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SEPARATION OF ANIONS ON POLAR STATIONARY PHASES IN HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

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ABSTRACT

The need to detect anions and cations in various environmental water samples is increasing rapidly with increasing environmental problems, and the time it takes to obtain the proper analysis method is fast, simple, and can provide an accurate assessment. Hydrophilic Interaction Liquid Chromatography (HILIC) is a highly precise technique for separating polar or hydrophilic compounds. Various places have been making a lot of market columns and columns explicitly designed for HILIC. For that to be done, the appropriate column must be selected to separate compounds primarily for separating anions using polar aminopropyl silica stationary phases, HILIC imidazole, pyridine, and Polar Amide-80. Polar stationary phases are best for separating the aminopropyl silica (NH2-60) because they can separate the anions without adding salt. While Polar pyridine can separate anions at low pH (4.2) using acidic stationary phases, and at low pH, Polar pyridine can serve as an ion exchanger (ion exchange)

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INTRODUCTION

Ion chromatography is an analytical technique widely used in various fields of science to separate and determine the content of anions and cations in a sample. This technique is fundamental in analyzing natural water samples to monitor environmental conditions. As environmental problems increase, the need to accurately and efficiently detect anions and cations in various water samples rapidly increases (Kalidas & M. V. Sangaranarayanan, 2023).

Several studies have been conducted to develop methods for analyzing anions with HILIC, including research on using HILIC columns to separate anions in wastewater samples.The results showed that this method effectively accurately separates various types of anions (Kiesilä et al., 2019; Prokopenko et al., 2018). Aminex A-25, a polar stationary phase column, separated anions in drinking water samples. The results showed that this method has high sensitivity and can detect anions in low concentrations. Although previous studies have shown promising results, some research gaps must be addressed. One of the research gaps is the expansion of the types of anions that HILIC can separate. The HILIC method is still limited to separating certain types of anions (X. Qiu et al., 2020). In addition, optimization of HILIC conditions for separating anions in various types of water samples has not been done. This is important to ensure that the HILIC method can be widely and effectively applied in various situations (Grumbach et al., 2021).

Increasing environmental problems drive the need to accurately and efficiently detect anions and cations in various water samples. Therefore, precise, rapid, and simple analytical methods are essential. One technique that fulfills these criteria is Hydrophilic Interaction Liquid Chromatography (HFCT)(Al-tannak et al., 2011). HILIC has proven effective in separating polar or hydrophilic compounds, including biologically active compounds such as pharmaceuticals, nucleosides, nucleotides, amino acids, peptides, proteins, oligosaccharides, and carbohydrates. Various column manufacturers currently provide columns designed explicitly for HILIC, allowing researchers to choose the right column to separate the desired compounds. Although HILIC has shown great potential in anion separation, some challenges still need to be overcome. One challenge is the development of more selective and efficient polar stationary phase columns to separate anions. In addition, further research needs to be done to understand the mechanism of anion separation in the HILIC system. This is important to optimize the HILIC conditions and improve the accuracy and sensitivity of the analysis (Nuijs et al., 2011).

This study evaluates the characteristics of NH_2-60, HILIC Imidazole, Polar Pyridine, and Amid-80 columns as polar stationary phases for anion separation in the HILIC process (Kambhampati et al., 2019). This study also aims to determine the optimum conditions of flow rate, mobile phase concentration, ammonium acetate salt concentration, and pH to separate anions effectively (Karayannakidis & Kalogiannis, 2016; Nakatani et al., 2022). This research is expected to provide valuable information about the characteristics of polar stationary phase columns for anion separation in HILIC and the optimum conditions for separating anions in the HILIC process (Heaton & Mccalley, 2016). The results of this study can be used to develop a more accurate, fast, and simple anion analysis method, which can be applied to various types of water samples.

This research is expected to increase understanding of the characteristics of polar stationary phase columns for anion separation in HILIC, provide information on the optimum conditions for separating anions in the HILIC process, and offer a more accurate, fast, and simple analytical method for detecting anions in water samples. The impact of this research

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can help better monitor environmental conditions through more accurate water analysis, support the development of more efficient analytical methods for various samples, and improve understanding of anion behavior in HILIC systems.

RESEARCH METHODS

Materials

The materials used in this study include Ammonium Propyl Silica (Sepax Technologies, Japan), HILIC Imidazole (Sepax Technologies, Japan), Polar Pyridine (Sepax Technologies, Japan), Amide-80 (Sepax Technologies, Japan), Acetonitrile (Kanto Chemical, Japan), Methanol (Wako Pure Chemical Industries), IC Water, Ammonium acetate (Nacalai Tesque, Japan), Acetic acid (Nacalai Tesque, Japan), Sodium acetate (Nacarai Chemicals, Japan), Hydrochloric acid (Nacalai Tesque, Japan), Sodium chloride (Nacalai Tesque, Japan), Tri Fluoro Acid (Nacalai Tesque, Japan), Sodium bicarbonate (Nacalai Tesque, Japan), Sodium Bromate (Nakalai Tesque, Japan), Sodium Bromide (Nakarai Tesque, Japan), Sodium Iodide (Nakarai Tesque, Japan), Sodium Nitrate (Nakarai Tesque, Japan), Sodium Nitrite (Nakarai Tesque, Japan), Sodium Thiocyanate (Nakalai Tesque, Japan).

Tools

The tools used include a CDS Data Processor (Shimadzu, Kyoto, Japan), UV Detector (Jasco, Tokyo, Japan), 0.2 0.2 µL Volume Injector (Rheodyne, Cotati, CA, USA), Microfeder (L. TEX Corporation, Tokyo, Japan), silica column (100 mm x 0.32 mm ID x 0.75 mm OD), PTFE (0.25 mm x 1/16 mm), (0.26 mm x 2 mm), (2 mm x 1 mm), (4 mm x 2 mm), stainless steel, cotton swab, syringe 0.5 mL; 0.1 mL; 0.25 mL (Ito, Fuji, Japan), balance and other glassware.

A 10 cm long capillary column that has been filled with polar stationary phase using connectors (Fig 8), without air cavities, was closed at both ends using Teflon Tube (PTFE) as many as 3 layers with each size 0.25 mm x 1/16 mm, 2 mm x 1 mm and 4 mm x 2 mm so that the column is not leaking on one of the parts given Quartz Glass Wool so that the stationary phase that has been filled does not come out. After that, at the end of the Teflon tube, stainless steel tubes are used as a connector to the HPLC equipment system.

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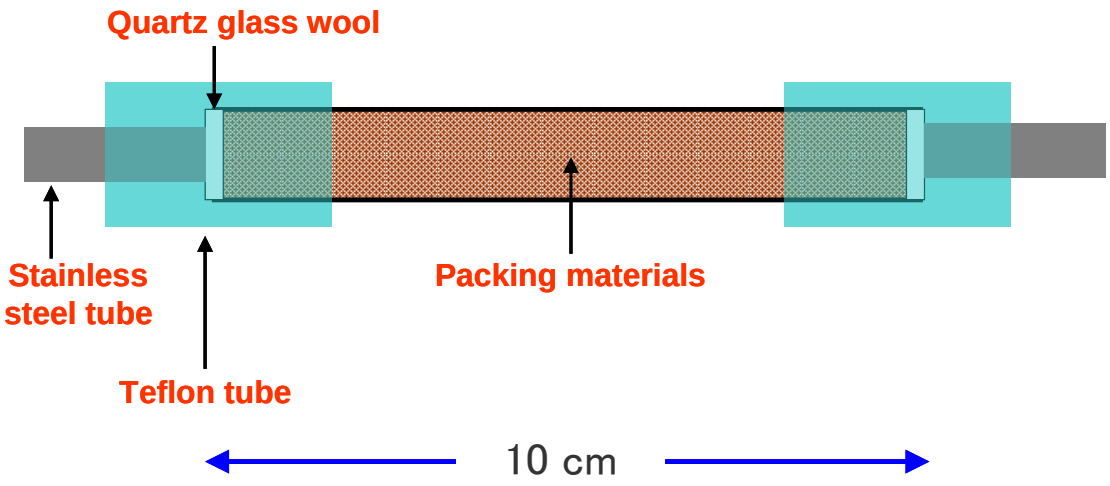

Fig 1. Capillary Column

Column Preparation

Polar stationary phases (NH_2-60, HILIC Imidazole, Polar Pyridine, Amid-80) were prepared sufficiently in a small bottle, then dissolved using 100% methanol, stirring well to form a slurry (not too dilute and not too concentrated). After that, the stationary phase is entered into a silica column made with a length of 10 cm and a diameter of 0.32 mm using a connector, as shown in Fig 8. After the silica column is finished packing, the column is installed on the HPLC system to be used (Mayaserli, 2015; Takeuchi et al., 2010).

Eluent Preparation

100% acetonitrile as eluent was dissolved in IC (Ion Chromatography) water, which is water specifically used to dissolve in chromatography in a ratio of 7:3, 8:2, 9:1. If using ammonium acetate salt, a variation of ammonium acetate concentration of 10, 20, 30, 40, 50 mM was made. Then, the ratio between acetonitrile and ammonium acetate was made 7:3, 8:2, 9:1. Eluent was put into a 0.5 mL syringe without air bubbles. The syringe was then attached to the HPLC system (Mayaserli, 2015; Takeuchi et al., 2010).

RESULTS AND DISCUSSION

Separation of Anions Using Amminopropyl Silica

Separation of anions using Aminopropyl Silica stationary phase with acetonitrile mobile phase and the addition of ammonium acetate salt is found in chromatogram 1. Where the concentration of ammonium acetate is varied 10, 20, 30, 40, and 50 mM. The chromatogram of Fig 1 shows that the best concentration of ammonium acetate salt to separate 6 anions is 50 mM. However, Br^- and BrO_3^- are not perfectly separated. Adding ammonium acetate increases the sensitivity and selectivity of the separation (Y. Guo & Gaiki, 2005; Mayaserli, 2015), so that the peaks that were initially small and still broad become more shape / sharp, and the time to separate the anion becomes faster.

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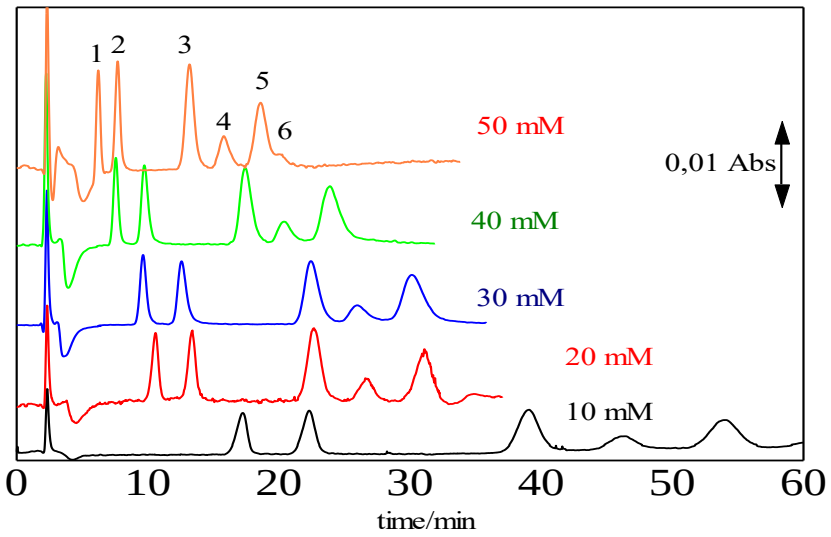

Fig 1. Chromatogram of anion separation using Ammino Propyl Silica (100 x 0.32 mm ID). Mobile phase: Acetonitrile (80%) in ammonium acetate (a) 10 mM, (b) 20 mM, (c) 30 mM, (d) 40 mM, (e) 50 mM. Flow Rate 3.0 µL/min. Injection Volume 0.02 µL. UV Detector at 210 nm. Anions: (1) SCN^- (2) I^- (3) NO_2^- (4) NO_3^- (5) Br^- (6) BrO_3^- 1mM of each analyte

The mobile phase variations used were acetonitrile: ammonium acetate (6:4, 7:3, and 8:2). Ammonium acetate concentration was varied to see the optimum condition of acetonitrile concentration for anion separation. The separation of 6 anions (SCN^-, I^-, NO_2^-, NO_3^-, Br^-, BrO_3^-) using aminopropyl silica is seen in the chromatogram of Fig 13. The anions can be separated perfectly, although Br^- and BrO_3^- are slightly clustered. The first anion to come out was SCN^- with Rt 6.2 minutes. BrO_3^- with Rt 20.5 minutes, the anion that took the longest to come out.

The Aminopropyl Silica stationary phase is the best because it can separate 6 anions only by using the acetonitrile mobile phase with water (without salt/additive mixture), although the chromatogram is not shape and short. However, the resulting chromatogram is better and more shaped after using ammonium acetate salt.

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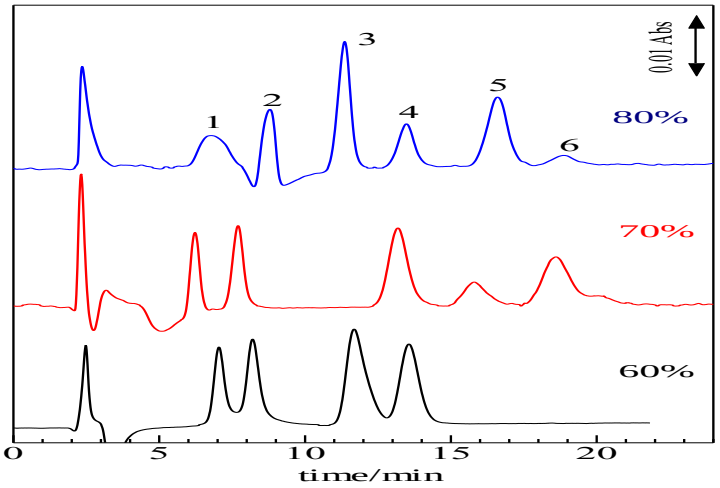

Fig 2. Chromatogram of anion separation using Ammonium Propyl Silica (100 x 0.32 mm ID). Mobile phase: Acetonitrile in 50 mM ammonium acetate. (a) 60%, (b) 70%, (c) 80%. Flow rate 3.0 µL/min. Injection volume 0.02 µL. UV Detector at 210 nm. Anions: (1) SCN^- (2) I^- (3) NO_2^- (4) NO_3^- (5) Br^- (6) BrO_3^- 1mM of each analyte.

In the HILIC process, using a polar stationary phase, Aminopropyl Silica can increase the selectivity and sensitivity of the separation. Due to the hydrophilic interaction between the stationary phase, mobile phase, and anion analyte, the strong hydrophilic interaction breaks the analyte bond so that it pushes out of the column (D. Guo et al., 2020; H. Qiu et al., 2006).

Separation of anions using HILIC Imidazole

Anion separation using HILIC Imidazol can be seen in the chromatogram of Fig 3. Anions can be separated even though BrO_3 only forms a small, broad peak. The time taken to separate the 6 anions was faster than using the Aminopropyl Silica stationary phase. The time required to separate anions using HILIC Imidazol is less than 12 minutes. While using Aminopropyl Silica, the time required to separate 6 anions is 20 minutes.

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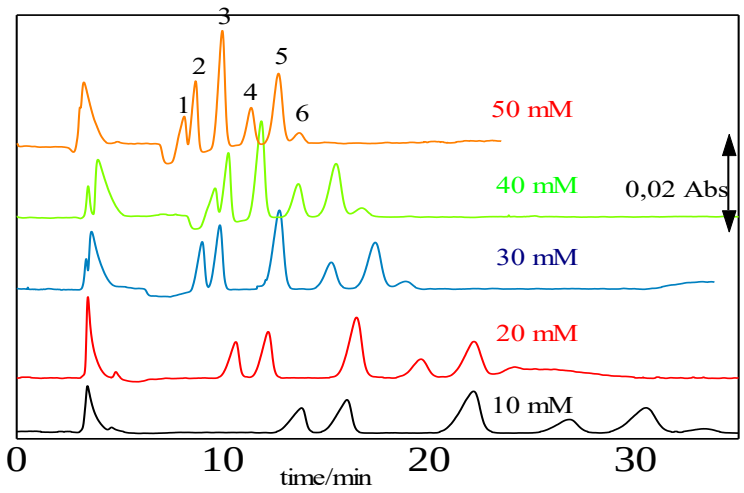

Fig 3. Separation of anions using HILIC Imidazole (100 x 0.32 mm ID). Mobile Phase: Acetonitrile (80%) in ammonium acetate (a) 10 mM, (b) 20 mM, (c) 30 mM, (d) 40 mM, (e) 50 mM. Flow rate 3.0µL/min. Injection volume 0.02 µL. UV Detector at 210 nm. Anions: (1) SCN^- (2) I^- (3) NO_3^- (4) Br^- (5) NO_2^- (6) BrO_3^- 1mM of each analyte.

Although the separation time is faster, the mobile phase used for anion separation must be supplemented with ammonium acetate salt. Only one peak is formed if the ammonium acetate salt is not added. In other words, the anion is not separated. Ammonium acetate can increase the selectivity and sensitivity of the separation (H. Qiu et al., 2007; Zein et al., 1990). The concentration of ammonium acetate varied to see which was suitable for anion separation. The variations used were 10, 20, 30, 40, and 50 mM. The chromatogram shows that the best concentration of ammonium acetate for separation is 50 mM. The resulting peak is sharper, and the retention time is faster (Akayama et al., 2017; A. J. Alpert, 2008; Ding et al., 2009).

The separation of anions using HILIC Imidazole can be seen in the chromatogram of Fig 4. The mobile phase variation used was acetonitrile: ammonium acetate (6:4, 7:3, and 8:2). The best optimum condition for the separation of 6 analytes using HILIC Imidazole is acetonitrile: ammonium acetate (8:2), the optimum concentration of ammonium acetate is 50 mM, with a flow rate of 3.0 µL/min and the concentration of each analyte is 1 mM.

In the chromatogram of Fig 4, it can be seen that the best separation is at 80% acetonitrile concentration. However, the time needed to separate the anions is longer than the 70% and 60% acetonitrile concentrations, namely with Rt 13.5 minutes. While at concentrations of 60% and 70%, the time is faster, which is about 11 minutes, the separation is not perfect because there are still anions that stick together.

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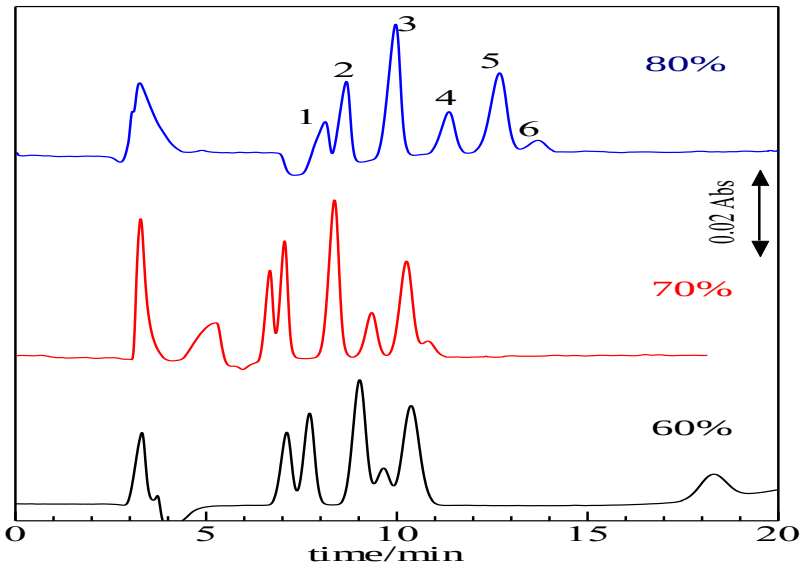

Fig 4. Separation of anions using HILIC Imidazole (100 x 0.32 mm ID). Mobile Phase: Acetonitrile in 50 mM Ammonium Acetate (a) 60% (b) 70% (c) 80%. Flow Rate 3.0µL/min. Injection volume 0.02 µL. UV Detector at 210 nm. Anions: (1) SCN^- (2) I^- (3) NO_3^- (4) Br^- (5) NO_2^- (6) BrO_3^- 1mM of each analyte.

Anions can be separated using HILIC Imidazole due to the hydrophilic interaction between the stationary, mobile, and analyte. The principle of HILIC Imidazol is the same as Aminopropyl Silica, but the order of separated anions is different between HILIC Imidazol and Aminopropyl Silica (Takeuchi et al., 2010).

Separation of anions using polar pyridine

The difference between anion separation using Polar Pyridine with Aminopropyl Silica and HILIC Imidazole is the separation conditions at low pH and the mobile phase used. Because of the use of acetonitrile with water or ammonium acetate, the anion cannot be separated, only forming several peaks.

In the chromatogram, it can be seen that the mobile phases used are 70% acetonitrile, acetonitrile + 50 mM Sodium Acetate in 50 mM Acetic Acid (1:9) and 50 mM Sodium Acetate in 50 mM Acetic Acid.

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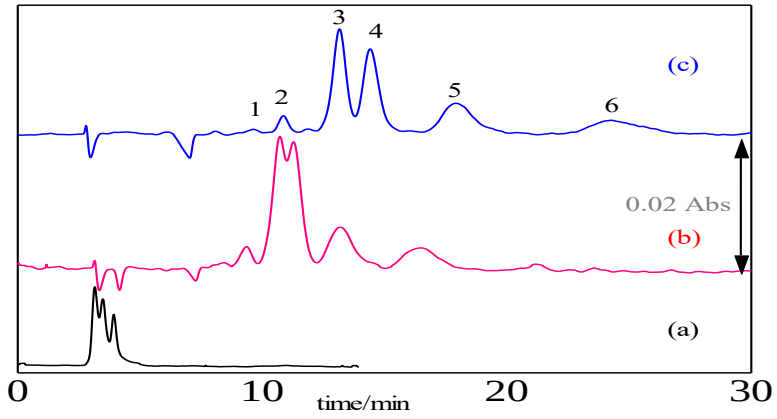

Fig 5. Separation of anions using Polar Pyridine (100 x 0.32 mm ID). Mobile Phase: (a). 70% Acetonitrile (b) Acetonitrile + 50 mM Sodium Acetate in 50 mM Acetic Acid (c) 50 mM Sodium Acetate in 50 mM Acetic Acid. Flow Rate 2.5 µL/min. Injection volume 0.02 µL. UV Detector at 210 nm. (1)BrO_3^- (2)NO_2^- (3)Br^- (4)NO_3^- (5)I^- (6) SCN^- 1 mM of each analyte.

For this reason, it is necessary to adjust the pH so that the anion can be separated, namely by replacing the acetonitrile mobile phase with 50 mM sodium acetate in 50 mM acetic acid so that the pH of the mobile phase becomes 4.2. Under these conditions, the anions can be separated well. The time required to separate 6 anions is 25 minutes.

The Polar Pyridine stationary phase is protonated because the separation occurs at low pH. So that the process that occurs is ion exchange (ion exchange)(Linda et al., 2013; Takeuchi et al., 2010). When a small amount of acetonitrile is added to 50 mM sodium acetate in 50 mM acetic acid, the anions can be separated but not perfectly separated. The Br^- and BrO_3^- peaks are close, but the NO_3^- and NO_2^- peaks are not perfectly separated. While I^- and SCN^- are separated, they have broad peaks (Al-tannak et al., 2011; Jandera, 2011).

As for the variation of acetonitrile concentration, 50 mM Sodium Acetate in 50 mM Acetic Acid was done to see the effect of additional concentration on anion separation.

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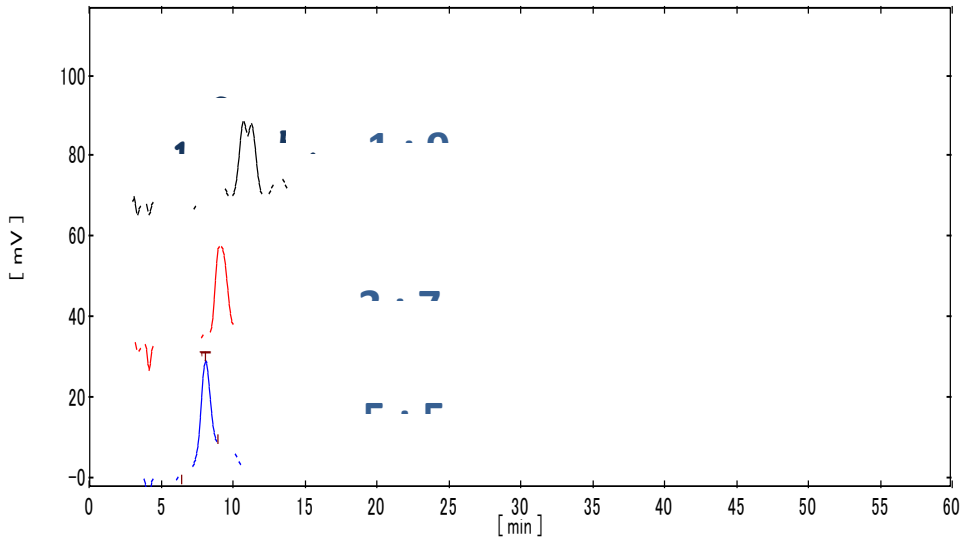

Fig 6. Separation of anions using Polar Pyridine (100 x 0.32 mm ID). Acetonitrile Mobile Phase: 50 mM Sodium Acetate in 50 mM Acetic Acid (a) 5:5 (b) 3:7 (c) 1:9. Flow Rate 2.5 µL/min. Injection volume 0.02 µL. UV Detector at 210 nm. (1)BrO_3^- (2)NO_2^- (3)Br^- (4)NO_3^- (5)I^- (6)SCN^- 1 mM of each analyte.

From the chromatogram of Fig 6, the concentration of acetonitrile separation is not good. If the concentration of Acetonitrile is lowered, then the separation will get better even though there are still those that are not perfectly separated. This may be due to the influence of pH (Ding et al., 2009; Ikegami et al., 2008; Zhao et al., 2022). pH of polar pyridine must be set in acidic conditions (pH 3 - 4) so that the anion can be separated. While using acetonitrile with a high concentration, the pH ranges from 6 - 8.

Separation of anions using amide-80

Separation of anions using Amide-80 with mobile phase Acetonitrile coupled with water and Ammonium Acetate can be seen in the chromatogram of Fig 7.

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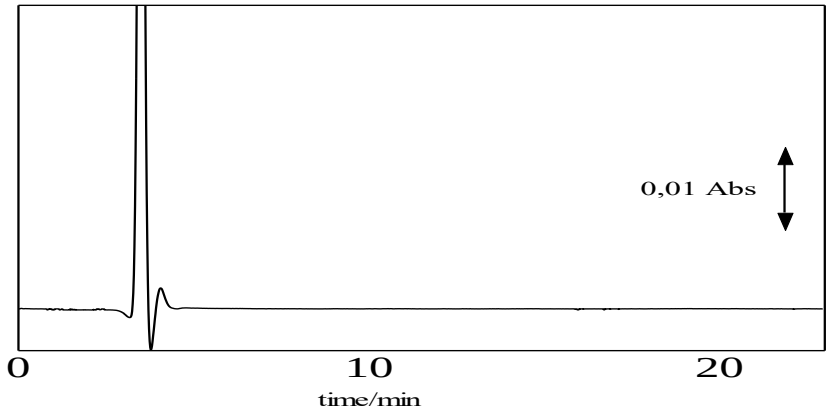

Fig 7. Separation of anions using Amide-80 (100 x 0.32 mm ID). Mobile Phase: 70% Acetonitrile + 50 mM Ammonium Acetate. Flow rate 2.5 µL/min. Injection volume 0.02 µL. UV Detector at 210 nm. BrO_3^-, Br^-, NO_2^-, NO_3^-, I^-, SCN^- 1 mM each analyte.

In previous studies, Amide-80 is widely used to separate organic compounds such as carbohydrates and proteins and for drugs. No research has tried to use Amide-80 to separate inorganic compounds (anions) in HILIC conditions and ion exchange (J. Alpert, 1990; Y. Guo & Gaiki, 2005; Yoshida & Okada, 1999). Amide-80 can be used to separate compounds in HILIC conditions, such as for the separation of polar compounds and hydrophilic by using acetonitrile mobile phase with the addition of ammonium acetate salt, pH ranging from 3 - 6.

CONCLUSION

Aminopropyl Silicate and HILIC Imidazole can separate SCN^-, NO_3^-, NO_2^-, Br^-, BrO_3^- and I^- anions using Acetonitrile mobile phase with the addition of Ammonium Acetate salt in the mobile phase, which can increase the sensitivity and selectivity of anions so that the resulting peak is sharper and more enormous. Polar pyridine can separate anions at low pH (4.2) using an acidic mobile phase (acidic eluent). Polar Pyridine can function at low pH as an ion exchanger (ion exchange). Amide-80 cannot separate anions using acetonitrile either with water or salt.

Further research was conducted to separate anions using Polar Pyridine and Amide-80 in the HILIC process using acetonitrile mobile phase with the addition of other salts (other than ammonium acetate). In further research, the separation of anions using polar stationary phases will be expected to be applied to environmental waste samples and other natural samples.

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