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Arsenosugar extracted from algae: Isolation by anionic exchange solid-phase extraction

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ABSTRACT

Obtaining reliable speciation data for evaluating dietary exposure, and increasing understanding of arsenic biochemistry in algae, are hindered by the availability of suitable standards of arsenosugars, the major species in these types of samples. Moreover, chemical syntheses of such compounds have been reported to be complex and tedious. The aim of this work was to investigate the feasibility of the anionic exchange SPE cartridges (SAX and WAX) as an easy and quick alternative for the isolation and preconcentration of arsenosugars. Two commercial silica-based SPE cartridges strong anion exchange sorbent (DSC-SAX) and weak anion exchange sorbent (DSC-NH2) were compared for the SPE of three arsenosugars (PO_4-Sug, SO_3-Sug and SO_4-Sug). The effect of pH, ionic strength, type of salt and elution solvent on the elution protocols of these arsenosugars are studied. Eluted solutions from SPE were analyzed by ICP-MS for total arsenic content and IC-ICP-MS for the study of arsenic speciation.

The developed SPE procedure allows to obtain a solution containing the three arsenosugars isolated from other arsenic species with recoveries over 75% for SO_3-Sug and SO_4-Sug, whereas for PO_4-Sug were around 45%.

1. Introduction

Arsenic is present in the environment from natural sources as well as human activities and has been identified as a public health problem because it has serious toxic effects even at low exposure levels. It is well known that the simple knowledge of total arsenic content in real samples is far from enough to learn about their associated toxicity. Toxicity of arsenic depends very much on its chemical forms [1,2]. Several investigations showed that inorganic arsenic species are more toxic than the organic ones. In general, organometallic compounds (i.e. methylated species) are more toxic than their corresponding inorganic species except in the case of arsenic [3–5]. Arsenic species such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide are present in marine aquatic organisms. Arsenobetaine (AsB) is the major species in fish and seafood, and arsenocholine (AsC) has been suggested as a precursor of AsB, which is the end product of marine arsenic metabolism. Arsenosugars, ribose derivatives, are the major arsenic compounds in marine algae and seaweed, although the metabolism and toxicology of these compounds is still not clear [3] and there is a lack of toxicity and chronic exposure data as well as human population studies [6].

Obtaining reliable speciation data for evaluating dietary exposure, and increasing understanding of arsenic biochemistry in algae, are hindered by the availability of suitable standards that need to be obtained for each study at small scale [7]. Chemical syntheses of some arsenosugars have been reported but they are complex and tedious. As an example, the described synthetic routes for arsenosugar sulphonate (SO_3-Sug) and arsenosugar sulfate (SO_4- Sug) provided a 5% overall yield and involved 10 steps [8]. Some attempts to prepare stock solutions by extracting different algae sources are also described, followed by purification and clean-up steps yielding milligrams of pure compounds making the approach inappropriate for routine application [9]. At present

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there are no arsenosugar calibration standards commercially available. Additionally, the availability of certified reference materials for method validation purposes is scarce and published data can only be found regarding contents of arsenosugar phosphate (PO_4-Sug) and arsenosugars sulphonate (SO_3-Sug) in a kelp dietary supplement (Thallus laminariae) (SRM 3232) from NIST [10,11], and first results on a new candidate reference material (Hijiki seaweed) for arsenosugars were reported recently [12]. Moreover, some recent reviews highlight that the availability of standards and reference materials for organic arsenic compounds are crucial for filling the data gap needed to address the human health risk from organic arsenic exposure [6,13-14].

Different approaches for sample treatment to analyze arsenic compounds have been developed as a cost- and time-saving alternative to the traditional extraction techniques [4,13,15] such as the use of resins [16], novel functionalized miniaturized membranes [17] or matrix solid-phase dispersion [3]. Solid phase extraction (SPE) has been developed as an alternative to other extraction techniques [5,18-28] and has been widely used for the separation, clean-up and concentration of several arsenic species. The retention efficiency on the SPE cartridges would be governed by the diverse pKa values and different ionic characters of the arsenic compounds and their hydrophobic interaction with the sorbent materials on the SPE cartridges and can be affected by the sample matrix and pH to a certain extent dependent on the retention mechanism of the analytes on the sorbents. The most widely studied compounds are arsenite, arsenate, MMA, DMA, AsB, AsC, trimethylarsine oxide (TMAO) or TMAI [2,16,19]. However, there are no methods for the clean-up and pre-concentration of organic arsenic species such as arsenolipids and arsenosugars. In this way, anionic exchange SPE aliphatic quaternary amine group (SAX) or aliphatic aminopropyl group considered weak anionic exchanged (WAX) can be used for such compounds that are negatively charged in aqueous solution.

The aim of this research was to investigate the feasibility of the anionic exchange SPE cartridges (SAX and WAX) as an easy and quick alternative for the isolation of arsenosugars present in algae that can be used as analytical standards for the correct identification and quantification of such compounds. This will be helpful for a better assessment of the environmental impact and potential health risks from arsenosugars in algae.

2. Experimental procedure

2.1. Reagents and materials

Analytical grade reagents were used exclusively. Ammonium dihydrogenphosphate 99.99% (Merck, Germany), 25% aqueous ammonia solution (Merck, Germany), ammonium hydrogencarbonate 99% (Fisher scientific, Spain), formic acid 98% (PanReac, Spain), ammonium formate 99.99% (Sigma-Aldrich-Merck, Germany), ammonium chloride 99.8% (Merck, Germany) and methanol 99.9% (PanReac, Spain).

2.2. Preparation of standard and working solutions

The stock standards used for inorganic arsenic species were a solution of As (III) with a certified concentration of 1002 ± 4 mg L^{-1} (Inorganic Ventures, USA) and a solution of As (V) with a certified concentration of 1002 ± 7 mg L^{-1} (Inorganic Ventures, USA), both traceable to NIST (National Institute of Standards and Technology).

Other stock standard solutions (500 mg As L^{-1}) were aqueous solutions prepared from $(CH_3)AsO(ONa)_2{\cdot}6H_2O$ (Carlo Erba, Germany) for methylarsonic acid (MMA), from $(CH_3)_2AsNaO_2{\cdot}3H_2O$ (Fluka-Fisher Scientific, Spain) for dimethylarsonic acid (DMA). These solutions were standardized against As (III) certified standard solutions. All stock solutions were kept at 4 °C in polyethylene containers. Further diluted solutions for analysis were prepared daily.

All solutions were prepared with doubly deionized water obtained from Millipore water purification system (18.2 MΩ cm^{-1} resistivity and total organic carbon < 30 $\mu g\ L^{-1}$).

2.3. Instrumentation and apparatus

For measuring total arsenic contents an Agilent 7500ce ICP-MS (Agilent, Germany) with a Burgener Ari Mist HP type nebuliser were used. For As species determination, HPLC-ICP-MS was used with an Agilent 1200 LC quaternary pump, equipped with an auto sampler and an analytical column Hamilton PRP-X100 (250 x 4.1 mm, 10 μm, Hamilton, USA). Analytical column was protected by guard column (20 mm × 2.0 mm id, 10 μm particle size) with the same characteristics. The outlet of the LC column was connected via PEEK capillary tubing to the nebulizer of the ICP-MS system.

A microwave digestor (Milestone Ethos Touch Control, Italy) was used for sample digestion before total arsenic determination.

A CRISON 2002 potentiometer (±0.1 mV) (Barcelona, Spain) equipped with a CRISON 5203 combined pH electrode from Orion Research (Boston, MA, USA) was used to measure the pH of the solutions; a centrifuge 460R of HettichZentrifugen (Tuttlingen, Germany) was used for arsenic species extraction. An analytical balance with a precision of ±0.1 mg was also used.

A Genevac™ miVac Centrifugal Concentrator (Ipswich, England), a TurboVap LV system from Caliper LifeSciences (Hopkinton, MA, USA) with nitrogen stream and a Lyophilizer Telstar Lyoquest 80 (Tokyo, Japan) were used to evaporate the eluents when needed.

Solid phase extraction (SPE) was performed using a 12-port SPE Supelco VisiprepTM vacuum manifold (Bellefonte, PA, USA). Silica-based SPE cartridges were purchased from Supelco (Merck, Germany), containing different types of sorbent materials (DSC-NH2, aminopropyl, weak interaction; DSC-SAX, quaternary amine, strong interaction) and capacities (0.5 and 1 g of bed weight).

2.4. Procedures

2.4.1. Sample preparation

Fucus Vesiculosus dietary supplement tablets were purchased at a local shop in Barcelona (Spain). The tablets were finely powdered in an agate mortar. The resulting powder was manually homogenized and stored in closed polyethylene containers at room temperature until analysis. For extracting As species, 0.25 g of the sample were weighed into centrifuge tubes and 10 mL of doubly deionized water were added. Samples were extracted using an end-over-end shaker at 30 rpm for 16 h at room temperature. The suspensions were centrifuged at 3000 rpm for 20 min and supernatant extracts were filtered through 0.45 μm nylon filters and kept at 4 °C until analysis.

2.4.2. Sample characterization: total arsenic content and arsenic speciation

The total arsenic content and the arsenic species in the sample was determined in triplicate by ICP-MS and IC-ICP-MS, respectively, following the procedures previously described [29]. In these conditions the LOQ for total arsenic is 0.04 $\mu g\,L^{-1}$ by ICP-MS, and for arsenosugars the following values of LOQ have been obtained by IC-ICP-MS: PO_4-Sug 0.1 $\mu g\,L^{-1}$; SO_3-Sug 0.4 $\mu g\,L^{-1}$; SO_4-Sug 0.6 $\mu g\,L^{-1}$.

2.4.3. Instrumental conditions ICP-MS

For arsenic quantification, ion intensity at *m/z* 75 (^{75}As) was considered. Additionally, ion intensities at *m/z* 77 ($^{40}Ar^{37}Cl$) and *m/z* 35 (^{35}Cl) were monitored to detect possible chloride interference ($^{40}Ar^{35}Cl$) at *m/z* 75. For total analysis a solution of ^{9}Be,^{103}Rh and ^{205}Tl was used as the internal standard and the samples were quantified by means of an external calibration curve from As (V) standards (0 - 50 $\mu g\ L^{-1}$). For speciation analysis peak assignment was in agreement with results in previous work [29]. Quantification was performed by external calibration curves to the nearest eluted standard. SO_3-Sug and SO_4-sug were quantified with As (V) standard whereas PO_4-Sug was quantified with MMA standard.

2.4.4. Chromatographic studies

The developed chromatographic method used a binary gradient elution program with 30 mM $NH_4H_2PO_4$ pH= 5.8 (as solvent A) and 30 mM $NH_4H_2PO_4$ pH= 8.0 (as a solvent B), both adjusted with aqueous ammonia. After optimization of the chromatographic separation (see Section 3.2) the gradient elution program used in this study started with a 3 min isocratic step at 100% solvent A and followed by a linear gradient elution up to 100% solvent B in 1 min, and an isocratic step at these last conditions for 9 min. Finally, solvent A was linearly increased up to 100% in 1 min, turning back to the initial conditions. The mobile phase flow rate was 1.5 mL min^{-1}, the injection volume was 100 μL, and the column was operated at room temperature.

2.4.5. SPE studies

For SPE preliminary studies, isolated fractions [29] containing separately SO_4-Sug and SO_3-Sug were used as testing solutions.

After optimization of the SPE procedure (see Section 3.3), silica-based SPE cartridges (DSC-SAX) with 1 g of capacity were selected. The optimized procedure was as follows: conditioning of the cartridge was made using 6 mL of MeOH, followed by 6 mL of 30 mM NH_4HCO_3 pH 8.0 in 1% MeOH. 3 mL of arsenosugar fraction was used to flow through the cartridge. A washing step with 2 mL of doubly deionized water was followed by elution step with 4 mL NH_4HCOO 0.5% in H_2O. All eluates from loading (L), washing (W), and elution procedures (E) were collected separately for subsequent analysis to determine total arsenic content by ICP-MS. All the experiments were carried out by triplicate.

2.5. Support software

ACD/pKa program from ACD/Labs (Toronto, Canada) with GALAS algorithm was used to predict acid dissociation constants of arsenosugars.

ChemDraw software from PerkinElmer Informatics, Inc. (Waltham, MA, USA) was used to estimate the log $P_{o/w}$ values.

3. Results and discussion

3.1. Sample characterization

In previous studies from the research group [29], various samples of different species of edible algae were characterized with the aim of selecting the best material for identification, separation, and isolation of arsenosugars. *Fucus Vesiculosus* was the selected algae species, as it presents the arsenosugars of interest.

The total arsenic content in the samples was determined by ICP-MS after microwave digestion as stated in the experimental section. For quality control purposes, the certified reference material ERM-CD 200 was also measured, and no significant differences were observed when comparing obtained values with certified values using a t-test at 95% confidence level. The total arsenic content was 85 ± 3 mg As kg^{-1} of sample.

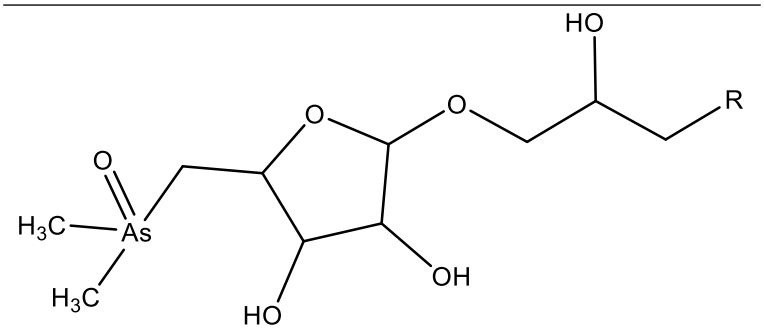
Arsenic species analysis was performed by HPLC-ICP-MS. Water was chosen as the solvent for arsenic species extraction as arsenosugars are polar and extremely soluble in water [30]. The extraction efficiency is calculated as the ratio of total arsenic present in the aqueous extracts to the total arsenic in the solutions resulting from acid digestion. Extraction efficiency was 89% (calculated as the ratio of the total content in the aqueous extract to the total arsenic content after microwave digestion) which is in accordance with previous studies [31]. Thus, it can be corroborated that water proved to be an effective solvent in the extraction of arsenic species. Column recovery was 80%, which was calculated as the ratio of the sum of species eluted from the chromatographic column to the total arsenic content in the aqueous extract injected into the column.

Concentrations expressed as mg As·kg^{-1} on dry mass, mean (SD), $n = 3$, of arsenic species in a *Fucus Vesiculosus* sample were as follows: As (III)+cations, 4.8 (0.3); DMA, 1.8 (0.1); PO_4-Sug, 4.1 (0.2); As (V), 1.7 (0.1); SO_3-Sug, 35 (1); SO_4-Sug, 13.4 (0.8). Anionic arsenosugars are the main arsenic compounds in the sample extracts, comprising the 85% of the extracted arsenic species. SO_3-Sug is the predominant species in the selected sample, accounting for 57% of the extracted arsenic. Lower concentrations of SO_4-Sug and PO_4-Sug were obtained with percentages of extracted arsenic of 22% and 7%, respectively. These results make the sample suitable for the following studies.

3.2. Optimization of the chromatographic separation

Considering their structure and pK_a values (Table 1), SO_4-Sug, SO_3-Sug and PO_4-Sug are anions at most pH values and among the typical separation mechanism (reversed phase, normal phase, ion exchange or adsorption), the ionic exchange seems to be the best choice for the separation of charged analytes from aqueous solution. Arsenosugars species analysis was performed by HPLC-ICP-MS using an anionic exchange column. The initial separation was made according with a method previously used [29] with a mobile phase consisted of 20 mM $NH_4H_2PO_4$ at pH = 5.8 adjusted with aqueous ammonia in isocratic conditions. The flow rate was adjusted to 1.5 mL min^{-1} and the injection volume was 100 µL in all analyses. In these conditions, the separation of the arsenosugars and the four available standards (Arsenite, Arsenate, DMA and MMA) is achieved in 40 min. To reduce the analysis time of arsenic species, several elution conditions were evaluated considering two factors that can be important for the separation (ionic strength and pH). Firstly, the concentration of the $NH_4H_2PO_4$ at mobile phase was studied at four levels (20, 40, 60 and 80 mM) maintaining pH at 5.8. pH was studied at three levels (5.8, 7.0 and 8.0) maintaining salt concentration at 20 mM. Fig. 1 shows the separation of the three arsenosugars in an aqueous extract of the sample studied to which the four available standards have been added. Specifically, Fig. 1A shows the influence of the ionic strength while Fig. 1B shoes the influence of the pH in this separation. As can be observed in Fig. 1A, as expected, the analysis time decrease when the ionic strength of the mobile phase increase, but the increase of the concentration of the $NH_4H_2PO_4$ (from 20 to 80 mM) at mobile phase impairs the separation of the mentioned standards. Fig. 1B shows the effect of the pH on the separation of the arsenocompounds at a concentration of the $NH_4H_2PO_4$ at mobile phase of 20 mM. In this case, the increase of the pH reduces the analysis time, but also impairs the separation of the standards. From these results several combinations salt concentration/pH were tested in order to select the best conditions for a gradient elution to achieve the baseline separation of all the compounds. Finally, an optimized gradient of pH made at 30 mM, as is explained in Section 2.4, was selected. Fig. 1C shows the baseline separation of three arsenosugars and four standards in less than 20 min.

Table 1
Structure and properties of arsenosugars.



PO_4-Sug		SO_3-Sug		SO_4-Sug	
R= $OPO_3CH_2CH(OH)CH_2OH$		R= SO_3H		R=OSO_3H	
pK_{a1}	1.2 ± 0.4	pK_{a1}	1.1 ± 0.4	pK_{a1}	- 3.3 ± 0.4
log $P_{o/w}$	−2.95	log $P_{o/w}$	−3.00	log $P_{o/w}$	−1.82

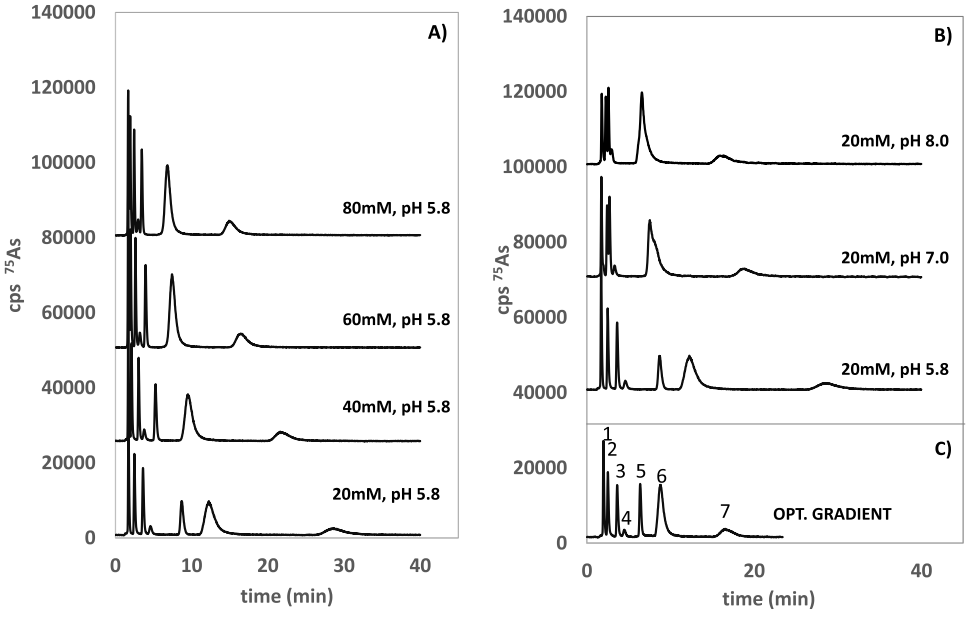


Fig. 1. Chromatographic separation of arsenic compounds by HPLC-ICP-MS. **A)** Effect of the Ionic strength of mobile phase on separation; **B)** Effect of the pH of mobile phase on separation **C)** Optimized gradient separation. Elution order: **1**. Arsenite + Cations; **2**. DMA; **3**. MMA; **4**. PO_4-Sug; **5**. Arsenate; **6**. SO_3-Sug; **7**. SO_4-Sug.

3.3. SPE studies

SPE materials range from the chemically bonded silica (with C_8 or C_{18} organic group among others) and the carbon or ion-exchange materials to the polymeric based on styrene-divinylbenzene. SPE based on polymeric resins obtained good results to extract polar compounds from aqueous samples. However, the main disadvantage of using highly crosslinked sorbents is their hydrophobicity, which, in the extraction of the most polar compounds, leads to poor retention [32]. This could be the case of arsenosugars as can be inferred from the log $P_{o/w}$ values summarized in Table 1. In addition, the arsenosugars are anions at most pH values as stated before (Table 1). So, anionic exchange cartridges were selected (DSC-SAX and DSC-NH2) as a best option considering the studied compounds as anion with a high hydrophilicity.

To study the interaction with the selected sorbent, for the SPE optimization and due to the low concentration of PO_4-Sug in the corresponding fractions only those containing SO_4-Sug, SO_3-Sug were used. Retention of the arsenosugars by different silica-based SPE cartridges (DSC-SAX and DSC-NH2) with different capacities (0.5 and 1 g) were tried. It was observed that cartridges with 1 g of sorbent retained from 4 to 6 times more than the ones with 0.5 g of sorbent. In addition, several pH values (6–10) of the samples were studied. The retention of arsenosugars was more efficient at pH 8. Fig. 2A shows the distribution of SO_4-Sug, SO_3-Sug among the SPE steps at pH 8. As it can be observed, approximately 30% of the SO_3-Sug is lost in the loading and washing steps and near 5% of the SO_4-Sug is lost in the washing step when DSC-NH2 cartridges were used, while DSC-SAX cartridges are more effective for both arsenosugars.

After the washing step with 2 mL of doubly deionized water, diverse elution solvents were assayed: 2 mL of NH_4Cl 2% followed by 2 mL of NH_4Cl 5%; 4 mL of NH_4Cl 5%, and 4 mL of NH_4HCOO 5%. The percentage of SO_3-Sug eluted is near 90% with NH_4Cl and near 100% for SO_4-Sug, while the use of NH_4HCOO 5% improves the result of SO_3-Sug up to 100%. Using a solution that contain both arsenosugars, there are no remarkable differences in the behavior of the two arsenosugars using DSC-SAX cartridge and using NH_4HCOO 5% in the elution step. However, the high concentration of salt in the eluent used (NH_4HCOO 5%) give some problems with the IC-ICP-MS system in the analysis step. Therefore, the concentration of NH_4HCOO (5, 1 and 0.5%) in the elution solvent was also

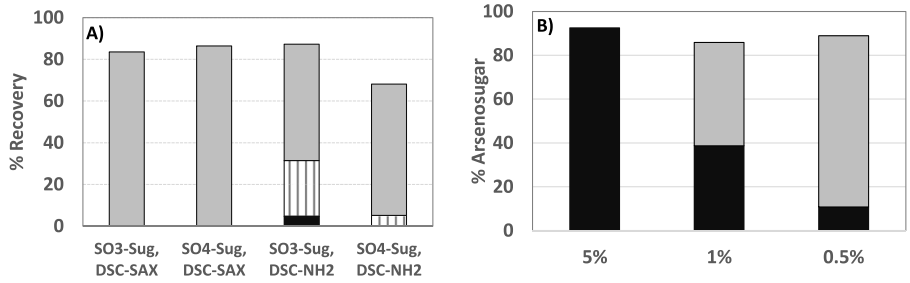


Fig. 2. Preliminary studies of SPE. **A)** Behavior of arsenosugars in DSC-SAX and DSC-NH2 cartridges: Loading step ■ ; Washing step ▥ ; E: Elution step ▒; **B)** Effect of the NH_4HCOO concentration on the elution of arsenosugars: Elution step 1 ■; Elution step 2 ▒.

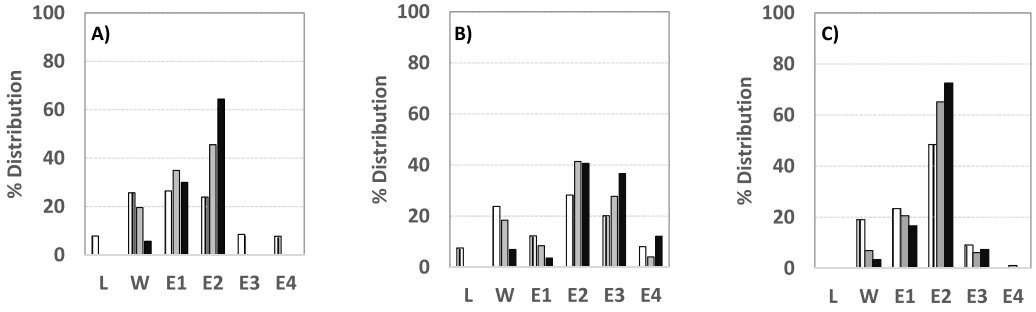


Fig. 3. Distribution of arsenosugars (%) in each step of SPE using DSC-SAX cartridges. **A)** NH_4HCOO 0.5% in H_2O; **B)** NH_4HCOO 0.5% in MeOH:H_2O (9:1); **C)** HCOOH 0.5% in MeOH:H_2O (9:1); Elution steps (**E1, E2, E3, E4**) with 2 mL each elution step. PO_4-Sug ▥; SO_3-Sug ▒; SO_4-Sug ■.

tested. Two elution steps, using 2 mL of NH_4HCOO each step, were considered. The percentage of eluted arsenic for each elution step is shown in Fig. 2B**.** Good reproducibility was achieved with RSD% values below 9%. As it can be seen in this figure, when varying the concentration of NH_4HCOO there is no significant difference between percentages of eluted arsenic considering both elution steps together. However, when using NH_4HCOO 5%, arsenosugars elute almost exclusively with the first elution volume, while with NH_4HCOO 1%, the eluted arsenosugars are distributed between the two elution steps (38% and 47% for the first and second elution, respectively). In contrast, when using NH_4HCOO 0.5% as the eluent, most arsenosugars elutes in the second elution step instead of the first one.

With the final objective of obtaining a clean and concentrated extract of the three main arsenosugars, the modification of the elution step using an easy-to-evaporate solvent such as methanol instead of water was studied. An extract from the sample that contain the three arsenosugars is used for this study and in subsequent studies. Fig. 3 shows the distribution of the arsenosugars (%) in the different SPE steps. Fig. 3A shows the profile of arsenosugars when steps (E1 to E4) of 2 mL NH_4HCOO 0.5% prepared in H_2O was used for elution. The most part of the SO_3-Sug and SO_4-Sug are obtained in the elution steps (E1+E2), a little part in the washing step (W), while PO_4-Sug appears in all the SPE steps. Fig. 3B, shows the profile of arsenosugars when 4 steps (E1 to E4) of 2 mL NH_4HCOO 0.5% prepared in MeOH:H_2O (9:1) were used to elute compounds of interest. The presence of the organic modifier changes the profile of arsenosugars that are distributed in all the SPE steps but mostly eluted in the second and third elution steps (E2+E3), showing that 6 mL of solvent elution are necessary to mostly recover the arsenosugars.

These results show that H_2O is the solvent preferred to elute arsenosugars from the SPE cartridges, as befits its polar nature, but to evaporate solvent and preconcentrate the extract the use of MeOH:H_2O mixture is the better option although the method is slightly long because it is necessary to collect a larger volume to completely elute the compounds.

Additionally, Fig. 3C shows the profile of arsenosugars when 4 steps (E1 to E4) of 2 mL HCOOH 0.5% prepared in MeOH:H_2O (9:1) were used to elute compounds of interest. To reduce the volume of the elution solvent, the use of HCOOH 0,5% in MeOH-water was tested because a change in the retention of the studied compound is expected as they will be more protonated, disrupting the electrostatic interaction with the anion exchange sorbent and then making easier its elution. Fig. 3C shows that the use of HCOOH makes that the profile changes, obtaining a profile more similar that those obtained in Fig. 3A, being arsenosugars mostly eluted in the elution steps (E1+E2). From Fig. 3 it can be deduced the different behavior of arsenosugars depending on the use of the salt or the acid in water or MeOH-water solvents. This can be explained considering that the electrostatic interaction disruption is only partial due to the strong acidic character of these compounds. The use of ACD/pKa software with a GALAS algorithm predicts accurately pKa values lower than 1.5 (Table 1), confirm that these arsenosugars are slightly protonated in acidic pH.

In addition, a study of the recovery was made using DSC-SAX cartridges, in the conditions optimized previously. Table 2 shows the absolute amount (ng) of the three arsenosugars, the RSD (%) and the recoveries for each arseno-compound obtained with different elution solvents (6 mL of acidic/ basic media) in water or MeOH-water solvents, made in triplicate. These recoveries were calculated by comparing the analytical results for extracted samples by SPE with the same sample but unextracted representing 100%. The amount of each arsenosugar obtained is comparable when different elution conditions are used. Good recoveries were obtained with all the four procedures tested for SO_3-Sug and SO_4-Sug, being from 77 to 91% and from 84 to 94%, respectively. For PO_4-Sug recoveries were around 45% in all cases.

Considering that all the tested SPE conditions have similar performances, it was important to check the purity of the fractions

Table 2
Arsenosugar recoveries obtained with DSC-SAX cartridges and different elution solvents conditions.

	As-PO_4 (ng)	RSD (%)	Recovery (%)	As-SO_3 (ng)	RSD (%)	Recovery (%)	As-SO_4 (ng)	RSD (%)	Recovery (%)
NH_4HCOO 0.5% in H_2O	158	6	45	2342	4	84	970	3	92
NH_4HCOO 0.5% in MeOH:H_2O (9:1)	162	19	46	2320	5	84	898	5	85
HCOOH 0.5% in H_2O	173	1	49	2539	2	91	998	7	94
HCOOH 0.5% in MeOH:H_2O (9:1)	150	5	43	2116	7	77	874	6	84

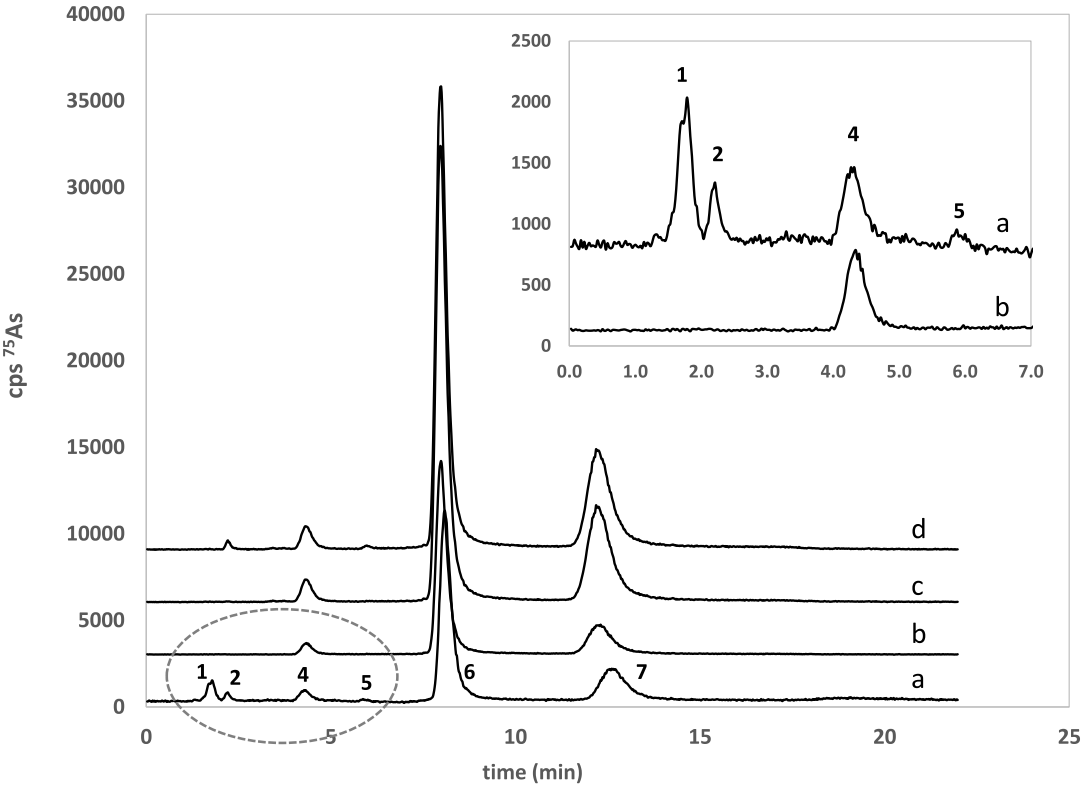


Fig. 4. Clean up obtained using different amounts of NH_4HCOO 0.5% in H_2O. **a.** Chromatogram of the extract not treated with SPE; **b.** SPE fraction obtained using 2 mL eluent; **c.** SPE fraction obtained using 4 mL eluent; **d** SPE fraction obtained using 6 mL eluent. Elution order as in Fig. 1:**1**. Arsenite + Cations; **2**. DMA; **4**. PO_4-Sug; **5**. Arsenate; **6**. SO_3-Sug; **7**. SO_4-Sug.

obtained keeping in mind the obtention of a clean solution containing the three arsenosugars isolated from other arsenic species. A careful inspection of the chromatograms shows that the cleanest solutions are obtained when NH_4HCOO 0.5% in H_2O is used as the eluent.

Fig. 4 shows the chromatograms obtained in such conditions collecting different elution volumes (2, 4 and 6 mL) to show the clean-up achieved. For comparison purposes, a chromatogram of the direct extracted sample (without SPE clean-up) is also included. It should be noted that the aqueous extract of the sample chromatogram (a) was more diluted (1/10) than the solutions obtained from SPE (3/10). Therefore, the direct comparison between chromatogram (a) and the other chromatograms (b,c,d) with quantitative purposes is not possible. The insert shows an enlargement of chromatograms of the direct extract (a) and the eluted solution with 2 mL (b). As can be observed the first part of the chromatogram (up to 7 min) is free of other arsenic species such as arsenite, arsenate, methylated forms or cations. This is also the case when eluting with 4 mL, but when 6 mL are used small amounts of dimethylated forms can be detected.

Finally, to preconcentrate, the corresponding effluents were evaporated near to dryness using diverse systems (vacuum, lyophilization, nitrogen stream). For vacuum and nitrogen stream systems, a study to evaluate the better temperature for eliminating the solvent was made using temperatures (20–80 °C) in 2 h. The higher temperatures studied (60–80 °C) seemed to degrade a part of arsenosugars and lower temperatures than 30 °C do not evaporate enough solvent in a short time. Thus, 40 °C was selected as the better option for both systems. From these two systems, nitrogen stream at 40 °C was much faster. Regarding lyophilization needs long processing time when MeOH is present, but is fast enough to get dryness of aqueous eluates. Fraction residues were reconstituted with mobile phase before analysis. Thus, isolation of arsenosugars by using SPE can be achieved with 4 mL of NH_4HCOO 0.5% in H_2O as elution solvent and a further lyophilization step allows an easily preconcentration.

4. Conclusions

In relation to the behavior of the studied arsenosugars on strong anion exchange sorbents, its character, as strong anions, has been verified that it agrees with the highly polar character of these substances. So, a decrease in retention is observed when both the polarity or acidity of the eluent are increased.

Similar arsenosugar recoveries were obtained when different elution conditions are used. In all cases, recoveries over 75% were obtained for SO_3-Sug and SO_4-Sug, whereas for PO_4-Sug recoveries

were around 45%. Additionally, a further lyophilization step allows an easily preconcentration.

The procedure developed in this work, using a strong anion exchange SPE cartridges, allows to isolate SO_3-Sug, SO_4-Sug and PO_4-Sug as the only arsenic species present in the solution. This is a preliminary step to advance for obtaining the analytical standards that are claimed in the literature.

CRediT authorship contribution statement

Alba Morales-Rodríguez: Investigation, Formal analysis, Writing - Original Draft, Visualization. **Miquel Pérez-López:** Investigation, Formal analysis. **Elle Puigpelat:** Investigation, Formal analysis. **Àngels Sahuquillo:** Conceptualization, Writing - Review & Editing. **Dolores Barrón:** Conceptualization, Writing - Original Draft, Visualization, Writing - Review & Editing, Supervision. **José Fermín López-Sánchez:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The autors declare that they have no known competint finantial interest or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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