High Performance Liquid Chromatography--Determination of Different Speciations of Arsenic in Cosmetics by Inductively Coupled Plasma Mass Spectrometry

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Abstract. There is a way to determine four arsenic speciations simultaneously in cosmetic by using liquid chromatography (HPLC) with inductively coupled plasma mass spectrometer (ICP-MS). After being extracted by phosphoric acid solution, the samples were separated by PRP-X100 anion chromatographic column. The mobile phase was aqueous solution (pH=6.0) containing 1% methanol (V/V), 2.0 mmol/L NaH2PO4 and 0.2 mmol/L ethylene diamine tetraacetic acid (EDTA). The octopole reaction cell was adopted to eliminate mass spectrum interferences. The separation of AsIII, DMA, MMA and As V was achieved within 12 min with flow rate of 1.00 mL/min and sample size of 100 µL. The recovery rates of AsIII, DMA, MMA and As V ranged from 80.6% to 107.2% and the relative standard deviations were less than 6.24% when the spiked amount was 0.10~ 1.00 µg in different kinds of cosmetic samples. The developed method has the advantage of simplicity, sensitivity and good reproducibility, and can be used for the determination of arsenic speciations simultaneously in cosmetics.

Keywords: Liquid Chromatography, Inductively Coupled Plasma Mass Spectrometry, Cosmetic, Inorganic Arsenic, Speciation

1. Introduction
Arsenic is a non-metallic element that you can find in nature. Large amounts of arsenic compounds exist in water, sediments, soil, plants, marine life and human bodies in various speciations, and can be converted to and from various arsenic compounds [1]. The toxicity of arsenic to humans and ecosystems is not only related to the total amount of the element, but also closely related to its chemical speciations. Since various arsenic speciations have different physical and chemical properties, their toxicity is different. Based on the median lethal dose LD50 of arsenic compounds, the toxicity is AsIII, As V, and Monomethylarsonic acid (MMA), dimethylarsine acid (DMA), arsenocholine (AsC), arszenobetaine (AsB), from the strongest to the weakest. Inorganic arsenic is the most toxic, while organic arsenic is less toxic. Among them, AsC and AsB are often considered non-toxic [2-4].
Cosmetics are light chemical products that are closely related to people's daily life. They are applied to the skin, hair, nails, lips and other human surfaces to achieve the purpose of cleaning, eliminating bad smell, skin care, beauty and embellishment [10]. In recent years, with the development of the beauty industry, various countries have gradually tightened their regulatory measures on cosmetics. Various relevant regulations have been issued to strictly regulate the hygienic chemical indicators and prohibited and restricted substances in cosmetics. Therefore, the inspection of harmful ingredients is particularly necessary. The lack of standards and methods in this area in China has seriously affected the safe use of cosmetics, especially the determination methods of different speciations of harmful heavy metal elements. In the field of cosmetics research, there are currently many literature studies on lead, arsenic, mercury, cadmium and rare earth elements in cosmetics, but there are no reports on the analysis methods of arsenic speciations in cosmetics.

Liquid chromatography or capillary electrophoresis separation, plasma mass spectrometry (ICP-MS) [5], atomic fluorescence spectroscopy (AFS) [6], atomic absorption spectroscopy (AAS) [7] and other technologies are often used in arsenic speciation analysis. Among them, high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) can simultaneously perform online analysis of trace elements in different speciations. Meanwhile, it has advantages like simple interfaces, low detection limits, wide linear ranges, and short analysis time, which makes it become one of the most promising ways in the analysis of the separation of arsenic speciations. It has been used in biological and environmental samples such as seafood, urine, Chinese medicine [8-10] and water sources [11]. In the analysis of arsenic speciations, due to the complex matrix and the low content of arsenic in each speciation, the samples need to be separated and enriched. Moreover, the speciations cannot be changed during the process. Neither dry ashing nor wet acid digestion is suitable for the analysis. In recent years, ultrasonic and microwave-assisted extraction have been widely used in sample pretreatment, which can ensure that various arsenic speciations in the extraction process are effectively dissolved without being destroyed. In this paper, a way for the determination of inorganic arsenic in cosmetics by HPLC-ICP-MS technology was established by using microwave assisted extraction technology.

2. Experiment Process

2.1. Instruments and reagents

7700X inductively coupled plasma mass spectrometer (Agilent, USA); LC 1200 high performance liquid chromatography system (Agilent, USA), equipped with Ailent G1328A pump, Rheodyne 9725 injection valve and 100µL injection loop; ETHOS I microwave digestion system (Milstone, Italy).

Nitric acid (guaranteed reagent), hydrochloric acid (guaranteed reagent), acetic acid (guaranteed reagent), phosphoric acid (guaranteed reagent), sodium hydroxide (guaranteed reagent) and methanol (chromatographically pure) were all bought from Tianjin Kemiou Chemical Reagent Co., Ltd.; EDTA (guaranteed reagent) was purchased from Amresco, USA; experimental water was 18.2 MΩ·cm ultrapure water. Arsenate (AsIII), arsenite (AsV), monomethylarsine acid (MMA) and dimethylarsine acid (DMA) were all purchased from the National Standard Material Research Center. The solutions in the experiment were filtered with 0.45µm microporous membrane before sample injection.

2.2. Experiment Method

2.2.1. Sample pretreatment. Weigh 0.5~1.0 g of sample and put into a centrifuge tube and add 10 mL of 10% (V/V) phosphoric acid solution. After vortexing, transfer to a polytetrafluoroethylene digestion tank, and extract at 60°C for 5 min by microwave. After cooling, transfer to the centrifuge tube. Wash the inner tank with a small amount of pure water, centrifuge with 9000 r/min at 4°C for 10 min, dilute volume to 25 mL with pure water, filter with a 0.45 µm membrane, and test the inorganic arsenic on the equipment.
Weigh 0.50 g of the sample and put into a polytetrafluoroethylene digestion tank. After adding 6.0 mL concentrated HNO$_3$ and soaking for 1 h, digest the sample by microwave. After cooling, take out the digestive solution, and dilute volume to 25 mL with pure water for the determination of total arsenic by ICP-MS.

2.2.2. LC and ICP-MS conditions. Chromatographic column: Anion guard column IonPac AG19 (50 mm×4 mm); anion analytical column IonPac AS19 (250 mm×4 mm); flow rate: 1.0 mL/min; sample size: 100 µL.

The conditions of ICP-MS were optimized based on the indicators of sensitivity, oxide and double charge yield. Through the tuning program set by the instrument, 1 µg/L 7Li, 89 Y, 140Ce, 205Tl tuning solution was used to optimize the ICP-MS parameters to obtain the prime SNR (Signal to Noise Ratio) and the stability of the baseline. The collision reaction cell was used to eliminate the mass spectrum interference of 40Ar35Cl$^{2+}$ on 75As. The optimized ICP-MS parameters are shown in Table 1.

| Parameters                    | Value  |
|-------------------------------|--------|
| RF power/W                    | 1250   |
| Reflected power/W             | 2      |
| Carrier flow/L·min$^{-1}$     | 0.85   |
| Make up flow/L·min$^{-1}$     | 0.15   |
| Nebulization temperature/℃    | 2      |
| Sampling cone/mm              | 1.0    |
| Skimmer cone/mm               | 0.4    |
| Lifting speed/mL·min$^{-1}$   | 1.0    |
| Number mass                   | 75     |
| He/ mL·min$^{-1}$             | 4.2    |

3. Results and Discussion

3.1. Selection of Pretreatment Conditions

The experiment investigated the extraction effects of pure water and aqueous solution with methanol of 50% volume ratio, aqueous solution with hydrochloric acid of 10% volume ratio, aqueous solution with acetic acid of 10% volume ratio, and aqueous solution with phosphoric acid of 10% volume ratio. The results revealed that the extraction efficiency of inorganic arsenic reached more than 60%, and the extraction efficiency of pure water was relatively low. When extracting from methanol solution, nitrogen sweeping or heating was required to remove methanol, which was cumbersome. When hydrochloric acid solution was put into ICP-MS, chlorine and argon interference appeared. When 10% acetic acid was used, a large amount of carbon was caused and likely to produce a matrix sensitization effect on arsenic [12]. Finally, the 10% phosphoric acid solution was selected as the extraction solvent.

The extraction temperature is a significant factor in resolving the extraction efficiency of inorganic arsenic as well. This paper examined the traditional water bath temperature conditions when a 10% phosphoric acid solution was used as the extracting solution. The results show that when the temperature is 60°C, the extraction efficiency of inorganic arsenic that needs to be extracted for 16 to 18 hours is the highest. Microwave extraction is a fast, efficient and energy-saving sample preparation method using microwave as energy. The study investigated the extraction efficiency of inorganic arsenic under different microwave conditions, and it was found that microwave extraction at 60°C for
5 min can achieve better extraction efficiency, and different speciations of arsenic compounds can be extracted simultaneously.

3.2. Selection of Chromatographic Conditions
At present, the separation of arsenic speciations mostly uses ion exchange mechanism. In this paper, an anion exchange column was selected for separation, and the phosphate solution was chosen to do isocratic eluting. Experiments show that the concentration of NaH$_2$PO$_4$ in the mobile phase had little effect on the chromatographic separation of arsenic speciations. For avoiding the effect of excessive sodium salt on the plasma moment, the concentration of NaH$_2$PO$_4$ was determined to be 2 mmol/L. Adding EDTA to the mobile phase could eliminate the loss of arsenic in the chromatographic separation process. The study examined the influence of different concentrations of EDTA on the peak time of different speciations of arsenic. It can be seen from Figure 1 that the increase of EDTA concentration shortened the retention time of DMA and AsIII, when 0.2mmol/LEDTA was added to the mobile phase, the separation effect was the best.

![Figure 1](image1.png)

**Figure 1.** Effect of EDTA on retention time of arsenic speciations

![Figure 2](image2.png)

**Figure 2.** Effect of pH value on retention time of arsenic speciations
With 2 mmol/L NaH$_2$PO$_4$ and 0.2 mmol/LEDTA as mobile phases, the retention time of different arsenic speciations under the condition of pH 4.0~10.0 was investigated. It can be seen from Figure 2 that pH value had little effect on the retention time of As$_{III}$, because the pKa of As$_{III}$ was 9.2. Before pH reached 9.2, As$_{III}$ existed in the form of H$_3$AsO$_3$, and there was almost no retention in the column. When pH>8.0, the retention time of DMA shortened with the increase of pH value. When pH>10.0, its peak time was after As$_{III}$. The retention time of DMA shortened with the increase of pH value when the value of pH was between 4.0 and 8.0, remained basically unchanged when the value of pH was between 6.0 to 8.0, and increased with the increase of pH value when pH value was above 8.0. The retention time of As$_{V}$ gradually decreased in the range of pH 4.0 to 6.0, and increased in the range of pH 6.0 to 8.0. Thus, the final pH value of the mobile phase was set as 6.0. Phosphoric acid and sodium hydroxide were used to adjust the pH value to avoid matrix interference. In addition, the addition of methanol has effectively improved the sensitivity and shorten the peak time. It was determined that the methanol content in the eluent was 1% (V/V). As shown in Figure 2, the four arsenic speciations in the standard solution have been completely separated within 12 minutes.

**Figure 3.** HPLC-ICP-MS chromatogram of five kinds of arsenic speciations

### 3.3. Linear Relationship and Detection Limit

ICP-MS has a wide linear range. The experiment performed HPLC-ICP-MS analysis on mixed standard solutions of different speciations of arsenic at concentrations of 5, 10, 20, 50 and 100 µg/L. The researchers took different concentrations as the abscissa, the peak area after deducting the mobile phase blank as the ordinate, and drew the working curve according to the results. The regression equation, correlation coefficient and detection limit are listed in Table 2.

| Arsenic speciations | Regression equation | Correlation coefficient | Concentration range /µg·L$^{-1}$ | LOD /µg·L$^{-1}$ |
|---------------------|---------------------|-------------------------|-----------------------------------|-----------------|
| As$_{III}$          | $Y = 566124X^2 + 92698$ | 0.9994                  | 5~100                             | 0.3             |
| DMA                 | $Y = 986256X^2 + 24960$ | 0.9998                  | 5~100                             | 0.2             |
| MMA                 | $Y = 584043X^2 + 61255$ | 0.9996                  | 5~100                             | 0.6             |
| As$_{V}$            | $Y = 434018X^2 + 51724$ | 0.9992                  | 5~100                             | 0.5             |
### Table 3. Precision and recovery rates (n=6)

| Sample          | Arsenic speciations | Added /µg | Recovery /% | RSD /% |
|-----------------|---------------------|-----------|-------------|--------|
|                 | AsIII               | 0.10      | 85.1 ~ 89.6 | 3.81   |
|                 |                     | 0.50      | 88.8 ~ 101.3| 4.83   |
|                 |                     | 1.00      | 90.6 ~ 103.2| 5.48   |
| (Smoothing toner)| DMA                 | 0.10      | 85.8 ~ 96.2 | 2.96   |
|                 |                     | 0.50      | 81.7 ~ 104.8| 5.62   |
|                 |                     | 1.00      | 90.2 ~ 101.6| 5.08   |
|                 | MMA                 | 0.10      | 81.2 ~ 90.8 | 2.81   |
|                 |                     | 0.50      | 88.8 ~ 101.3| 4.83   |
|                 |                     | 1.00      | 90.6 ~ 103.2| 5.48   |
|                 | AsV                 | 0.10      | 82.4 ~ 98.2 | 3.16   |
|                 |                     | 0.50      | 88.7 ~ 106.8| 6.24   |
|                 |                     | 1.00      | 90.2 ~ 103.6| 5.26   |
| (Skin lotion)   | AsIII               | 0.10      | 88.6 ~ 93.5 | 3.24   |
|                 |                     | 0.50      | 89.6 ~ 102.1| 5.44   |
|                 |                     | 1.00      | 90.8 ~ 103.8| 4.57   |
|                 | DMA                 | 0.10      | 89.7 ~ 102.2| 2.52   |
|                 |                     | 0.50      | 92.1 ~ 104.7| 4.31   |
|                 |                     | 1.00      | 92.5 ~ 107.2| 3.95   |
|                 | MMA                 | 0.10      | 80.6 ~ 92.5 | 3.42   |
|                 |                     | 0.50      | 84.2 ~ 101.5| 5.68   |
|                 |                     | 1.00      | 90.8 ~ 103.8| 4.57   |
|                 | AsV                 | 0.10      | 82.7 ~ 96.5 | 3.12   |
|                 |                     | 0.50      | 88.6 ~ 104.7| 5.69   |
|                 |                     | 1.00      | 92.4 ~ 106.5| 4.95   |

#### 3.4. Method’s Precision and Recovery Rates

In the experiment, multiple cosmetic samples were accurately weighed, and mixed standard solutions of different arsenic speciations with different concentrations were added. The recovery rates of different speciations of arsenic were calculated by chromatographic peak integral quantification. The results are shown in Table 3. When the spiked amount in the sample was 0.10~1.00 µg, the recovery rates of AsIII, DMA, MMA and AsV ranged from 85.1%~103.8%, 85.8 ~ 107.2%, 80.6%~103.8%, 82.4%~106.5%, RSD was less than 6.24%. The experiment shows that in the sample processing and separation analysis process of this method, there wasn’t mutual conversion of arsenic speciations. The arsenic in the cosmetic samples selected for the experiment mostly existed in the form of inorganic arsenic.
4. Conclusion
In this paper, high performance liquid chromatography and inductively coupled plasma mass spectrometry were combined and used to isolate and determine the different speciations of arsenic in cosmetics online. In the experiment, 10% phosphoric acid solution was used as the extraction solvent, and the collision cell technology was applied to eliminate the spectroscopic interference of $^{40}\text{Ar}^{35}\text{Cl}^+$ on As during the ICP-MS determination. The experiment investigated the effects of mobile phase and pH value on the separation determination of different arsenic speciations of As$\text{III}$, DMA, MMA and AsV, and the detection limits were 0.3µg/L, 0.2µg/L, 0.6µg/L, 0.5µg/L, respectively. The actual sample recovery rate ranged from 80.6% to 107.2%. In the process of sample processing and separation analysis, there was no mutual conversion phenomenon of arsenic speciations, which could be used for simultaneous analysis of different arsenic speciations in cosmetics.

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