri toplandıkları bölgede kullanılan sudaki arsenik seviyelerine göre gruplandırılmış ve her bir grup için arsenik türlerinin ve toplam arseniğin ortalamaları hesaplanarak Tablo 13'te verilmiştir. Her bir grubun deneysel ortalamalarına t-testi uygulandığında ortalamalar arasındaki farkın %95 güven seviyesinde anlamlı olduğu bulunmuştur. Üç grupta da türleme analizlerinde derişimi en yüksek olan tür DMA dır. Ayrıca, organik arsenik türlerinin derişimlerinin toplamının inorganik arsenik türlerinin toplamından daha fazla olduğu görülmüştür. Sonuçlar HPLC–HG–AFS ve HG–AFS yöntemlerinin idrar ve su numunelerinde arsenik türleme analizlerinde ve toplam arsenik tayininde oldukça duyarlı, tekrarlanabilir ve doğru teknikler olduğunu göstermektedir.

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10 APPENDIX

Appendix 1. Arsenic speciation results in Nevşehir area (arsenic level in drinking water above 50 µg L^{-1}; all concentrations are in µg L^{-1}).

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
1	5.15	0.5	81.34	1.91	10.51	0.22	<LOD	–	97	1.99	93.7	19.66
2	4.45	0.14	81.64	0.17	2.74	0.35	3.17	0.63	92	0.75	110.03	5.36
3	7.42	0.12	70.32	0.24	6.07	0.35	<LOD	–	83.81	0.44	83.5	3.54
4	7.64	1.24	77.95	2	8.7	0.05	<LOD	–	94.29	2.35	116.28	9.44
5	<LOD	–	42.4	0.59	4.39	0.08	<LOD	–	46.79	0.6	106.88	8.23
6	15.11	0.43	102.88	0.47	11.87	0.42	2.52	–	132.38	0.77	153.8	10.29
7	<LOD	–	40.8	2.63	36.1	3.18	<LOD	–	76.9	4.13	70.3	4.35
8	4.3	0.02	100.2	2.04	24.42	0.72	<LOD	–	128.92	2.16	141.2	7.6
9	4.64	0.03	76.4	0.4	5.62	0.02	<LOD	–	86.66	0.4	86.33	6.02
10	28.16	0.26	27.63	0.85	8.26	0.1	<LOD	–	64.05	0.89	173.45	9.92
11	15.1	0.75	168.7	1.87	17.7	0.18	1.45	0.39	202.95	2.06	257.85	16.37
12	1.34	0.04	20.63	1.37	<LOD	–	<LOD	–	21.97	1.37	40.78	3.01
13	3.05	0.18	84.69	2.43	11	0.08	6.25	0.65	104.99	2.52	99.7	1.17
14	<LOD	–	29.43	0.5	6.43	0.19	2.61	0.1	38.47	0.54	151.13	0.9
15	7.45	0.25	93.41	0.67	<LOD	–	<LOD	–	100.86	0.72	93.48	6.32
16	4.48	0.2	82.9	0.65	17.01	0.94	10.75	2.3	115.14	2.58	122.8	6.38
17	4.44	1.01	57.4	1.58	4.71	1.95	<LOD	–	66.55	2.71	126.35	5.44
18	13.57	1.82	151.41	0.94	23.57	1.33	<LOD	–	188.55	2.44	201.45	12.81
19	6.18	0.04	46.81	2.31	9.72	0.23	<LOD	–	62.71	2.32	80.08	3.33
20	3.07	0.19	132.9	0.26	15.5	0.28	<LOD	–	151.47	0.43	133.1	17.27
21	1.52	0.24	20.97	1.92	2.57	0.51	<LOD	–	25.06	2	24.22	1.74
22	11.06	0.79	114.9	1.99	16.8	0.91	<LOD	–	142.76	2.33	193.91	8.88
23	2.12	0.11	27.17	1.22	<LOD	–	<LOD	–	29.29	1.22	39.76	3.19
24	5.23	0.5	56.2	2.95	9.79	1.23	<LOD	–	71.22	3.24	69.5	6.08
25	14.11	0.33	52.31	1.86	19.23	0.33	<LOD	–	85.65	1.92	117.13	7.23
26	9.76	0.46	90.02	3.06	20.79	0.49	<LOD	–	120.57	3.13	137.95	2.47
27	5.54	0.42	107.8	4.22	19.16	0.63	<LOD	–	132.5	4.29	177.63	7.85
28	7.72	0.45	69.68	1.63	13.53	0.01	<LOD	–	90.93	1.69	90.64	1.91
29	14.76	0.57	935.2	0.96	176.6	0.59	<LOD	–	1126.56	1.26	1155.4	35.54
30	4.64	0.01	85.88	1.49	9.97	1.31	<LOD	–	100.49	1.98	128.1	4.09
31	6.18	0.02	78.9	0.57	10.4	0.04	<LOD	–	95.48	0.57	117.65	7.34
32	3.75	0.16	33.65	1.32	2.96	0.87	<LOD	–	40.36	1.59	50.17	6.09
33	4.95	0.35	95.8	3.55	7.86	0.72	<LOD	–	108.61	3.64	101.23	7.77
34	2.79	0.9	52.94	0.87	5.7	1.54	<LOD	–	61.43	1.98	103.35	10.75

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
35	16.94	1.27	116.8	3.45	16.6	0.43	1.57	0.04	151.91	3.7	249.1	5.69
36	9.09	0.51	133.2	4.13	18.9	0.76	5.48	0.43	166.67	4.25	264.25	15.49
37	<LOD	–	30.02	0.17	5.66	0.65	<LOD	–	35.68	0.67	44.68	6.28
38	1.49	0.01	84.85	1.16	6.62	0.24	<LOD	–	92.96	1.18	91.06	11.68
39	12.79	0.14	147.1	2.79	17.7	0.74	<LOD	–	177.59	2.89	168.94	8.21
40	10.2	0.06	90.7	4.72	28	2.28	7.57	1.99	136.47	5.61	148.08	9.77
41	2.04	0.32	59.12	2.7	8.89	0.09	<LOD	–	70.05	2.72	69.52	9.51
42	6.93	0.18	142.1	2.41	7.73	0.26	<LOD	–	156.76	2.43	162.63	2.33
43	4.12	0.83	147.48	11.36	16.97	3.09	<LOD	–	168.57	11.8	172.56	9.02
44	3.63	0.74	120.3	0.71	36.1	1.48	<LOD	–	160.03	1.8	170.2	8.66
45	9.65	0.1	205.09	0.56	26.14	1.85	<LOD	–	240.88	1.94	264.2	11.1
46	15.39	0.46	79.24	12.84	9.02	0.26	4.9	0.14	108.55	12.85	105.6	6.4
47	5.8	0.53	63.39	6.29	9.39	0.92	1.42	0.13	80	6.38	93.66	14.37
48	7.18	0.41	60.2	0.84	5.26	0.74	<LOD	–	72.64	1.19	67.4	1.86
49	7.9	0.21	18.55	0.3	2.76	0.25	<LOD	–	29.21	0.44	58.32	2.97
50	6.3	0.1	239.42	1.16	22.11	0.67	<LOD	–	267.83	1.34	265.42	5.89
51	<LOD	–	38.1	1.18	1.23	0.78	9.68	1.76	49.01	2.26	66.6	0.78
52	0.3	0.01	37.85	1.47	2.21	0.11	<LOD	–	40.36	1.47	54.35	12.3
53	5.63	0.27	31.58	1.37	1.85	0.18	<LOD	–	39.06	1.41	45.56	5.13
54	<LOD	–	72.04	6.13	<LOD	–	<LOD	–	72.04	6.13	85.56	6.78
55	1.28	0.2	85.28	1.07	11.81	0.49	<LOD	–	98.37	1.19	120.9	3.3
56	<LOD	–	73.97	4.1	<LOD	–	<LOD	–	73.97	4.1	78.14	4.38
57	2.84	0.51	83.2	2.08	14.3	1.23	<LOD	–	100.34	2.47	103.84	6.17
58	14.3	0.1	110.9	0.49	17.75	0.81	3.79	0.3	146.74	1	188.87	6.77
59	5.37	0.69	32.8	0.21	2.59	0.52	<LOD	–	40.76	0.89	45.73	3.92
60	<LOD	–	121.9	10.91	<LOD	–	<LOD	–	121.9	10.91	122.3	5.6
61	15	0.02	51.3	0.76	<LOD	–	<LOD	–	66.3	0.76	63.4	2.1
62	<LOD	–	73.18	1.64	<LOD	–	<LOD	–	73.18	1.64	75.56	3.92
63	3.29	0.05	163.6	1.03	8.49	0.17	3.9	0.02	179.28	1.05	169.4	7.65
64	3.75	0.31	68	0.03	8.4	0.16	<LOD	–	80.15	0.35	106.3	4.1
65	5.52	0.28	358	5.37	<LOD	–	<LOD	–	363.52	5.38	317.26	13.71
66	<LOD	–	99.55	0.43	<LOD	–	<LOD	–	99.55	0.43	84.95	15.91
67	<LOD	–	77.93	5.24	5.87	0.26	<LOD	–	83.8	5.25	106.13	6.9
68	<LOD	–	420	19.7	<LOD	–	9.4	0.44	429.4	19.7	371.97	16.8
69	3.09	0.47	124.2	0.45	8.56	0.4	6.01	0.18	141.86	0.78	165.67	11.05
70	<LOD	–	128.2	1.12	<LOD	–	<LOD	–	128.2	1.12	114.67	7.9
71	<LOD	–	168.2	1.28	7.81	0.78	<LOD	–	176.01	1.5	192.65	13.48
72	4.6	0.25	97.04	0.1	1.38	0.04	<LOD	–	103.02	0.27	61.57	11.5
73	7.41	0.03	305.4	1.19	3.44	0.02	<LOD	–	316.25	1.19	287.6	14.5
74	4.49	0.21	127.8	1.05	<LOD	–	<LOD	–	132.29	1.07	127.56	8.1

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
178	4.54	0.06	49.59	0.41	20.49	0.02	<LOD	–	74.62	0.41	93.83	7.21
179	10.03	0.05	47.22	0.15	20.11	0.1	<LOD	–	77.36	0.19	71.6	3.09
181	39.72	0.11	194.5	0.61	162.1	0.61	41.78	0.3	438.1	0.92	605.43	32.63
182	35.58	0.07	195.9	0.73	70.58	0.33	<LOD	–	302.06	0.8	454.87	4.1
183	3.71	0.05	13.18	0.12	<LOD	–	<LOD	–	16.89	0.13	26.23	0.27
184	12.99	0.05	75.07	0.63	59.44	0.03	<LOD	–	147.5	0.63	134.07	2.12
185	5.29	0.09	105.8	0.22	49.56	0.26	<LOD	–	160.65	0.35	486.71	29.44
186	17.25	0.18	79.96	0.36	49.79	0.39	<LOD	–	147	0.56	293.45	16.29
187	13.25	0.16	75.56	0.61	28.61	0.18	<LOD	–	117.42	0.66	192.23	8.73
188	15.34	0.03	62.7	0.12	37.55	0.06	<LOD	–	115.59	0.14	178.41	10.26
189	13.39	0.32	101	0.96	27.52	0.44	<LOD	–	141.91	1.1	300.94	7.91
190	9.87	0.12	60.9	0.27	43.33	0.44	<LOD	–	114.1	0.53	180.12	4.92
191	8.83	0.04	55.95	0.39	21.99	0.3	<LOD	–	86.77	0.49	215.34	5.81
192	13.25	0.23	75.03	0.06	32.94	–	18.76	0.34	139.98	0.41	166.68	2.52
193	27.13	0.06	110.2	0.87	59.06	0.83	<LOD	–	196.39	1.2	227.93	3.35
194	11.33	0.37	57.18	0.24	20.22	0.23	<LOD	–	88.73	0.5	254.71	11.58
195	6.89	0.11	85.73	0.12	14.54	0.02	<LOD	–	107.16	0.16	224.51	13.02
196	20.24	0.36	157.7	0.6	50.4	0.19	<LOD	–	228.34	0.73	424.5	13.07
197	2.54	0.04	22.59	0.09	9.18	0.06	<LOD	–	34.31	0.12	71.60	3.01
198	3.71	0.05	54.88	0.08	14.45	0.06	<LOD	–	73.04	0.11	128.01	6.3
199	14.39	0.01	92.2	0.1	32.32	0.07	<LOD	–	138.91	0.12	221.11	9.89
200	11.18	0.23	59.79	1	20.61	0.19	<LOD	–	91.58	1.04	176.84	1.64
201	26.45	0.06	98.81	0.77	55.66	0.27	13.15	0.37	194.07	0.9	252.14	8.54
202	<LOD	–	14.81	0.09	12.29	0.04	<LOD	–	27.1	0.1	45.15	4.16
203	6.81	0.05	95.49	0.15	60.63	0.66	<LOD	–	162.93	0.68	313.96	3.48
204	<LOD	–	55.43	0.26	13.35	0.18	<LOD	–	68.78	0.32	125.39	1.01
205	3.55	0.15	51.59	0.27	24.13	0.02	<LOD	–	79.27	0.31	137.21	1.55
206	7.58	0.15	70.06	0.07	20.05	0.02	<LOD	–	97.69	0.17	351.01	13.12
207	11.19	0.05	72.44	0.29	21.82	0.15	<LOD	–	105.45	0.33	182.14	4.49
208	4.82	0.06	35.19	0.13	12.82	0.22	<LOD	–	52.83	0.26	93.12	5.78
209	11.21	0.34	42.81	1.11	15.37	0.13	<LOD	–	69.39	1.17	74.53	0.94
210	21.79	0.29	102.6	0.6	55.06	0.06	<LOD	–	179.45	0.67	310.57	7.39
211	4.33	0.11	37.6	0.01	42.52	0.32	<LOD	–	84.45	0.34	211.81	4.08
212	23.31	0.12	37.11	0.04	29.81	0.07	6.9	0.18	97.13	0.23	106.15	4.57
213	10.28	0.2	64.5	0.06	21.11	0.26	<LOD	–	95.89	0.33	187.07	2.35
214	8.4	0.08	64.86	0.45	22.25	0.27	<LOD	–	95.51	0.53	182.68	9.1
215	6.45	0.05	58.83	0.18	47.73	0.52	<LOD	–	113.01	0.55	208.71	16.47
216	13.96	0.07	64.49	0.21	23.18	0.02	<LOD	–	101.63	0.22	154.48	4.76
217	12.49	0.01	146.7	0.22	54.71	0.28	<LOD	–	213.9	0.36	281.18	20.91
218	3.38	0.02	61.86	0.52	38.28	0.55	<LOD	–	103.52	0.76	224.14	2.51

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
219	7.76	0.05	56.15	0.17	26.31	0.05	<LOD	–	90.22	0.18	193.49	17.87
220	9.62	0.28	75.23	0.67	51.76	0.02	<LOD	–	136.61	0.73	179.68	5.81
221	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	367.03	21.98
222	2.54	0.05	47.95	0.43	49.2	0.13	<LOD	–	99.69	0.45	127.81	5.34
223	10.85	0.08	64.97	0.61	23.73	0.47	<LOD	–	99.55	0.77	175.12	12.96
223	21.76	0.27	72.78	0.28	36.94	0.08	<LOD	–	131.48	0.4	218.15	15.69
224	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	46.75	1.68
225	8.74	0.48	42.55	0.79	13.76	0.24	<LOD	–	65.05	0.96	90.3	0.7
226	7.15	0.01	42.31	0.19	32.78	0.02	<LOD	–	82.24	0.19	69.21	8.32
227	2.65	0.03	53.13	0.01	14.36	0.08	<LOD	–	70.14	0.09	142.77	5.52
228	5.69	0.06	48.6	0.96	17.79	0.48	<LOD	–	72.08	1.07	125.54	6.37
229	3.41	0.08	36.48	0.28	23.07	0.06	62.46	0.06	125.42	0.3	79.61	4.52
230	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	21.18	0.39
231	7.13	0.14	14.97	0.13	11.46	0.1	8.11	0.01	41.68	0.22	136.13	9.64
232	36.95	0.1	215.3	0.81	71.01	1.3	<LOD	–	323.26	1.53	310.98	16.56
233	15.39	0.03	67.48	0.15	45.25	0.55	28.5	0.6	156.62	0.83	218.13	15.68
234	23.35	0.38	22.08	0.24	18.75	0.19	11.16	0.63	75.34	0.8	233.58	15.41
235	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	265.56	12.98
236	3.57	0.07	25.16	0.17	14.71	0.34	<LOD	–	43.44	0.39	51.34	1.03
237	14.2	0.15	99.39	2.95	13.24	0.01	<LOD	–	126.83	2.95	140.42	7.12
238	49.39	0.51	136.5	0.49	81.23	0.48	36.98	0.6	304.1	1.04	309.81	22.88
239	10.66	0.03	33.39	0.28	13.78	0.25	19.2	0.19	77.03	0.42	74.89	5.65
240	10.56	0.22	31.64	0.15	19.32	0.15	<LOD	–	61.52	0.31	138.73	6.05
241	23.07	0.06	104.4	0.02	36.97	0.01	<LOD	–	164.44	0.06	361.51	22.76
242	5.67	0.13	41.47	0.04	31.26	0.43	<LOD	–	78.4	0.45	168.92	6.11
243	7.54	0.2	26.74	0.33	26.08	0.21	<LOD	–	60.36	0.44	49.27	1.12
244	23.96	0.25	85.35	0.61	23.27	0.19	24.49	0.15	157.07	0.7	197.94	28.49
245	27.77	0.01	44.83	0.2	24.95	0.17	8.54	0.18	106.09	0.32	118.15	10.51
246	19.04	0.38	86.39	1.45	26.7	0.63	<LOD	–	132.13	1.63	203.63	10.28
400	1.74	0.09	5.85	0.17	3.07	0.3	<LOD	–	10.66	0.36	48.23	8.47
401	1	0.02	9.19	0.12	4.51	0.14	<LOD	–	14.7	0.19	186.89	5.56
402	<LOD	–	4.65	0.06	1.52	0.07	<LOD	–	6.17	0.09	74.27	0.47
403	<LOD	–	7.81	0.11	2.24	0.26	<LOD	–	10.05	0.28	141.26	0.23
404	<LOD	–	5.22	0.04	1.22	0.13	<LOD	–	6.44	0.14	84.34	8.21
405	0.63	0.15	4.11	0.58	2.55	0.1	<LOD	–	7.29	0.61	78.34	6.23
406	1.95	0.05	8.86	0.09	4.31	0.3	2.39	0.16	17.51	0.36	164.4	7.22
407	<LOD	–	2.84	0.06	1.13	0.02	<LOD	–	3.97	0.06	41.41	0.31
408	3.11	0.02	11.79	0.03	5.24	0.01	<LOD	–	20.14	0.04	154.71	11.68
409	2.39	0.23	7.57	0.31	5.1	0.14	<LOD	–	15.06	0.41	201.2	6.95
410	0.34	0.01	4.28	0.27	2	0.21	<LOD	–	6.62	0.34	71.99	4.25

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
411	0.87	0.08	3.49	0.06	1.62	0.2	<LOD	–	5.98	0.22	25.29	0.94
412	<LOD	–	3.03	0.36	1.11	0.05	<LOD	–	4.14	0.36	65.83	1.22
413	<LOD	–	5.18	0.07	2.92	0.02	1.48	0.33	9.58	0.34	124.9	7.2
414	<LOD	–	1.86	0.01	0.99	0.05	<LOD	–	2.85	0.05	28.17	3.51
415	1.33	0.11	8.18	0.25	3.68	0.53	0.9	0.07	14.09	0.6	145.25	3.6
416	<LOD	–	3.2	0.14	1.28	0.05	<LOD	–	4.48	0.15	87.38	1.9
417	<LOD	–	6.84	0.12	2.86	0.03	<LOD	–	9.7	0.12	141.02	8.28
418	0.68	0.05	6.42	0.18	2.62	0.04	<LOD	–	9.72	0.19	8.21	0.54
420	1.59	0.01	15.11	0.96	3.93	0.24	<LOD	–	20.63	0.99	280.43	17.90
421	<LOD	–	3.56	0.08	1.63	0.05	<LOD	–	5.19	0.09	65.09	3.18
422	<LOD	–	6.09	0.08	1.49	0.23	<LOD	–	7.58	0.24	84.57	5.45
423	1.03	0.01	10.38	0.11	4.97	0.14	4.26	0.75	20.64	0.77	227.96	9.54
424	0.69	0.02	7.22	0.51	3.29	0.1	<LOD	–	11.2	0.52	146.93	1.38
425	0.46	0.02	9.46	0.21	2.96	0.08	0.99	0.3	13.87	0.38	111.23	3.51
427	<LOD	–	3.24	0.17	1.85	0.09	<LOD	–	5.09	0.19	79.45	3.2
428	0.77	0.08	6.68	0.28	3.8	0.29	<LOD	–	11.25	0.41	128.45	3.78
429	<LOD	–	6.17	0.04	3.22	0.06	<LOD	–	9.39	0.07	81.72	0.85
430	0.48	0.06	6.89	0.18	3.29	0.16	<LOD	–	10.66	0.25	156.18	1.49
431	<LOD	–	6.28	0.19	3.43	0.04	<LOD	–	9.71	0.19	130.5	3.53
432	0.63	0.02	4.97	0.13	2.03	0.1	<LOD	–	7.63	0.17	103.96	1.89

Appendix 2. Arsenic speciation results in Nevşehir area (arsenic level in drinking water in the range of 10-50 µg L^{-1}; all concentrations are in µg L^{-1}).

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
75	1.23	0.07	32.86	0.88	<LOD	–	<LOD	–	34.09	0.88	63.33	2.17
76	<LOD	–	75.9	0.53	<LOD	–	<LOD	–	75.9	0.53	65.09	7.78
77	<LOD	–	66.52	2.82	<LOD	–	<LOD	–	66.52	2.82	117.75	9.45
78	<LOD	–	69.95	5.24	<LOD	–	<LOD	–	69.95	5.24	66.75	4.94
79	<LOD	–	36.15	1.92	3.4	0.52	<LOD	–	39.55	1.99	38.47	3.32
80	<LOD	–	16.81	2.53	1.15	0.01	<LOD	–	17.96	2.53	43.23	4.39
81	<LOD	–	126.9	16.33	<LOD	–	<LOD	–	126.9	16.33	135.67	5.31
82	<LOD	–	38.08	3.62	<LOD	–	14.54	1.68	52.62	3.99	66.3	3.2
83	<LOD	–	80.95	3.12	6.39	0.16	<LOD	–	87.34	3.12	81.61	4.78
84	<LOD	–	169.7	1.43	<LOD	–	<LOD	–	169.7	1.43	157.4	6.5
85	<LOD	–	162.3	5.41	<LOD	–	<LOD	–	162.3	5.41	173.7	9.2
87	<LOD	–	183.1	1.34	<LOD	–	<LOD	–	183.1	1.34	187.4	9.6
89	<LOD	–	36.22	0.7	<LOD	–	19.05	1.22	55.27	1.41	64.3	3.5
90	<LOD	–	28.86	1.54	<LOD	–	<LOD	–	28.86	1.54	57.6	3.2
91	<LOD	–	49.59	1.52	<LOD	–	<LOD	–	49.59	1.52	113.3	6.1
91	<LOD	–	12.58	0.06	<LOD	–	<LOD	–	12.58	0.06	187.4	9.6
92	<LOD	–	77.96	5.37	<LOD	–	<LOD	–	77.96	5.37	120.1	8.9
93	<LOD	–	53.75	0.42	<LOD	–	<LOD	–	53.75	0.42	55.1	4.9
94	<LOD	–	31.32	0.82	3.06	0.51	<LOD	–	34.38	0.97	22.51	0.25
95	<LOD	–	48.94	1.09	<LOD	–	<LOD	–	48.94	1.09	41.4	3.2
96	<LOD	–	145.2	11.95	11.78	1.28	<LOD	–	156.98	12.02	149.54	5.83
97	<LOD	–	99.6	2.44	<LOD	–	<LOD	–	99.6	2.44	106.9	3.1
97	<LOD	–	430.8	14.7	<LOD	–	5.91	0.04	436.71	14.7	441.87	11.20
99	<LOD	–	87.18	4.54	<LOD	–	<LOD	–	87.18	4.54	112.6	6.9
100	4.81	0.08	93.83	4.63	19.49	2.57	3.68	0.48	121.81	5.32	117.5	6.1
101	<LOD	–	11.49	0.11	<LOD	–	<LOD	–	11.49	0.11	19.4	2.1
102	<LOD	–	184.1	5.44	<LOD	–	4.25	0.21	188.35	5.44	198.3	7.5
103	<LOD	–	32.62	0.48	<LOD	–	<LOD	–	32.62	0.48	65.6	2.1
104	<LOD	–	59.19	1.59	<LOD	–	<LOD	–	59.19	1.59	67.7	6.8
105	<LOD	–	33.57	3.16	<LOD	–	<LOD	–	33.57	3.16	40.71	3.76
107	<LOD	–	92.63	6.31	<LOD	–	<LOD	–	92.63	6.31	95.9	7.3
108	<LOD	–	73.6	3.69	<LOD	–	<LOD	–	73.6	3.69	71.5	3.9
108	2	0.11	81.74	0.05	<LOD	–	14.02	0.04	97.76	0.13	105.61	6.07
109	<LOD	–	33.31	0.48	<LOD	–	<LOD	–	33.31	–	117.79	0.97
109	<LOD	–	236.4	0.1	<LOD	–	<LOD	–	236.4	0.1	300.2	17.1
110	<LOD	–	95.66	0.24	<LOD	–	<LOD	–	95.66	0.24	106.9	7.3
111	<LOD	–	23.85	2.36	<LOD	–	<LOD	–	23.85	2.36	52.9	1

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
112	<LOD	–	16.2	2	<LOD	–	<LOD	–	16.2	2	21.3	1.8
113	<LOD	–	56.92	3.16	<LOD	–	<LOD	–	56.92	3.16	54.4	4.4
114	<LOD	–	108.9	0.33	<LOD	–	<LOD	–	108.9	–	170.2	8.5
115	<LOD	–	136.8	0.01	<LOD	–	<LOD	–	136.8	–	152	8.5
116	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	15.48	1.31
117	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	19.9	2.54
118	<LOD	–	11.76	0.21	<LOD	–	<LOD	–	11.76	0.21	12.1	2
119	3.77	0.36	81.62	2.64	<LOD	–	<LOD	–	85.39	2.66	115.3	11.1
120	<LOD	–	50.9	2.75	<LOD	–	<LOD	–	50.9	2.75	63.6	3.5
121	<LOD	–	27.78	0.2	<LOD	–	<LOD	–	27.78	0.2	49.9	6.5
122	<LOD	–	110.9	3.5	<LOD	–	<LOD	–	110.9	3.5	108.4	9.8
123	<LOD	–	10.8	0.09	<LOD	–	<LOD	–	10.8	0.09	36.2	3.01
124	<LOD	–	14.91	0.06	<LOD	–	<LOD	–	14.91	0.06	40.01	2.08
125	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	28.15	1.19
126	<LOD	–	180.8	1.44	<LOD	–	<LOD	–	180.8	1.44	169.9	7.5
127	<LOD	–	214.1	1.95	<LOD	–	<LOD	–	214.1	1.95	207	8.9
128	<LOD	–	102.7	2.7	<LOD	–	<LOD	–	102.7	2.7	72.9	4.1
129	<LOD	–	220.8	0.1	<LOD	–	<LOD	–	220.8	0.1	287	7.2
130	<LOD	–	126.4	0.59	<LOD	–	<LOD	–	126.4	0.59	121.1	4.9
131	<LOD	–	35.09	0.74	<LOD	–	<LOD	–	35.09	0.74	32.3	3.8
132	<LOD	–	11.28	0.03	<LOD	–	<LOD	–	11.28	0.03	42.8	1.78
134	<LOD	–	12.95	0.16	<LOD	–	<LOD	–	12.95	0.16	53.7	4.81
135	<LOD	–	19.56	0.17	<LOD	–	<LOD	–	19.56	0.17	89.2	4.75
136	<LOD	–	171.9	4.49	<LOD	–	<LOD	–	171.9	4.49	197	9.5
137	<LOD	–	112	2.46	<LOD	–	<LOD	–	112	2.46	114.9	6.4
138	<LOD	–	105.4	2.85	<LOD	–	<LOD	–	105.4	2.85	129.8	4.1
139	<LOD	–	129.1	7.34	<LOD	–	<LOD	–	129.1	7.34	117.1	13.2
140	<LOD	–	11	0.04	<LOD	–	<LOD	–	11	0.04	25.88	2.65
141	<LOD	–	2.05	0.13	1.88	0.3	<LOD	–	3.93	0.33	38.25	1.42
143	<LOD	–	9.14	0.01	4.72	0.04	<LOD	–	13.86	0.04	81	4.17
144	0.85	0.04	10.18	0.11	7.71	0.03	<LOD	–	18.74	0.12	24.4	0.97
145	<LOD	–	8.98	0.13	<LOD	–	<LOD	–	8.98	0.13	49.94	2.58
146	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	64.52	3.92
146	<LOD	–	3.61	0.04	1.84	0.01	<LOD	–	5.45	0.04	72.52	3.92
147	<LOD	–	8.71	0.01	<LOD	–	<LOD	–	8.71	0.01	34.91	0.51
148	<LOD	–	60.92	0.37	<LOD	–	<LOD	–	60.92	0.37	75.1	4.8
149	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	21.64	0.59
150	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	27.7	0.73
152	<LOD	–	7.1	0.09	<LOD	–	<LOD	–	7.1	0.09	23.55	1.71
153	<LOD	–	9.74	0.01	<LOD	–	<LOD	–	9.74	0.01	50.43	1.67

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
154	<LOD	–	6.65	0.08	5.07	0.1	<LOD	–	11.72	0.13	50.13	2.14
156	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	23.24	1.46
156	<LOD	–	2	0.23	1.24	0.04	<LOD	–	3.24	0.23	37.81	6.19
158	<LOD	–	14.24	0.08	<LOD	–	<LOD	–	14.24	0.08	27.09	1.92
159	<LOD	–	115.1	2.56	<LOD	–	<LOD	–	115.1	2.56	238	5.1
160	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	39.43	2.64
160	<LOD	–	3.11	0.24	1.41	0.04	<LOD	–	4.52	0.24	50.42	3.46
161	<LOD	–	12.26	0.13	8.73	0.11	<LOD	–	20.99	0.17	48.06	1.88
162	<LOD	–	10.05	0.08	5.69	0.17	<LOD	–	15.74	0.19	51.17	0.63
163	<LOD	–	11.77	0.06	6.52	0.26	<LOD	–	18.29	0.27	47.76	0.98
164	<LOD	–	5.58	0.01	3.81	0.01	<LOD	–	9.39	0.01	33.21	0.9
165	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	35.92	2.37
165	<LOD	–	4.85	0.31	1.93	0.01	<LOD	–	6.78	0.31	45.67	3.88
166	<LOD	–	5.79	0.04	5.14	0.17	<LOD	–	10.93	0.17	60.89	0.45
167	2.26	0.12	32	0.45	<LOD	–	<LOD	–	34.26	0.47	101	4.3
168	<LOD	–	81.68	0.6	<LOD	–	<LOD	–	81.68	0.6	139	11.9
169	<LOD	–	15.01	0.11	<LOD	–	<LOD	–	15.01	0.11	30.78	2.64
170	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	10.8	0.03
171	<LOD	–	7.71	0.12	5.49	0.01	<LOD	–	13.2	0.12	57.33	1.71
172	<LOD	–	220.1	3.3	<LOD	–	<LOD	–	220.1	3.3	203.9	11.1
174	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	37.71	6.15
247	13.18	0.31	17.54	0.09	12.58	0.11	12.64	0.48	55.94	0.59	67.8	1.91
248	<LOD	–	17.74	0.1	8.08	0.21	<LOD	–	25.82	0.23	184.52	13.2
249	<LOD	–	15.95	0.36	<LOD	–	<LOD	–	15.95	0.36	41.82	0.38
249	<LOD	–	14.28	0.49	3.1	0.12	<LOD	–	17.38	0.5	112.25	11.17
250	5.04	0.02	15.73	0.05	5.59	0.06	<LOD	–	26.36	0.08	26.31	2.42
251	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	29.51	0.1
252	<LOD	–	10.78	0.01	6.61	0.08	<LOD	–	17.39	0.08	76.12	1.13
252	4.47	0.13	19.08	0.35	7.19	0.11	<LOD	–	30.74	0.39	101.25	8.27
253	<LOD	–	15.57	0.07	6.58	0.09	<LOD	–	22.15	0.11	51	1.04
255	<LOD	–	11.48	0.04	4.67	0.07	<LOD	–	16.15	0.08	35.71	3.54
256	8.16	0.42	35.16	0.32	16.3	0.21	<LOD	–	59.62	0.57	77.54	4.39
257	2.37	0.08	37.55	0.28	12.32	0.17	<LOD	–	52.24	0.34	53.85	4.91
258	<LOD	–	7.08	0.05	5.63	0.01	<LOD	–	12.71	0.05	27.8	1.55
259	5.11	0.03	37.9	0.23	14.78	0.01	7.21	0.07	65	0.24	100.62	1.74
261	4.96	0.1	31.95	0.25	9.77	0.05	5.51	0.03	52.19	0.28	59.81	5.67
262	11.06	0.04	54.36	0.11	23.43	0.04	8.98	0.15	97.83	0.19	92.47	1.54
263	<LOD	–	5.69	0.03	4.27	0.02	<LOD	–	9.96	0.04	17.67	0.6
264	<LOD	–	7.66	0.02	5.14	0.12	<LOD	–	12.8	0.12	58.7	8.49
265	<LOD	–	17.07	0.38	6.45	0.27	<LOD	–	23.52	0.47	61.32	5.4

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
266	<LOD	–	10.59	0.01	6.1	0.05	<LOD	–	16.69	0.05	16.2	1.83
266	5.31	0.12	42.79	0.29	11.95	0.04	<LOD	–	60.05	0.32	56.28	3.91
267	<LOD	–	23.96	0.22	9.22	0.01	<LOD	–	33.18	0.22	99.75	2.42
268	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	15.02	2.29
270	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	44.09	2.06
271	<LOD	–	8.67	0.04	5.63	0.04	<LOD	–	14.3	0.06	69.69	2.7
272	9.43	0.15	11.59	0.11	7.81	0.04	6.81	0.31	35.64	0.36	38.89	2.86
273	8.8	0.19	36.7	0.01	10.44	0.12	19.86	0.81	75.8	0.84	134.21	10.24
274	4.58	0.21	20.66	0.03	7.16	0.07	3.3	0.57	35.7	0.61	62.74	2.56
275	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	114.53	1.7
276	6.25	0.07	28.36	1.02	7.74	0.07	<LOD	–	42.35	1.02	44.9	2.13
278	4.18	0.01	50.82	0.44	16.73	0.14	<LOD	–	71.73	0.46	52.5	2.24
279	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	29.55	2.08
280	<LOD	–	4.95	0.09	3.89	0.05	<LOD	–	8.84	0.1	33.13	2.87
282	<LOD	–	10.24	0.07	5.78	0.03	<LOD	–	16.02	0.08	55.04	0.51
283	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	9.61	0.21
284	<LOD	–	4.25	0.03	3.6	0.03	<LOD	–	7.85	0.04	63.12	4.98
284	3.25	0.13	107.9	0.31	23.3	0.4	<LOD	–	134.45	0.52	216.66	21.63
285	5.76	0.13	51.74	0.19	17.15	0.15	<LOD	–	74.65	0.27	93.31	2.22
286	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	28.63	0.44
287	1.68	0.01	16.56	0.22	7.39	0.19	<LOD	–	25.63	0.29	60.72	4.91
288	3.73	0.01	35.09	0.32	23.35	0.03	<LOD	–	62.17	0.32	78.72	1.56
289	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	26.54	4.41
289	<LOD	–	3.11	0.15	1.3	0.17	<LOD	–	4.41	0.23	34.24	4.41
290	3.34	0.12	6.81	0.03	5.83	0.01	<LOD	–	15.98	0.12	26.73	2.81
291	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	18.53	0.83
292	0.35	0.01	17.5	0.04	8.18	0.01	<LOD	–	26.03	0.04	171.34	2.83
293	3.95	0.01	19.68	0.1	9.79	0.17	<LOD	–	33.42	0.2	54.69	1.53
294	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	37.6	2.41
295	1.83	0.06	20.41	0.16	9.75	0.11	<LOD	–	31.99	0.2	34.08	1.28
296	6.11	0.03	50.2	0.13	21.6	0.01	<LOD	–	77.91	0.13	118.24	1.73
297	4.21	0.23	22.33	0.01	5.69	0.01	5.66	0.15	37.89	0.27	40.67	0.7
298	<LOD	–	5.78	0.41	2.09	0.09	<LOD	–	7.87	0.42	72.83	2.92
300	6.11	0.2	30.22	0.18	10.12	0.21	<LOD	–	46.45	0.34	163.07	5.41
301	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	26.74	2.69
302	3.89	0.08	7.2	0.09	<LOD	–	6.9	0.78	17.99	0.79	15.22	2.05
303	<LOD	–	15.86	0.11	8.43	0.27	<LOD	–	24.29	0.29	52.33	0.11
304	4.32	0.09	34.16	0.22	17.7	0.34	<LOD	–	56.18	0.41	50.36	3.18
305	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	44.85	0.12
306	6.88	0.04	28.15	0.24	16.8	0.26	<LOD	–	51.83	0.36	74.01	1.78

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
307	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	38.7	2.13
308	1.64	0.05	20.55	0.06	11.28	0.03	<LOD	–	33.47	0.08	35.1	3.61
309	1.52	0.02	19.37	0.05	12.31	0.08	<LOD	–	33.2	0.1	202.45	5.48
310	<LOD	–	14.4	0.01	8.28	0.01	<LOD	–	22.68	0.01	56.71	9.1
311	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	17.73	3.41
312	<LOD	–	10.59	0.16	<LOD	–	<LOD	–	10.59	0.16	38.87	2.11
313	<LOD	–	9.62	0.02	<LOD	–	<LOD	–	9.62	0.02	49.81	3.05
314	<LOD	–	6.63	0.02	<LOD	–	<LOD	–	6.63	0.02	26.82	1.32
315	3.03	0.08	14.82	0.05	7.95	0.02	<LOD	–	25.8	0.1	52.54	3.06
316	7.01	0.11	23.9	0.7	16.64	0.06	15.33	0.47	62.88	0.85	55.61	3.7
317	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	24.53	0.85
318	3.22	0.01	19.15	0.16	9.94	0.05	<LOD	–	32.31	0.17	51.06	2.66
319	6.55	0.08	31.64	0.22	12.71	0.13	<LOD	–	50.9	0.27	73.29	4.44
320	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	21.47	0.13
321	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	118.67	17.50
322	17.45	0.36	51.72	0.13	31.7	0.03	<LOD	–	100.87	0.38	121.19	0.34
323	<LOD	–	7.03	0.01	<LOD	–	<LOD	–	7.03	0.01	51.43	5.65
324	5.93	0.08	32.14	0.18	13.77	0.08	<LOD	–	51.84	0.21	91.81	4.17
325	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	16.47	1.68
326	<LOD	–	12.42	0.03	<LOD	–	<LOD	–	12.42	0.03	47.14	6.51
327	<LOD	–	28.2	0.13	<LOD	–	<LOD	–	28.2	0.13	156.5	8.87
328	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	111.16	2.45
329	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	69.02	2.97
330	2.18	0.09	21.71	0.1	13.52	0.21	<LOD	–	37.41	0.25	37.3	2.15
331	<LOD	–	10.96	0.03	<LOD	–	<LOD	–	10.96	0.03	90.61	7.62
332	<LOD	–	6.97	0.09	<LOD	–	<LOD	–	6.97	0.09	28.89	1.4
333	<LOD	–	10.11	0.02	<LOD	–	<LOD	–	10.11	0.02	26.54	1.89
334	<LOD	–	13.07	0.08	6.18	0.1	<LOD	–	19.25	0.13	110.98	2.86
335	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	24.98	0.44
336	<LOD	–	14.08	0.02	9.4	0.23	<LOD	–	23.48	0.23	75.69	0.21
337	9.13	0.04	47.88	0.04	15.38	0.19	<LOD	–	72.39	0.2	113.07	1.4
338	13.27	0.04	21.44	0.06	15.09	0.2	<LOD	–	49.8	0.21	118.04	6.47
339	<LOD	–	10.95	0.13	5.86	0.09	<LOD	–	16.81	0.16	88.01	1.5
340	<LOD	–	4.37	0.08	4.41	0.01	<LOD	–	8.78	0.08	24.79	0.6
341	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	13.44	0.96
342	1.74	0.01	21.08	0.17	14.49	0.09	<LOD	–	37.31	0.19	64.47	1.4
343	<LOD	–	13.99	0.06	6.75	0.03	<LOD	–	20.74	0.07	21.32	1.16
345	7.17	0.04	30.84	0.2	13.34	0.04	<LOD	–	51.35	0.2	93.84	2.41
348	4.58	0.13	24.9	0.19	12.54	0.2	<LOD	–	42.02	0.3	85.94	0.93
350	<LOD	–	5.92	0.04	4.11	0.02	<LOD	–	10.03	0.04	38.23	3.16

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
351	<LOD	–	9.18	0.06	6.95	0.01	<LOD	–	16.13	0.06	98.39	6.9
352	8.27	0.08	11.44	0.15	11.37	0.17	<LOD	–	31.08	0.24	64.13	1.26
353	<LOD	–	7.19	0.01	4.63	0.05	<LOD	–	11.82	0.05	61.75	0.2
354	<LOD	–	36.97	0.45	11.62	0.16	<LOD	–	48.59	0.48	46.68	8.78
355	<LOD	–	6.23	0.09	4.11	0.04	<LOD	–	10.34	0.1	61.6	0.6
356	7.24	0.05	22.42	0.02	26.5	0.09	<LOD	–	56.16	0.1	107.7	4.72
433	<LOD	–	1.94	0.03	1.46	0.14	<LOD	–	3.4	0.14	39.35	2.42
434	<LOD	–	2.33	0.08	0.81	0.06	<LOD	–	3.14	0.1	42.27	4.21
435	<LOD	–	2.68	0.04	1.05	0.01	<LOD	–	3.73	0.04	26.96	0.34
437	<LOD	–	1.2	0.06	0.69	0.04	<LOD	–	1.89	0.07	61.15	1.90
438	<LOD	–	1.96	0.4	1.2	0.05	<LOD	–	3.16	0.4	31.68	1.25
439	<LOD	–	2.38	0.03	1.83	0.03	<LOD	–	4.21	0.04	49.91	0.12
440	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	10.07	0.13
442	<LOD	–	4.9	0.34	1.57	0.24	<LOD	–	6.47	0.42	38.82	4.96
443	<LOD	–	4.86	0.24	1.56	0.03	<LOD	–	6.42	0.24	72.45	4.66
444	<LOD	–	2.17	0.14	1.01	0.07	<LOD	–	3.18	0.16	46.97	5.67
445	<LOD	–	2.54	0.04	1.15	0.23	<LOD	–	3.69	0.23	58.86	0.59
446	<LOD	–	1.76	0.06	1.17	0.13	<LOD	–	2.93	0.14	7.10	0.45
447	<LOD	–	5.64	0.1	1.62	0.05	<LOD	–	7.26	0.11	72.46	2.97
448	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	33.13	6.52
449	0.23	0.01	2.84	0.01	1.04	0.03	<LOD	–	4.11	0.03	49.39	1.59
450	<LOD	–	2.09	0.1	<LOD	–	<LOD	–	2.09	0.1	15.50	0.67
451	<LOD	–	4.02	0.02	1.38	0.01	<LOD	–	5.4	0.02	51.62	1.76
452	<LOD	–	4.27	0.02	1.41	0.07	<LOD	–	5.68	0.07	47.98	8.38
453	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	33.25	1.36
455	<LOD	–	2.31	0.03	1.39	0.25	<LOD	–	3.7	0.25	36.54	1.48
456	0.55	0.02	3.13	0.04	1.43	0.01	<LOD	–	5.11	0.05	55.43	0.38
457	<LOD	–	4.92	0.13	1.77	0.01	<LOD	–	6.69	0.13	71.27	10.32
458	<LOD	–	4.59	0.06	1.82	0.05	<LOD	–	6.41	0.08	106.65	3.15
459	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	88.7	2.46
460	<LOD	–	3.79	0.02	1.95	0.03	<LOD	–	5.74	0.04	71.9	3.57
461	<LOD	–	1.65	0.02	0.81	0.1	<LOD	–	2.46	–	32.44	0.11
462	<LOD	–	1.56	0.1	0.73	0.06	<LOD	–	2.29	0.12	30.53	1.44
463	<LOD	–	3.1	0.2	1.77	0.22	<LOD	–	4.87	0.3	105.08	1.17
464	<LOD	–	2.81	0.11	1.11	0.04	<LOD	–	3.92	0.12	33.73	2.89
465	<LOD	–	4.33	0.01	1.89	0.11	<LOD	–	6.22	0.11	70.16	6.07
466	<LOD	–	2.56	0.07	1.11	0.06	<LOD	–	3.67	0.09	16.41	2.08
467	0.74	0.03	6.13	0.33	1.98	0.12	<LOD	–	8.85	0.35	84.03	2.69
468	<LOD	–	4.72	0.47	2.18	0.1	<LOD	–	6.9	0.48	96.21	9.65
469	<LOD	–	2.52	0.21	1.29	0.34	<LOD	–	3.81	0.4	51.18	9.16

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
470	0.47	0.18	3.94	0.43	1.37	0.15	<LOD	–	5.78	0.49	86.47	1.67
471	<LOD	–	6.64	0.1	2.94	0.07	1.04	0.12	10.62	0.17	100.79	3.35
472	<LOD	–	2.18	0.09	1.27	0.28	<LOD	–	3.45	0.29	34.5	3.03
473	<LOD	–	1.55	0.09	0.07	0.04	<LOD	–	1.62	0.1	22.39	0.96
474	<LOD	–	3.11	0.11	1.55	0.04	<LOD	–	4.66	0.12	30.87	1.24
475	<LOD	–	4.16	0.94	1.78	0.54	<LOD	–	5.94	1.08	64.32	3.02
477	<LOD	–	3.78	0.35	1.38	0.02	<LOD	–	5.16	0.35	72.79	4.78
478	<LOD	–	4.5	0.28	1.38	0.02	<LOD	–	5.88	0.28	69.72	1.25
479	<LOD	–	3.12	0.08	1.72	0.15	<LOD	–	4.84	0.17	53.6	3.69
480	<LOD	–	2.36	0.2	0.91	0.21	<LOD	–	3.27	0.29	65.55	0.45
481	<LOD	–	5.07	0.18	1.42	0.07	<LOD	–	6.49	0.19	106.42	0.17
482	<LOD	–	3.23	0.23	1.95	0.01	<LOD	–	5.18	0.23	68.87	1.08
483	<LOD	–	3.13	0.3	1.85	0.05	<LOD	–	4.98	0.3	55.17	1.37
484	<LOD	–	2.31	0.02	1.17	0.04	<LOD	–	3.48	0.04	15.90	0.10
485	<LOD	–	2.31	0.14	0.9	0.11	<LOD	–	3.21	0.18	61.2	8.89
486	<LOD	–	5.22	0.1	1.81	0.07	<LOD	–	7.03	0.12	56.27	0.42
488	1.28	0.11	5.31	0.09	2.08	0.09	<LOD	–	8.67	0.17	72.74	7.5
489	<LOD	–	1.98	0.04	1.39	0.06	<LOD	–	3.37	0.07	58.59	1.92
490	0.81	0.07	3.74	0.07	1.5	0.2	<LOD	–	6.05	0.22	54.8	1.88

Appendix 3. Arsenic speciation results in Nevşehir area (arsenic level in drinking water less than 10 µg L^{-1}; all concentrations are in µg L^{-1}).

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
500	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	10.48	0.64
501	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	1.25	0.05
502	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	0.82	0.03
503	<LOD	–	1.25	0.01	<LOD	–	<LOD	–	1.25	0.01	9.34	1.01
503	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	10.62	0.74
505	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	8.53	1.71
506	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	8.82	0.48
508	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	5.77	0.07
509	<LOD	–	1.95	0.19	<LOD	–	<LOD	–	1.95	0.19	18.12	1.07
510	<LOD	–	1.96	0.51	<LOD	–	<LOD	–	1.96	0.51	6.91	0.08
512	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	16.93	0.58
513	<LOD	–	1.08	0.02	<LOD	–	<LOD	–	1.08	0.02	8.57	0.09
514	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	14.67	1.76
515	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	2.59	0.04
516	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	20.94	3.27
517	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	36.03	0.85
520	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.13	0.19
521	<LOD	–	2.05	0.25	0.77	0.01	<LOD	–	2.82	0.25	18.06	1.21
522	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	12.85	0.31
523	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	6.39	0.12
524	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	14.93	0.66
525	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	11.30	0.12
526	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	14.64	0.76
527	<LOD	–	2.41	0.01	1.02	0.05	<LOD	–	3.43	0.05	19.63	1.28
528	<LOD	–	1.37	0.11	<LOD	–	<LOD	–	1.37	0.11	15.14	0.01
529	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	1.71	0.21
530	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	2.98	0.12
531	<LOD	–	4.54	0.2	<LOD	–	<LOD	–	4.54	0.2	12.52	0.17
532	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	9.67	1.39
533	<LOD	–	1.77	0.19	<LOD	–	<LOD	–	1.77	0.19	17.08	0.54
534	<LOD	–	1.51	0.17	0.79	0.18	<LOD	–	2.3	0.25	11.47	0.94
535	<LOD	–	1.12	0.02	<LOD	–	<LOD	–	1.12	0.02	9.80	0.62
536	1.33	0.1	3.8	0.23	2.07	0.62	<LOD	–	7.2	0.67	15.89	0.88
538	<LOD	–	1.78	0.08	0.9	0.11	<LOD	–	2.68	0.14	11.76	0.84
540	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.78	0.04
541	<LOD	–	2.07	0.13	1.04	0.03	<LOD	–	3.11	0.13	3.2	0.35
542	<LOD	–	3.18	0.04	1.39	0.03	<LOD	–	4.57	0.05	27.46	1.81

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
543	<LOD	–	2.42	0.31	2.07	0.23	<LOD	–	4.49	0.39	3.98	0.28
544	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	10.62	1.48
545	<LOD	–	1.58	0.02	0.74	0.11	<LOD	–	2.32	0.11	15.16	1.03
546	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	5.39	0.53
547	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.73	0.44
548	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.83	0.16
549	<LOD	–	2.18	0.46	<LOD	–	<LOD	–	2.18	0.46	7.27	0.08
550	<LOD	–	3.95	0.19	<LOD	–	<LOD	–	3.95	0.19	33.61	2.59
551	<LOD	–	2.65	0.13	0.97	0.03	<LOD	–	3.62	0.13	6.21	0.38
552	<LOD	–	1.41	0.13	<LOD	–	<LOD	–	1.41	0.13	18.48	0.55
553	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.45	0.09
554	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	0.67	0.07
555	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	11.65	0.86
556	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	5.31	0.45
557	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	3.54	0.28
558	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	2.61	0.02
559	<LOD	–	1.28	0.22	<LOD	–	<LOD	–	1.28	0.22	13.80	0.22
560	<LOD	–	2.91	0.06	1.84	0.31	<LOD	–	4.75	0.32	10.2	0.24
561	<LOD	–	1.61	0.08	0.84	0.07	<LOD	–	2.45	0.11	7.59	0.63
562	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	0.9	0.11
563	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	2.51	0.27
564	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	10.26	0.18
565	<LOD	–	1.52	0.11	1.09	0.01	<LOD	–	2.61	0.11	26.15	2.25
566	<LOD	–	2.45	0.21	1.12	0.09	<LOD	–	3.57	0.23	18.75	0.24
567	<LOD	–	3.85	0.06	<LOD	–	<LOD	–	3.85	0.06	20.17	0.14
568	<LOD	–	1.35	0.01	<LOD	–	<LOD	–	1.35	0.01	18.47	1.58
569	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.49	0.39
570	<LOD	–	2.11	0.04	0.92	0.04	<LOD	–	3.03	0.06	14.30	0.48
571	<LOD	–	1.34	0.09	1	0.03	<LOD	–	2.34	0.09	12.18	0.59
572	<LOD	–	1.68	0.11	0.61	0.01	<LOD	–	2.29	0.11	6.69	0.49
573	<LOD	–	1.25	0.18	<LOD	–	<LOD	–	1.25	0.18	13.43	0.18
574	<LOD	–	1.74	0.04	1.05	0.13	<LOD	–	2.79	0.14	104.39	0.55
575	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	6.81	0.32
576	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	3.22	0.09
577	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.81	0.42
578	<LOD	–	1.67	0.01	0.86	0.01	<LOD	–	2.53	0.01	8.54	0.47
578	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	12.46	1.35
579	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	2.71	0.21
580	<LOD	–	1.71	0.12	<LOD	–	<LOD	–	1.71	0.12	14.49	0.02
581	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	27.96	1.02

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
582	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	6.91	0.89
583	<LOD	–	1.36	0.01	0.86	0.05	<LOD	–	2.22	0.05	14.05	0.29
584	<LOD	–	1.58	0.02	<LOD	–	<LOD	–	1.58	0.02	8.24	0.14
585	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	5.01	0.23
586	<LOD	–	3.68	0.28	<LOD	–	<LOD	–	3.68	0.28	12.83	0.21
587	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	7.71	0.73
588	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	10.21	0.85
589	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	3.85	0.17
590	<LOD	–	1.17	0.03	0.75	0.05	<LOD	–	1.92	0.06	12.5	0.41
591	<LOD	–	1.2	0.13	0.91	0.08	<LOD	–	2.11	0.15	11.61	0.99
592	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	6.26	0.30
593	<LOD	–	4.77	0.01	1.52	0.01	<LOD	–	6.29	0.01	56.68	1.97
600	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	0.62	0.10
601	<LOD	–	1.94	0.24	0.8	0.1	<LOD	–	2.74	0.26	39.38	1.58
602	<LOD	–	0	–	<LOD	–	<LOD	–	<LOD	–	7.90	0.12
604	0.65	0.07	2.11	0.22	<LOD	–	<LOD	–	2.76	0.23	12.87	0.55
605	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	6.50	0.24
606	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	11.44	0.38
607	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	5.89	0.16
608	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	9.65	0.06
609	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	23.91	2.42
610	<LOD	–	2.13	0.3	0.89	0.04	<LOD	–	3.02	0.3	40.26	1.74
612	<LOD	–	1.34	0.04	<LOD	–	<LOD	–	1.34	0.04	16.22	0.83
614	<LOD	–	1.8	0.01	0.92	0.11	<LOD	–	2.72	0.11	12.11	0.43
615	<LOD	–	3.28	0.07	1.51	0.02	<LOD	–	4.79	0.07	19.85	0.29
616	<LOD	–	1.53	0.02	0.52	0.07	<LOD	–	2.05	0.07	3.55	0.27
617	<LOD	–	1.73	0.17	<LOD	–	<LOD	–	1.73	0.17	44.37	0.73
618	<LOD	–	4.59	0.61	1.35	0.15	<LOD	–	5.94	0.63	44.96	1.41
619	<LOD	–	3.36	0.01	<LOD	–	<LOD	–	3.36	0.01	20.95	0.19
620	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	23.27	0.67
621	<LOD	–	5.55	0.86	<LOD	–	<LOD	–	5.55	0.86	147.86	6.13
625	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	13.43	0.62
626	<LOD	–	1.77	0.16	0.89	0.04	<LOD	–	2.66	0.16	14.92	0.12
627	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	0.85	0.09
628	<LOD	–	2.16	0.21	1.09	0.09	<LOD	–	3.25	0.23	105.88	4.18
630	<LOD	–	1.52	0.04	0.62	0.09	<LOD	–	2.14	0.1	13.61	0.27
631	<LOD	–	2.18	0.15	<LOD	–	<LOD	–	2.18	0.15	16.97	0.34
633	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	16.33	0.47
634	<LOD	–	2.33	0.17	0.96	0.1	<LOD	–	3.29	0.2	14.97	0.46
635	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	1.66	0.27

Code	As(III)	SD(±)	DMA	SD(±)	MMA	SD(±)	As(V)	SD(±)	Sum	SD(±)	Total As	SD(±)
636	<LOD	–	1.21	0.05	0.6	0.11	<LOD	–	1.81	0.12	6.95	0.85
637	<LOD	–	4.67	0.08	1.49	0.1	<LOD	–	6.16	0.13	17.8	2.12
639	<LOD	–	1.1	0.01	<LOD	–	<LOD	–	1.1	0.01	3.56	0.18
640	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	3.31	0.48
641	<LOD	–	1.48	0.12	<LOD	–	<LOD	–	1.48	0.12	11.54	0.59
642	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	67.70	1.30
643	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	9.92	0.47
645	<LOD	–	2.9	0.51	0.57	0.04	<LOD	–	3.47	0.51	194.63	0.89
646	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	8.84	0.96
647	<LOD	–	2.72	0.57	1.07	0.23	<LOD	–	3.79	0.61	138.45	6.96
648	<LOD	–	1.67	0.01	0.95	0.18	<LOD	–	2.62	0.18	20.90	2.55
649	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	5.87	0.20
650	<LOD	–	1.59	0.05	0.65	0.01	<LOD	–	2.24	0.05	45.43	4.32
651	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	53.41	2.44
652	<LOD	–	1.86	0.31	0.96	0.02	<LOD	–	2.82	0.31	12.20	1.04
653	0.65	0.05	3.31	0.09	1.48	0.12	<LOD	–	5.44	0.16	26.87	1.52
654	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	13.68	0.41
655	<LOD	–	2.17	0.23	<LOD	–	<LOD	–	2.17	0.23	9.95	1.09
656	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	4.41	0.12
657	1.02	0.12	3.26	0.46	1.55	0.01	<LOD	–	5.83	0.48	59.41	4.33
658	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	27.05	0.41
659	<LOD	–	1.61	0.2	0.83	0.04	<LOD	–	2.44	0.2	13.59	0.67
660	<LOD	–	3.49	0.2	<LOD	–	<LOD	–	3.49	0.2	3.28	0.17
661	<LOD	–	2.6	0.3	1.17	0.33	<LOD	–	3.77	0.45	9.29	0.10
662	<LOD	–	1.03	0.02	<LOD	–	<LOD	–	1.03	0.02	11.08	0.26
663	<LOD	–	1.56	0.25	1.03	0.1	<LOD	–	2.59	0.27	10.89	0.38
664	<LOD	–	<LOD	–	<LOD	–	<LOD	–	<LOD	–	7.35	0.47
665	<LOD	–	1.52	0.02	<LOD	–	<LOD	–	1.52	0.02	2.33	0.13
666	<LOD	–	4.41	0.02	1.59	0.05	<LOD	–	6	0.05	37.80	0.90
667	<LOD	–	3	0.62	<LOD	–	<LOD	–	3	0.62	68.96	0.06

11 CURRICULUM VITAE

PERSONAL INFORMATION	Marital status Nationality Date of birth Place of birth	Married and has a daughter (F. Lina, ~4 years) and a son (Ahmed, ~10 months) Palestinian December 18, 1977 Khanyounis, Gaza Strip, Palestine
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	September 1997 – August 2001	BS in chemistry (CGPA: 2.63/4.00) Chemistry Department Middle East Technical University (METU), Ankara, TURKEY * 2000 -2001, Analytical Chemistry Option.
	September 2001 – December 2004	MS in analytical chemistry (CGPA: 3.00/4.00) Chemistry Department Middle East Technical University (METU), Ankara, TURKEY Supervisor: Prof. Dr. Sezer Aygün <u>Thesis title:</u> Separation and quantitation of some platinum group metals by RP-HPLC
	September 2007 – March 2013	Ph.D. in analytical chemistry (CGPA: 4.00/4.00) Department of Analytical Chemistry Faculty of Pharmacy Gazi University Ankara, TURKEY Supervisor: Prof. Dr. Nusret Ertaş Co-supervisor: Prof. Dr. O. Yavuz Ataman <u>Thesis title:</u> Speciation studies by capillary electrophoresis (CE) and high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS)

LANGUAGES	Arabic (Native) English (Fluent) Turkish (Fluent)	
WORK EXPERIENCE	June – July, 2000	Summer Practice, Emergency Unit, Biochemistry Laboratory. Cerrahpaşa Faculty of Medicine, Istanbul University Istanbul, Turkey
	2002 – 2007	Delta Translation Office, Ankara, Turkey English – Arabic Arabic – English Turkish – Arabic * May 2004, Translator for the Organization of the Islamic Conference (OIC), Antalya, Turkey
RESEARCH INTERESTS	Chromatographic and electrodriven separation sciences, Elemental speciation analysis, Microextractions, Ultratrace determination of organic and inorganic substances of health and environmental concern.	
TECHNICAL EXPERIENCE	Capillary Electrophoresis (CE) High Performance Liquid Chromatography (HPLC) Hydride Generation-Atomic Fluorescence Spectrometry (HG–AFS) Inductively Coupled Plasma-Mass Spectrometry (ICP–MS) Gas Chromatography (GC) Atomic Absorption and Emission Spectrophotometry (AAS and AES) Inductively Coupled Plasma-Optical Emission Spectrometry (ICP–OES) Ultraviolet/Visible spectrophotometry (UV/VIS) Fourier Transform Infrared Spectroscopy (FTIR)	
PUBLICATIONS	**JOURNAL PAPERS** 1. **U. Alshana**, I. Lubbad, N. G. Göğer, İ. Çok, U. Tamer, N. Ertaş, Dispersive liquid-liquid microextraction based on solidification of floating organic drop combined with counter-electroosmotic flow normal stacking mode in capillary electrophoresis for the determination of bisphenol A in water and urine samples. J. Liq. Chromatogr. R. T., In press.	

2. M. F. Us, **U. Alshana**, I. Lubbad, N. G. Göğer, N. Ertaş, Dispersive liquid-liquid microextraction based on solidification of floating organic drop combined with field-amplified sample injection in capillary electrophoresis for the determination of beta(2)-agonists in bovine urine. Electrophoresis, 34 (2013) 854–861.

3. **U. Alshana**, N. G. Göğer, N. Ertaş, Dispersive liquid-liquid microextraction combined with field-amplified sample stacking in capillary electrophoresis for the determination of non-steroidal anti-inflammatory drugs in milk and dairy products. Food Chemistry, 138 (2013) 890–897.

4. **U. Alshana**, N. G. Göğer, N. Ertaş, Ultrasound-assisted emulsification microextraction for the determination of ephedrines in human urine by capillary electrophoresis with direct injection. Comparison with dispersive liquid–liquid microextraction. J. Sep. Sci. 35 (2012) 2114–2121.

5. **U. Alshana**, and R. S. Aygun, Determination of platinum and palladium in soil as their chelates with N,N-Diethyl-N '-benzoylthiourea by RP-HPLC. J. Liq. Chromatogr. R. T. 34 (2011) 1326–1339.

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1. **U. Alshana**, N. G. Göğer, and N. Ertaş, Dispersive liquid-liquid microextraction combined with field-amplified sample stacking in capillary electrophoresis for the determination of non-steroidal anti-inflammatory drugs in milk and dairy products. The 8th Aegean Analytical Chemistry Days (AACD) (Izmir, Turkey: 16-20 September, 2012).

2. M. F. Us, **U. Alshana**, I. Lubbad, and N. Ertaş, Determination of four beta(2)-agonists in bovine urine using dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry. The 8th Aegean Analytical Chemistry Days (AACD) (İzmir, Turkey: 16-20 September, 2012).

3. **U. Alshana**, N. Ertaş, O. Y. Ataman, E. Kadıoğlu, N. D. Hisarlı, E. Aşık, G. Ç. Demircigil, C. R. Çelebi, E. Atabey, H. Serçe, N. Bilir, A. M. Tuncer, and S. Burgaz, Speciation of urinary arsenic in Nevşehir area, Turkey, by high performance liquid chromatography coupled to hydride-generation atomic fluorescence spectrometry. The 8th Aegean Analytical Chemistry Days (AACD): 16-20 September, 2012).

4. **U. Alshana**, N. G. Göğer, and N. Ertaş, Dispersive liquid-liquid microextraction combined with field-amplified sample stacking-capillary electrophoresis for the determination of non-steroidal anti-inflammatory drugs in milk and dairy. VI. Ulusal Analitik Kimya Kongresi (Hatay, Turkey: 03-07 September 2012).

5. M. F. Us, **U. Alshana**, I. Lubbad, N. G. Göğer, and N. Ertaş, A novel combination of dispersive liquid-liquid microextraction based on solidification of floating organic drop with field-amplified sample injection capillary electrophoresis for preconcentration and determination of beta(2)-agonists in bovine urine. VI. Ulusal Analitik Kimya Kongresi (Hatay, Turkey: 03-07 September 2012).

6. **U. Alshana**, I. Lubbad, N. G. Göğer, İ. Çok, U. Tamer, and N. Ertaş, Dispersive liquid-liquid microextraction based on solidification of floating organic drop combined with counter-electroosmotic flow normal stacking mode in capillary electrophoresis for the determination of bisphenol A in water and urine samples. VI. Ulusal Analitik Kimya Kongresi (Hatay, Turkey: 03-07 September 2012).

7. E. Kadıoğlu, N. D. Hisarlı, E. Aşık, G. Ç. Demircigil, **U. Alshana**, N. Ertaş, C. R. Çelebi, E. Atabey, O. Y. Ataman, H. Serçe, N. Bilir, A. M. Tuncer, and S. Burgaz, AS3MT gene polymorphism in residents exposed to inorganic arsenic via drinking water. EUROTOX 2012 (Stockholm, Sweden: 17-20 June 2012).

8. **U. Alshana**, N. G. Göğer, and N. Ertaş, Solidification of organic drop microextraction followed by online back-extraction in capillary electrophoresis. IMA 2011-Instrumental Methods of Analysis-Modern Trends and Applications (Chania, Crete, Greece: 18-22 September 2011).

9. **U. Alshana**, I. Lubbad, N. G. Göğer, İ. Çok, U. Tamer, and N. Ertaş, Solidification of organic drop microextraction combined with capillary electrophoresis for preconcentration and determination of bisphenol A in water samples. IMA 2011-Instrumental Methods of Analysis-Modern Trends and Applications (Chania, Crete, Greece: 18-22 September 2011).

10. S. Yarımkaya, **U. Alshana**, N. Ertaş, and N. G. Göğer, Separation and determination of ephedrine, norephedrine and guaifenesin in pharmaceutical syrup and biological samples using micellar electrokinetic chromatography. IMA 2011-Instrumental Methods of Analysis-Modern Trends and Applications (Chania, Crete, Greece: 18-22 September 2011).

11. **U. Alshana** and N. Ertaş, Speciation of arsenic and antimony by capillary electrophoresis. The 7th Aegean Analytical Chemistry Days (AACD) (Lesvos, Greece: 29 September-3 October, 2010).

12. **U. Alshana** and N. Ertaş, Separation, on-line preconcentration and speciation of arsenic and antimony by micellar electrokinetic chromatography. The 5th National Analytical Chemistry Congress (Erzurum, Turkey: 21-25 June, 2010).

13. **U. Alshana** and R. S. Aygün, Separation and quantitation of Pt(II) and Pd(II) chelates with N,N-diethyl-N'-benzoylthiourea by reversed-phase high performance liquid chromatography. The 35th International Symposium on High Performance Liquid Phase Separations and Related Techniques 'HPLC 2010' (Boston, MA, USA: 19-24 June, 2010).

14. **U. Alshana** and R. S. Aygün, Optimization of a HPLC method for separation of some platinum group metals. The 3rd Black Sea Basin Conference on Analytical Chemistry, Process Related Environmental Analytical Chemistry (Constanta, Romania: 12-14 September, 2005).

15. **U. Alshana** and R. S. Aygün, Separation and enrichment of platinum and palladium in soil, adapted to their determination by RP-HPLC. The 1st International Conference on Air Pollution and Combustion (Ankara, Turkey: 22-25 June, 2005).

16. **U. Alshana**, H. Refiker, S. Tuncel, and R. S. Aygün, Separation and quantitation of some platinum group metals by high performance liquid chromatography. The 4th Aegean Analytical Chemistry Days (AACD) (Aydın, Turkey: 29 September-3 October 2004).

17. H. Refiker, **U. Alshana**, M. Merdivan, and R. S. Aygün, Preconcentration of some precious metals using N,N-diethyl-N'-benzoylthiourea impregnated resin. The 4th Aegean Analytical Chemistry Days (AACD) (Aydın, Turkey: 29 September-3 October 2004).

REFEREE ACTIVITIES

Contribution to peer review activities for the following journals:

- Journal of Liquid Chromatography & Related Technologies,
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