CHAPTER 2

EXPERIMENTAL

The objective of this work is to study the effect of common chromatographic variables such as; type and concentration of ion interaction reagent, concentration of organic modifier, flow rate and pH of the mobile phase and wavelength of detection for each anion, which have influence on the separation and retention time of some anions (iodate, bromate, nitrate, iodide and thiocyanate) by ion interaction high performance liquid chromatographic technique. Therefore, the influence of these variables is verified by the experiments as follows.

2.1 Apparatus and Chemicals

2.1.1 Apparatus

1) High performance liquid chromatographic system, manufactured by Agilent, Germany, consisting of :
    a) Isocratic pump, Agilent series 1100, Germany
    b) Fixed loop 20 μL, Rheodyne, U.S.A.
    c) Variable wavelength detector, Agilent series 1100, Germany
    d) Integrator, Hewlette Packard series III, U.S.A.
2) Guard column, Phenomenex, U.S.A.
3) Analytical column, Microsorb MV-100 C_{18} (3 μm, 4.6 X 50 mm), Varian, U.S.A.
4) 25 μL Syringe, Hamilton, U.S.A.
5) Vacuum pump, Millipore, U.S.A.
6) Ultrasonicator model 8891, Cole-Parmer Instrument Co., U.S.A.
7) Filter unit, Millipore, U.S.A.

8) Filter membrane, 0.45 µm, Sartorius, Germany
9) Micropipette, Nichiryo model 5000, Japan
10) Magnetic stirrer model MR 3001, Heidolph, Germany
11) pH meter model pH 900, Precisa, Switzerland
12) UV-Vis spectrophotometer model U-2000, Hitachi, Japan

2.1.2 Chemicals

1) Potassium nitrate, purum, Fluka, Switzerland
2) Potassium iodate, GR grade, Merck, Germany
3) Potassium bromate, GR grade, Merck, Germany
4) Potassium iodide, Anala R, BDH Chemicals, England
5) Potassium thiocyanate, Anala R, BDH Chemicals, England
6) Hexylamine 99%, Aldrich, U.S.A.
7) Heptylamine, purum, Fluka, Switzerland
8) Octylamine 99%, Aldrich, U.S.A.
9) Methanol, HPLC grade, Merck, Germany
10) Phosphoric acid, Anala R, BDH Chemicals, England
11) Sodium hydroxide, GR grade, Merck, Germany

2.2 Preparation of the solutions

2.2.1 Preparation of the mobile phase

The investigation of different varieties of mobile phase was carried out using a binary solvent system where methanol was mixed with alkylamine-phosphate solution. Preparation of alkylamine-phosphate was done by dissolving an appropriate amount of alkylamine compound in deionized water and adjusting the pH with phosphoric acid.

For example, the preparation of 1 L of 5.0 mM hexylamine-phosphate at pH 6.4±0.3 was done by pipetting hexylamine 660 µL into a 1000 mL beaker and diluting it near the mark with deionized water. Then, the solution was adjusted to pH 6.4 by phosphoric acid and the solution was finally adjusted to 1000 mL.

In case of the solutions of 5.0 mM heptylamine-phosphate and octylamine-phosphate, the preparation were done similarly to the hexylamine-phosphate solution except the volumes of heptylamine and octylamine used were 743 µL and 826 µL, respectively.

Each of the solutions was filtered by means of a filtering unit with 0.45 µm filter membrane and a vacuum pump. Finally, the prepared solvent was degassed for 30 minutes in an ultrasonicator prior to its use as a mobile phase. The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal was obtained, a minimum of 2 hours was necessary. After finishing each experiment, the column should be washed by 50 % methanol.

2.2.2 Preparation of the standard anion stock solutions, 100 ppm

Each stock solution of standard anions was prepared by dissolving appropriate weight of potassium salt of the desired anion in deionized distilled water to make up the solution of 100 mL.

The following potassium salts were weighed out in order to prepare 100 mL of 100 ppm of nitrate, iodate, bromate, iodide and thiocyanate, respectively.

Salt	Weight Used (g)
KNO_3	0.1631
KIO_3	0.1224
$KBrO_3$	0.1306
KI	0.1308
KSCN	0.1673

2.3 Optimization of HPLC conditions for the separation of anions

2.3.1 Types of ion interaction reagent

Three types of ion interaction reagent that affected the separation of anions were investigated, namely; hexylamine-phosphate, heptylamine-phosphate and octylamine-phosphate. A standard mixture consisting of 5 ppm each of IO_3^-, BrO_3^-, NO_3^-, I^- and SCN^- was injected into the ion interaction chromatographic system at preselected conditions. The injection volume was 20 µL. This condition was set as follows: 5.0 mM alkylamine-phosphate, 5% MeOH (v/v) at pH 6.4±0.3 was used as a mobile phase at a flow rate of 0.5 mL/min and the wavelength of detection was set at 200 nm. The separating column used was a Microsorb MV-100 C_{18} (3 µm, 4.6 X 50 mm).

2.3.2 Wavelength of detection

A standard mixture containing 5 ppm each of IO_3^-, BrO_3^-, NO_3^-, I^- and SCN^- was injected into the ion interaction chromatographic system. The injection volume was 20 µL. The mobile phase used was 5.0 mM heptylamine-phosphate, 5% MeOH (v/v) at pH 6.4±0.3 with a flow rate of 0.5 mL/min. The detection wavelengths in the range of 200 - 230 nm were utilized.

2.3.3 Flow rate

By varying the flow rate, the optimum flow rate would be obtained and it should provide good separation and short analysis time. Therefore, a standard mixture at 5 ppm each of IO_3^-, BrO_3^-, NO_3^-, I^- and SCN^- was injected into the ion interaction chromatographic system. The injection volume was 20 µL. The mobile phase used was 5.0 mM heptylamine-phosphate, 5% MeOH (v/v) at pH 6.4±0.3 with the wavelength of detection at 200 nm. The flow rates used in the experiment ranging from 0.5 to 0.8 mL/min.

2.3.4 Concentration of the ion interaction reagent

A standard mixture consisting of 5 ppm each of IO_3^-, BrO_3^-, NO_3^-, I^- and SCN^- was injected into the ion interaction chromatographic system using various ion interaction reagent concentrations ranging from 5.0-20.0 mM. The injection volume was 20 μL. The mobile phase used was heptylamine-phosphate, 5% MeOH (v/v) at pH 6.4±0.3 with a flow rate of 0.5 mL/min and 200 nm was used as a wavelength of detection.

2.3.5 pH of the mobile phase

This part of the study followed the same procedure and conditions used in 2.3.3 except the pH was varied in the range of 4.4 -7.4 at a flow rate of 0.5 mL/min.

2.3.6 Concentration of organic modifier

The selected organic modifier is methanol. A standard mixture at 5 ppm each of IO_3^-, BrO_3^-, NO_3^-, I^- and SCN^-) was injected into the ion interaction chromatographic system at various methanol concentration in the range 0-15 % (v/v). The injection volume was 20 μL. The mobile phase used was 5.0 mM heptylamine-phosphate at pH 6.4±0.3 with a flow rate 0.5 mL/min and wavelength of detection 200 nm.

2.4 Characteristics of the analysis

2.4.1 Linearity range

A standard solution of mixed anions at 5 ppm each of IO_3^-, BrO_3^-, NO_3^-, I^- and SCN^- in the range 0.4 -100 ppm was injected into the ion interaction chromatographic system. The injection volume was 20 μL. The chromatographic conditions used were the same as previously described.

2.4.2 Detection limit

A definition of detection limit in this work is based on the concentration which give signal equal to the blank signal plus three standard deviations of the blank. The detection limit was calculated from the calibration curve by means of the blank signal, which can be used as an estimate of the calculated intercept, plus three standard deviations of the blank. It can be used as an estimation of the calculated value from the regression line.

A mixture of standard anions solution containing 5 ppm each of anions were prepared in the range of 0.04 -0.8 ppm and 20 μL volume of each concentration was injected into the ion interaction chromatographic system. The chromatographic conditions used were the same as previously described.

2.4.3 Reproducibility

A standard mixture at 5 ppm each of IO_3^-, BrO_3^-, NO_3^-, I^- and SCN^- was injected (five times) into the ion interaction chromatographic system. The injection volume was 20 μL. The chromatographic conditions used were the same as previously described. The attenuations of the instrument were set at 32, 64 and 128, respectively, for investigation. The precision of the instrument can be illustrated by standard deviation (S.D.) and percent relative standard deviation (% R.S.D) of the retention times and the peak area of each anion.

2.4.4 Recovery assay

The chromatographic peaks produced from standard solutions were qualitatively confirmed using the spike method. The recoveries of some anions were examined at four levels of concentration generally found in tap water from Chiang Mai dormitory. Water sample were spiked with a mixture of anion at the following concentrations: 1, 2, 3 and 4 ppm, respectively. The chromatographic conditions used were the same as previously described.

2.4.5 Analysis of anions in water sample

Six water samples were collected from various water resources. After collecting, the samples were filtered through the cellulose acetate filtering membrane with the pore size 0.45 micron and injected by triplicately into the ion interaction chromatographic system. The injection volume was 20 μL. The chromatographic conditions used was 5.0 mM heptylamine-phosphate, 5% MeOH (v/v) at pH 6.4±0.3 with a flow rate of 0.5 mL/min, along at the wavelength of detection of 200 nm. The separating column used was a Microsorb MV-100 C_{18} (3 μm, 4.6X50 mm). From the obtained chromatograms the amounts of anions present in the water sample were determined from the appropriate calibration curve of each anion.