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Speciation of chromium and arsenic metal ions by RP-HPLC Coupled with AAS

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Abstract:

The speciation of arsenic [As (III) and As (V)] and chromium [Cr (III) and Cr (VI)] was carried out by high performance liquid chromatography. The column used was Econosil C18 (250 * 4:6 mm i.d., particle size 10 µm). The mobile phases consisted of water–acetonitrile (80:20, v/v) for arsenic and 10 mM ammonium acetate buffer (6.0 pH)–acetonitrile (10:90, v/v) for chromium speciation separately and respectively. The detection was carried out by UV–Vis at 410 nm and atomic absorption spectrometer (AAS) respectively and separately. The values of a and Rs of As(III) and As(V) species were 1.4 and 1.5 respectively while the values of a and Rs for Cr(III) and Cr(VI) were 1.35 and 0.2 respectively. The effect of the acetonitrile percentages was also carried out on the speciation of arsenic only. The relative standard deviation and limit of detection were in the range of 0.01–0.02 and 0.4–1:0 l g/ml respectively.

Keywords: Chromium, Arsenic, Speciation, HPLC,AAS

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1. Introduction:

Metal ions are the most dangerous water pollutants due to their acute toxicity and carcinogenicity [1,2]. Some of the metal ions are cumulative poison capable of being assimilated, absorbed in the tissues of the organism causing noticeable adverse physiological effects [3]. It has been reported that the same metal ion may possess different toxicity in its different oxidation states which are responsible for their different physico-chemical and biological activities [4]. The phenomenon of the separation and identification of the different oxidation states, of a particular metal ion, is called as speciation [5-7]. Thus, in order to obtain the information on toxicity and biotransformation of elements in aquatic and biological systems, the speciation of metal ions is of great importance.

Arsenic and chromium have been reported as the acute toxic elements at lower concentrations [8-10]. The toxicity of As(III) is greater than the toxicity of As(V) [9,11-13]. On the other hand, Cr(III) is an essential nutrient while Cr(VI) is carcinogenic and mutagenic agent [14] .The chromatographic methods such as gas chromatography (GC) and high performance liquid chromatography (HPLC) have been used for the speciation of metal ions [15,16]. The costly instruments such as atomic absorption spectrometer, inductively coupled plasma and electrospray mass spectrometry have been coupled with these techniques as the detectors. Keeping all these points into consideration, the objective of this work is to develop an alternative analytical methodology (HPLC method) for the speciation of arsenic and chromium metal ions. Therefore attempts have been made to develop a fast and reliable reversed phase HPLC method for the speciation of arsenic and chromium using UV– Vis detector.

2. Experimental

2.1. Chemicals and reagents

Sodium arsenate (Na_2HAsO_4 .$7H_2O$) and sodium arsenite ($NaAsO_2$) were obtained from Sigma Chemical Co., St Louis, MO, USA. Chromic anhydride (CrO_3) Co., Milwaukee, WI, USA. Chromic chloride ($CrCl_3.6H_2O$) and acetonitrile were obtained from Fisher Scientific Co., Fairlawn NJ, USA. The solutions (0.1 mg/ml) of the individual species of arsenic and chromium were prepared in deionised water.

2.2. Instruments used

The HPLC system consisting of water solvent delivery pump (model 510, Milford, Massachusetts, USA), water injector (model WISP 710B), water tunable absorbence detector (model 484) and water integrator (model 740) was used in this study. Econosil C_{18} column (250 * 4:6 mm i.d., particle size 10 lm) was used and obtained from Alltech Associates Inc. (Deerfield, IL, USA). An alternative detection was also carried out using

atomic absorption spectrometer (AAS) obtained from Perkin Elmer, USA (model 3100).

2.3. Chromatographic conditions

A 20 µl of a mixture of the species of these metal ions were injected on to a HPLC system as described above. The mobile phases used in this study were water–acetonitrile (80:20, v/v) for arsenic and 10 mM ammonium acetate buffer (6.0 pH)–acetonitrile (10:90, v/v) for chromium speciation separately and respectively. The mobile phase was filtered and degassed before the use. The flow rate of the mobile phases was 1.0 ml/min throughout this study. The chart speed was kept constant at 0.1 cm/min. All the experiments were carried out at 23±C^0. The detection was carried out at 410 nm. An alternative detection was also confirmed by using atomic absorption spectrometer. Arsenic and chromium species were determined at 193.7 and 357.9 nm wavelengths separately and respectively using an air acetylene flame (energy source hollow cathode lamps). For this purpose, solutions of various concentrations of sodium arsenate (Na_2HAsO_4.7H2O), sodium arsenite (Na-AsO_2), chromic anhydride (CrO_3) and chromic chloride ($CrCl_3.6H_2O$) were prepared and their absorbences were recorded by AAS. Calibration plots were established, and from these, the concentrations of the arsenic and chromium species were determined. The chromatographic parameters such as capacity factor (k), separation factor (a) and resolution factor (Rs) were calculated. The identification of the separated species of arsenic and chromium was confirmed by running the chromatograms of the individual species under the identical chromatographic conditions. The internal addition method is useful for the identification of a particular species especially in natural water sample and, therefore, the identification of the separated species of arsenic and chromium was confirmed by the internal addition method.

3. Results and discussion

The capacity (k), separation (a) and resolution (Rs) factors of the separated species of arsenic and chromium under the reported chromatographic conditions are presented in Table 1. The typical chromatograms of the separated species of these metal ions are shown in Fig.1A. The order of elution of these metal ion species was confirmed by running the chromatograms of the individual species of the metal ions under identical chromatographic conditions. The confirmation of the species in the mixture was also carried out by the internal addition method. The order of the elution was As (V) >As (III) and Cr (VI) > Cr (III). Sometimes, the other pollutants present in natural water interfere with the detection of arsenic and chromium species by UV–Vis detector and, therefore, AAS was coupled with the HPLC system described above and was used as the detector. It has been observed that the detection of arsenic and chromium species by AAS was quite good. To optimize the chromatographic conditions, mixtures of various alcohols and acetonitrile were tested. As a result of extensive experiments the optimized chromatographic conditions were developed and reported herein.

Fig. 1A shows the base line separation of arsenic species while the separation of chromium species is partial. Table 1 reports the chromatographic parameters namely retention, separation and resolution factors for these metal ions species. The ionic radii of As(III),As(V), Cr(III) and Cr(VI) are 0.58, 0.46, 0.63 and 0.52 A^o respectively [17]. It is interesting to note that the difference of ionic radii of As(III) and As(V) is greater than the difference of the ionic radii of Cr(III) and Cr(VI). Accordingly, the partial resolution of chromium may be explained on the basis of the smaller ionic radii difference. It has also been observed that the speciation of arsenic is controlled by the amount of acetonitrile in the mobile phase. Therefore, the effect of the percentage of acetonitrile in the mobile phase on separation (a) and resolution (Rs) factors was studied. The results are presented in Fig. 2 which show that the values of separation and resolution factors first increase, then decrease and finally became constant. Fig. 2 also shows that the higher values of separation and resolution factors were observed when using water–acetonitrile (80:20 v/v) mobile phase. Therefore, this mobile phase has been chosen for arsenic speciation in this study. The separation of chromium species was partial (Fig. 1A) and, therefore, the effect of acetonitrile on the speciation of chromium was not carried out.

The stationary phase in Econosil C18 column is octadecyl silane bonded to silica gel. Therefore, the separation of the metal ions species on this column may be due to the adsorption and partition of the metal ions species between the mobile and stationary phases. The metal ion species, posses different physical and chemical properties, have different adsorption and partition properties which are responsible for their separation under the reported chromatographic conditions. It may be assumed that the two metal ion species form different van der Waals forces of various magnitudes with the stationary phase. It is very interesting to observe that As(V) and Cr(VI) are eluting first followed by As(III) and Cr(III) which is due to the smaller cationic sizes of As(V) and Cr(VI) species in comparison to As(III) and Cr(III)

species respectively. The relative standard deviation (RSD) was also calculated for the speciation of arsenic and chromium and was in the range of 0.01–0.02. The limit of detection of arsenic and chromium species was also determined and it varied from 0.4 to 1:0 lg/ml. The efficiency of the developed HPLC method was tested by analyzing the species of arsenic and chromium in natural water as shown in Fig. 1B

Table 1. The capacity (k), separation (a) and resolution (Rs) factors for the separated species of arsenic and chromium on Econosil C18 column (250 4:6 mm) using mobile phases water–acetonitrile (80:20, v/v) for arsenic and 10 mM ammonium acetate buffer (pH 6.0)–acetonitrile (10:90, v/v) for chromium species at 1.0 ml/min flow rate.

Metal ions	K_1	K_2	α	Rs
As	4.8[As(V)]	6.1 [As(III)]	1.40	1.5
Cr	2.8 [Cr (VI)]	3.8 [Cr(III)]	1.35	0.2

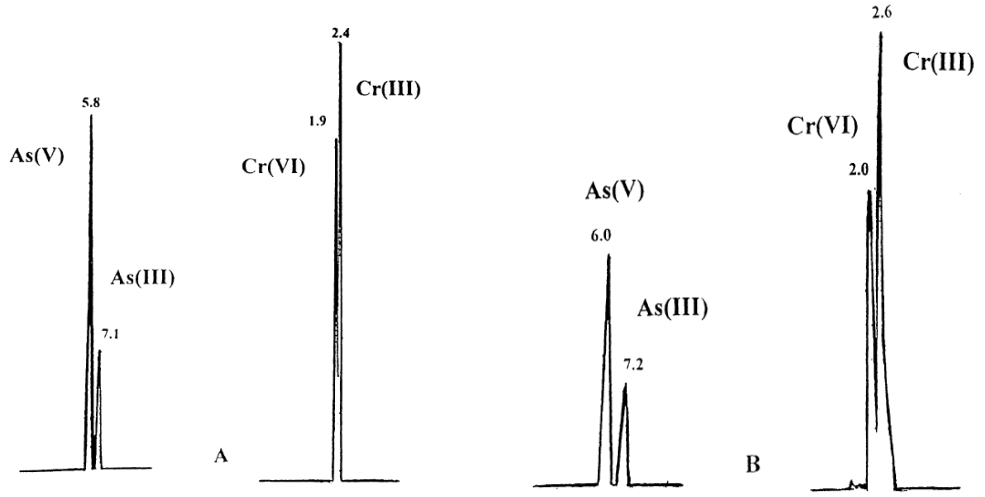

Fig. 1. The chromatograms of the separated species of arsenic and chromium metal ions in (A) synthetic and (B) natural water samples on Econosil C18 column (250 4:6 mm i.d., particle size 10 lm) using mobile phase water–acetonitrile (80:20, v/v) for arsenic and 10 mM ammonium acetate buffer (6.0 pH)– acetonitrile (10:90, v/v) for chromium at 1.0 ml/min flow rate.

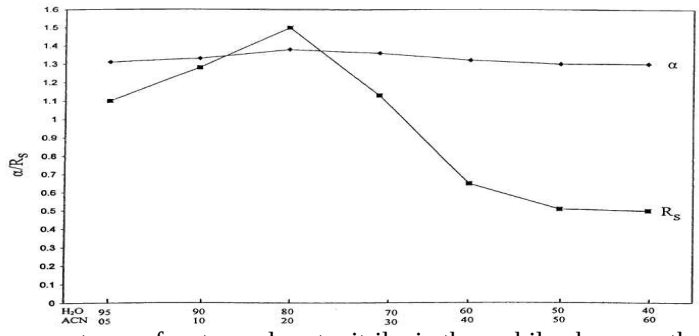

Fig. 2. The effect of the percentages of water and acetonitrile, in the mobile phase, on the speciation of arsenic on Econosil C18 column (250 * 4:6 mm i.d., particle size 10 lm) at 1.0 ml/min flow rate.

4. Conclusions

A simple, fast and reliable HPLC method was developed for the detection of the species of arsenic and chromium ions. The speciation of arsenic has been achieved at base line while the speciation of chromium was partial. Arsenic speciation is controlled by acetonitrile content. The method has been used for the detection of arsenic and chromium species in natural water. Therefore, the proposed HPLC method can be used for the speciation of arsenic and chromium in unknown environmental samples.

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