



Determination of UV active inorganic anions in potable and high salinity water by ion pair reversed phase liquid chromatography

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ABSTRACT

Reversed phase column was dynamically modified into anion exchange column using various types of tetraalkylammonium salts as ion pair reagents (IPRs) for the separation and quantification of toxic anions such as nitrite, bromate, bromide and nitrate in potable and high salinity water. Various chromatographic parameters such as types and concentration of IPRs, concentration of organic modifier, phosphate buffer and mobile phase pH were optimized for the base-line separation of anions. The lowest detection limits (LDLs) were 0.2 for nitrate and nitrite, $0.6 \mu\text{g ml}^{-1}$ for bromate and bromide respectively for potable water samples. NaCl and Na₂SO₄ were incorporated in the mobile phase for the analysis of high salinity water samples to minimize matrix interferences. This has resulted in change in elution order of anions, better tolerance of matrix anions such as chloride and sulphate. The developed method was successfully utilized for analysis of anions in potable, high salinity and sea water samples.

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1. Introduction

Inorganic anions such as nitrite, bromate, bromide and nitrate are of great environmental concern due to their toxicity and health related impact. Moreover, nitrite and nitrate are found at tracer concentration in the bulk matrices such as in high salinity, sea water, industrial and agricultural water. Anthropogenic activities are main source of these anions. Nitrate and nitrite are run-off of nitrogenous fertilizer, leaching from septic tanks and sewages. Nitrate can induce methaemoglobinemia [1]. Nitrite may react with secondary and tertiary amines forming highly carcinogenic nitrosamines [2]. Therefore, the permissible level of nitrite and nitrate in potable water is 0.1 and $10 \mu\text{g ml}^{-1}$, respectively [3]. The source of bromide in environmental matrices is brominated pesticides such as methyl bromide which is used as fumigant on plants and from sea intrusion in the coastal areas [4]. For bromide the acceptable daily intake (ADI) is 0.4 mg kg^{-1} of the body weight [5]. The bromate is produced from the ozonation of source water that contains naturally occurring bromide from anthropogenic activity such as burning of coal [6]. For bromate the World Health Organization (WHO) set a guideline value of $25 \mu\text{g l}^{-1}$ [7]. The available methods of analysis for the above mentioned anions are colorimetry, gravimetry, potentiometry and amperometry. These are based on single element detection, limited sensitivity, suffer from interferences and are time consuming [8,9].

High performance liquid chromatography (HPLC) is a technique of choice for anions analysis. Among its various forms ion-chromatography (IC) and ion-pair chromatography (IPC) is suitable for determination of anions, cations, organometallic and coordination compounds [10–13]. The other added advantages of HPLC are simultaneous determination of cations and anions in single run by IPC and minimum matrix interferences [14]. Based on the above mentioned advantages of IC and IPC, the USA Environmental Protection Agency (EPA), American Society for Testing and Materials (ASTM) and the International Standard Organization (ISO), has approved chromatographic methods for effective monitoring of anions in drinking and wastewater [15].

IC with conductometric detection is sensitive to matrix effects and base-line fluctuation with temperature variations. It is difficult to measure low nitrite, bromide and bromate concentration in the presence of bulk chloride, such as in sea and wastewater. Moreover, in suppressed IC mode, oxidation of nitrite to nitrate may occur under acidic conditions [16]. The suppressed IC though very efficient and sensitive however, requires regeneration or replacement of suppressor in order to overcome the problem of high background. Various new stationary phases such as hydrophobic C₃₀ and poly (ethylene oxide) have been successfully utilized for separation of anions [17,18]. Capillary zone electrophoresis (CZE) is also a potential technique for anion analysis, but has certain associated problems such as instability and irreproducibility of migration times and peak areas, moderate sensitivity, difficult to manipulate separation selectivity and limited detection options [19,20]. Multi-dimensional IC has been utilized for determination of trace level of UV active and other anions in chloride and sulphate matrix such as in sea water, but it adds complexity to the

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HPLC hardware system [21–23]. Electrostatic IC with an electrolyte as an eluent has also been used for bromide, nitrate and iodide quantification in saline matrix [24]. Tandem usage of detector such as UV and conductivity in saline water sample analysis is also reported for multiple information about samples [25]. NaCl and Na₂SO₄ are incorporated in the mobile phase to eliminate chloride and sulphate matrix interferences [26,27].

IPC used in the current investigation has the advantages of its ability to separate samples containing neutral and ionized solutes simultaneously with minimum peak tailing and band broadening. Tetraalkylammonium salts are frequently used for ion pairing with anions, such as nitrite and nitrate. Various mechanisms of solute retention in IPC have been proposed. These are ion pair formation, dynamic ion exchange and ion interaction mechanisms [28,29]. In continuation of our previous work [9,30] the present IPC method has been reported for the simultaneous separation and determination of nitrite, bromate, bromide and nitrate using IPC mode. Mobile phase consisting of phosphate buffer and tetraalkylammonium salts as ion-pairing reagent has been optimized using UV-detection. Interference of expected ions regarding analysis of potable and saline water has also been investigated. The developed method is capable of analyzing bromide ions in coastal area sample G-6 in the presence of 458-fold concentration of chloride with a RSD ($n=5$) of 0.029.

2. Experimental

2.1. Chemicals

Tetrabutylammonium perchlorate (TBAP), tetrabutylammonium hydroxide (TBAOH) and tetrabutylammonium chloride (TBACl) were purchased from Fluka AG (Switzerland). Sodium dihydrogenphosphate, phosphoric acid, sodium chloride, and other salts to prepare standard solutions for anions were of analytical grade from E. Merck (Germany). Standard solution of 1000 $\mu\text{g ml}^{-1}$ of each anion was prepared in double deionized distilled water which was prepared using Sybron deionizer (USA).

2.2. Instrumentation

A Perkin-Elmer series 10 solvent delivery system (USA) fitted with a 20 μl loop and Rheodyne 7120 sample injector, variable UV detector DuPont (USA) and data processor D-2500, Hitachi (Japan) were used. The analytical column was reversed phase C₁₈ (250 mm \times 4.6 mm) particle size (5 μm) Eurosphere from Macherey Nagel Germany. A Metrohm 780 pH meter (Switzerland) was calibrated using two standard buffers and mobile phase pH was adjusted with 0.5 M phosphoric acid. All the measurements were carried out at room temperature (24 ± 2 °C).

2.3. Sample collection and preparation

Water samples were collected from Simly dam, Rawal dam near Islamabad; saline and sea water was from coastal area Karachi and Arabian Sea, respectively. These samples were filtered through a 0.25 μm Millipore syringe filter and stored at 10 °C in refrigerator before HPLC analysis.

3. Results and discussion

3.1. Optimization of mobile phase

3.1.1. Effect of ion pair reagents (IPRs)

The currently employed reversed phase column was packed with C₁₈ silica having pore size 0.01 μm , surface area 350 $\text{m}^2 \text{g}^{-1}$

and pore volume of 0.9 ml g^{-1} , respectively [30]. Tetraalkylammonium salts are adsorbed on RP column by hydrophobic interaction [29]. Since the TBA⁺ ions are used as IPR in the pH range of 2–4, the anions nitrite, nitrate, bromate and bromide form ion pairs with TBA⁺ which act both as ion-pairing and ion exchanger sites. This may be the basis for retention and separation of anions on RP column coated with IPR. The chromatographic conditions were optimized by using TBACl (1–5 mM) in water, phosphate buffer NaH₂PO₄/H₃PO₄ at 10 mM concentration at pH 2 and UV 220 nm, and the elution order was nitrite, bromate, bromide and nitrate with total analysis time of 20 min. The separation factor of four anions was enhanced by increasing TBACl amount in the mobile phase from 1 to 5 mM. This trend was in accordance with the findings regarding the regulation of retention of anions on reversed phase column employing IPC [31].

$$\alpha = E_{QX} [Q^+]_{aq} V_s V_m^{-1}$$

here α is the separation factor, E_{QX} is the extraction constant, $[Q^+]$ counter ion (TBA⁺), X (anion) and (V_s), (V_m) are volume of stationary and mobile phase, respectively. Above equation indicates that for fixed E_{QX} the increase in $[Q^+]$ will tend to increase the α value, the same trend of increasing α is observed when TBA⁺ concentration was increased. The standard chromatogram showing the baseline separation of four anions using TBACl is shown in Fig. 1.

3.1.2. Effect of anion of IPR

TBAP and TBAOH were also used to see the effect of anions of IPRs on the resolution of four anions. The resolution was poor between nitrite and bromate in case of TBAP and for nitrite and nitrate in case of TBAOH, respectively. The decreased resolution in case of TBAP may be due to greater partitioning of TBAP on the column surface.

3.1.3. Effect of buffer

A variation of 10 to 60 mM NaH₂PO₄ for a fixed concentration of IPR (5 mM) at pH 2 tends to decrease the separation factor between nitrite and bromate. This may be due to increased concentration of H₂PO₄⁻ ions thus increasing mobile phase elution strength. For further studies 20 mM NaH₂PO₄ was found to be optimum concentration.

3.1.4. Effect of eluent pH

The variation of mobile phase pH is an important parameter in IPC. A variation in pH can help in improving separation selectivity and sensitivity. The mobile phase pH was varied from 2 to 4 in steps at fixed concentration of IPR and NaH₂PO₄. At pH 2 all the four anions peaks were well separated while at pH=3 the separation factor decreases between nitrite and bromate and at pH 4 these two peaks were merged due to increase ionization at higher pH.

A gradual increase in the resolution for anions was observed by decreasing the pH of mobile phase while keeping the concentration of IPR and buffer salt as constant. For further investigation pH 2 was opted for better resolution. The order of elution, retention times, linear ranges, lowest detection limits (LDL) and the linear regression analysis of the chromatographic data of the analyzed anions using TBACl as IPR and optimized mobile phase are shown in Table 1.

3.2. Analysis of real samples

The efficiency of the developed method was tested by analyzing water samples collected from dams. These samples were analyzed after clean up procedure such as filtration and dilution. The analysis of Simly dam water is shown in Fig. 2.

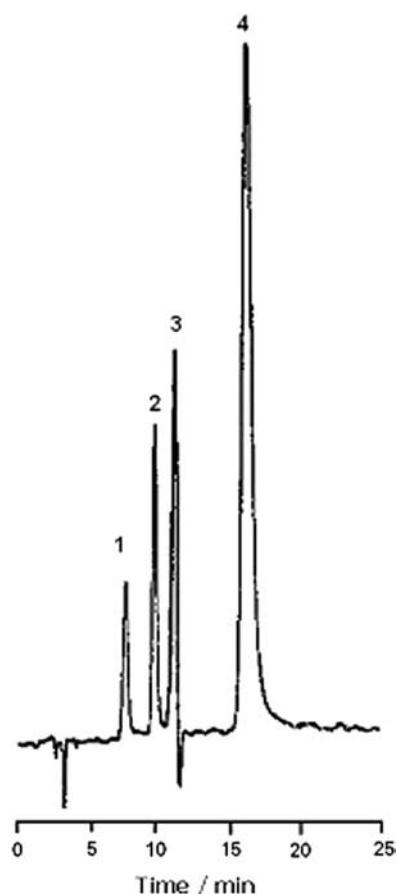


Fig. 1. Separation of the standard of four anions using TBACl as an IPR, peak identification: 1 = NO_2^- , 2 = BrO_3^- , 3 = Br^- , 4 = NO_3^- ($10 \mu\text{g ml}^{-1}$ each), chromatographic conditions = TBACl (5 mM), NaH_2PO_4 (10 mM), pH = 2, Column = C₁₈ (250 mm × 4.6 mm), UV = 220 nm, flow rate = 0.8 ml min^{-1} .

Table 1
Chromatographic data and regression analysis data of four anions.

Anions	Retention time (min)	Detection limits	Linear ranges	Slope	Intercept	Correlation coefficient
NO_2^-	9.8	0.20	0.2–5	16.27 ± 1.0	1.64 ± 2.5	0.982
BrO_3^-	12.5	0.60	0.6–20	2.97 ± 0.1	0.68 ± 1.5	0.987
Br^-	14.3	0.60	0.6–20	4.49 ± 0.2	5.20 ± 2.0	0.990
NO_3^-	20.8	0.20	0.2–5	18.14 ± 0.6	1.24 ± 1.5	0.995

3.3. Analysis of UV active anions in high salinity water

3.3.1. Study of matrix effect

Interference of non UV active anions such as chloride and sulphate in high salinity water was investigated for the developed method. The physical parameters such as conductivity, pH, major cations and anions in high salinity water collected in coastal area of Arabian Sea are shown in Table 2. The presence of 458 fold chloride did not affect the resolution of four anions however the recovery of these anions were decreased by increasing the concentration of chloride. The recoveries of four anions are 83.4, 75, 85 and 80% for BrO_3^- , Br^- , NO_2^- and NO_3^- ions, respectively.

3.4. Study of matrix elimination for high salinity water

The presence of bulk chloride and sulphate can interfere in the determination of four anions in high salinity water. Therefore, mobile

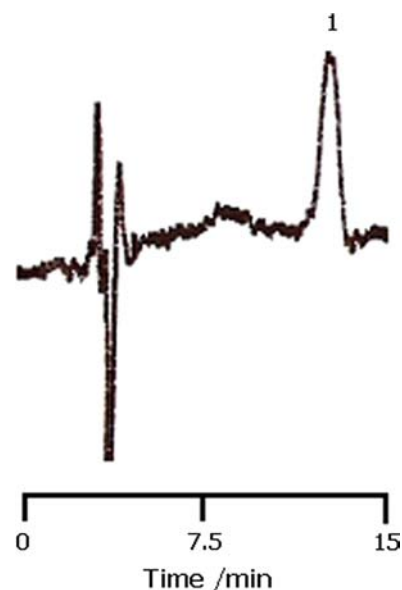


Fig. 2. Analysis of sample from Simly Dam, peak identification: 1 = NO_3^- chromatographic conditions as per Fig. 1.

Table 2
Physico-chemical characteristic of coastal area water (conc. $\mu\text{g ml}^{-1}$).

Sample code	^a Na^+	^a K^+	^a Mg^{+2}	^a Ca^{+2}	^b SO_4^{-2}	^b Cl^-	pH	^c Cond.
G-1	60	20	40	30	15	100	8.3	0.883
G-2	5720	250	180	760	138	8000	8.2	33.0
G-3	1810	270	280	300	98	2200	7.4	12.8
G-4	1920	140	120	280	57	2300	7.3	12.43
G-5	680	60	70	130	50	700	8.3	4.86
G-6	8470	70	610	610	213	9400	7.8	38.2
G-7	8830	100	640	660	190	8300	7.9	37.9
G-8	5710	20	340	380	168	5900	7.6	29.9

^a Concentration of cations measured by ICP-OES after appropriate dilution.

^b Concentration of Cl^- measured by titration and SO_4^- by spectrometry after appropriate dilution.

^c Conductivity is ms cm^{-1} .

phase was modified using NaCl addition in the mobile phase for analysis of these four anions in high salinity water samples. The incorporation of NaCl in the mobile phase resulted in change in elution order i.e. bromate, bromide, nitrite, and nitrate as shown in Fig. 3. The elution order was changed for first two anions i.e. bromate come first and then bromide followed by nitrite and nitrate, respectively. This may be due to the use of reversed phase column and separation of anions involves mixed mode of interaction in the current investigation i.e. ion interactions and hydrophobic interactions of anions with the reversed phase surface. However this new elution order may be helpful for analysis of bromate and bromide in water samples with high concentration of nitrate. Total elution time was also reduced to 9.5 min. The reduction in elution time may be due to the competing effect of the inorganic anions of the adding salt (NaCl) present in high concentration with the analyte anion. The capacity factor of the relevant anion decreases with the decrease in total elution time [32]. This was in contrast to the findings of Xiao et al. [26]. This may be due to use of normal phase pure silica column by the author. Addition of 50 mM of Na_2SO_4 in the NaCl based eluent has resulted in decrease of separation factor between nitrite and nitrate. This may be due to low hydrophobicity of symmetrical IPRs such as TBA^+ ion and competition of sulphate anion for active sites of the ion exchange sites dynamically created on reversed column by TBA^+ ion. However, addition of Na_2SO_4 works well in non symmetrical IPRs for separation of UV active anions [26]. The order of elution, retention times, linear ranges,

lowest detection limits (LDL) and the linear regression analysis of the chromatographic data of the analyzed anions using optimized mobile phase is shown in Table 3.

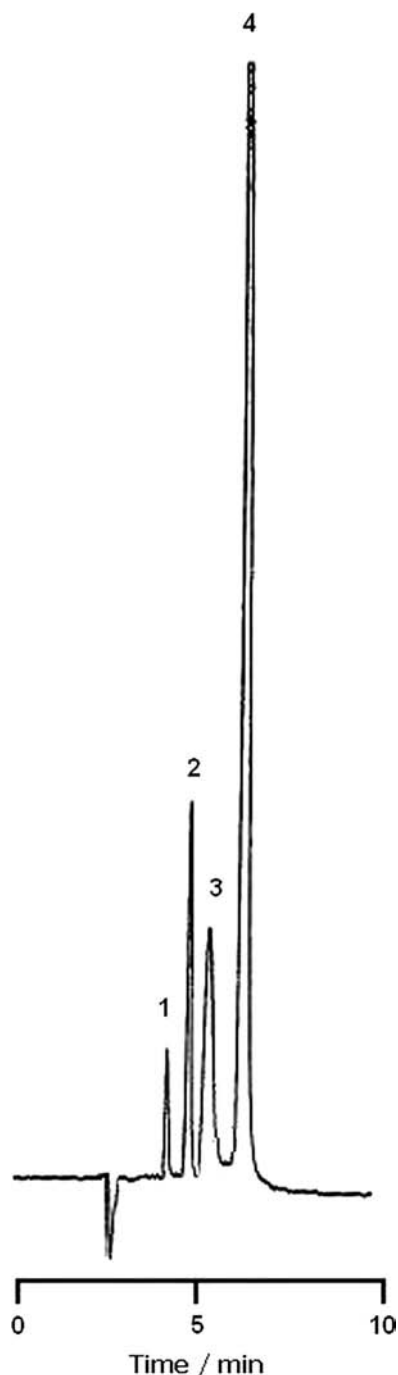


Fig. 3. Separation of the standard of four anions using TBACl as IPR, peak identification: 1=BrO₃⁻, 2=Br⁻, 3=NO₂⁻, 4=NO₃⁻, (10 μg ml⁻¹ each), Mobile phase=TBACl (5 mM), NaH₂PO₄ (5 mM), NaCl (100 mM), Column=C₁₈ (250 mm × 4.6 mm), pH=3, UV=210 nm, flow rate=0.8 ml min⁻¹.

3.5. Analysis of high salinity water

The efficiency of the developed method was tested by analyzing water samples collected from seawater, and coastal area water as shown in Fig. 4. These samples were analyzed after clean up procedure such as filtration and dilution. The high salinity water cannot be analyzed by IC due to high chloride contents in samples as it will mask the nitrite and bromate that are eluted immediately after the chloride. The dilution of these samples will impair the LDL of these anions. Whereas, the developed IPC method can

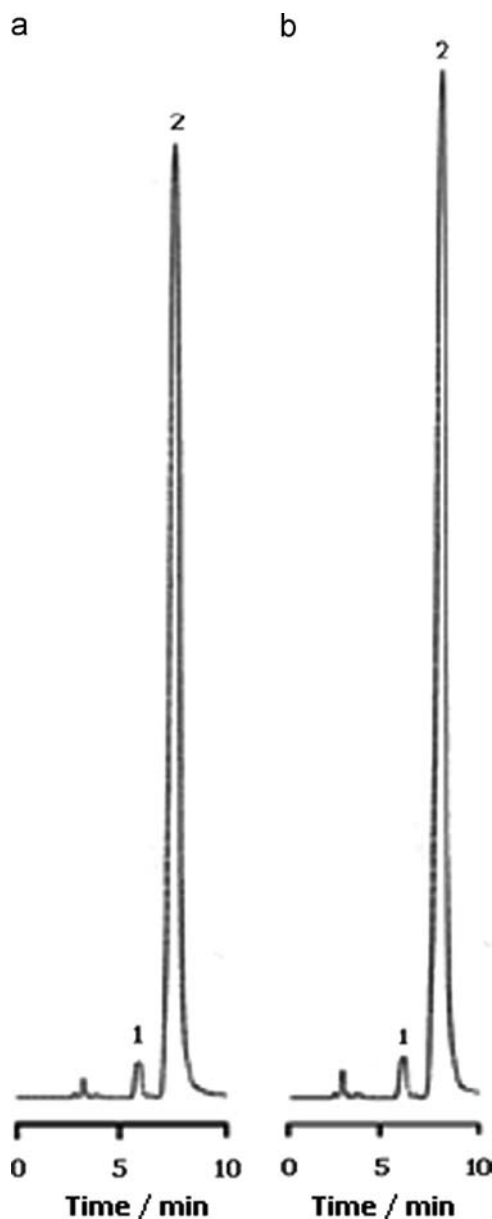


Fig. 4. Analysis of (a) G-2 and (b) G-6 samples, peak identification: 1=Bromide, 2=NO₃, chromatographic conditions as per Fig. 3.

Table 3

Chromatographic data and regression analysis data of four anions with addition of NaCl in mobile phase at pH=3.

Anions	Retention time (min)	Detection limits	Linear ranges	Slope	Intercept	Correlation coefficient
BrO ₃ ⁻	6.5	1.3	1.3–20	6.12 ± 0.1	-2.33 ± 1.1	0.999
Br ⁻	7.5	1.3	1.3–20	10.45 ± 0.3	18.46 ± 4.0	0.995
NO ₂ ⁻	8.4	0.3	0.3–5	14.09 ± 0.7	-2.29 ± 1.8	0.992
NO ₃ ⁻	9.5	0.3	0.3–5	47.88 ± 3.5	-24.36 ± 9.2	0.983

Table 4
Concentration of anions in various types of water determined by IPC ($\mu\text{g ml}^{-1}$).

Sample code	Br^-	NO_3^-
Simly dam	nd	2.48 ± 0.03
Rawal dam	nd	1.5 ± 0.02
G-1	3.91 ± 0.07	30.26 ± 0.92
G-2	22.66 ± 0.41	216.66 ± 2.51
G-5	3.1 ± 0.1	70.33 ± 1.04
G-6	20.5 ± 0.6	82.5 ± 0.5
G-8	32.1 ± 0.4	342.6 ± 2.5
Sea water	44.2 ± 0.7	71.2 ± 1.1

G (1–8)=Karachi coastal area high salinity water samples.

Sea water=Arabian Sea water.

nd=Not detected.

G-1 to G-8 samples and Sea water analysis by adding NaCl in the mobile phase.

NO_2^- and BrO_3^- were not detected in any sample.

tolerate up to 458 fold of chloride in the samples without affecting the detection of four anions due to better sensitivity achieved in this method. Another advantage of currently reported method is the detection of bromide in sample of coastal area water which could not be detected by IC due to high contents of chloride. The concentration of four anions determined by currently developed method for Simly dam, Rawal dam, Sea water and Karachi coastal area water are shown in Table 4. The presence of bromide in the coastal area water samples may be due to sea water intrusion and excess nitrate may be due to the agricultural activity incorporating nitrogen fertilizer.

4. Conclusions

The reported method is applicable for potable and high salinity water samples. For potable water from dams there is no need to modify the eluent. However the mobile phase modified with NaCl can tolerate high salinity matrix and there is no need for matching standard to cater matrix effects. The robustness of the developed method was tested by varying the mobile phase pH from 2.3 to 2.6 the recoveries of all four anions were between 98.5 and 99%. The buffer concentration was also varied from 10 to 20 mM resulted in recovery of all anions in the range of 98.2–98.4%. This method is sensitive, selective and can be applied to other environmental matrices such as estimation of bromate in aquaculture and determination of low levels of nutrients (nitrate and nitrite) in pollution associated with eutrophication and blooms in sea water. The used symmetrical IPR has fast equilibration with RP column

and its washing from column surface is also easy as compared to unsymmetrical IPRs. There is no alteration in column performance with prolonged use of symmetrical IPRs in the mobile phase.

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