P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : *https://tci-thaijo.org/index.php/SciTechAsia* **Science & Technology Asia**
Vol. 23 No.3 July - September 2018 Page: [35-43]

Original research article

Ion-Interaction Chromatography with Ion-Trap Mass Spectrometry Detection for the Analysis of Perchlorate in Drinking and Natural Waters

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Received 10 March 2018; Received in revised form 19 June 2018
Accepted 21 June 2018; Available online 25 September 2018

ABSTRACT

In this work, liquid chromatography with mass spectrometry (LC-MS/MS) for the determination of perchlorate in natural waters was developed. The method was based on ion-interaction (ion-pair) chromatography with the cationic ion-pairing reagent, hexamethonium bromide. Separation of the perchlorate was carried out using methanol and 1 mmol L^{-1} hexamethonium bromide (10:90, v:v) as the mobile phase at a flow rate of 0.40 mL min^{-1} and 100 µL injection volume. Under the optimal condition, the linearity range of perchlorate was 4 – 1000 µg L^{-1} with a coefficient of determination (r^2) of 0.9998. The detection limit was 2 µg L^{-1}, within U.S. EPA requirements (EPA methods 314.1 and 331.0). This method was effectively applied to the quantitation of perchlorate in drinking water and natural water samples.

Keywords: Cationic ion-pairing reagent; Drinking water; Ion-interaction chromatography; Natural water; Perchlorate

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1. Introduction

Perchlorate (ClO_4^-) is the anion of an oxyhalide salt containing a single chlorine atom and four oxygen atoms. These salts are soluble in water. Perchlorates are found in explosives, automobile airbags and fireworks [1] and thus lead to contaminants in water [2-4], soil [2, 4-5], fertilizers [6], plants [7] and food [1, 4]. The presence of perchlorate in the environment and food supplies poses a considerable human health risk, even at trace levels, as it can interfere with the thyroid gland's ability to produce thyroid hormones. Perchlorate is known to inhibit the transport of iodide by the sodium-iodide symporter (NIS) present in the thyroid and mammary glands [8-10]. The U.S. Environmental Protection Agency's Candidate Contaminant List (CCL) is therefore considering a regulatory limit for perchlorate of 4 $\mu g\ L^{-1}$ in water [2]. Therefore, an effective and sensitive method for the determination of perchlorate in environmental samples such as drinking water, groundwater and soil is required. From the literature, analytical methods for achieving this can be divided into two groups based on detection technique. The first group is screening methods, while the second is confirmatory methods that use mass spectrometric detection.

The screening methods for perchlorate analysis are spectrophotometric methods [11-12], electrochemical methods (ion selective electrode, ISE) [13] and chromatographic methods, i.e. ion chromatography [14-16] and capillary electrophoresis [17-18]. These methods lack specificity with the need for confirmatory of identity.

Ion-pairing extraction [19] and post column ion-pairing complexation [20-22] have been employed to improve specificity and sensitivity. This improvement is based on monitoring the ion-pair complex of perchlorate with an *m/z* value much greater than that of perchlorate itself, and avoids measuring in the low *m/z* range which usually has relatively high background signals. For example, post column ion-pairing complexation of perchlorate and other hydrophobic ions with a dicationic agent (D^{2+}) was reported by Martinelango and Dasgupta [21].

In this work, a confirmatory and quantitative LC-MS/MS method, with an on-column ion-pairing formation, was developed for perchlorate analysis. Chlorine isotopic pattern and oxygen isotope-labeled sodium perchlorate, $NaCl^{18}O_4$ as an internal standard were used for improvement in specificity and sensitivity. The selection of the ion-pairing reagent was studied. The final optimized method was applied to the determination of the perchlorate ion in natural waters, such as drinking water and natural water.

2. Materials and Methods

Reagents and chemicals

All chemicals and reagents were use of analytical-reagent grade. Sodium perchlorate was purchased from Acros, USA. Oxygen-labeled sodium perchlorate was purchased from Cambridge Isotope Laboratories, Inc., USA. Sodium sulphate and sodium chloride were purchased from Carlo Erba, Italy. Di-sodium hydrogen orthophosphate was purchased from Ajax Chemicals, Australia. Glacial acetic acid was purchased from Merck, Germany. Nitric acid was purchased from RCI Labscan Ltd., Thailand. Hexamethonium bromide, HMB and decyltrimethyl-ammonium bromide, DTAB were purchased from Sigma Aldrich, Switzerland. Hexadecyltrimethylammonium bromide, CTAB was purchased from Fluka, Switzerland. Dihexylammonium acetate, DHAA was purchased from Tokyo Chemical Industry Co., Ltd., Japan. HPLC-grade methanol was purchased from Merck, Germany. Water was deionized and purified on a deionized water purification system (ELGASTAT Option3A, Elga Ltd., England) and used to prepare all solutions.

All glassware for reagent storage were cleaned and soaked in 10% (w/w) nitric acid before use.

Natural waters

This method was applied to the determination of the perchlorate ion in drinking waters and natural waters. Drinking waters were purchased from a convenience store in Pathum Thani province. Natural waters were collected from Chiang Mai and Pathum Thani provinces. Water samples were stored in 500 mL precleaned polyethylene bottles.

All water samples were stored at 4 ^{o}C until analysis. No preservation was required as from the literature perchlorate is stable at room temperature for up to 50 days. A 28-day holding time is recommended by USEPA guidelines for anion analysis [2-3].

Instrumentation and chromatographic conditions

Finnigan SpectraSYSTEM liquid chromatograph (Thermo Finnigan, USA) was used with a binary gradient pump (P2000), in-line degasser (SCM1000), and variable-loop autosampler (AS3000). The analytical column was a Zorbax SB-C18 (50 x 4.6 mm i.d., 5 µm) column from Agilent Technologies (USA). The mobile phase was methanol and 1 mmol L^{-1} hexamethonium bromide (10:90, v:v) at a flow rate of 0.40 mL min^{-1} and 100 µL injection volume. All solutions were filtered through a 0.20 µm nylon membrane filter (Chromex Scientific, United Kingdom). Detection was by MS/MS in the positive ion mode using a LCQ Advantage ion-trap mass spectrometer (Thermo Finnigan, USA) with Xcalibur (version 1.3) software. The electrospray voltage and desolvation temperature were set at 4.5 kV and 300 ^{o}C, respectively. The following conditions were found to provide the optimum signal: sheath gas at 40 L h^{-1}, auxiliary gas at 10 L h^{-1}, capillary voltage at 44 V and collision energy at 30 eV. Moreover, chlorine isotopic pattern and oxygen-labeled sodium perchlorate ($NaCl^{18}O_4$) as internal standard were used in this method for the improvement in specificity and sensitivity. Quantitation of the perchlorate was based on the peak area ratio of perchlorate and internal standard. Detection of the ions was carried out in the full scan mode, with *m/z* 301.2 and 309.2 as precursor ions for the perchlorate and internal standard ion-pair, respectively. The product ions for quantitation were *m/z* 242.0 and *m/z* 250.0 for the perchlorate and internal standard ion-pair, respectively.

Standard solution preparation

The primary stock standard solution of perchlorate at a concentration of 1000 mg L^{-1} was prepared by accurately weighing 61.6 mg of sodium perchlorate and diluting to 50.0 mL with deionized water. The secondary stock solution (500 µg L^{-1}) was prepared by diluting the primary stock solution of perchlorate using deionized water. Stock oxygen-labeled sodium perchlorate, $Cl^{18}O_4^-$ which was used as an internal standard, was prepared at a concentration of 500 µg L^{-1} by diluting 100 mg L^{-1} using deionized water. The stock solutions were stored in a refrigerator at 4 ^{o}C. The working standard solutions of perchlorate (4, 10, 20, 30, 40 and 50 µg L^{-1}) were freshly prepared by further dilution of the secondary stock solution with deionized water. These solutions were spiked with the internal standard at a final concentration of 50 µg L^{-1} prior to analysis for construction of calibration curve.

Sample preparation

All water samples were spiked with the internal standard, ^{18}O-labeled perchlorate at a final concentration of 50 µg L^{-1} and filtered through a 0.20 µm nylon syringe filter before injection into the LC-MS/MS system.

3. Results and Discussion

Investigation of ion-pairing reagent

Since anions are not retained on a reverse phase column, an ion-interaction (ion-pairing) reagent that has an opposite charge, was therefore added to the mobile phase. Moreover, this method has an added advantage of converting a low molecular weight analyte (perchlorate, MW 99.5) to a higher-mass species by forming an adduct with the ion-pairing reagent. The selected ion-pairing reagents in this work were hexadecyltrimethylammonium bromide (CTAB), decyltrimethylammonium bromide (DTAB), dihexylammonium acetate (DHAA) and hexamethonium bromide (HMB).

When solutions of perchlorate at concentration of 20 mg L^{-1} and CTAB, DTAB and DHAA at concentration of 2 mmol L^{-1} were directly infused into the spray chamber of the mass spectrometer. The mass spectra showed only the molecular ions of CTAB, DTAB and DHAA. Therefore CTAB, DTAB and DHAA did not form a positively charged ion-pair adduct with perchlorate anion. On the other hand, HMB, which gives 2+ cationic species (D^{2+}, *m/z* 202.2), was found to form an ion-pair adduct with the perchlorate anion ($DClO_4^+$), with the molecular ion of *m/z* 301.2 and 303.2, as shown in Fig. 1 (a). The *m/z* 303.2 peak, at 30 % of *m/z* 301.2 peak, is the characteristic isotope peak for a species with one chlorine atom. The MS/MS mass spectrum for the precursor ion *m/z* 301.2 showed three product ions at *m/z* 128.2, 187.1 and 242.0, respectively (Fig. 1(b)). The quantifying ion was *m/z* 242.0 (base peak) and the qualifying ions were *m/z* 187.1 and 128.2.

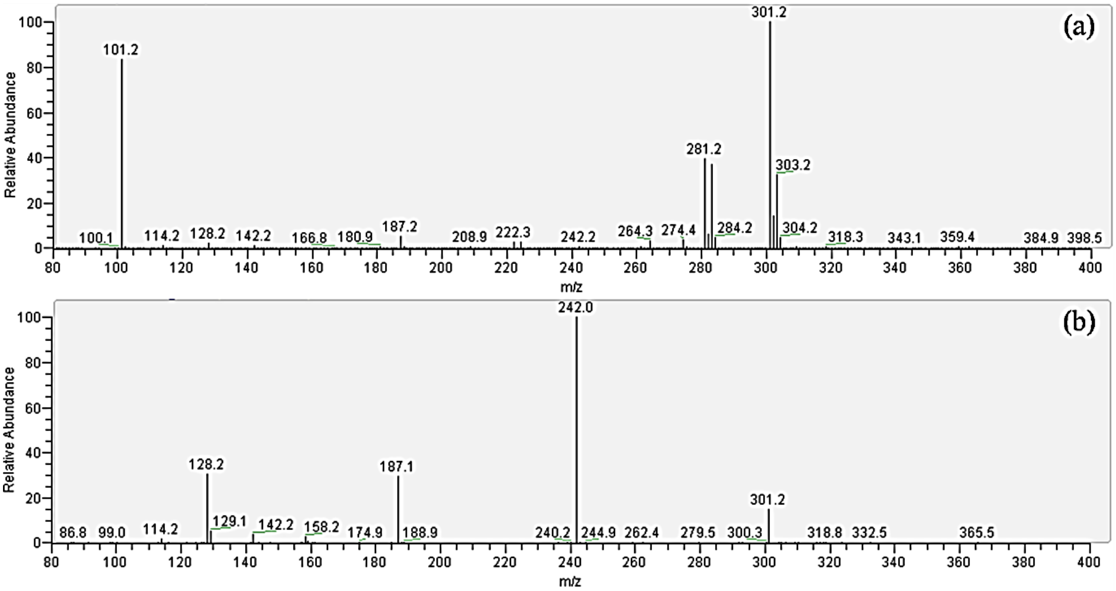

Fig. 1. (a) Full scan mass spectrum and (b) MS/MS spectrum of the ion-pairing reagent (HMB) with perchlorate. The sample solution was directly infused into the spray chamber. In Figure (b) the precursor ion is *m/z* 301.2.

Optimization of the LC condition

Selection of ion-pairing formation

In this work, the types of ion-pairing formation (pre-column and on-column) in the liquid chromatographic system were investigated. In the first method, the mobile phase did not contain the ion-pairing reagent but the reagent was added directly to the sample which was then injected onto the LC column. This method is called ‘pre-column ion-pairing formation’. In the second method, the ion-pairing reagent is a component of the mobile phase. This is the normal ion-interaction mode of ion-liquid chromatography. The results showed that pre-column ion-pairing formation gave peaks for both the ion-pairing reagent and the ion-pair (Fig. 2 (a)). However, the peak area for the perchlorate complex did not correlate with the concentration of the sample. There is an on-column dissociation of the complex as it moves down the column. The ion-interaction mode gave a single peak for the perchlorate complex whose area increased with the concentration of the sample (Fig. 2(b)). Therefore, this latter on-column method was selected for optimization of the chromatography condition.

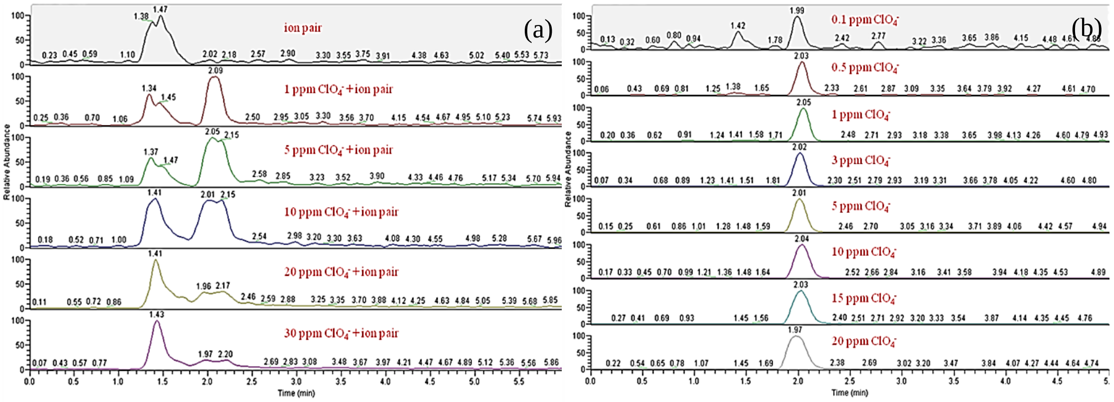

Fig. 2. LC/MS chromatograms for ion-pair formation between HMB and perchlorate, (a) pre-column ion-pair formation using methanol and 0.1 % (v/v) acetic acid (10:90) as the mobile phase and (b) on-column ion-pair formation using methanol and 1 mmol L^{-1} HMB in 0.1 % (v/v) acetic acid (10:90) as the mobile phase. (The mass detection was *m/z* 301.2.)

Composition of the mobile phase

The composition of the mobile phase was investigated. These parameters include an organic modifier and acid.

(i) Effect of organic modifier

Electrospray ionization (ESI) requires a volatile component in the mobile phase. However, an increase of the organic modifier (methanol was used in this work) for a reverse phase column leads to decreased retention. Perchlorate and other anions such as chloride, sulphate and phosphate were therefore investigated for the study of elution on the C18 column (*m/z* 301.2, 237.2, 299.2, 299.2 for perchlorate, chloride, sulphate and phosphate, respectively). Fig. 3 shows the effect of increasing the percentage of methanol in the mobile phase on the retention times t_R of the anions, perchlorate, chloride, sulphate and phosphate, respectively. The methanol content was 0, 5, 10, 15 and 20 % (v/v). However, the methanol content also affects the electrospray ionization process. Fig. 4 shows the peak area ratio for the 4 anions with various methanol compositions.

As a compromise between retention and ionization efficiency, the methanol content was set at 10% (v/v).

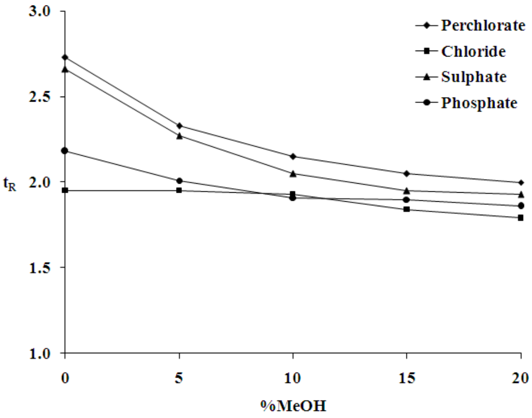

Fig. 3. Retention times (t_R) of perchlorate, chloride, sulphate and phosphate at various methanol contents in the mobile phase. (The mass detections of the perchlorate, chloride, sulphate and phosphate anions were *m/z* 301.2, 237.2, 299.2, 299.2, respectively.)

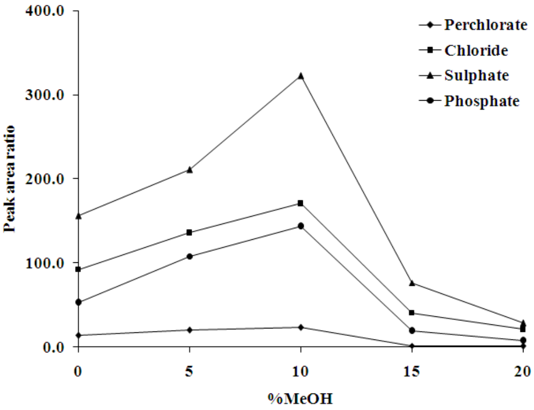

Fig. 4. Peak area ratios of perchlorate, chloride, sulphate and phosphate versus internal standard at various methanol contents in the mobile phase. (The mass detections of the perchlorate, chloride, sulphate and phosphate anions were *m/z* 301.2, 237.2, 299.2, 299.2, respectively.)

(ii) Effect of acid

For an ESI process, a small amount of acid (0.1 – 1 %) is usually added to the mobile phase to promote the proton transfer process to give the protonated species of the analyte $(M+H)^+$ necessary for mass detection. However, in this work, the positively charged species of the perchlorate analyte was produced by an ion-pairing formation with the dicationic hexamethonium ion. Nevertheless, it was found that increasing the concentration of acetic acid (0.0 – 0.10 % (v/v)) in the mobile phase led to a decrease in the response of the perchlorate ion-pair (Fig. 5). The acetate anion could be competing for the hexamethonium cation in the aerosol, thereby reducing the effective amount binding with the perchlorate ion. Thus no acid was added to the mobile phase.

Optimization of the MS/MS parameters

The collision-induced dissociation (CID) step for MS/MS was optimized. The collision energies were varied from 26.0 – 35.0 eV with full scan mode in order to obtain the maximum response of the fragment ions. The results showed that the suitable collision energy that produced the highest yield of product ions with a minimum precursor ion was 30.0 eV, as shown in Fig. 6. This collision energy had about 10 % relative abundance of the precursor ion (*m/z* 301.2) remaining.

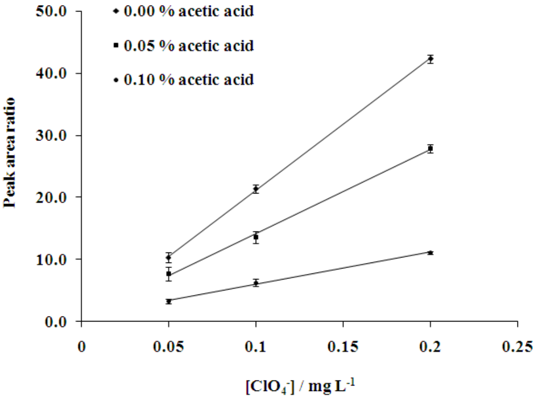

Fig. 5. Peak area ratio and the concentration of perchlorate at various acetic acid contents in the mobile phase. LC conditions; methanol and 1 mmol L^{-1} HMB (10:90) as the mobile phase with a flow rate of 0.40 mL min^{-1} at 100 μL injection volume.

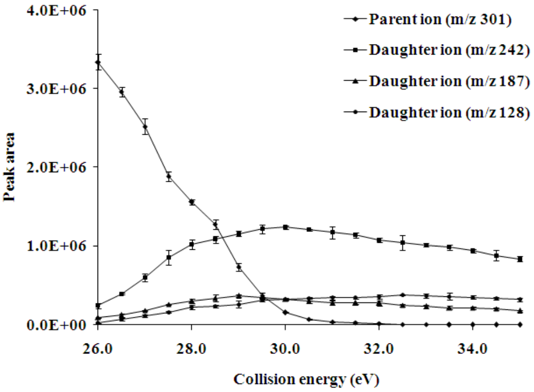

Fig. 6. Ion abundances of precursor ion and fragment ions (expressed as peak area) as a function of the collision energy for the HBS-perchlorate ion-pair complex.

Validation study

The linearity range, repeatability, recovery and limit of detection, LOD were studied for validation of the method.

(i) Linearity and working range

Peak area ratios of twenty concentrations of perchlorate (in the range of 0.5 – 50,000 µg L^{-1}) and internal standard (50 µg L^{-1}) were plotted against concentrations of perchlorate. The results showed that the linearity range of the method was 4.00 – 1,000 µg L^{-1} of perchlorate with a coefficient of determination (r^2) of 0.9998. For analysis of the samples in this work the working range of perchlorate was limited to only 4.00 – 50.0 µg L^{-1}).

(ii) Repeatability

Repeatability of the determination of perchlorate was studied for the instrumental system precision by injecting 10 times the same sample solutions that added known amount of perchlorate. The results showed that the %RSD values of perchlorate content in drinking water, Water-1 and natural water, Water-6 were 9.80 and 15.54 %, respectively. This proposed method indicated good repeatability that agreed with the EPA method 331.0 (%RSD must be ≤ 20 %, n=7) [23].

(iii) Recovery study

Recovery study was carried out by spiking two samples, drinking water, Water-1 and natural water, Water-6, with perchlorate at a concentration of 50 µg L^{-1}. The recoveries of drinking water, Water-1 and natural water, Water-6 were 86.59 and 80.10 %, respectively. These recoveries conform to EPA method 331.0 with mean recovery 80 – 120 % of the true value [23].

(iv) Limit of detection (LOD)

The limit of detection (LOD) of the proposed method was performed as the lowest concentration that still obtained the same ion fragments and the same relative intensity of fragments (±20 % if relative intensity of base peak > 50 %) [24]. In this method, the detection limit was 2 µg L^{-1}, which is below the regulatory limit for perchlorate of 4 µg L^{-1} in water. At this limit, the ratio of the abundance of the qualifying ions to the quantitation ion still agrees with that at higher concentrations.

Analysis of natural waters

The final optimized method was applied to the analysis of the 5 samples of drinking water (Water-1 – Water-5) and 5 samples of natural water (Water-6 – Water-10). The results showed that perchlorate was not detected in all the samples (LOD was 2 µg L^{-1}).

4. Conclusion

In this work, a LC-MS/MS method for the determination of perchlorate in drinking waters and natural waters was developed. Using reversed phase C18 column, the di-cationic ion-pairing reagent, hexamethonium ion, was added to the mobile phase. The reagent serves a dual role as an ion-interacting reagent for retention of the ionic perchlorate analyte and to form a singly positively charged ion-pair complex for mass spectrometry detection. The limit of detection of the method was 2 µg L^{-1}.

All analytical performances conform to U.S. EPA requirements (EPA method 314.1 and 331.0).

Acknowledgements

The authors gratefully acknowledge the financial support provided by Thammasat University Research Fund under the TU Research Scholar, Contract No. 21/2555. The authors would also like to thank the Central Scientific Instrument Center (CSIC) and Center of Scientific Equipment for Advanced Research (TU-CSEAR), Department of Chemistry, Faculty of Science and Technology, Thammasat University, for facility and equipment support throughout this research.

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