A RP-HPLC Method Using α-Hydroxy Isobutyric Acid for Preconcentration and Determination of Uranium in Seawater

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Abstract

A high-performance liquid chromatographic (HPLC) method is developed for the determination of uranium (U), present at ppb levels, in seawater. α-Hydroxy isobutyric acid is used as the complexing agent for preconcentration as well as separation of U from other interfering metal ions (Mo, V, Ti, Ni etc.). A C$_{18}$ reversed-phase monolithic column and α-HIBA of pH 6-7 are employed for the selective preconcentration of U. Mobile phase consisting of α-HIBA of pH 2 and MeOH is used for elution of adsorbed U which is monitored after post-column derivatisation with Arsenazo(III), using a spectrophotometric detector. The methodology is validated by using simulated as well as seawater samples. Quantitative recovery for U is possible with linear response over a concentration range of 1 to 30 ppb of U. Metal ions which have been previously reported to give interferences do not give any problem in the present approach. The developed HPLC methodology is validated by comparing the results with those of isotope dilution-thermal ionization mass spectrometry.

Introduction

Seawater is considered as a possible source of uranium (U) due to its limited reserves available in the earth's crust worldwide (1,2). Concentration of U, present at ppb levels, in seawater varies with water salinity, depth, temperature, etc. (3). Studies are being pursued by different research groups to explore the feasibility of recovering U from seawater in an economic way. These studies demand the availability of a method for determination of U at different stages of preconcentration and separation. In addition, determining the fate of U in natural water systems including seawater is important for environmental monitoring (4). However, U determination in seawater is a challenging task because of the high salt content, presence of bio-fouling agents and low ppb concentrations of U. A variety of analytical techniques commonly employed for the determination of U at ppb levels are inductively coupled plasma–mass spectrometry (5–7), inductively coupled plasma-atomic emission spectroscopy (8), spectrophotometry (9), differential pulse polarography (10), solid state nuclear track detector (11), X-ray fluorescence (12), laser fluorimetry (13), radiometry (14,15), etc. Low U concentrations and large amounts of other dissolved species in seawater necessitate chemical separation of U prior to its determination. Commonly used separation procedures are precipitation (5), solid phase extraction (7,10), liquid–liquid extraction (12,15), ion-exchange (15), and supercritical fluid extraction (16).

A few attempts are reported in literature to develop high-performance liquid chromatography (HPLC) for the determination of U in natural water systems because of its high separation efficiency, transportability, and relatively low instrumentation cost (17–21). Cassidy et al. used α-hydroxy isobutyric acid (α-HIBA) for the determination of U in groundwater and simulated urine samples with a bonded-phase cation exchange stationary phase (17). Kerr et al. also used α-HIBA for the selective preconcentration of U on a reversed-phase (RP) column for U determination in groundwater samples (18). These authors included a dynamic cationic modifier in the mobile phase along with α-HIBA to improve the peak shape during elution. Hao and Haddad studied the retention behaviour of α-HIBA complexes of Th(IV) and UO$_2^{2+}$ with an objective to understand the mechanism of their elution pattern under reversed-phase conditions (19). Hao et al. also compared glycolic acid, α-HIBA and mandelic acid for the on-line preconcentration of Th and U (20) and observed poor recovery (40%) by α-HIBA. Subsequently, Shaw et al. reported the utilisation of chelation ion chromatography based on 2,6-pyridine dicarboxylic acid as the chelating agent for the determination of U in spiked seawater samples (21). The majority of these studies were performed using spiked samples containing significantly large amounts of U added externally compared to the amount present in seawater.

The present work was undertaken with the following objectives. Our aim was to develop an HPLC method using α-HIBA for the selective preconcentration as well as determination of U in seawater employing the commonly used post-column reagent, that is, Arsenazo (III). Since this reagent is neither specific nor

highly sensitive to U, optimized conditions were developed for the separation of U from other metal ions present in seawater as well as for its preconcentration. The combination of α-HIBA as ligand and C_{18} as stationary phase satisfied the requirements of preconcentration step (20). Experimental parameters were optimized for quantitative recovery and fast elution with a good peak shape for quantification. The present work differs from the studies reported previously (18–20) in many ways. Firstly, we have used α-HIBA of pH ≥ 6.0 as it provides better retention characteristics than at pH 4.0 used by Haddad et al. (19,20). This also leads to efficient recovery of U by RP stationary phase. In contrast to the work reported by Kerr et al., we have not employed any dynamic ion interaction reagent in the mobile phase. In addition, we employed the same column for the preconcentration and separation of U, whereas Kerr et al. used a cartridge for preconcentration and an analytical column for separation (18). Finally, the developed method was validated by determining U, present at ppb levels, in simulated and actual seawater samples by comparing the results from isotope dilution-thermal ionization mass spectrometry (ID–TIMS). At the time of the study there was no report available in the literature on the determination of U in actual seawater by HPLC method and the results substantiated by an independent analytical methodology.

Experimental

Reagents

Freshly de-ionised water purified with a Milli-Q system (Millipore, Bengaluru, India) was used for all the dissolutions and dilutions. α-HIBA used as a chelating agent was obtained from Lancaster (Lancashire, UK). High purity reagents such as HNO_3, NH_4OH (Suprapure grade from E. Merck, Darmstadt, Germany), etc. were used during sample treatment. MeOH (gradient grade, Merck) was used as the organic modifier for the mobile phase. Arsenazo (III) (Fluka, Buchs, Switzerland) was used as the post-column reagent (PCR). NaCl, KCl, $Mg(NO_3)_2$ and $Ca(NO_3)_2$ (Thomas Baker, Mumbai, India) were used for preparing simulated seawater (Table I). U stock solution was prepared from uranyl nitrate after the standardization procedure mentioned elsewhere (25). 30% (v/v) solution of tributyl phosphate (TBP) was prepared by mixing appropriate volumes of TBP and CCl_4 (S.D. Fine Chemicals, Mumbai, India). The seawater samples were received from the Desalination Division of BARC.

Apparatus

Chromatographic studies were carried out using an HPLC system consisting of a L-7100 gradient pump (Merck Hitachi, Tokyo, Japan), Rheodyne injector (Model 7725i, IDEX Health & Science, Oak Harbor, WA) with 20 µL and a custom made 2.4 mL sample loops, monolithic C_{18} RP column of dimensions 50 mm × 4.6 mm (Merck) and L-7450A (Merck Hitachi) diode array detector (DAD). The effluent from the column was monitored after reaction with a post-column reagent (PCR) which was added with a Hurst piston pump (Princeton, Indiana) into a low dead volume-mixing tee (Valco, Houston, Texas). The signal from the detector was processed by HSM 7000 software package and

the chromatograms were monitored on a PC. The HPLC system was computer controlled through interface D-7000 (Merck Hitachi). An isocratic pump with all SS contact parts (Model 501, Waters Corporation, Milford, MA) was used as the concentrator pump for delivering the sample solution through the column. Finnigan MAT-261 (Thermo Electron, Bremen, Germany) thermal ionization mass spectrometer equipped with multi-Faraday cup detection system was employed for isotope dilution experiments.

Procedure

The glass-wares used were cleaned by immersing them in 7 M HNO_3 overnight and then boiling in 3 M HNO_3. An appropriate quantity of α-HIBA was dissolved in water to prepare 0.5 M solution. Different solutions were adjusted to the desired pH using NH_4OH and HNO_3 and were filtered through 0.45-µm Millipore membrane filters. Unless otherwise mentioned, all the HPLC studies were carried out at a flow rate of 1 mL/min. The PCR solution was prepared as mentioned elsewhere (24) and was delivered at a flow rate of 0.3 mL/min.

The seawater sample was acidified to pH 2–3 using HNO_3 and was heated to boiling for 15 min. The solution was cooled to room temperature and filtered through 0.45-µm filters. The filtered solution was made-up to known volume and was divided into two portions; one for HPLC analysis and the other for ID–TIMS analysis.

In the portion used for HPLC experiments, 0.025 M α-HIBA was added and the pH of the solution was adjusted to 6–7. The HPLC column was conditioned with 10 mL of 0.025 M α-HIBA solution of pH 6–7 using HPLC pump. The conditioned column was disconnected from the system and was connected to the concentrator pump. A known quantity (25–50 mL) of the treated

Table I. Composition of Simulated Seawater Sample*

Element	Concentration (µg/g)
Sodium (Na)	10,800
Magnesium (Mg)	1,290
Potassium (K)	392
Calcium (Ca)	411

* References: http://www.seafriends.org.nz/oceano/seawater.htm
http://www.oceanplasma.org/documents/chemistry.html

Table II. Optimized Gradient Condition for the Elution of U*

Time (min)	α-HIBA (pH 6–7) [M]	α-HIBA (pH 2.5) [M]	MeOH (v/v) %
0.0	0.025	0	0
2.0	0.025	0	0
2.5	0.025	0.2	35
10	0.025	0.2	35

* Chromatographic conditions: 50 × 4.6 mm monolith C_{18} column, mobile phase flow rate: 1 mL/min and post-column reagent [0.15mM Arsenazo (III) and 0.01M urea in 0.1M HNO_3] flow rate: 0.3 mL/min.

seawater was passed through the column at a flow rate of 1.5 mL/min. The effluents from the column were collected and weighed to determine the exact amount of sample passed though the column. The loaded column was then connected again with the HPLC system and washing was done with 10 mL of 0.025 M α-HIBA solution of pH 6–7 to remove the salts. The adsorbed U was eluted from the column by using (α-HIBA + MeOH) gradient (Table II). The experiments for determining the blank with 0.025 M HIBA of pH 6–7 was performed before and after the sample analysis under identical conditions. Quantitation of U was based on the area of peak from chromatogram.

The fraction for ID–TIMS analysis was mixed with a known amount of precalibrated ^{233}U spike. The mixture was treated with 8 M HNO_3 and evaporated to near dryness. The treatment with 8 M HNO_3 was repeated three times to ensure proper isotopic exchange between sample and the spike isotopes. The spiked mixture was then dried and dissolved in 8 M HNO_3 to carry out the solvent extraction of U using an equal volume of 30% TBP solution. This extraction procedure was repeated three times and the 8 M HNO_3 aqueous phase was discarded. U was stripped from the organic phase by back-extracting successively four times with water. The aqueous phase was collected and was evaporated dryness. The residue was dissolved in 1 M HNO_3 for loading onto the sample filament of a double rhenium filament assembly for TIMS analysis. The sample and the ionization filaments were heated to temperatures corresponding to heating currents of 2.2A and 6A, respectively. The mean value of ^{233}U/^{238}U atom ratio was determined by taking run summary from three blocks, each block consisting of 10–12 scans.

Results

Optimization of parameters for U preconcentration

Initially, for studying the effect of change in the concentration of α-HIBA on the retention of U, pH of the mobile phase used was maintained at 3. The results are shown in Figure 1. It is seen that
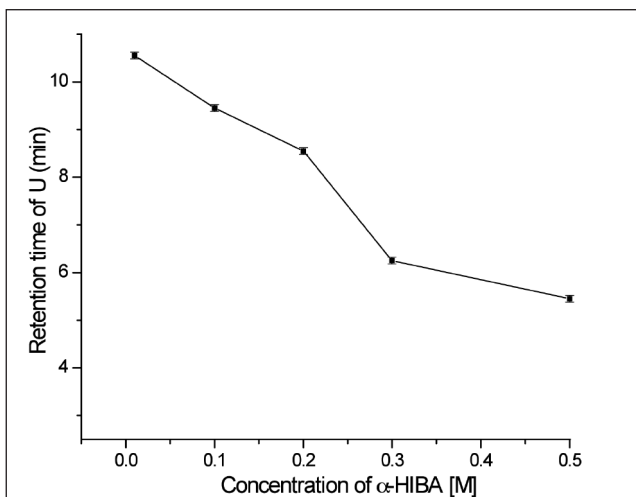

Figure 1. Effect of concentration α-HIBA of pH 3.0 on the retention of U. Chromatographic conditions: C$_{18}$ (50 mm × 4.6 mm) monolith column; mobile phase: α-HIBA of pH 3.0; mobile phase flow rate: 1 mL/min; post-column reagent [0.15 mM Arsenazo (III) and 0.01M urea in 0.1M HNO3] flow rate: 0.3mL/min. 10 µg/g of U. Detection at 650 nm.

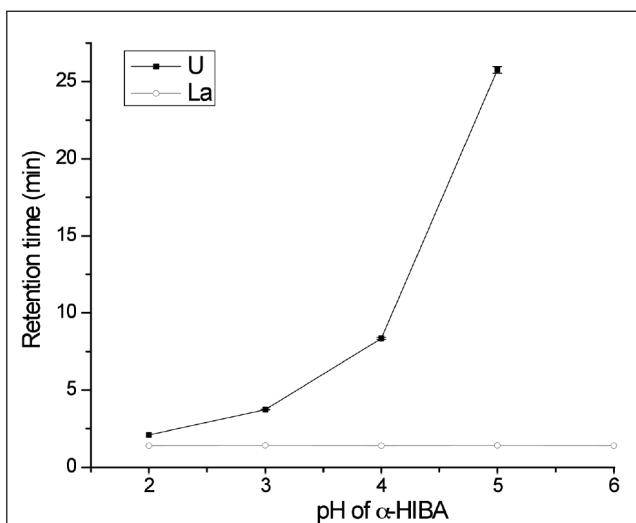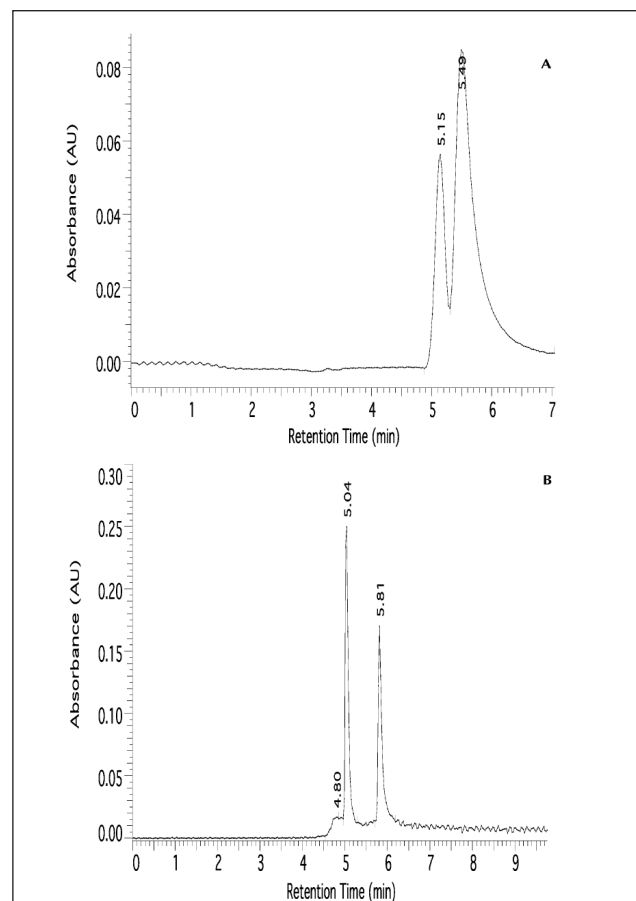**Figure 2.** Effect of pH of α-HIBA on the retention of U and La. Chromatographic conditions: 0.5 M α-HIBA; 10 µg/g each of U and La. Other conditions same as in Figure 1.

Figure 3. (A) Chromatogram of isocratic separation of U. Chromatographic conditions: mobile phase: 0.2M α-HIBA of pH 2.5; injected 10 µg/g of U through 20 µL loop. Other conditions same as in Figure 1. (B) Chromatogram of separation of U in under the gradient condition. Chromatographic conditions: mobile phase as per Table II; injected 10 µg/g of U through 20 µL loop. Other conditions same as in Figure 1.

the retention of U decreases with the increase in concentration of α-HIBA in the mobile phase. Since the retention of U is significant even at 0.5M α-HIBA, the effect of change in pH of the mobile phase was studied at this concentration. Figure 2 shows the effect of pH of the mobile phase on the retention of U. In Figure 2, the retention time of U is compared with that of La since the latter does not form any hydrophobic complex with α-HIBA. It is seen that increase in pH increases the retention of U significantly whereas there is no effect on La, as expected. U could not be eluted with solution of pH 6 by passing a mobile phase solution equal to 80 times the column volume. Thus 0.025M α-HIBA of pH 6–7 was used for carrying out the preconcentration of uranyl ion on the C_{18} surface.

Optimization of parameters for U separation

Figure 3A shows the chromatogram obtained for injection of 20 μL of aqueous solution containing 10 ppm of U. Elution was carried out isocratically using 0.2 M α-HIBA of pH 2.5. The chromatogram shows two closely eluting peaks. The peak height of the second peak decreased with sample dilution with no effect on the first peak, which was found to depend on the volume of the condition solution passed and sample solution injected. Thus the second peak was identified to be that of U. The first peak was also seen during the blank experiments and probably corresponds to the elution of α-HIBA sorbed onto the stationary phase during the conditioning, loading and washing stages. Since the two peaks were eluting quite closely, studies were performed to improve the resolution between the two peaks. It was observed during previous studies that the peak shape and the response of U peak improve with the increase in the methanol content in the mobile phase (23). Hence, a concentration gradient of methanol and α-HIBA as given in Table II was selected for the separation of U. Figure 3B shows the chromatographic separation of U under optimized gradient conditions.
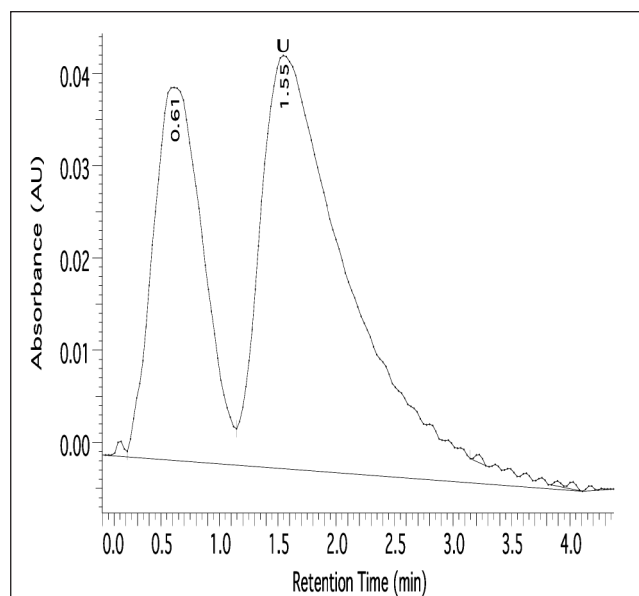
Preconcentration studies

Since a larger volume of the seawater sample needed to be injected for preconcentration of U, efforts were made to introduce the sample into the column using one of the inlets of quaternary gradient pump. This had the advantage of accurate sample delivery and ease of operation by programming all the steps to be executed in sequence. However, no elution peak for U could be seen using this procedure. A detailed examination revealed that during sample loading step, U gets adsorbed onto the walls of PTFE tubing used as solvent inlets and, therefore, does not reach the RP column. This was checked by flushing the inlet tubing with pH 2 α-HIBA as shown in Figure 4. The studies indicated appreciable (> 80% for 50 mL solution with 100 ppb of U) loss of U during the on-line preconcentration. Though the method offered linear response for aqueous samples with U amounts ranging from 10 ppb to 1000 ppb, it was abandoned for further studies due to the fact that multiple injection procedure is laborious and consumes copious volume of samples during the injection process.

A high-pressure pump with all stainless tubing connection was therefore used as a concentrator pump for delivering the sample into the column. Samples were prepared in 0.025M α-HIBA and a definite quantity of the sample was fed through the column after adjusting the pH to 6–7. The effluent from the column was collected and weighed to determine the actual amount of the sample solution passed through the column. The column was then connected to the HPLC system for U separation. Under the optimized conditions, most of the interfering metal ions (10 ppm of each of V, Ni, Ti, Mo) were not retained on the column. The separation of U was then carried out using (MeOH + pH 2.5 α-HIBA) gradient and the chromatographic run was completed in 6 min. This arrangement was found to give satisfactory recovery of U.
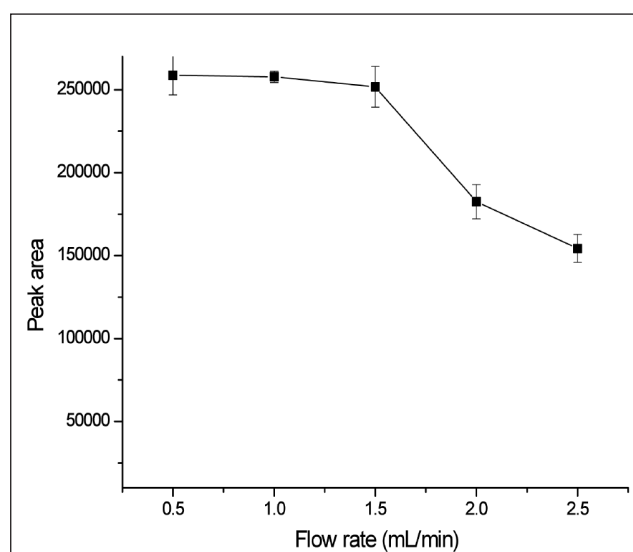

Figure 4. Chromatogram of elution of U adsorbed onto the PTFE tubing. Loading condition: 50 mL of 100 ppb U in 0.025 M a-HIBA passed through the PTFE tubing. Elution condition: 0.2 M a-HIBA of pH 2.5. Detection: same as Figure 1.

Figure 5. Recovery of U as function of loading flow rate. Chromatographic conditions: 50 mL of 10 ppb U in simulated seawater solution containing 0.025 M a-HIBA and pH 6–7 was fed through the preconditioned 50 mm × 4.6 mm column. The column was washed with 10 mL of 0.025 M α-HIBA of pH 6–7. Elution carried out as per the conditions given in Table II.

Studies with simulated samples

Simulated seawater sample containing 10 ppb of U was prepared and 50 mL of this solution was fed to the column at different flow rates. The effect of sample loading flow rate on the recovery was studied by loading at 0.5 to 2.5 mL/min. Figure 5 shows the effect of flow rate on the recovery of U by the RP column. It was seen that recovery of U remains independent of flow rate in the range of 0.5–1.5mL/min and at higher flow rates there was a steady loss on the amount of U retained by the column. Hence, a flow rate of 1.5 mL/min was chosen for carrying out the sample loading studies. Recovery of U was found to be 94 ± 5% for 50 mL of 5 ppb of U in simulated seawater sample. Linearity studies were carried out using simulated seawater sample. 50 mL of solutions containing U in the range 1 to 50 ppb were analysed by the developed procedure. Good linearity was observed for the peak area response as a function of U concentration from 1 to 30 ppb (Figure 6) and R^2 is 0.998. Detection
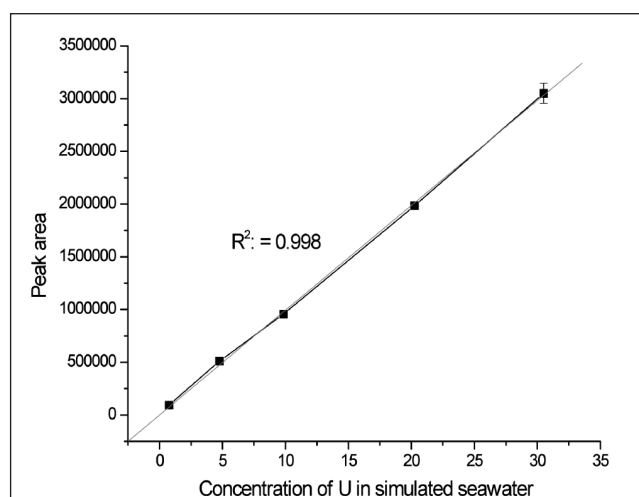

Figure 6. Peak area of U as function of its concentration in simulated sea water. Chromatographic conditions: Sample loading through the column at 1.5 mL/min. Other conditions same as in Figure 5.
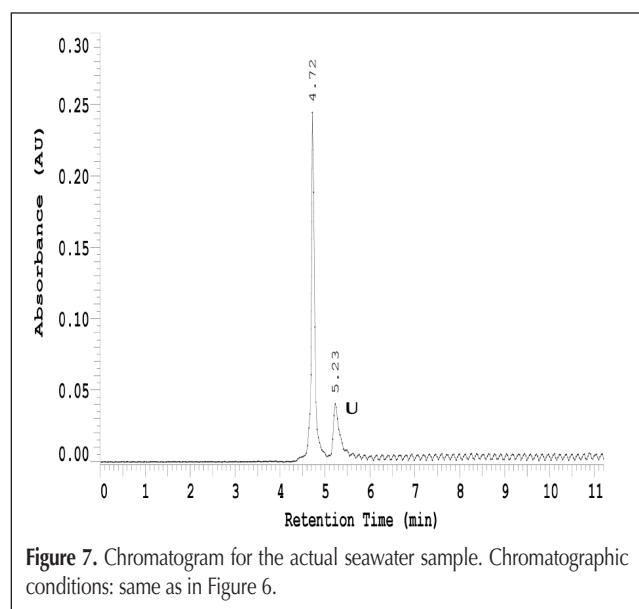

Figure 7. Chromatogram for the actual seawater sample. Chromatographic conditions: same as in Figure 6.

limit of U in simulated seawater was found to be 0.2 ppb employing 100 mL of sample with a S/N ratio of 3 (27). Intraday precision of the developed method was evaluated by analyzing ten replicates of simulated seawater sample containing 10 ppb of U and was found to be 7%.

Analysis of seawater samples

The seawater samples were acidified with HNO_3 to pH 2–3 and heated for 15 min to ensure complete dissociation of the carbonato complexes. pH of the treated samples was then adjusted to 6–7 and the HPLC analysis was performed as per the procedure discussed above. Twenty-five to fifty mililiters of the sample was used for preconcentration. Three seawater samples were analysed for U concentration by the above developed method. In the case of the samples obtained after the concentration of seawater by Reverse Osmosis (RO1 and RO2), the samples were diluted to twice the original volume to take care of the excessive salt content of the solution. Figure 7 shows the chromatogram obtained for one of the seawater samples. The concentration of U in seawater was determined by standard addition method employing HPLC. The results obtained by HPLC and ID–TIMS are given in Table III. It is seen that within the measurement uncertainty, the results compare well by the two methods.

Discussion

α-HIBA was selected for the preconcentration of U since our previous studies (25) had shown that uranyl-α-HIBA complex exhibits strong retention on a RP column due to the hydrophobic nature of this complex. We preferred to use a C_{18} monolith column in comparison to particulate RP column since the former offered the advantage of low back-pressure and fast separation.

Uranyl ion is known to form various complexes with α-HIBA such as $UO_2(HIBA)^+$; $UO_2(HIBA)_2$; $UO_2(HIBA)_3^-$; etc. The composition of the different complex species changes as a function of concentration of α-HIBA and pH of the medium (19,22). It was well established that uranyl ion forms stable complex with α-HIBA in the pH range 2–8. Complex formation constant value of 6.6 (log β_3) at pH 4.0 for $UO_2(HIBA)_3^-$ was reported (19,22). α-

Table III. Concentration of U in Seawater Determined by HPLC and ID-TIMS*

Sample code	U Concentration (ng/g)	
	HPLC[†]	ID-TIMS[‡]
SW-1	3.2 ± 9%	3.4 ± 7%
SW-2	3.4 ± 5%	3.2 ± 6%
SW-3	13.0 ± 7%	12.2 ± 5%
RO-1	5.3 ± 2%	–
RO-2	4.5 ± 3%	–

* Chromatographic conditions: same as given in Table II
[†] Concentration determined by standard addition method
[‡] Mean of three determinations

HIBA showed dominant bidentate coordination via oxygen atoms of carboxylic acid group in the pH range 2–4 and at pH ≥ 5, enhancement of chelation occurs due to the involvement of –OH group of the ligand in the complexation (22). Therefore, we performed the studies by varying the pH of α-HIBA between 2–6.

During preconcentration stage, efficient retention of uranyl ion on the C_{18} column using α-HIBA of pH 6–7 was attributed to (*i*) the hydrophobic nature of the uranyl-α-HIBA complex [UO_2 (α-HIBA)$_2$] and (*ii*) the increased complexation of U due to increased fraction of ionized α-HIBA at higher pH. Elution of U preconcentrated on the RP column using low pH (2.5). α-HIBA was explained on the basis of its existence as uranyl ion (UO_2^{2+}), which has no preference for the non-polar column.

The on-line sample preconcentration method using one of the inlets of quaternary gradient pump was found to result in appreciable loss of U. The studies revealed that the loss is due to the sorption of uranyl-HIBA complex onto the walls of PTFE tubing used as inlets. This is in sharp contrast to other reports published in literature which employed the hydrophobic interaction of uranyl complexes as basis of enrichment (19). Though multiple manual injections was offering good recovery and linear response for U in aqueous samples, it was not followed as the procedure is laborious. In addition, the injection loop needed to be flushed with sample solutions of at least 3 times the loop volume to ensure reproducible results (26). Finally, an off-line preconcentration method consisting of a high-pressure pump with all stainless steel tubing connection used as sample delivery system was employed. After passing a known amount of the sample through the column, it was then connected to the HPLC system for U separation. This arrangement was found to give satisfactory recovery of U with ease of operation.

Since U in seawater exists in the hexavalent state and anionic uranyl-carbonato complexes, $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$, acidification of seawater samples was carried out with HNO_3 and this treatment ensures complete dissociation of these carbonato complexes (28). Under the optimized conditions, metal ions viz. V, Ni, Ti, and Mo were not retained on the column and hence showed no interference during the elution of U. The presently developed method has been demonstrated for actual seawater samples by validating the HPLC results with those from internationally accepted technique of isotope dilution-thermal ionization mass spectrometry.

Conclusion

The preconcentration method based on the chelation of U by α-HIBA at pH 6–7 and its preferential adsorption on a RP column offers a simple approach for the quantification of U in seawater. Low pH (2.5) and MeOH were used for elution of adsorbed U from the column. The same column was used for the preconcentration and separation of U. The methodology was validated by simulated samples as well as by comparing the results obtained on seawater samples by HPLC and ID-TIMS. No interference was observed from other metal ions viz. V, Ni, Ti, and Mo. The methodology can be used routinely for the determination of U present at ppb levels in seawater.

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