

# Selective separation of arsenic species from aqueous solutions with immobilized macrocyclic material containing solid phase extraction columns

著者	Rahman Ismail M.M., Begum Zinnat A., Nakano Masayoshi, Furusho Yoshiaki, Maki Teruya, Hasegawa Hiroshi
journal or publication title	Chemosphere
volume	82
number	4
page range	549-556
year	2011-01-01
URL	<a href="http://hdl.handle.net/2297/25761">http://hdl.handle.net/2297/25761</a>

doi: 10.1016/j.chemosphere.2010.10.045



23 **Abstract**

24 A combination of solid phase extraction (SPE) columns was used for selective separation  
25 of water-soluble arsenic species: arsenite, arsenate, monomethylarsonic acid (MMA) and  
26 dimethylarsinic acid (DMA). The SPE columns, namely AnaLig TE-01 (TE-01), AnaLig  
27 AN-01 Si (AN-01) and AnaLig As-01 PA (As-01), contain immobilized macrocyclic material  
28 as the sorbent and commonly known as molecular recognition technology (MRT) gel. The  
29 retention, extraction and recovery behavior of the MRT gel SPE columns were studied at pH  
30 4–10. Fortified deionized water spiked with 100  $\mu\text{M}$  of arsenic species were treated at the  
31 flow rate of 0.2 mL  $\text{min}^{-1}$ .  $\text{HNO}_3$  (1.0 and 6.0 M) was used as eluent to recover the retained  
32 arsenic species from TE-01 and AN-01 SPE columns. Arsenic species retained in the As-01  
33 column were eluted with  $\text{HNO}_3$  (0.1 M) followed by  $\text{NaOH}$  (2.0 M). Likely interference from  
34 the various coexisting ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{ClO}_4^-$ )  
35 (10 mM) were negligible. Quantitative separation of As(III), As(V), MMA and DMA was  
36 achieved based on the differences in extraction and recovery behavior of the MRT gel SPE  
37 columns with pH for different arsenic species. Complexation between arsenic species and  
38 MRT gel is the core phenomenon of the proposed technique as the complexation of MRT  
39 gels is expected to be stronger than the resin-based separation processes. MRT gel SPE  
40 columns are advantageous as compared with other reported SPE columns in terms of its  
41 performance with As(III). Effortless regeneration and unaltered separation performance of  
42 the sorbent materials for more than 100 loading and elution cycles are other sturdy  
43 characteristics to consider the MRT gel SPE columns for sensitive and selective arsenic  
44 species separation.

45

46 **Keywords:** Solid phase extraction; Molecular recognition technology gel; water-soluble  
47 arsenic; selective separation; pH

## 48 **1.0 Introduction**

49 Arsenic, a ubiquitous toxic trace element, has raised a major toxicological and  
50 environmental concerns (WHO, 2001). The concentration levels, oxidation and binding states,  
51 ionic and molecular forms and metabolic pathways of As vary strongly in different  
52 environmental compartments, food chains and ultimately in humans (Mandal and Suzuki,  
53 2002). Widespread human exposure to high levels of As is reported to occur via drinking  
54 water and contaminated water irrigated food causing both cancerous and non-cancerous  
55 health effects (Karim, 2000; Rahman et al., 2008).

56 Arsenite (oxidation state + III), arsenate (oxidation state + V), monomethylarsonic acid  
57 (MMA) and dimethylarsinic acid (DMA) are common water-soluble arsenic species existing  
58 in natural water systems-a major pathway of arsenic ingestion to humans (Smedley and  
59 Kinniburgh, 2002). Arsenic toxicity in human depends strongly on its chemical form. As(III)  
60 is 10 times more toxic than As(V) while almost 70 times more toxic than the methylated  
61 forms, MMA and DMA (Squibb and Fowler, 1983). As(III), having successive acid  
62 dissociation constants ( $pK_a$ ) of 9.2, 12.2 and 13.4, is not dissociated at neutral pH and is  
63 present as a neutral species. As(V) and MMA has a wide range of  $pK_a$  values [As(V): 2.2, 6.9,  
64 11.5; MMA: 4.1, 8.7], and exist mainly as anionic species at almost all pH. DMA with a  $pK_a$   
65 value of 6.2 subsists as a cation in acidic medium (Committee on Medical and Biologic  
66 Effects of Environmental Pollutants, 1977). The United States Environmental Protection  
67 Agency proposed a maximum contaminant level of  $10 \mu\text{g L}^{-1}$  arsenic for the community  
68 water systems (USEPA, 2002). Because of increasingly stringent environmental regulations,  
69 selective and accurate measurement of arsenic species is required for precise monitoring and  
70 understanding the extent of arsenic contamination.

71 In natural waters, As usually exists at trace levels and several techniques are proposed for  
72 selective quantification and speciation analysis of arsenic species at trace levels (Barra et al.,

73 2000; Munoz and Palmero, 2005; Terlecka, 2005; Kumar and Riyazuddin, 2007; Mays and  
74 Hussam, 2009). Ion chromatography and high performance liquid chromatography separation  
75 followed by sensitive detection such as inductively coupled plasma mass spectrometry  
76 (Lintschinger et al., 1998; Bissen and Frimmel, 2000), atomic absorption spectrometry (AAS)  
77 with hydride generation interface (Hasegawa et al., 1999; Kumar and Riyazuddin, 2007) and  
78 electrospray/nanospray mass spectrometry (Pergantis et al., 1997; Ritsema et al., 1998) are  
79 some potential techniques. However, concerns related to the use of element-selective  
80 detectors to interface the chromatographic methods limit the efficiency of these techniques  
81 (Yu et al., 2003).

82 Separation and preconcentration of contaminant ions using solid sorbent materials, known  
83 as solid phase extraction (SPE) systems, have increased in popularity since the 1980s (Hosten  
84 and Welz, 1999). The technique has been developed as a cost- and time-saving alternative to  
85 the traditional extraction techniques featuring the capability to interact with a variety of metal  
86 ions including the fairly specific selectivity to a particular ion (Nickson et al., 1995; Ghaedi  
87 et al., 2008). Ion exchange resins (Leal et al., 2004; Jitmanee et al., 2005), silica gel bonded  
88 with octadecyl functional groups (Pozebon et al., 1998), yeast immobilized on controlled  
89 pore glass (Koh et al., 2005), activated alumina (Karthikeyan et al., 1999), open tubes knotted  
90 reactors (Yan et al., 2002; Herbello-Hermelo et al., 2005), polytetrafluoroethylene turnings-  
91 packed micro-columns (Anthemidis et al., 2010) have been employed as SPE sorbent  
92 material. One group of SPE materials includes the macrocyclic chelants, such as crown ethers,  
93 immobilized on a silica or polymer support (Hosten and Welz, 1999). Ion-selective behavior  
94 of SPE-type systems with immobilized macrocyclic materials has been mentioned for  
95 preconcentration and separation of metals (Bradshaw et al., 1988; Izatt et al., 1994;  
96 Hasegawa et al., 2010). SPE techniques have been applied for the quantitative  
97 analysis/speciation/separation of various trace elements including arsenic (Yalcin and Le,

98 2001; Yu et al., 2003; Liang et al., 2004; Long et al., 2006; Sanchez et al., 2009). Reports on  
99 the retention behavior of different arsenic species with some SPE systems (silica-based or  
100 resin-based) at pH 5.5 (Yalcin and Le, 2001) and pH 5.6 (Yu et al., 2003) were available. It  
101 was observed that the hydrophobic interaction of the arsenic species with the SPE materials,  
102  $pK_a$  values and ionic characters are important factors which may control the retention  
103 efficiency of the SPE columns (Yu et al., 2003). Though quantitative retention was achieved  
104 with the SPE columns for the water-soluble arsenic species (As(III), As(V), MMA and  
105 DMA), elution of the retained species was quiet difficult or sometimes unachievable for some  
106 species particularly with As(III) (Yalcin and Le, 2001; Yu et al., 2003).

107 The objective of the work is to investigate the feasibility of an ion-selective immobilized  
108 macrocyclic material attached to a solid phase, commonly known as a molecular recognition  
109 technology (MRT) gel, for the selective separation of As(III), As(V), MMA and DMA from  
110 aqueous solutions followed by graphite furnace AAS determination. We used following MRT  
111 gel SPE columns: AnaLig TE-01, AnaLig AN-01 Si and AnaLig As-01 PA. Specific MRT  
112 gel SPE columns have the advantage of the selective retention of the mentioned arsenic  
113 species followed by quantitative recovery. Most importantly, As(III) was quantitatively  
114 retained and recovered with the AnaLig As-01 PA SPE column.

## 115 **2.0 Experimental**

### 116 **2.1 Instruments**

117 A PerkinElmer model AAnalyst 600 AAS (PerkinElmer, Massachusetts, USA) including  
118 the AS-800 autosampler equipped with a transverse-heated graphite atomizer with integrated,  
119 pyrolytic graphite coated platform (THGA) and longitudinal Zeeman-effect background  
120 corrector was used. End-capped THGA tubes were used for better sensitivity and improved  
121 precision. An electrodeless discharge lamp (EDL) powered by EDL System II operated at

122 380 mA was employed as light source. The wavelength was set at the 193.7 nm resonance  
123 line and the monochromator spectral bandpass at 0.7 nm. Baseline offset correction time was  
124 set to 2.0 s and the read delay at 0.0 s. Argon was used as purge gas and the flow rate was set  
125 to 250 mL min<sup>-1</sup>. A temperature program was performed and the different steps were: first  
126 and second dry at 110 and 130 °C, ashing at 1200 °C and atomization at 2000 °C held at 30,  
127 30, 20 and 5 s respectively. After a calibration with 5 standards (0.5–2.5 μM), 20 μL of  
128 sample and 10 μL of Pd–Mg matrix modifier were introduced in the graphite furnace with  
129 three replicates of each measurement. The pH of the sample solutions was measured with a  
130 Navi F-52 pH meter (Horiba Instruments, Japan) and a combination electrode.

## 131 ***2.2 Reagents and materials***

132 Analytical grade commercial products were used. Stock solutions (10 mM) of As(III),  
133 As(V), MMA and DMA were prepared from sodium arsenite (NaAsO<sub>2</sub>) (Kanto Chemical,  
134 Japan), sodium arsenate heptahydrate (Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O), monomethylarsonic acid  
135 (CH<sub>3</sub>AsO(OH)<sub>2</sub>), dimethylarsinic acid sodium salt trihydrate (C<sub>2</sub>H<sub>6</sub>AsNaO<sub>2</sub>·3H<sub>2</sub>O) (Nacali  
136 Tesque, Japan). Working standards of metal solutions in the range of μM to mM were  
137 prepared by dilution on a weight basis. Deionized water prepared with a Barnstead 4 housing  
138 E-Pure systems was used to prepare all solutions and is referred to as EPW hereafter.

139 The experimental pH range was 4–10, and adjusted using either 1 M HCl or 1 M NaOH.  
140 MES (2-(*N*-morpholino) ethanesulfonic acid monohydrate, C<sub>6</sub>H<sub>13</sub>NO<sub>4</sub>S·H<sub>2</sub>O) (Sigma–Aldrich,  
141 USA), HEPES (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid, C<sub>8</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S) (Nacali  
142 Tesque, Japan), and TAPS (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid,  
143 C<sub>7</sub>H<sub>17</sub>NO<sub>6</sub>S) (MP Biomedicals, USA) were used as buffer reagents for pH 4–6, 7–8 and 9–10,  
144 respectively.

145 NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> were used as a source of cations while the Na-salt of anions  
146 (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, ClO<sub>4</sub><sup>-</sup>) (Nacali Tesque, Japan) were used to study the

147 effect of coexisting ions. Working solutions of 10 mM concentration were prepared in H<sub>2</sub>O  
148 matrix and pH was maintained to 7.0. The final solutions were allowed to equilibrate for 24 h  
149 before use. The interference study were carried out in a non-competitive environment by  
150 applying 4 mL of fortified deionized water at the optimized flow rate with subsequent  
151 collection using appropriate eluent.

152 Experimental variables, *e.g.* sample loading flow rate, selection of eluent and eluent  
153 concentration were optimized using As(V) spiked solutions (100 μM) in H<sub>2</sub>O matrix with pH  
154 maintained at 5.0. The MRT gel SPE columns were fed with 4 mL of sample solutions at  
155 varying flow rates, and the retention percentage of the As-species into the columns was  
156 determined. Different eluent (individual or combinations), 0.1–6.0 M HNO<sub>3</sub> and 0.1–4.0 M  
157 NaOH, was checked to select the most appropriate eluent or eluent combinations that were  
158 suitable for optimum recovery of the ‘captured’ species.

159 Certified reference materials (CRMs): BCR-713 (effluent wastewater) and BCR-610  
160 (groundwater) from EC-JRC-IRMM (European Commission Joint Research Centre, Institute  
161 of Reference Materials and Measurements), fortified samples of ‘real’ waters: tap water  
162 sample from our laboratory in Kakuma campus, Kanazawa University (Kanazawa, Japan)  
163 and river water sample from Asano River (Kanazawa, Japan) were analyzed to validate the  
164 proposed separation process. Each of the real water samples was filtered through the cellulose  
165 membrane filter of 0.45 μm pore size (Advantec, Japan) before the analysis.

166 Low-density polyethylene bottles (Nalge, USA), perfluoroalkoxy (PFA) tubes and  
167 micropipette tips (Nichiryo, Japan) were used throughout. The laboratory wares were cleaned  
168 following the sequence: (a) soaking in an alkaline detergent (Scat 20X-PF, Nacali Tesque,  
169 Japan) overnight, (b) rinsed with EPW, (c) soaking in 4 M HCl overnight, and (d) rinsed with  
170 EPW. PFA tubes and micropipette tips were cleaned according to the procedure described by  
171 Sohrin et al. (1998).



## 172 **2.3 Separation procedure**

### 173 *2.3.1 Column cleaning and conditioning*

174 MRT gel SPE columns: AnaLig TE-01 (TE-01), AnaLig AN-01 Si (AN-01), AnaLig As-  
175 01 PA (As-01) were purchased from GL Sciences, Japan. The SPE sorbents are proprietary  
176 polymeric organic materials comprised of ion-selective sequestering property. The sorption  
177 ability of the SPE materials is based on the molecular recognition and macrocyclic chemistry.  
178 SPE materials packed in 3 mL columns were used in the experiments. Column cleaning was  
179 conducted with 8 mL of 1.0 M HNO<sub>3</sub> and 6 mL of EPW. Appropriate buffer solution (5 mL)  
180 was allowed to follow through the column to ensure the desired pH condition (4–10).

### 181 *2.3.2 Retention, extraction and recovery of arsenic species with MRT gel columns*

182 The work-flow sequence for the separation of As(III), As(V), MMA and DMA using  
183 MRT gel SPE columns followed by GF-AAS determination is summarized in Table 1.  
184 Sample solution (4 mL) was passed through the SPE column at the optimized pre-set flow  
185 rate of 0.2 mL min<sup>-1</sup>. The pH of the sample solution was pre-adjusted with 0.1 M buffer  
186 solution (MES, HEPES or TAPS, whichever appropriate). The column effluent was collected.  
187 The MRT gel SPE columns were then washed with EPW to remove the analyte that is not  
188 captured by the immobilized macrocyclic material in SPE columns. The total analyte  
189 concentration in the column effluent and EPW wash solution represent the unretained  
190 concentration of analyte in the SPE system. The final step is the elution of analyte from the  
191 SPE systems. HNO<sub>3</sub> (1.0 and 6.0 M) was used to elute the arsenic species retained in TE-01  
192 and AN-01 SPE columns, and analytes retained in As-01 column were eluted with 0.1 M  
193 HNO<sub>3</sub> followed by 2.0 M NaOH. The arsenic concentrations in the sample, effluents and  
194 eluent solutions were measured with GF-AAS. Three replicate measurements per sample  
195 were made in all instances. The peak height of the reported signal was proportional to the  
196 concentration of the respective arsenic species and was used for all measurements.

197 The terms, retention, extraction and recovery, were used to explain the separation  
198 performance of the SPE systems. The retention ratio was calculated comparing the analyte  
199 concentration in the sample solution loaded in SPE columns with that in the solution passed  
200 through the columns providing only the information about the concentration of analyte sorbed  
201 in the SPE systems. On the other hand, the analyte concentrations in the column effluent,  
202 EPW wash solution and eluent were measured to understand the extraction and recovery  
203 behavior of the SPE columns. The extraction ratio of each column for the individual species  
204 was calculated by comparing the numbers of mol of analyte in the eluent with the cumulative  
205 number of mol of analyte present in the total effluents. Numbers of mol of analyte recovered  
206 in all fractions were compared with the numbers of mol of analyte in the solution loaded to  
207 the column to calculate the recovery ratio.

## 208 **3.0 Results and discussion**

### 209 ***3.1 Optimization of variables***

#### 210 ***3.1.1 Sample loading flow rate***

211 The flow rate of the metal-rich sample solution has a reasonable impact on the metal  
212 retention rate in SPE columns (Bag et al., 1998). Effect of sample loading flow rates adjusted  
213 in the range of 0.2–4.0 mL min<sup>-1</sup> (Table 2) was checked at the optimum conditions.  
214 Quantitative retention up to the flow rates of 0.25 mL min<sup>-1</sup> was observed. The retention  
215 capacities decrease gradually with the increase of flow rates above 0.25 mL min<sup>-1</sup>. Such  
216 behavior indicates the constant retaining capability of the MRT gel at the initial loading  
217 period. Therefore, a flow rate of 0.2 mL min<sup>-1</sup> was applied to ensure maximum retention of  
218 the analyte from MRT Gel SPE columns.

### 219 3.1.2 Selection of eluent and eluent concentration

220 The eluent should be able to extract the analyte without affecting the quantitative  
221 determination of analytes (Chen et al., 2009). Analytes retained in the TE-01 and AN-01 SPE  
222 columns were eluted with HNO<sub>3</sub> (4 mL) of varying concentrations (0.1–6.0 M). The recovery  
223 patterns were similar and the recovery rates became constant for the eluent concentration  
224 above 0.5 M (Figs. 1a and 1b). However, greater than or equal to 5.0 M acids were  
225 recommended for the elution of bound ions in TE-01 and AN-01 SPE columns (IBC  
226 Advanced Technologies, 2007, 2009). Hence, a combination of 1.0 M HNO<sub>3</sub> (2 mL) and 6.0  
227 M HNO<sub>3</sub> (1 mL) was selected as eluent for the subsequent experiments to ensure the  
228 complete elution of the analyte when treated with TE-01 or AN-01. On the other hand, only  
229 0.1–4.0 M NaOH (2 mL) or 0.1–6.0 M HNO<sub>3</sub> (2 mL) was found unsuitable for the elution of  
230 analytes from As-01. Combinations of 0.1–4.0 M NaOH (1 mL) followed by 2.0 M HNO<sub>3</sub> (1  
231 mL) and vice-versa were used to check the elution of arsenic species from the As-01 column  
232 (Figs. 1c and 1d). The recovery was achieved at quantitative maximum for the following  
233 eluent combination: 0.1 M HNO<sub>3</sub> (1 mL) + 2.0 M NaOH (1 mL), and was applied for the next  
234 experiments with As-01 MRT gel column.

### 235 3.2 Retention behavior of the MRT gel SPE columns

236 The retention efficiency of the MRT gel SPE columns for different arsenic species at  
237 varying pH is illustrated in Fig. 2. The retention (%) of As(III) was negligible with TE-01 and  
238 AN-01 SPE columns. Average retention efficiency (%) of 92±3.7 was observed with As-01  
239 column at the pH 4 to 10 while it was highest at pH 7 (96±1.2). As(III) mainly exists as a  
240 neutral species, As(OH)<sub>3</sub>, at the entire range of the studied pH. Thus, the macrocyclic  
241 materials immobilized in the TE-01 and AN-01 columns were not capable of retaining the  
242 neutral form of As(III). Almost complete retention of As(V) and MMA was achieved at pH 4  
243 to 7 with all the MRT gel SPE columns. As(V) and MMA remain in the anionic form within

244 that pH range, as evident from the corresponding  $pK_a$  values. Therefore, all of the MRT gel  
245 columns investigated can retain the anionic form of As(V) and MMA. DMA, which exists as  
246 a cation in the acidic medium, was retained at an average efficiency (%) of  $94\pm 3.3$  with As-  
247 01 column between pH 4 and 6 while the retention was not that notable with TE-01 and AN-  
248 01 columns.

249 Data evaluation showed that the most significant finding of our work was with As(III).  
250 Yu et al. (2003) checked 11 SPE systems at pH 5.6 and found that none of them were capable  
251 of retaining As(III) quantitatively. Yalcin and Le (2001) worked with 7 SPE systems and  
252 reported that Alumina-A, -B and -N (normal phase in acidic, basic, and neutral activity; from  
253 Millipore-Waters, Missisauga, ON, Canada) and silica-based LC-SCX (sulfonic acid-bonded;  
254 from Supelco, Bellefonte, PA, USA) columns can retain As(III) at the pH of 5.5. None of  
255 those SPE systems were recommended for As(III) separation considering the difficulty in  
256 elution. In our study, at pH 7, As(III) was completely retained at As-01 SPE column followed  
257 by quantitative recovery.

### 258 ***3.3 Extraction and recovery behavior of the MRT gel SPE columns***

259 The extraction behavior of the MRT gel SPE columns with four arsenic species is  
260 illustrated in Fig. 3. A similar extraction pattern was observed with TE-01 and AN-01 SPE  
261 columns; As(III) was not captured, As(V) was captured at an average rate (%) of  $99\pm 0.5$  until  
262 pH 8, MMA extracted at an average percent rate of  $99\pm 0.60$  at pH 6 and 7, and the highest  
263 extraction (%) of  $71\pm 4.6$  was observed at pH 7 for DMA. With As-01 SPE columns, the  
264 average extraction (%) was  $96\pm 3.2$  at pH 4–6 for As(V), MMA and DMA, while it was  
265  $99\pm 1.1$  at pH 7–9 for As(III).

266 Recovery (%) of the arsenic species with the MRT gel SPE columns is shown in Fig. 4.  
267 TE-01 SPE columns showed quantitative recovery performance at the entire studied pH range  
268 for all the arsenic species. AN-01 SPE columns showed similar behavior with As(III), As(V)

269 and DMA while fluctuating recovery was achieved for MMA at different pH. A gradual  
270 increase in the recovery (%) was observed from pH 4 to pH 10 with As-01 SPE columns, and  
271 expected maximum recoveries were achieved for all the arsenic species at pH 7.

272 The extraction and recovery behavior of the MRT gel SPE columns leads us to the  
273 following assumptions: (i) TE-01 and AN-01 columns are not effective for As(III) separation  
274 but can be used to separate other target species (As(V), MMA and DMA) quantitatively at  
275 varying pH conditions; (ii) selective separation and complete elution of As(III) is possible  
276 with the As-01 column; (iii) the As-01 column can also be used to preconcentrate the targeted  
277 water-soluble arsenic species for the determination of total arsenic content in the samples, if  
278 selective separation is not desired; and (iv) column regeneration process is simple because the  
279 retained analytes are completely eluted.

### 280 ***3.4 Interference studies***

281 Cations of alkaline and alkaline earth metals are always found in water samples and have  
282 the capability to compete with the target metal ions during the binding with the SPE material,  
283 and common anions have the ability to bind with the target metal ions. In their presence, the  
284 efficiency of the SPE material to bind the target ions may be reduced resulting in a reduction  
285 of the recovery. The effects of matrix ions in water samples on the recovery of the spiked  
286 sample solutions of 100  $\mu\text{M}$  As(III), As(V), MMA and DMA were investigated. The  
287 recovery of different arsenic species in the presence of 10 mM of different ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  
288  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{ClO}_4^-$ ) in the water samples were observed  
289 in the range of  $95\pm 2.7$ – $100\pm 3.2\%$ . Therefore, there is limited possibility of the interference  
290 from the matrix ions commonly found in sample waters, which is may be due to the selective  
291 separation capability of the MRT gel SPE materials.

### 292 **3.5 Retention capacity and regeneration of the SPE columns**

293 Retention capacity of the MRT gel SPE columns is important for determining the stability  
294 of the MRT gel SPE columns during the separation process. Analyte concentration and  
295 breakthrough volume (the volume of sample that causes the target analyte to be eluted from  
296 the SPE columns) were used to find out the retention capacity (Yu et al., 2003). After arsenic-  
297 spiked sample solutions were passed through the SPE columns, the retention capacity was  
298 expressed in terms of mmol of analyte captured in one gram of SPE material. The retention  
299 capacities of the MRT gel SPE columns at pH 7 were calculated as follows:  $0.40 \pm 0.02$  mmol  
300  $\text{g}^{-1}$  TE-01,  $0.39 \pm 0.02$  mmol  $\text{g}^{-1}$  AN-01 and  $0.31 \pm 0.01$  mmol  $\text{g}^{-1}$  As-01 (sample solution– 10  
301 mM of As(V), matrix–  $\text{H}_2\text{O}$ , flow rate–  $0.2 \text{ mL min}^{-1}$ , elution– 2 mL of  $\text{HNO}_3$  + 1 mL of 6  
302 M  $\text{HNO}_3$  + 1 mL of EPW, for TE-01 and AN-01 SPE columns and 1 mL of 0.1 M  $\text{HNO}_3$  + 1  
303 mL of 2.0 M NaOH + 2 mL of EPW). The result was in good agreement with the certified  
304 values for the MRT gel SPE columns (IBC Advanced Technologies, 2006, 2007, 2009). The  
305 regeneration ability of the MRT gel SPE columns was investigated, and it was observed that  
306 more than 100 loading and elution cycles can be performed without the loss of analytical  
307 performance. SPE systems with macrocycles attached onto solid supports allow selective  
308 separation of analytes from matrix facilitating the repeated use of the macrocycles (Bradshaw  
309 et al., 1988; Horwitz et al., 1992; Izatt, 1997).

### 310 **3.6 Scheme for selective separation of arsenic species**

311 The differences in extraction and recovery pattern of MRT gel SPE columns for different  
312 arsenic species enabled us to propose a selective separation method. The method is based on  
313 the selective retention of the arsenic species followed by quantitative selective recovery at the  
314 elution step. Retention, extraction and recovery behavior of three MRT gel SPE columns:  
315 TE-01, AN-01 and As-01 were studied and combined to design a multi-step separation  
316 technique for quantitative measurement of As(III), As(V), MMA and DMA. Another MRT

317 gel SPE column, AnaLig AN-02, was also checked. The retention, extraction and recovery  
318 behaviors of the AN-02 column were somewhat similar with those of AN-01 column.  
319 Therefore, AN-02 column can be considered as an alternative of AN-01 column in the  
320 separation process. The scheme for selective separation with subsequent quantitative  
321 measurement of the arsenic species by GF-AAS technique is shown in Fig. 5.

322 At pH 5, As(V) and MMA were quantitatively retained in the TE-01 SPE column while  
323 As(III) and DMA remained in the column effluent. The captured species was eluted with  
324 HNO<sub>3</sub>. The eluted solution was separated into two equal portions, and pH was adjusted to 5  
325 and 8 respectively. When each of the pH-adjusted portions independently treated with AN-01  
326 SPE columns, As(V) and MMA were quantitatively extracted and recovered from the eluted  
327 solution, respectively, at pH 5 and pH 8. The column effluent containing As(III) and DMA  
328 were adjusted to pH 9, and treated with As-01 SPE column. DMA remained in the solution  
329 that passed through the SPE material while As(III) was selectively captured. Captured As(III)  
330 was eluted with the eluent combination of 0.1 M HNO<sub>3</sub> followed by 2.0 M NaOH. GF-AAS  
331 were used to determine the concentration of the individual arsenic species.

### 332 **3.7 Analytical characteristics**

333 The concentrations of As(III), As(V), MMA and DMA in the treated solutions from MRT  
334 gel SPE columns were measured using GF-AAS. At optimum conditions, the linear range  
335 was found to be 0.01–0.32 µg mL<sup>-1</sup> As(III), 0.01–0.78 µg mL<sup>-1</sup> As(V), 0.01–0.35 µg mL<sup>-1</sup>  
336 MMA and 0.01–0.54 µg mL<sup>-1</sup> DMA. The method detection limits were calculated by three  
337 times the standard deviation ( $n = 15$ ) of the blank. The values were 0.06 µg L<sup>-1</sup> for As(III)  
338 and As(V), and 0.05 µg L<sup>-1</sup> for MMA and DMA. The precision of the method was also  
339 studied. The repeatability, as relative standard deviation, was 0.65, 2.93, 2.25 and 1.20%,  
340 calculated from 10 replicate measurements at the 1.0 µM of As(III), As(V), MMA and DMA  
341 respectively.

### 342 **3.8 Accuracy and applications**

343 The accuracy of the proposed separation scheme was evaluated by analyzing two EC-  
344 JRC-IRMM CRMs, namely BCR-713 (effluent wastewater) and BCR-610 (groundwater)  
345 (Table 3). None of the arsenic species measured in this work has either certified or literature  
346 values. Our values for the total of all arsenic species determined for both BCR-713 and BCR-  
347 610 were in good agreement with the certified value, the calculated recoveries, 97% for BCR-  
348 713 and 94% for BCR-610, were satisfactory. The proposed separation scheme was also  
349 applied to the analysis of local natural water samples (tap water and river water) and was  
350 validated by spiking the samples with known amounts of As(III), As(V), MMA and DMA  
351 (Table 4). The recoveries from spiked solutions were varied in the range  $98\pm 1.6$ – $102\pm 1.7\%$ .

### 352 **4.0 Conclusions**

353 The application of three MRT gel SPE columns (TE-01, AN-01 and As-01) for selective  
354 separation of four different arsenic species (As(III), As(V), MMA and DMA) was  
355 demonstrated. Retention behaviors of the arsenic species were varied with the change of pH  
356 at the range of 4 to 10. TE-01 and AN-01 SPE columns were unable to retain As(III) while  
357 As-01 showed the ability to retain all the species at a certain pH quantitatively. Either HNO<sub>3</sub>  
358 or a combination of HNO<sub>3</sub> and NaOH were used as eluent to recover the ‘captured’ species  
359 from the MRT gel structure. However, the recovery ratio was also found to depend on the pH.  
360 pH-dependent retention and recovery behavior of the MRT gel SPE columns were used to  
361 design a selective separation scheme for quantitative determination of a particular arsenic  
362 species in the sample solution. It is possible to overcome the tedious preconcentration process  
363 by following the proposed selective separation technique. To the best of our knowledge, it is  
364 the first ever report dealing with SPE columns equipped with immobilized macrocyclic  
365 material as sorbent material for selective determination of arsenic in water. In addition,  
366 quantitative retention followed by recovery of As(III) was achieved with As-01 column



367 which was previously not achieved with any other reported SPE systems. Easy operation,  
368 virtually unlimited loading and elution capability of the sorbent material without losing the  
369 analytical performance and high-sensitive separation ability can make the proposed technique  
370 as a useful one for selective separation of arsenic species from natural waters.

371

## 372 **Acknowledgement**

373 This research was partly supported by Grants-in-Aid for Scientific Research (K22042)  
374 from Ministry of the Environment, Japan.

375

376

377

378

379

380

381

382

383

384

385

386

387

388

389 **References**

- 390 Anthemidis, A.N., Zachariadis, G.A., Stratis, J.A., 2010. On-line preconcentration and determination  
391 of nickel and zinc in natural water samples by flow injection - flame atomic absorption  
392 spectrometry using PTFE-turnings for column packing. *Int. J. Environ. An. Ch.* 90, 127-136.
- 393 Bag, H., Lale, M., Turker, A.R., 1998. Determination of iron and nickel by flame atomic absorption  
394 spectrophotometry after preconcentration on *Saccharomyces cerevisiae* immobilized sepiolite.  
395 *Talanta* 47, 689-696.
- 396 Barra, C.M., Santelli, R.E., Abrao, J.J., de la Guardia, M., 2000. Arsenic speciation - A review. *Quim.*  
397 *Nova* 23, 58-70.
- 398 Bissen, M., Frimmel, F.H., 2000. Speciation of As(III), As(V), MMA and DMA in contaminated soil  
399 extracts by HPLC-ICP/MS. *Fresen. J. Anal. Chem.* 367, 51-55.
- 400 Bradshaw, J.S., Bruening, R.L., Krakowiak, K.E., Tarbet, B.J., Bruening, M.L., Izatt, R.M.,  
401 Christensen, J.J., 1988. Preparation of silica gel-bound macrocycles and their cation-binding  
402 properties. *J. Chem. Soc. Chem. Comm.* 812-814.
- 403 Chen, D., Huang, C., He, M., Hu, B., 2009. Separation and preconcentration of inorganic arsenic  
404 species in natural water samples with 3-(2-aminoethylamino) propyltrimethoxysilane  
405 modified ordered mesoporous silica micro-column and their determination by inductively  
406 coupled plasma optical emission spectrometry. *J. Hazard. Mater.* 164, 1146-1151.
- 407 Committee on Medical and Biologic Effects of Environmental Pollutants, 1977. Arsenic: Medical and  
408 Biologic Effects of Environmental Pollutants. National Academy of Sciences, Washington,  
409 D.C.
- 410 Ghaedi, M., Shokrollahi, A., Kianfar, A.H., Mirsadeghi, A.S., Pourfarokhi, A., Soylak, M., 2008. The  
411 determination of some heavy metals in food samples by flame atomic absorption  
412 spectrometry after their separation-preconcentration on bis salicyl aldehyde, 1,3 propan  
413 diimine (BSPDI) loaded on activated carbon. *J. Hazard. Mater.* 154, 128-134.
- 414 Hasegawa, H., Matsui, M., Okamura, S., Hojo, M., Iwasaki, N., Sohrin, Y., 1999. Arsenic speciation  
415 including 'hidden' arsenic in natural waters. *Appl. Organomet. Chem.* 13, 113-119.
- 416 Hasegawa, H., Rahman, I.M.M., Kinoshita, S., Maki, T., Furusho, Y., 2010. Non-destructive  
417 separation of metal ions from wastewater containing excess aminopolycarboxylate chelant in  
418 solution with an ion-selective immobilized macrocyclic material. *Chemosphere* 79, 193-198.
- 419 Herbello-Hermelo, P., Barciela-Alonso, M.C., Bermejo-Barrera, A., Bermejo-Barrera, P., 2005. Flow  
420 on-line sorption preconcentration in a knotted reactor coupled with electrothermal atomic  
421 absorption spectrometry for selective AS(III) determination in sea-water samples. *J. Anal.*  
422 *Atom. Spectrom.* 20, 662-664.

423 Horwitz, E., Dietz, M., Chiarizia, R., 1992. The application of novel extraction chromatographic  
424 materials to the characterization of radioactive waste solutions. *J. Radioanal. Nucl. Ch.* 161,  
425 575-583.

426 Hosten, E., Welz, B., 1999. Evaluation of an immobilised macrocyclic material for on-line column  
427 preconcentration and separation of cadmium, copper and lead for electrothermal atomic  
428 absorption spectrometry. *Anal. Chim. Acta* 392, 55-65.

429 IBC Advanced Technologies, 2006. AnaLig® Data Sheet: As-01 PA. IBC Advanced Technologies,  
430 Inc., Utah, USA.

431 IBC Advanced Technologies, 2007. AnaLig® Data Sheet: TE-01 and TE-02. IBC Advanced  
432 Technologies, Inc., Utah, USA.

433 IBC Advanced Technologies, 2009. AnaLig® Data Sheet: AN-01 Si. IBC Advanced Technologies,  
434 Inc., Utah, USA.

435 Izatt, R.M., 1997. Review of selective ion separations at BYU using liquid membrane and solid phase  
436 extraction procedures. *J. Incl. Phenom. Macro.* 29, 197-220.

437 Izatt, R.M., Bradshaw, J.S., Bruening, R.L., Bruening, M.L., 1994. Solid phase extraction of ions of  
438 analytical interest using molecular recognition technology. *Am. Lab.* 26, 28C-28M

439 Jitmanee, K., Oshima, M., Motomizu, S., 2005. Speciation of arsenic(III) and arsenic(V) by  
440 inductively coupled plasma-atomic emission spectrometry coupled with preconcentration  
441 system. *Talanta* 66, 529-533.

442 Karim, M., 2000. Arsenic in groundwater and health problems in Bangladesh. *Water Res.* 34, 304-310.

443 Karthikeyan, S., Prasada Rao, T., Iyer, C.S.P., 1999. Determination of arsenic in sea water by sorbent  
444 extraction with hydride generation atomic absorption spectrometry. *Talanta* 49, 523-530.

445 Koh, J., Kwon, Y., Pak, Y.-N., 2005. Separation and sensitive determination of arsenic species  
446 ( $As^{3+}/As^{5+}$ ) using the yeast-immobilized column and hydride generation in ICP-AES.  
447 *Microchem. J.* 80, 195-199.

448 Kumar, A.R., Riyazuddin, P., 2007. Non-chromatographic hydride generation atomic spectrometric  
449 techniques for the speciation analysis of arsenic, antimony, selenium, and tellurium in water  
450 samples - a review. *Int. J. Environ. An. Ch.* 87, 469-500.

451 Leal, L.O., Semenova, N.V., Forteza, R., Cerdà, V., 2004. Preconcentration and determination of  
452 inorganic arsenic using a multisyringe flow injection system and hydride generation-atomic  
453 fluorescence spectrometry. *Talanta* 64, 1335-1342.

454 Liang, P., Liu, Y., Guo, L., Zeng, J., Lu, H.B., 2004. Multiwalled carbon nanotubes as solid-phase  
455 extraction adsorbent for the preconcentration of trace metal ions and their determination by  
456 inductively coupled plasma atomic emission spectrometry. *J. Anal. Atom. Spectrom.* 19,  
457 1489-1492.

458 Lintschinger, J., Schramel, P., Hatalak-Rauscher, A., Wendler, I., Michalke, B., 1998. A new method  
459 for the analysis of arsenic species in urine by using HPLC-ICP-MS. *Fresen. J. Anal. Chem.*  
460 362, 313-318.

461 Long, X.B., Miro, M., Hansen, E.H., Estela, J.M., Cerda, V., 2006. Hyphenating multisyringe flow  
462 injection lab-on-valve analysis with atomic fluorescence spectrometry for on-line bead  
463 injection preconcentration and determination of trace levels of hydride-forming elements in  
464 environmental samples. *Anal. Chem.* 78, 8290-8298.

465 Mandal, B.K., Suzuki, K.T., 2002. Arsenic round the world: A review. *Talanta* 58, 201-235.

466 Mays, D.E., Hussam, A., 2009. Voltammetric methods for determination and speciation of inorganic  
467 arsenic in the environment-A review. *Anal. Chim. Acta* 646, 6-16.

468 Munoz, E., Palmero, S., 2005. Analysis and speciation of arsenic by stripping potentiometry: a review.  
469 *Talanta* 65, 613-620.

470 Nickson, R.A., Hill, S.J., Worsfold, P.J., 1995. Analytical perspective. Solid phase techniques for the  
471 preconcentration of trace metals from natural waters. *Anal. Proc.* 32, 387-395.

472 Pergantis, S.A., Winnik, W., Betowski, D., 1997. Determination of ten organoarsenic compounds  
473 using microbore high-performance liquid chromatography coupled with electrospray mass  
474 spectrometry mass spectrometry. *J. Anal. Atom. Spectrom.* 12, 531-536.

475 Pozebon, D., Dressler, V.L., Gomes Neto, J.A., Curtius, A.J., 1998. Determination of arsenic(III) and  
476 arsenic(V) by electrothermal atomic absorption spectrometry after complexation and sorption  
477 on a C-18 bonded silica column. *Talanta* 45, 1167-1175.

478 Rahman, I.M.M., Nazim Uddin, M., Hasan, M.T., Hossain, M.M., 2008. Assimilation of arsenic into  
479 edible plants grown in soil irrigated with contaminated groundwater. In: Bundschuh, J.,  
480 Armienta, M.A., Birkle, P., Bhattacharya, P., Matschullat, J., Mukherjee, A.B. (Eds.). *Natural*  
481 *Arsenic in Groundwaters of Latin America*. CRC Press/Balkema, Leiden, The Netherlands,  
482 pp. 351-358.

483 Ritsema, R., Dukan, L., Navarro, T.R.I., van Leeuwen, W., Oliveira, N., Wolfs, P., Lebret, E., 1998.  
484 Speciation of arsenic compounds in urine by LC-ICP MS. *Appl. Organomet. Chem.* 12, 591-  
485 599.

486 Sanchez, W.M., Zwicker, B., Chatt, A., 2009. Determination of As(III), As(V), MMA and DMA in  
487 drinking water by solid phase extraction and neutron activation. *J. Radioanal. Nucl. Ch.* 282,  
488 133-138.

489 Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic  
490 in natural waters. *Appl. Geochem.* 17, 517-568.

491 Sohrin, Y., Iwamoto, S.-i., Akiyama, S., Fujita, T., Kugii, T., Obata, H., Nakayama, E., Goda, S.,  
492 Fujishima, Y., Hasegawa, H., Ueda, K., Matsui, M., 1998. Determination of trace elements in  
493 seawater by fluorinated metal alkoxide glass-immobilized 8-hydroxyquinoline concentration

494 and high-resolution inductively coupled plasma mass spectrometry detection. *Anal. Chim.*  
495 *Acta* 363, 11-19.

496 Squibb, K.S., Fowler, B.A., 1983. The toxicity of arsenic and its compounds. In: Fowler, B.A. (Ed.).  
497 *Biological and Environmental Effects of Arsenic*. Elsevier Science Publishers B.V., New  
498 York, pp. 233-269.

499 Terlecka, E., 2005. Arsenic speciation analysis in water samples: A review of the hyphenated  
500 techniques. *Environ. Monit. Assess.* 107, 259-284.

501 USEPA, 2002. Implementation Guidance for the Arsenic Rule - Drinking Water Regulations for  
502 Arsenic and Clarifications to Compliance and New Source Contaminants Monitoring (EPA-  
503 816-K-02-018). United States Environmental Protection Agency (USEPA), Washington, DC.

504 WHO, 2001. Environmental Health Criteria 224: Arsenic and Arsenic Compounds World Health  
505 Organization (WHO), Geneva.

506 Yalcin, S., Le, X.C., 2001. Speciation of arsenic using solid phase extraction cartridges. *J. Environ.*  
507 *Monit.* 3, 81-85.

508 Yan, X.-P., Yin, X.-B., He, X.-W., Jiang, Y., 2002. Flow injection on-line sorption preconcentration  
509 coupled with hydride generation atomic fluorescence spectrometry for determination of  
510 (ultra)trace amounts of arsenic(III) and arsenic(V) in natural water samples. *Anal. Chem.* 74,  
511 2162-2166.

512 Yu, C.H., Cai, Q.T., Guo, Z.X., Yang, Z.G., Khoo, S.B., 2003. Inductively coupled plasma mass  
513 spectrometry study of the retention behavior of arsenic species on various solid phase  
514 extraction cartridges and its application in arsenic speciation. *Spectrochim. Acta B* 58, 1335-  
515 1349.

516

517

518

519

520

521

522

523

524

525 Table 1. Separation process of As(III), As(V), MMA and DMA using MRT gel SPE columns

Step	Function	Solution	Volume (mL)	Flow rate (mL min <sup>-1</sup> )
1	Rinsing 1	0.1 M HNO <sub>3</sub>	8	0.5
2	Rinsing 2	EPW	6	0.5
3	Conditioning	200 mM NaNO <sub>3</sub> + 0.1 M buffer solution*	32–40	0.2–0.5
4	Collection	100 μM As-species spiked sample solution	4	0.2
5	Washing	EPW	4	0.2
6	Elution 1	<i>For TE-01 or AN-01 SPE columns</i> 1 M HNO <sub>3</sub>	2	0.2
		<i>For As-01 SPE column</i> 0.1 M HNO <sub>3</sub>	1	0.2
7	Elution 2	<i>For TE-01 or AN-01 SPE columns</i> 6 M HNO <sub>3</sub>	1	0.2
		<i>For As-01 SPE column</i> 2.0 M NaOH	1	0.2
8	Elution 3	<i>For TE-01 or AN-01 SPE columns</i> EPW	1	0.2
		<i>For As-01 SPE column</i> EPW	2	0.2

526 \*MES Buffer (pH 4–6), HEPES Buffer (pH 7–8), TAPS Buffer (pH 9–10)

527

528

529

530

531

532

533

534

535 Table 2. Effect of the sample loading flow-rates on the retention capacities (%) of the MRT  
536 gel SPE columns

Flow rate (mL min <sup>-1</sup> )	TE-01	AN-01	As-01
0.20	101±3.8	100±3.7	101±4.6
0.25	99±3.0	100±3.4	100±4.3
0.30	88±2.8	82±2.6	92±3.8
0.50	75±2.4	71±2.7	87±2.9
1.00	74±1.8	68±3.2	82±2.7
2.00	65±2.6	62±1.6	71±3.4
4.00	62±3.2	59±1.8	68±2.2

537

538

539

540

541

542

543

544

545

546

547

548

549

550

551 Table 3. Analysis of EC-JRC-IRMM CRMs for arsenic species

Arsenic species	Effluent Wastewater CRM		Groundwater CRM	
	BCR-713 ( $\mu\text{g L}^{-1}$ )		BCR-610 ( $\mu\text{g L}^{-1}$ )	
	This work	Certified value	This work	Certified value
As(III)	1.9±0.3	NR	3.3±0.6	NR
As(V)	7.1±1.2	NR	6.9±1.1	NR
MMA	BDL	NR	BDL	NR
DMA	0.4 ±0.1	NR	BDL	NR
$\Sigma$ (As-species)	9.4±1.4	9.7±1.1	10.2±1.6	10.8±0.4

552 \*'BDL' – Below Detectable Limit; 'NR'– Not reported

553

554

555

556

557

558

559

560

561

562

563

564

565

566



567 Table 4. Determination of arsenic species in the fortified samples of 'real' waters

Arsenic species	Tap water			River water		
	Added ( $\mu\text{g L}^{-1}$ )	Found ( $\mu\text{g L}^{-1}$ )	Recovery (%)	Added ( $\mu\text{g L}^{-1}$ )	Found ( $\mu\text{g L}^{-1}$ )	Recovery (%)
As(III)	0	BDL	–	0	0.7±0.12	–
	19.5	19.3±0.10	99±0.5	20	19.4±0.41	99±2.1
As(V)	0	BDL	–	0	1.3±0.15	–
	31.2	31.3±0.48	100±1.5	31.2	30.5±0.51	98±1.6
MMA	0	BDL	–	0	BDL	–
	21.0	21.3±0.34	102±1.7	21.0	20.7±0.43	99±2.0
DMA	0	BDL	–	0	0.1±0.01	–
	32.1	32.3±0.27	101±0.8	32.1	31.9±0.60	99±1.9

568 \*'BDL' – Below Detectable Limit

569

570

571

572

573

574

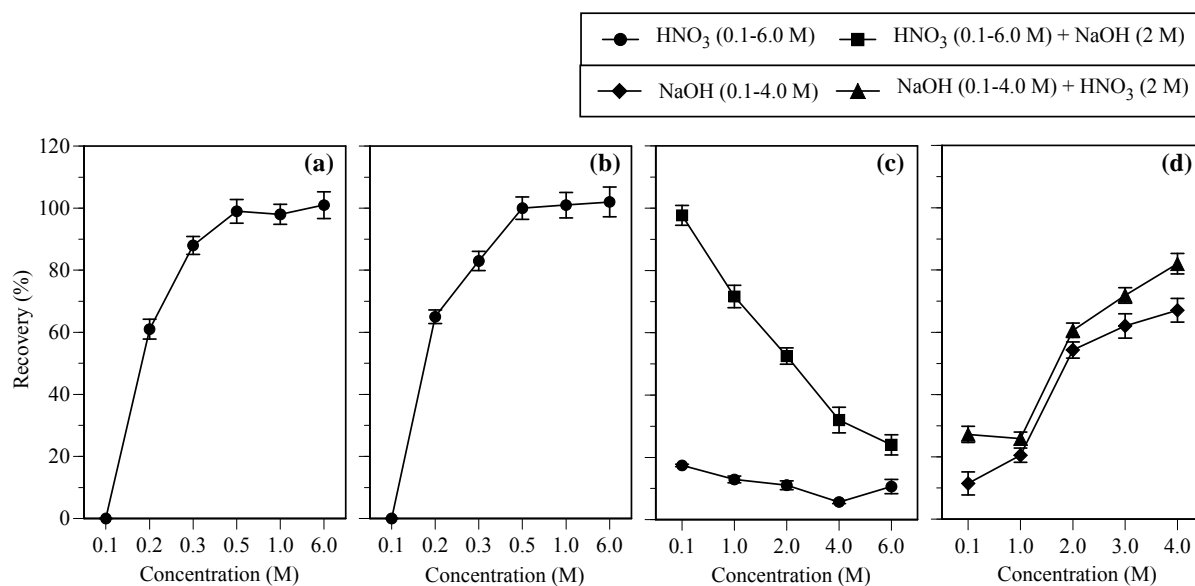
575

576

577

578

579



580

581 Figure 1: Selection of eluent and eluent concentration: (a) AnaLig TE-01 (HNO<sub>3</sub>-  
 582 0.1/0.2/0.3/0.5/1.0/6.0 M) (b) AnaLig AN-01 Si (HNO<sub>3</sub>- 0.1/0.2/0.3/0.5/1.0/6.0 M) (c)  
 583 AnaLig As-01 PA (HNO<sub>3</sub>- 0.1/1.0/2.0/4.0/6.0 M + NaOH- 2.0 M) (d) AnaLig As-01 PA  
 584 (NaOH- 0.1/1.0/2.0/3.0/4.0 M + HNO<sub>3</sub>- 2.0 M). Sample solution- As(V) (100 μM), matrix-  
 585 H<sub>2</sub>O, pH- 5, sample volume- 4 mL, flow rate- 0.2 mL min<sup>-1</sup> (n =3).

586

587

588

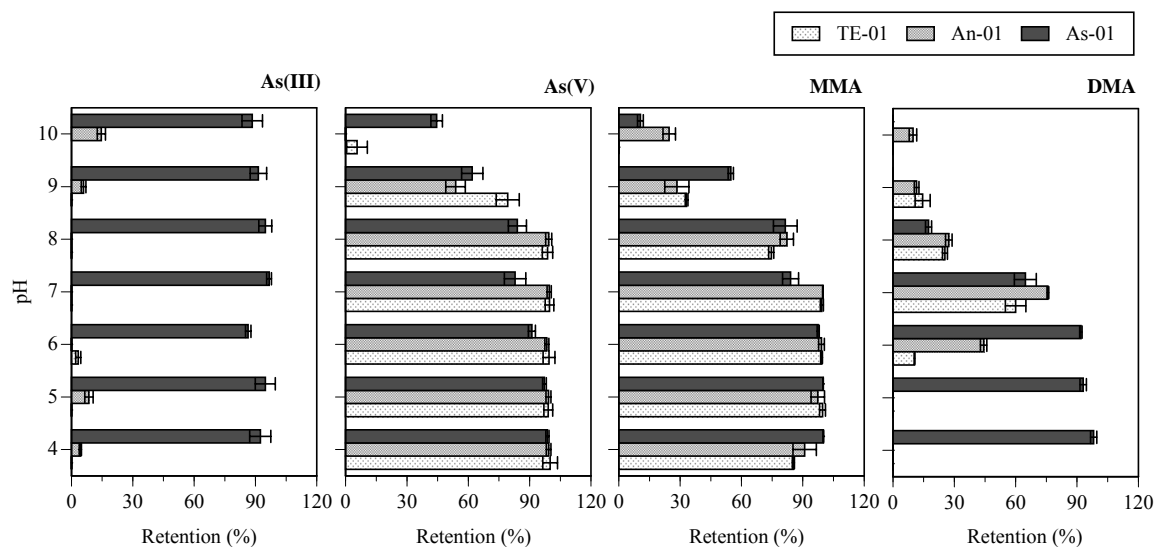
589

590

591

592

593



594

595 Figure 2: Retention behavior of the MRT gel SPE columns. Sample solution– As(III), As(V),  
 596 MMA and DMA (100  $\mu$ M), matrix– H<sub>2</sub>O, pH– 4 to 10, sample volume– 4 mL, flow rate– 0.2  
 597 mL min<sup>-1</sup>, elution– 1.0 M HNO<sub>3</sub> (2 mL) + 6.0 M HNO<sub>3</sub> (1 mL) + EPW (1 mL), for TE-01  
 598 and AN-01 SPE columns and 0.1 M HNO<sub>3</sub> (1 mL) + 2.0 M NaOH (1 mL) + EPW (2 mL), for  
 599 As-01 SPE column ( $n=3$ ).

600

601

602

603

604

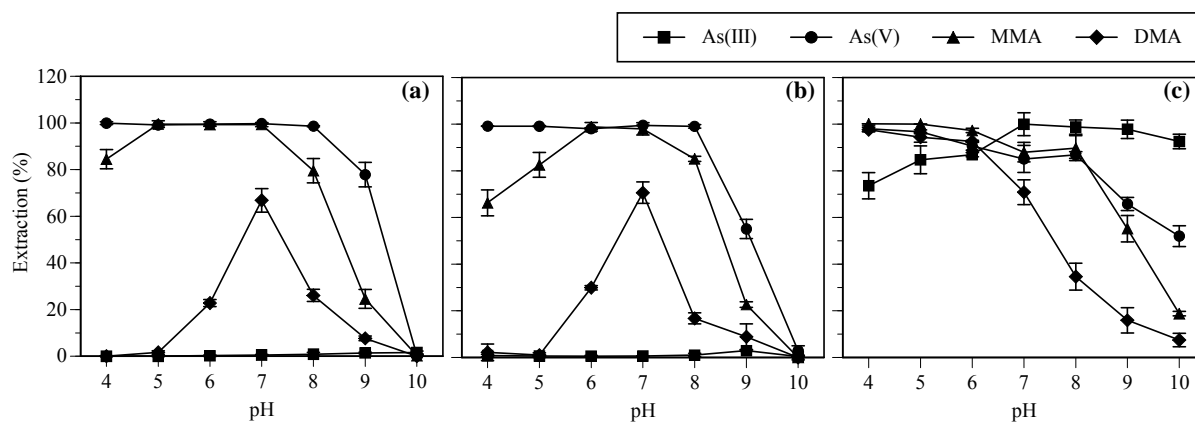
605

606

607

608

609



610

611 Figure 3: Extraction behavior of the MRT gel SPE columns: (a) AnaLig TE-01, (b) AnaLig  
 612 AN-01 Si and (c) AnaLig As-01 PA. Sample solution– As(III), As(V), MMA and DMA (100  
 613  $\mu\text{M}$ ), matrix–  $\text{H}_2\text{O}$ , pH– 4 to 10, sample volume– 4 mL, flow rate–  $0.2 \text{ mL min}^{-1}$ , elution–  
 614  $1.0 \text{ M HNO}_3$  (2 mL) +  $6.0 \text{ M HNO}_3$  (1 mL) + EPW (1 mL), for TE-01 and AN-01 SPE  
 615 columns and  $0.1 \text{ M HNO}_3$  (1 mL) +  $2.0 \text{ M NaOH}$  (1 mL) + EPW (2 mL), for As-01 SPE  
 616 column ( $n = 3$ ).

617

618

619

620

621

622

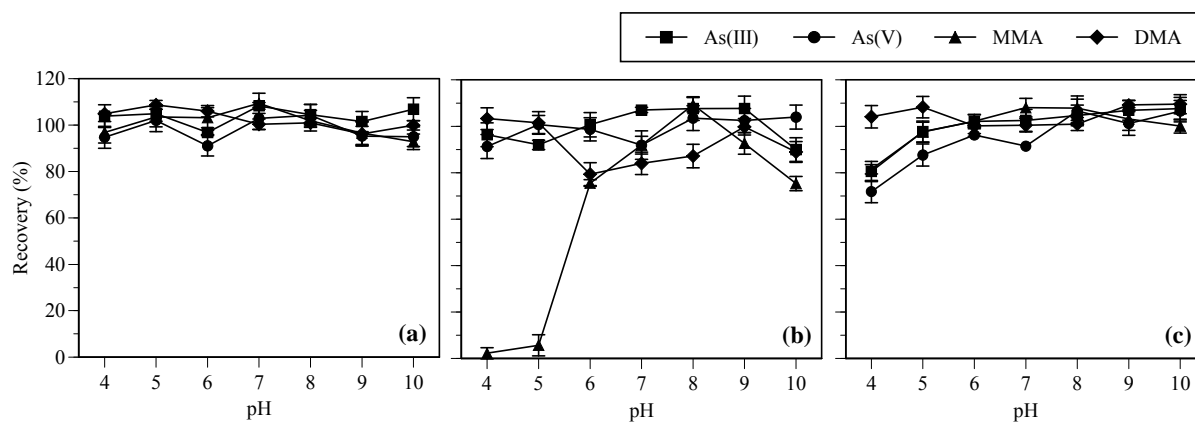
623

624

625

626

627



628

629 Figure 4: Recovery behavior of the MRT gel SPE columns: (a) AnaLig TE-01, (b) AnaLig  
 630 AN-01 Si and (c) AnaLig As-01 PA. Sample solution– As(III), As(V), MMA and DMA (100  
 631  $\mu\text{M}$ ), matrix–  $\text{H}_2\text{O}$ , pH– 4 to 10, sample volume– 4 mL, flow rate–  $0.2 \text{ mL min}^{-1}$ , elution–  
 632  $1.0 \text{ M HNO}_3$  (2 mL) +  $6.0 \text{ M HNO}_3$  (1 mL) + EPW (1 mL), for TE-01 and AN-01 SPE  
 633 columns and  $0.1 \text{ M HNO}_3$  (1 mL) +  $2.0 \text{ M NaOH}$  (1 mL) + EPW (2 mL), for As-01 SPE  
 634 column ( $n = 3$ ).

635

636

637

638

639

640

641

642

643

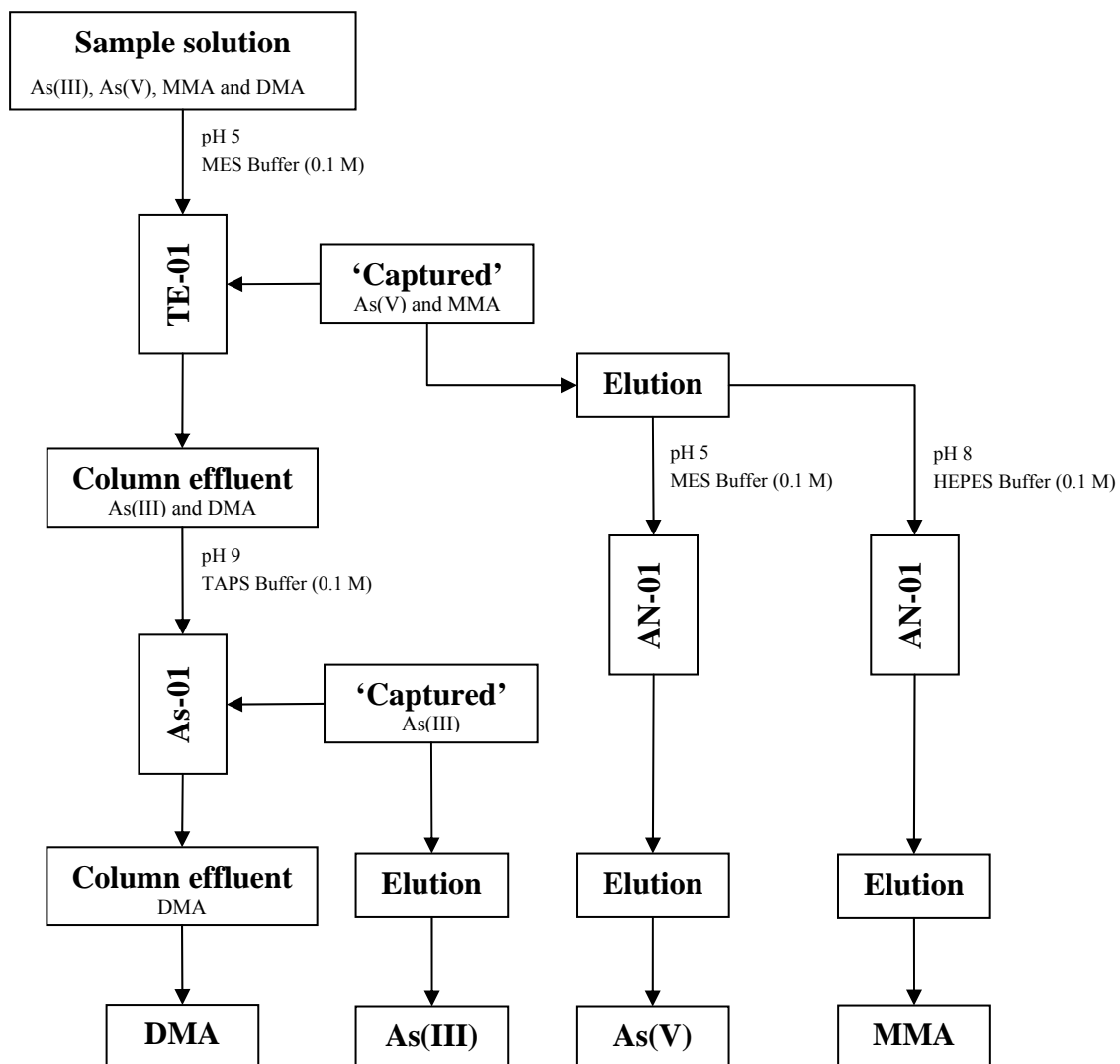


Figure 5: Scheme for selective separation of the arsenic species by MRT gel SPE columns