Simultaneous Determination of Arsenite and Arsenate in Arsenic Trioxide Injection by Dual Detection Ion Chromatography

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Abstract

This paper describes a rapid method to determine arsenite assay and arsenate impurity in Arsenic Trioxide Injection using a single conductivity detector. The arsenite assay was determined in a non-suppressed conductivity detection mode and arsenate impurity was quantified in a suppressed conductivity detection mode. Dual-conductivity detections were enabled by valve switching and time programming. The method was validated with respect to specificity, linearity, precision, accuracy, stability, and limit of quantification. The limit of detection and quantification for arsenite were 0.855 mg/L and 2.593 mg/L, and 0.044 mg/L and 0.133 mg/L for arsenate, respectively.

Introduction

Arsenic compounds have been used as medicinal agents for many centuries. Arsenic over time has been used in many preparations including external pastes for the treatment of skin and breast cancers, inhaled as vapor, injected hypodermically or intravenously, taken orally in liquid or solid form (usually as arsenous acid) and even given as enemas. In the 18th century, Dr. Thomas Fowler developed a therapeutic agent by mixing As_2O_3 (1 g) with potassium bicarbonate (1%, w/v, 100 mL). This oral agent was known as Fowler's Solution (1), and was used to treat various malignant diseases such as leukemia, Hodgkin's disease, and pernicious anemia, as well as non-malignant diseases such as eczema, asthma, pemphigus, and psoriasis. Until the introduction of chemotherapy and radiation therapy in the mid-1900's, arsenic was used as one of the standard treatments for chronic myeloid leukemia and other leukemias. Trivalent arsenic is much more cytotoxic than pentavalent arsenic and is, therefore, mostly responsible for the biological effects of this metalloid. In humans, it has been clearly demonstrated that arsenic interacts with the nervous system at several levels (2). However, with the development of chemotherapy and in conjunction with toxicity concerns of arsenical compounds, the use of arsenic diminished through the 20th century and was eventually abandoned.

Recently and worldwide, arsenic trioxide became accepted as the second-best choice for the treatment of acute promyelocytic leukemia (APL). Much is known about its mechanism of action and clinical application, particularly in APL (1,3). An important function of arsenic trioxide is the induction of apoptosis on the APL cells (4). Due to very good results obtained in the APL treatment, arsenic trioxide is also studied as a potential drug for other malignancies like multiple myeloma, an incurable malignancy of plasma cells (immune system cells in bone marrow that produce antibodies) (5).

The active substance specification for arsenic trioxide is relevant for it to be used in parenteral products, and comprises tests for arsenic(III) assay and content of arsenate, in addition to other parameters.

Analytical techniques for arsenic speciation

Several analytical instrumentation techniques are reported for inorganic arsenic speciation (6–8). Arsenic speciation by cathodic stripping voltammetry (9–18) has been reported for freshwater samples. It involves the quantification of arsenite followed by the determination of the total arsenic content, either by the oxidation of arsenite to total arsenate or by the reduction of the available arsenate to total arsenite. This is a time-consuming, indirect quantification method. The technique is sensitive down to the nanogram level. Quantification requires the proper adjustment of the pH value and the addition of various electrolytes in specified quantities, which is tedious and not suitable for automation. The instrument used for this determination is low-priced and utilizes a Hanging Mercury Drop Electrode (HMDE). Mercury vapours are poisonous and the disposal of mercury is a difficult task; therefore, this is not a preferred analytical technique in the pharmaceutical industry. Modified solid-state electrodes and rotating disk electrodes can be used instead of the HMDE. Anodic stripping voltammetry for the determination of arsenic on gold or gold film electrodes is described in various literatures (19–23). However, frequent polishing of the working electrode surface is necessary, and the reproducibility of these techniques is highly matrix-dependent.

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Arsenate can be determined by the molybdenum blue method based on the formation of an antimonyl-phosphomolybdate complex (24). The optimum time for the complex formation is 1 h at room temperature and is slightly favored in daylight. The total arsenic can be determined after the oxidation of arsenite to arsenate. However, phosphate present in a higher concentration must be removed from the matrix before the measurement. An automated photometric procedure allowing in situ arsenic analysis is described by Dasgupta et al. (25).

To a less extent, capillary electrophoresis has also been applied to arsenic speciation (26). Furthermore, the combination of liquid chromatography (LC) or ion chromatography (IC) with other atomic spectrometry detection, such as flame atomic absorption spectrometry (27,28), electrothermal atomic absorption spectrometry (29-31), inductively coupled plasma atomic emission spectrometry (ICP-AES) (32,33), or ICP-mass spectrometry (ICP-MS) (34–42) is used. All these sophisticated analytical techniques are very cost-intensive in terms of instrument acquisition and maintenance.

Butler (43) separated arsenite and arsenate by ion-exclusion chromatography and detected both species by UV detection at 200 nm, but the sensitivity obtained was poor. Tan and Dutrizac (44) determined arsenite and arsenate in metallurgical processing media, simultaneously using electrochemical and suppressed conductivity detection respectively. Sequential determination of arsenite and arsenate in water samples using IC with electrochemical detection and spectrophotometric detection was reported by Zong-li Li (45).

Incomplete suppressed IC is discussed by Huang et al. (46). He suggested to operate the suppressor in a mode such that the eluent is just barely neutralized, so that the arsenite will remain in an ionic state. However, it is very difficult to maintain such an operation condition with a constant background and low-noise environment. The achieved detection limit reported by this method is much lower than that of non-suppressed conductivity detection.

Sequential conductometric determination of arsenite and arsenate is reported by P.K. Dasgupta et al. (47), in which arsenate was determined by suppressed conductometric detection of an electrolytically generated hydroxide eluent and an electrolytic suppressor. Arsenite was determined by post-column potassium hydroxide addition. A second conductivity detector then measured the conductivity of the stream. In this case, two conductivity detectors were used.

In case of suppressed conductivity detection, both arsenite and arsenate exists in their protonated forms. Because the pK_a value of the arsenic acid is 2.1, it will dissociate to an arsenate ion; hence, suppressed conductivity detection is then possible. The pK_as value of arseneous acid is 9.23 and under these conditions it exists as a neutral, undissociated molecule; hence, suppressed conductivity detection is not possible. However, in the case of non-suppressed conductivity detection, there is no change in the pH value of the eluent entering the detector, and arsenite remains in the completely dissociated form, which makes conductivity detection possible. This means that arsenite requires non-suppressed conductivity detection, while arsenate requires suppressed conductivity detection.

In the dual-detection mode discussed in this work, arsenite is determined under non-suppressed conductivity detection, and arsenate by suppressed conductivity detection using a single conductivity detector.

Materials and Methods

Instrument

For all experiments, an advanced modular IC instrument from Metrohm, Herisau, Switzerland was used. The advanced modular IC instrument consisted of an 818 high pressure dual piston pump, an 820 IC separation centre with two injection valves, an 833 suppressor module, an 819 advanced conductivity detector, an 830 dual channel interface, and an 838 advanced sample processor. An 819 conductivity detector has a precisely thermostated conductivity detector block with a temperature stability of ± 0.01°C. This conductivity detector has features to choose different ranges, such as full-scale and auto zero functions. The Metrosep A Supp 5–150 column (150 mm × 4.0 mm, 5 µm particle size), containing polyvinyl alcohol with quaternary ammonium groups, in combination with the Metrosep A Supp 4/5 guard column, was also used. The instrument control and data collection were carried out with the software ICNet 2.3.

Chemicals and reagents

All solutions were prepared using deionized water (> 18 MΩ) purified by a Milli-Q gradient system (Millipore, Billerica, MA). Sodium carbonate (puriss grade, Fluka, 31432), sodium bicarbonate (puriss grade, Fluka 31437), sodium (meta) arsenite ($NaAsO_2$) (Fluka, 71273), sodium hydroxide (50% for IC, Fluka, 72064), and suprapure sulfuric acid (Fluka, 84716) were procured from Sigma-Aldrich, Bangalore, India. Disodium hydrogen arsenite ($Na_2HAsO_4.^7H_2O$) from Merck (order number 6284), Mumbai, India was used as received. 50 mmol/L of sulfuric acid was prepared and used as the suppressor regenerating solution.

Standard solution

The standard stock solution of As(III) was prepared by accurately weighing about 875.7 mg of sodium meta arsenite and diluted to 500 mL with water, which is equivalent to 1000 mg/L of As(III). The water used for dilution was purged with nitrogen for 30 min to remove the dissolved oxygen in it. The standard stock solution of 1000 mg/L of As(V) was prepared by accurately weighing 2.1248 g of disodium hydrogen arsenite and diluting it to 500 mL with water. The lower concentrations of mixed standards were prepared from standard stock solutions.

Mobile phase solution

The mobile phase containing 15 mmol/L sodium hydroxide and 2.0 mmol/L sodium carbonate was prepared by dissolving about 1.58 mL of sodium hydroxide and 214.1 mg of sodium carbonate in 2000 mL of water, and the solution was subjected to sonication for approximately 10 min to remove any air bubbles, and filtered through a 0.45 µm membrane filter.

Test solution

One mL of arsenic trioxide injection was accurately pipetted

out into a clean 10-mL volumetric flask and made up to the mark with water. The diluted sample solutions were filtered through a 0.45 µm membrane filter.

Method

In this experimental setup, a motorized six-port Injection Valve B was installed in between the column and the suppressor. The suppressor inlet and outlet were connected to the Positions 2 and 5 of the six-port injection valve. The column outlet was connected to Port 1 and the conductivity detector was connected to Port 6. Ports 3 and 4 were closed with stoppers.

When the Injection Valve B was in fill position, the eluent from the column outlet bypassed the suppressor; arsenite was detected in a non-suppressed conductivity mode. The polarity of the detector was set to negative so that the analyte was detected as a positive peak. After the arsenite was detected, the detector polarity was set to positive and the Injector Valve B was switched to the inject position so that the eluent flowed through the suppressor, and arsenate was detected in the suppressed conductivity detection mode. Furthermore, the zero allowed for viewing the entire chromatogram on the same scale. The operation was controlled by the software and can be automated. The instrumental setup is shown in Figures 1 and 2. The time program that was used is given in Table I.

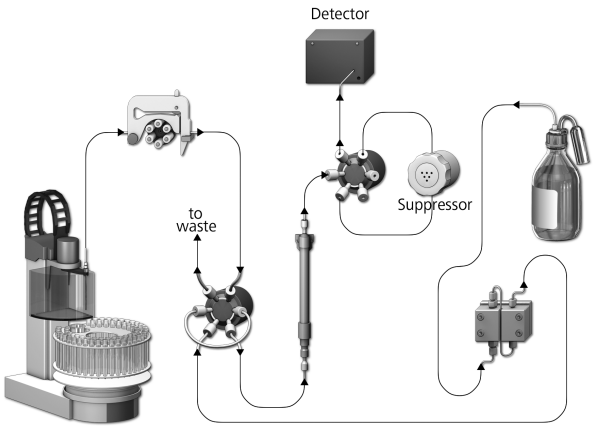

Figure 1. Non-suppressed conductivity detection of arsenite.

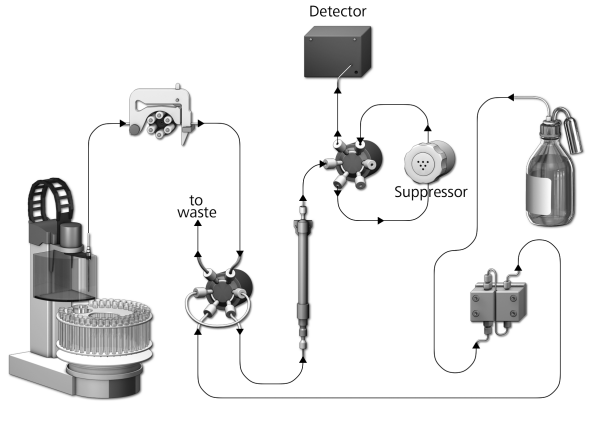

Figure 2. Suppressed conductivity detection of arsenate.

Results and Discussion

Chromatographic optimization

The separation was tried with the default eluent of 3.2 mmol/L sodium carbonate with 1.0 mmol/L sodium bicarbonate for the Metrosep A Supp 5 column. The background conductivity and the noise for this eluent composition under non-suppressed conductivity mode were 780 µS/cm and 1.2 nS/cm, respectively. The detector equilibration time was less than a minute while switching over from suppressed to non-suppressed mode. With this eluent, however, the arsenite peak was eluting closer to the system peak, which is insufficient for the precise quantification.

Hence, eluents with 5, 10, and 15 mmol/L of sodium hydroxide with 2.0 mmol/L sodium carbonate were tested. Although the 5 and 10 mmol/L sodium hydroxide eluents provide lower background conductivity and were suitable for the arsenite determination, a long run time up to 45 min was required. The 15 mmol/L sodium hydroxide eluent composition had a background conductivity of 3200 µS/cm with the noise of 7.5 nS/cm. The detector needed 4 min to produce a stable baseline after switching over from suppressed to non-suppressed mode. With this eluent composition, the arsenate eluted well before 21 min; thus, the total analysis time was reduced to 25 min.

Compared to the carbonate eluent, the sodium hydroxide based eluent gave a three-fold increase in sensitivity for arsenite. One reason for this higher sensitivity is that the equivalent conductance of the hydroxide ion is approximately 2.5 times higher than that of the carbonate ion, but correspondingly the background noise also increases. The other reason is the pH of the eluent. The percentage of ionization depends on the pH value of the medium and the pK_a of the analyte. For weakly acidic substances the percentage of ionization is given by:

$$\% \text{ ionized} = [100/(1+10^{pK_a\text{-}pH})]$$

From this equation, it is obvious that the pH of the eluent should be higher by at least 2 units than the pK_a value of the analyte to achieve more than 99% ionization. The pH value of the used sodium hydroxide and carbonate eluents were 12.3 and 10.5, respectively, which were higher by 3.27 units and 1.27 units, respectively, than the pK_a value of arsenite. Because of this, ionization of arsenite was almost 100% for the selected

Table I. Time Programming for Automated Suppressed and Non-suppressed Conductivity Detection by Valve-Switching

Time	Instrument	Function	Remark
0.00	820.0320 IC Separation Center: Valve B	Fill	non suppressed
0.00	820.0320 IC Separation Center: Valve A	Fill	Sample filling
0.00	819 IC Detector: Polarity	-ve	
2.50	819 IC Detector: Zero	on	
3.00	820.0320 IC Separation Center: Valve A	Inject	Sample inject
11.50	819 IC Detector: Polarity	+ve	
11.50	820.0320 IC Separation Center: Valve B	Inject	Suppressed
14.50	819 IC Detector: Zero	on	

hydroxide eluent, while it was less than 95% for the carbonate eluent. Hence, the peak area for arsenite is higher in the hydroxide eluent compared to the carbonate eluent.

Specificity

With this eluent composition, arsenite was well separated from the injection peak as well as chloride. The relative retention time for nitrate, phosphate, and sulphate with respect to arsenate is 0.31, 0.73, and 0.39, respectively. The selectivity was checked using a blank with water injection, a placebo, and an arsenic trioxide injection. A clear separation of arsenite from the chloride peak confirms the suitability of this chromatographic condition for the determination of arsenite and arsenate in the arsenic trioxide injection. Chromatograms of the placebo, arsenic standards, and the sample are shown in Figure 3A, 3B, and 3C, respectively. The dip between 8 min to 10 min was due to the change in conductivity while switching from the non-suppressed to suppressed mode. By optimizing the tube lengths between the column, Injector B, and the suppressor, the dead volume and the dip size was reduced.

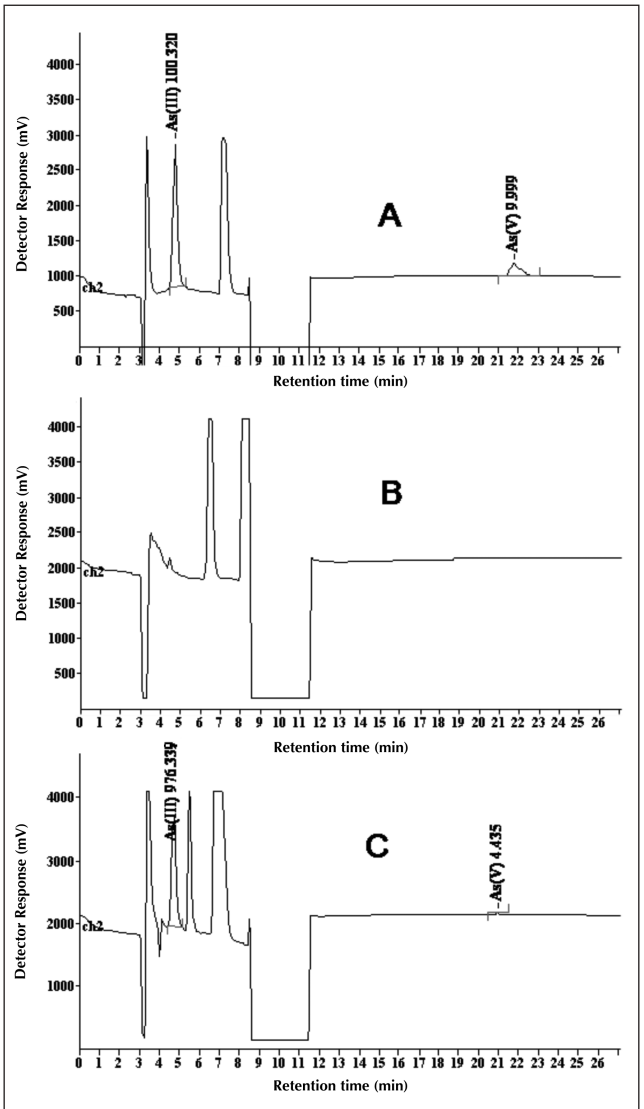

Figure 3. (A) mixed standard: 100 µg/mL arsenite and 10 µg/mL arsenate; (B) placebo; (C) sample: arsenox. Column: Metrosep A Supp 5–150. Eluent: 15 mmol/L sodium hydroxide, +2.0 mmol/L sodium carbonate. Flow rate: 0.7 mL/min. Injection volume: 20 µL.

Linearity

Mixed arsenic standards containing 2, 5, 10, and 100 mg/L of arsenite and 0.2, 0.5, 1.0, and 10 mg/L of arsenate standards were prepared by diluting stock standard solutions with ultrapure water. Prepared standards were injected in triplicate to check the precision and linearity. The relationship between the peak area (mV/s) response and the concentration was found to be linear with the correlation coefficient (r^2) of 0.999 for arsenite and arsenate. The slope (S) and y-intercept values were calculated from the linearity data and the values were 308.2 ± 0.9761 and –378.9 ± 49.12, respectively, for arsenite. The S and y-intercept for arsenate was, respectively, 603.6 ± 0.9808 and –5.197 ± 4.936. The residual standard deviation (SD) was 79.93 and 8.033, respectively, for arsenite and arsenate. The percentage relative standard deviation (RSD) for the response factor was 1.429% and 0.935%, respectively, for arsenite and arsenate.

Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were predicted using the S and the residual SD that were obtained from the linearity study. The formula used for the prediction of LOD and LOQ were 3.3 × SD/S and 10 × SD/S, respectively. The predicted LOD and LOQ levels for arsenite were found to be 0.855 mg/L and 2.593 mg/L, respectively, and for arsenate the predicted LOD and LOQ levels were 0.044 mg/L and 0.133 mg/L, respectively.

Table II. Recovery Data

S. No	Sample identification	Arsenite (µg/mL)	Arsenate (µg/mL)
1	B.No. ATI0001/10/04/07/Initial	1022.2	2.1
2	B.No. ATI0001/10/04/07/2 Week/60°C	1022.1	2.1
3	B.No. ATI0001/10/14/07/1 Month/60°C	1022.0	2.2
4	B.No. ATI0001/11/04/07/Initial	923.4	1.4
5	B.No. ATI0001/11/04/07/2 Week/60°C	923.6	1.5
6	B.No. ATI0001/11/04/07/1 Month/60°C	923.6	1.9
7	B.No. ATI0001/Arsenox initial	976.2	4.4
8	B.No. ATI0001/Arsenox/2 Week/60°C	976.2	4.5
9	B.No. ATI0001/Arsenox/1 month/60°C	976.3	4.8

Table III. Arsenic Trioxide Injection Thermal Stability

Recovery Data					
Arsenite			Arsenate		
Spiked conc. (mg/L)	Detected conc (mg/L)	Recovery (%)	Spiked conc. (mg/L)	Detected conc. (mg/L)	Recovery (%)
100	99.66	99.66	0.1	0.0998	99.80
100	99.73	99.73	0.1	0.0996	99.60
100	99.32	99.32	0.1	0.0992	99.2
Mean	99.57			0.0995	
RSD%	0.22			0.31	

Accuracy and precision

A spiking and recovery study was carried out to check the accuracy of the method. The recovery rate was evaluated by spiking the placebo with stock standards to get 100 mg/L of arsenite and 0.1 mg/L of arsenate. The spiked placebo sample was injected thrice to calculate the precision. A recovery ranging from 99.7% to 99.2% was obtained. The percentage RSD was 0.22% for arsenite, and 0.31% for arsenate.

Stability of sample solution

The stability of the sample solution at room temperature (~25°C) was evaluated by analyzing the sample solutions at different time intervals up to 24 h. The percentage difference between the results obtained from the initial and different time intervals was found to be less than 1 h, suggesting that the sample solution is stable for at least up to 24 h at room temperature (~25°C).

Sample analysis

Based on the validated method previously mentioned herein, several batches of arsenic trioxide stability samples were analyzed. The representative sample result is disclosed in the Table II.

Conclusions

The work described herein offers an economical and time-saving method to quantify the arsenic species in arsenic trioxide injection using only a single conductivity detector. The results obtained from the validation protocol prove that the method is selective, sensitive, linear, accurate, and precise. Hence, this optimized IC method can reliably be used to simultaneously determine arsenite and arsenate content in arsenic trioxide injections, both for quality control and thermal stability assessment.

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