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Arsenic (III) Determination by Spectrophotometry Coupled with Preconcentration Technique in Water Samples

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ABSTRACT

A micro organism Lactic acid bacteria as an immobilized cell on a solid support was presented as a new biosorbent in a simple and sensitive spectrophotometric determination of As(III) in various samples using IH-benzo [d] imidazole-2-thiol (max 256 nm) at pH 3.0 -4.0 . Beer's law was obeyed over the range of 0.01-0.1 μg L−1 . Here we show that arsenic reacts with IH-benzo[d]imidazole-2-thiol in acidic conditions to yield the As- IH benzo[d]imidazole-2-thiol complex. We studied the Beer's law at 256 nm, which showed linearity over the concentration range 0.2–1.0 μg /mL of arsenic. We have shown that molar absorptivity, Sandell's sensitivity and the detection limit of the method are 6.06 × 10^4 L/mol cm, 0.0012 μg / cm^2 and 0.060 μg / mL respectively. The detailed study of various interfering ions made the method more sensitive and selective. The recovery of As (III) from various samples range from 97.75 to 99.35%. The present method was successfully applied for the determination of As (III) in spiked, natural water samples.

Key Words: Arsenic(III), I*H*-benzo[*d*]imidazole-2-thiol, Solid phase extraction, Spectrophotometry, Amberlite-2010.

INTRODUCTION

Arsenic occurs naturally in the Earth's crust in its inorganic form, trivalent (arsenite) or pentavalent (arsenate). Erosion of arsenic containing surface rocks probably accounts for a significant amount of arsenic in water supplies. It is a ubiquitous element in water, soil and sediments. The occurrence of arsenic in plants and animals generally reflects its accumulation from the environment. The presence of arsenic in drinking water has reached calamitous

proportions in many parts of the world. There are numerous reports in the literature based on past and ongoing experience in various countries in Asia and South America concerning the higher risks of skin, bladder, lung, liver, and kidney cancer that result from continued consumption of elevated levels of arsenic in drinking water [1]. Consumption of even low levels of arsenic over a long period can cause a multitude of diseases. The maximum permissible limit for arsenic in drinking water is 0.05 mg/L as recommended by WHO [2]. In certain areas in India, Bangladesh, China, and Mongolia [3], arsenic levels in groundwater exceed 1 ng/ mL. Regarding inorganic arsenic, arsenic (III) is appreciably more toxic than arsenic (V). Usually these species of arsenic in natural water are found at the trace levels [10]. There are only a few analytical techniques available, which have sufficient sensitivity and selectivity to directly determine these species of arsenic, at the trace levels in natural water. Therefore, the development of sensitive and accurate methods for speciation and preconcentration of trace amounts of arsenic(III) and arsenic(V) is necessary. Recently, many kinds of conventional analytical techniques, such as hydride generatively coupled plasma atomic emission spectrometry(HG-ICP-AES) [5], capillary electrophoresis inductively coupled plasma mass spectrometry (CE-ICP-MS) [4], high performance liquid chromatography–inductively coupled plasma mass spectroscopy [5], electro thermal atomic absorption spectrometry(ETAAS) [6], hydride generation – atomic absorption spectrometry [7], hydride generation – atomic fluorescence spectrometry [8], cathodic stripping voltammetry [9], anodic stripping voltammetry [10], neutron activation analysis [11], photometric analysis [12], ionselective electrodes [13] and energy dispersive X-ray fluorescence spectrometry [14], have been used for the determination of low concentrations of arsenic. But all these techniques are costly and require trained staff. Recently, most of the spectrophotometric methods have been developed as an alternative for the determination of arsenic instead of conventional techniques. The imidazoles are widely used as chelating agents for the separation of trace heavy metals from matrix species as well as their preconcentration prior to determination. Among the imidazoles, are newly chelating agents which have found good application in the extraction of metal ions. Solvent extraction techniques are time consuming, tedious and usually involve harmful solvents. In this investigation the novel, facile and sensitive sorbent, Lactic acid bacteria was successfully anchored to amberlite resin -2010 for the preconcentration of As (III) in water samples.

Optimum experimental conditions were investigated with respect to a standard solution of the same matrix, in order to examine the possibility to obtaining the maximum extraction efficiency with minor sample treatment and minimal experimental conditions. Under these conditions, and the detection limit achieved was 0.06 μg L^{-1}. The method was successfully applied for the spectrophotometric determination of As (III) in various water samples using newly synthesized analytical reagent IH- benzo[d] imidazole-2-thiol.

EXPERIMENTAL SECTION

Apparatus

A HITACHI U 2001 spectrophotometer with 1.0 cm matched quartz cells were used for all absorbance measurements. An Elico Li-129 model pH meter with combined glass-calomel electrode was used for all pH measurements. Elemental analysis was carried out on Perkin-Elemer 240 elemental analyser.

Reagents

All reagent used were of analytical reagent grade. Double distilled water was used throughout the experiment. Solutions containing 1.0 M of nitric acid (Merck, Mumbai, India) were used as eluent. A stock solution of Standard arsenic solutions were prepared in the range of 0.2–1.0 mg/L. (E-Merck, Germany) in double distilled water in 1000 mL standard flask. Arsenic (III) working standard stock solutions were prepared freshly by appropriate dilution of the standard stock solution with double distilled water. 0.01M of reagent solution was prepared by dissolving 0.15 g of reagent in 100 mL of Methanol. This solution is further diluted, whenever necessary with double distilled water. Amberlite XAD-2010 (specific surface area 660 m^2g-1 and bead size, 20-60 mesh) and it was purchased from

(E-Merck, Germany). Nitric acid and KOH buffer was prepared. It was adjusted to Ph 3.0 . It was stored in clean 1L (metal free) polyethylene bottle.

Preparation of bacterial biomass

A solid MRS agar medium (HIMEDIA) was prepared by dissolving 55 g in 1L distilled water. The mixture was sterilized in the previously sterilized Petri dish at 121^oC and leaved to become solid the bacterium, Lactic acid bacteria, was inoculated on the solid medium and stored at 30-35^0C in order to growth bacterium. Liquid medium was prepared by mixing the substances mentioned above expect agar and sterilized at 120 ± 1^0C for about 30 min. Firstly, in order to prepare the starter culture, Lactic acid bacteria grown on the solid medium was implicated to 100 mL of liquid medium. Then, it is incubated for 48 h at 28 ± 2 ^0C on a shaker (about 200 rpm) for preparing the experimental culture, 200 mL of liquid medium was prepared and inoculated with 10 mL of the starter culture and incubated on shaker for 48 h at 28 ± 2^o C Then, the bacterium grown in the experimental culture was separated from the media using centrifugation (5000 × g for 5 min) to isolate the biomass. In order to obtain the dead and dry bacteria, 10 mL of 0.1 mol/L HCl was added to the isolated biomass after 10 min, the mixture was centrifuged and the acid solution was discarded. This procedure was repeated three times and then followed by rinsing the acid washed biomass in distilled water these rinsed bacteria were again centrifuged and the resulting biomass was lyophilized to yield a dry bacterial powder.

Immobilisation of bacteria onto Amberlite XAD-2010.Commercial available Amberlite XAD-2010 was prepared as substrate by washing successively with methanol, water 1mole of HNO_3 and water respectively, to remove organic and inorganic contaminant then the immobilization of the Lactic acid on the substrate was performed as follows. 150 mg of dry and bacteria powder was mixed with 1 g of Amberlite XAD-2010. The mixture was wetted with 2 mL of double distilled water and thoroughly mixed after mixing the past was heated in an oven at 105 ^{o} C for 1 h to dry the mixture. The wetting and dry step was repeated to maximize the contact between a Lactic acid and Amberlite XAD-2010, there by improving the immobilization efficiency, then the product obtained was ground to get original size (20-40mesh) and used as an adsorbent.

General procedure

Agro bacterium tumifacients (1.0 g) was first packed in a glass column (10 cm length × 10 mm internal diameter) between firsts, using the method recommended by the manufacturer. The column was treated with 1 M HNO_3 (25 mL) and washed with double distilled water until free from acid. A suitable aliquot of the sample solution containing As(III) in the concentration range

of 0.01-0.1 μgL−1 was passed through the column after adjusting its pH to the optimum range (Nitric acid buffer of pH 3.0 ± 0.2) with aflow rate of 2.0-4.0 mL min−1. The column was washed with double-distilled water to remove the free metal ion. The bound metal ion As(III) was stripped from the column with 1M HNO_3 (8 to 10 mL) passed at a flow rate of 2.0-4.0 mLmin−1. The eluent was then mixed with 0.01M IH-benzo[d] imidazole-2-thiol to form product, which was measured spectrophotometically at wavelength 256 nm against reagent blank as shown in **Figure 1.** Procedure for the determination of As (III) in spiked water samples. The extraction efficiency was studied using spiked water samples for the recovery of Arsenic. Doubly distilled water was spiked with known amounts of metal standards (20-80 ng L−1) and allowed to stand over night. The concentration of Arsenic in spiked water samples was determined and results were summarized in **Table 2.**

Procedure for the determination of As (III) in natural water samples.

Different water samples (river water and Tap water) were collected from various places in and around Tirupati, A.P., India. The samples (150 mL) were stored at 0-5^{o}C in metal free polyethylene bottles. Water samples were filtered through what man filter paper no. 41 and clean solution was collected into 250 mL beaker. The contents were diluted up to the mark with double distilled water. 15 mL of this solution was further diluted to get working solution for determination of As(III) as described in above procedure and compared with the reported method with statistical validation. The results were summarized in **Table3**.

RESULTS AND DISCUSSION

Effect of pH

The effect of pH on the peak height of As (III) at different concentrations was investigated with a fixed 0.01 M IH- benzo[d] imidazole-2-thiol concentration. The pH of acetate buffer was taken in the range of 2.5-6.0 and the peak height wave measured for each concentration level of As (III). At all concentration levels of As (III) maximum peak height were found between 3.0 to 3.5. Therefore, a pH 3.0 ± 0.2 of Nitric acid buffer system was chosen throughout in the study as represented graphically in **Figure 2.**

Analytical parameters

Beer's law was obeyed in the concentration range 0.01to 0.1 μg L−1 of As (III). The molar absorptivity and Sandell's sensitivity of complex at pH 3.0 was calculated as 6.06×10^{4} M and 0.0012 μg cm−2 respectively. The choice of selecting an eluent was a difficult problem. In addition to an eluent should not destroy the solid support in the column. Hence, for the determination of the preconcentrated As (III) by spectrophotometry, the elution was performed with 0.5-3.0 M HNO_3 and is dependent on the concentration of HNO_3 as shown in **Figure 3**, quantitatively As (III) was achieved for 4 mL of 1 M HNO_3. Hence, 4 mL of 1 M HNO_3 was chosen the optimum eluent for the As(III) determination and recoveries were higher than 99% and the results are shown .

Effect of volume of a sample on elution

The effect of the sample volume on the extraction of As (III) was studied by taking different volumes of water samples in the range of 100, 200, 300, 400, 500 and 600 mL. As the volume

of sample increases, the recovery of metal ion increases gradually up to 500 ml to obtain higher than 99%. Hence, the 500mL of water sample was chosen for the present study.

Effect of the reagent concentration

The effect of concentration of IH-benzo[d] imidazole-2-thiol on the peak height was investigated at pH 3.0 ± 0.2by using 2.0 and 3.5 μg L−1 As (III) solution of IH-benzo[d] imidazole-2-thiol was varied in the range 0.01 M-1.0 M. Maximum peak height was obtained at a concentration of 0.01 M of 4 mL IH-benzo[d] imidazole-2-thiol as reagent for lower concentration level of As(III) solution.

Effect of volume of an eluent on percentage of elution of As (III). The effect of volume of an eluent on elution of As (III) for various water samples on Lactic acid bacteria measured at 30-35^0C. It can be observed that the percentage of recovery increases with the increase in the volume of eluent to some extent. After increasing the volume of an eluent, the elution percentage slightly decreases. Thus 4.0 mL of 1 M HNO_3 was chosen for 100% recovery of As (III) ion.

Accuracy and Application of the Proposed Method

The calibration graph was linear in the concentration range 0.2–1.0 mg/L of As(III). We show that molar absorptivity, Sandell's sensitivity and the detection limit of the method are 6.06×10^4 L/mol cm, 0.0012 μg/cm2 and 0.060 μg/mL, respectively. The calibration graph of arsenic constructed by a UV-Visble spectrophotometer is linear up to 0.01 mg/L of arsenic .The calibration curve for As^{3+} constructed under optimum conditions show good linearity. The results obtained by using these techniques are given in **Table 2**.

Detection limit

Under optimum conditions the detection limits for the determination of As(III) in various environmental samples was found 5.0 μg mL−1 for a 500 mL volume As(III) in solution.

Effect of column performance

In order to examine the long term stability of the support, it was subjected to successive adsorption and adsorption cycles (6 runs in a day and the next 6 runs another day, and so on, total 30 runs) by 500 mL of metal solutions through the column. The stability and potential recycle ability of the column containing support was assessed by monitoring the change in the recoveries of the analyte. After 15 runs, the recoveries of the analytes lightly decreased to < 95%.

Interference studies

Although hexamethylene dithiocarbamate does not form complexes with alkali and alkaline earth metals, high concentrations of themwere tested, due to their presence in natural water. K and Na were tolerable up to 1 g/L while Mg and Ca upto 100 mg/L. Chloride anions do not cause any significant interference even in concentrations (5 g/L). On the other hand it is well known that transition metals form strong complexes with HMDTC, so the recovery of 0.1 mg/L of arsenic was tested in the presence of these metals under the optimum conditions described above. The elements Mn(II), Fe(III), and Cr(III)were found not to interfere at concentrations up to 50 mg/L, while Cu(II), Co(II), Cd(II), and Sn(II) were tolerable up to 5 mg/L.

Table 1. Optical characteristics, precision and accuracy of the proposed method

Parameter	Value
pH	3.0
λmax, nm	256
Beer's law limits, µg/mL	0.2–1.0
Limit of detection, µg/mL	0.060
Molar absorptivity, L/mol cm	6.06 × 104
Sandell's sensitivity, µg/cm2 per 0.001 absorbance unit	0.0012

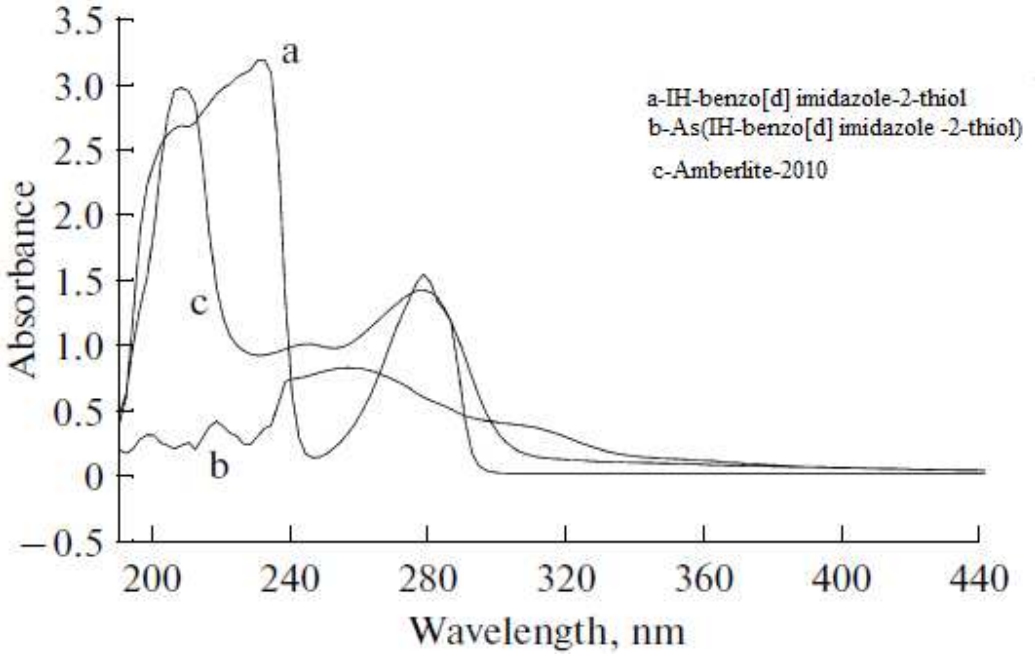

Figure1: a) absorptionSpectra of [As (III)IH-benzo[d] imidazole-2-thiol](1.0g/mL) in Amberlite -2010; Amberlite -2010 of 0.01 IH-benzo[d] imidazole-2-thiol + pH 3
b) Under the same conditions,only Amberlite -2010 without IH-benzo[d] imidazole-2-thiol
c) only IH-benzo[d] imidazole-2-thiol without Amberlite-2010

Table 2. Analytical data of the determination of arsenic(III) in real samples , Measured*, µg/mL

Sample	Spiked, µg/mL	Measured*, µg/mL	CV	Recovery, %
Tap Water	0.000	n.d.	-	-
(Tirupati)	0.500	0.495± 0.017	2.3	99
	1.000	0.990± 0.016	2.2	99
Tap water	0.000	nd	-	-
(Karakam badi)	0.500	0.524± 0.021	2.7	105
	1.000	1.022 ±0.022	1.9	102

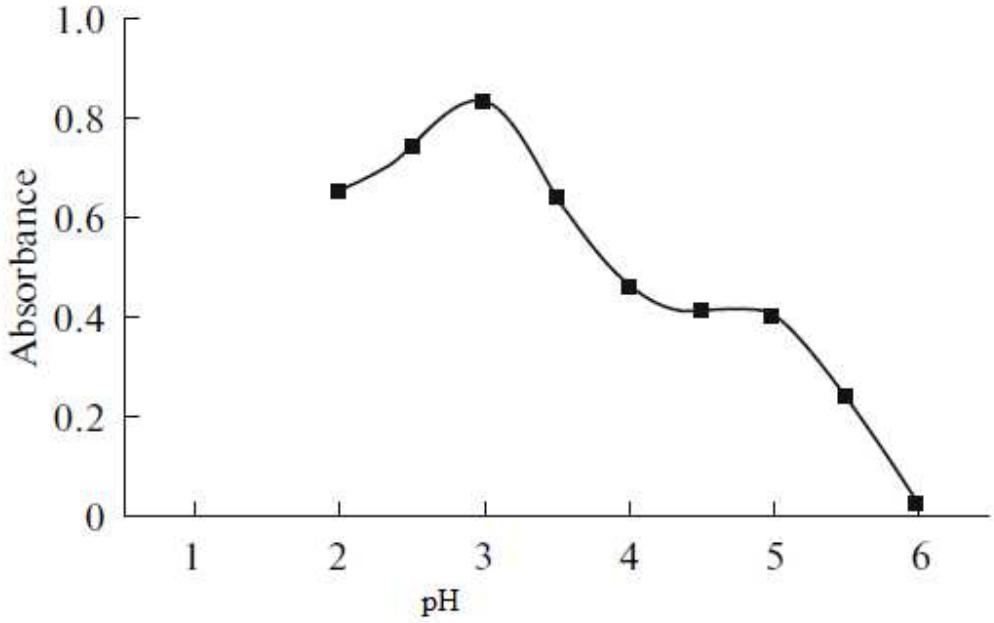

Figure 2: Effect of pH on absorption spectra of As(IH-benzo[d] imidazole-2-thiol (1.0 µg/mL); 1mL of 1% Amberlite-2010+ 0.5 mL of IH-benzo[d] imidazole-2-thiol

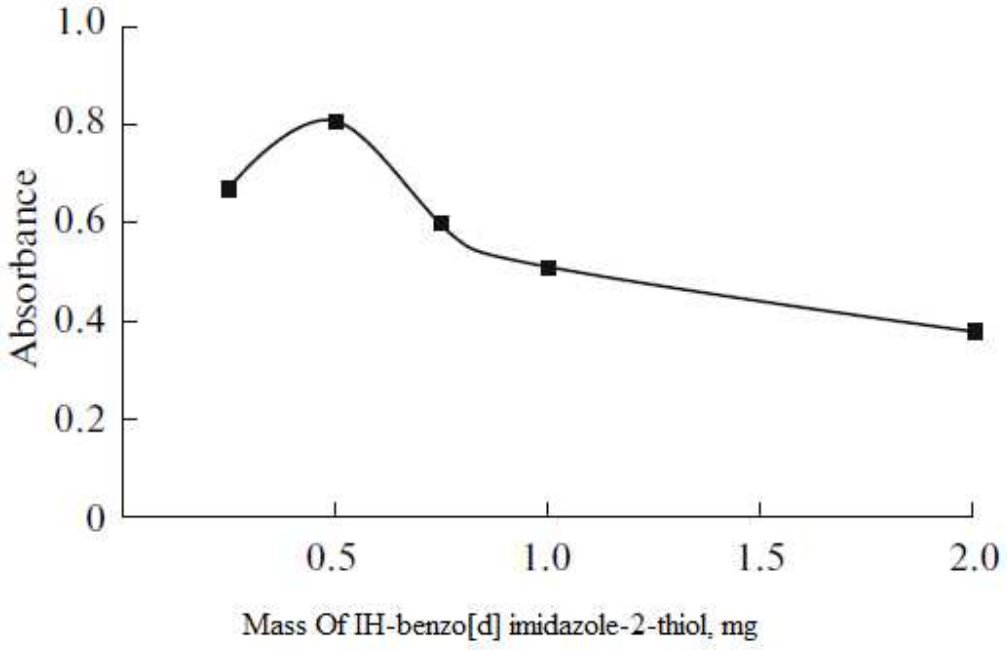

Figure 3: Effect of the amount of (IH-benzo[d] imidazole-2-thiol on sorption of As(IH-benzo[d] imidazole-2-thiol (1.0 µg/mL):1mL of 1% Amberlite-2010,pH 3

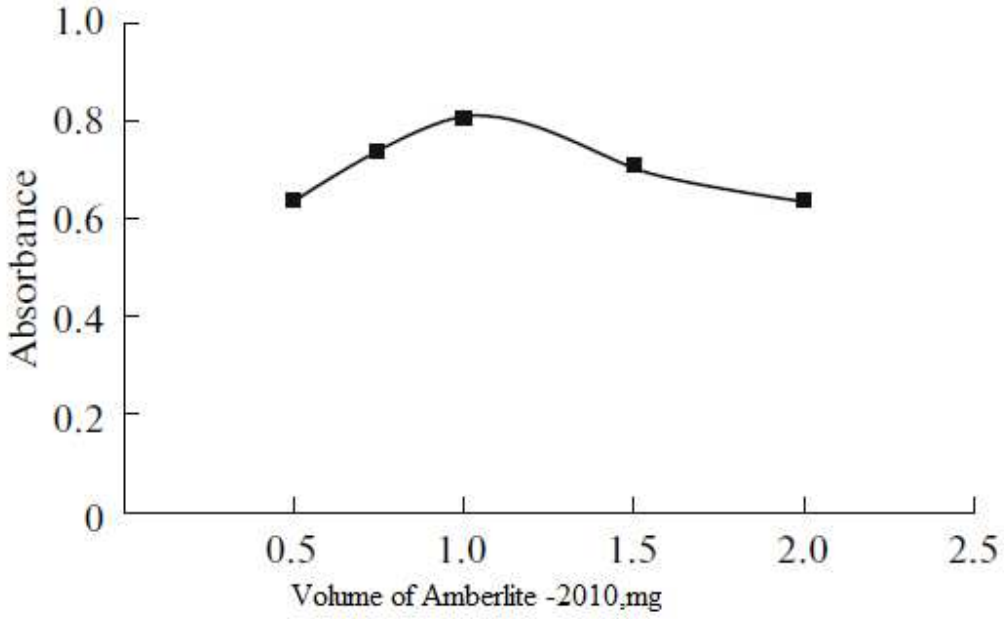

Figure 4.Effect of the Concentration of Amberlite-2010 on absorption spectra As (IH- benzo[d] imidazole-2-thiol);0.5 mL of0.1 %(IH- benzo[d] imidazole-2-thiol ,pH 3

Table 3. Comparison with other methods

.

NO	Reagents/reference	Wavelength	Range of determination, mg/L	Remarks
1	Ammonium molybdate + sodium metavanadate [17]	460	1–30	Phosphorus, silicon, interference; less
2	Silver diethyldithiocarbamate + piperidine [18]	500	0.03–0.24	sensitive
3	Ammonium molybdate + SDHA [19]	780	0.02–0.14	A toxic reagent used
4	IH-benzo[d] imidazole-2-thiol[Present Method]	256	0.2–1.0	Extraction required; time consuming Sensitive, simple, rapid; free from

CONCLUSION

The proposed preconcentration spectrophotometric method is simple, highly sensitive and selective for the determination of As (III) in Water samples. The limit of detection of the proposed method is superior when compare to reported method. The method has additional advantage over reported method owing to it's.

Complexing reagent employed in the present method *i.e.* IH-benzo[d] imidazole-2-thiol was economical and easy to prepare in an ordinary laboratories. Low reagent consumption, elimination of the analytical error, less interference and statistical analysis which made the method to be more sensitive and selective. Lactic acid bacteria used as a biological adsorbent is hightly selective with respect to As(III) determination.The performance of the column are simple and sensitive to As(III) determination by using eco friendly biological material (Lactic acid bacteria).

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