One-pot Facile Preparation of Amino-functionalized Silica Hybrid Monoliths for Mixed-mode Chromatography

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Abstract

Silica hybrid monolithic columns were prepared using two precursors, in which organo-functionalized trialkoxysilanes are mixed with tetraalkoxysilanes. In this study, several types of amino-functionalized silica hybrid monolithic columns were prepared via single-step “one-pot” approach, and the amount of silica precursors, porogens, as well as the reaction conditions were optimized. The preparation was carried out by mixing the silica precursors, i.e. tetraethoxysilane (TEOS) or tetramethoxysilane (TMOS) with amino precursors such as aminopropyltrimethoxysilane (APTES), aminooethylaminopropyl-trimethoxysilane (AEAPTMS), and phenylaminopropyltrimethoxysilane (PAPTMS) in a porogenic solution. The chromatographic performance of these hybrid monolithic columns was optimized by investigating several parameters through the separation of inorganic anions (IO₃⁻, BrO₃⁻, Br⁻, NO₂⁻, NO₃⁻, I⁻, SCN⁻) and some polar compounds (thymine, thymidine, adenosine, adenine, uridine). Results showed that the silica hybrid monolithic columns could be operated at higher flow-rate that favors rapid separation. The run-to-run repeatability of Si-APTES and Si-PAPTMS hybrid monolithic columns were satisfactory with relative standard deviations (n = 5) of less than 8% for all the analyte anions.

Amino-functionalized silica precursors was used in the preparation of hybrid monolithic column to reduce the preparation time via one-pot approach. The amine groups in the structure can provide weak anion exchange interaction and also hydrophilic interaction. The synthesized hybrid monolithic columns revealed good mechanical stability and good separation repeatability.

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81
Introduction

Ion Chromatography (IC) was firstly introduced by Small et al. [1] in 1975. They have developed ion-exchange chromatographic methods for anions and cations determination using a conductivity detector. Over the past 40 years, IC has become one of the most common analytical methods for the determination of inorganic anions and cations, as well as small organic anions and amino acids [2]. The most frequently found application of IC is for natural water analysis, i.e. drinking water, seawater, pond, and lake water. Inorganic anions are present in these waters, thus the determination of these inorganic anions could be a useful parameter in order to monitor the water quality and its safety for human activities.

There are two types of stationary phases that are commonly used for capillary ion chromatography; first, a particle-packed column with silica or polymer particles as packing materials. Silica-based packing materials have advantages such as resistance to organic solvents, and the silanol groups on the surface of silica allow easy modification with many functional groups. Some researchers have developed various stationary phases by modifying the silica surface. Poly(ethylene oxide)-bonded stationary phase was successfully prepared by chemically bonded with aminopropylsilica for anions separation by ion-exchange mode [3-5]. Kawase et al., have prepared a hyperbranched amine-modified capillary column by reacting aminopropylsilica with diethoxymidofication and ammonia repeatedly for separation of iodide, nitrate, and iodate, as well as hydrophobic compounds [6]. Kisiliva et al., have modified the silica particles with 3-(phenylamino)propyl trimethoxysilane in toluene for separation of phenols [7] and separation of anions using conductivity detector [8].

Another type of stationary phase for capillary ion chromatography is a porous monolithic column. The combination of high permeability and high separation efficiency has allowed utilization of these columns in IC, such as high-efficiency low pressure IC [9-11], excellent stability, and reproducibility [12,13]. Monolithic columns can be classified into three types; organic polymer-based monolithic columns, inorganic silica-based monolithic columns, and organic-inorganic hybrid monolithic columns. Organic polymer-based monolithic columns have good biocompatibility, excellent pH stability, but poor in mechanical stability due to the possibility of the swelling and shrinkage in the structure [14-18]. Inorganic silica-based monolithic columns, which can be prepared by immobilizing silica particles in a silica matrix or sol-gel method have high permeability and mechanical stability; however, the preparation of silica monoliths is time-consuming, and the preparation process is difficult to control and cannot be used in alkaline eluents due to the chemical instability [18-21]. Hybrid organic-inorganic monolithic columns have caused an interest due to their higher pH stability [22] and the ability to avoid the drawbacks of solvent swelling [23].

Sol-gel chemistry is the preparation of inorganic polymers from a solution through a transformation from liquid precursors to sol and finally to a network structure called a gel [24]. Sol-gel network offers high efficiency, high permeability, and high mechanical strength [25-27]. Basically, the sol-gel process involves the synthesis of the sol from hydrolysis and the formation of the gel via polycondensation of alkoxides in aqueous acid or base solution [25,28,29]. The preparation of silica hybrid monolithic columns is usually done by the post-modification method, which is the modification of the silica surface with functional monomers [30-32]. As previously mentioned, the preparation process for this method is time-consuming and the entire process is difficult to control. Initial incorporation of functional precursors together with the alkoxide precursors can reduce the preparation time. Hayes and Malik have prepared silica monolith with surface-bonded C18 in a single step by the sol-gel process [33]. L. Yan et al. mixed tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES) for the separation of organic acids and triterpenoids from Ganoderma lucidum in capillary electrochromatography (CEC). There is no shrinkage within the capillary and excellent mechanical stability was observed in the hybrid monolithic column [25].

In the present study, a single step preparation of silica hybrid monolithic column with an anion exchanger has been investigated for fabricating amino-functionalized silica hybrid monolithic columns. Three types of amino precursors were used in this study such as 3-aminopropyltriethoxysilane (APTES), aminoethylaminopropyltrimethoxysilane (AEAPTMS), and [3-(phenylamino)propyl] trimethoxysilane (PAPTS). The use of amino precursors could reduce the preparation time and simplify the preparation method. The presence of amine groups could act as a weak anion exchanger and a hydrophilic stationary phase. The presence of a benzene ring in PAPTS could also act as a
hydrophobic stationary phase. The chromatographic performance of these hybrid monolithic columns was optimized by investigating several parameters through the separation of inorganic anions and some polar compounds.

**Experimental Section**

**Materials and Apparatus**

Tetramethoxysilane (TMOS) was purchased from Shin-ETSu Chemical (Tokyo, Japan). TEOS, APTES, PAPTMS, AEAAPTMS were purchased from Tokyo Chemical Industry (TCI, Tokyo, Japan). Poly(ethylene glycol) (PEG, MW 10000) was purchased from Sigma Aldrich (Tokyo, Japan). Urea, acetic acid, sodium hydrosxide (NaOH), hydrochloric acid (HCl), sodium nitrate, sodium nitrite, sodium thiocyanate, sodium iodide, and sodium bromate were purchased from Nacalai Tesque (Kyoto, Japan). Cetyltrimethylammonium bromide (CTAB), methanol, ethanol, acetonitrile, sodium chloride, sodium sulfate, sodium dihydrogen phosphate, sulfuric acid, phosphoric acid, sodium bromide, and sodium iodate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ultrapure water was prepared in the laboratory by using a simplicity UV water purification system (Millipore, Molsheim, France), and all solutions used in this study were prepared using this ultrapure water. Capillaries with 0.32 mm I.D. x 0.45 mm O.D. were the products of GL Sciences (Tokyo, Japan).

All experiments were operated by using a CLC system which consists of a Microfeeder (L.TEX 8301, Tokyo, Japan) equipped with a gas-tight syringe (0.5 mL; Ito, Fuji, Japan) as a pump, an M-435 Micro Injection Valve with 0.2 µL injection volume (Upchurch Scientific, Oak Harbor, WA, USA) as an injector, a UV-2075 UV detector (JASCO, Tokyo, Japan), a data processor (CDS-Lite ver. 5.0; LA soft, Chiba, Japan) and the inlet pressure was monitored by an LTEX-8150 Pressure Sensor (L.TEX). Morphology of the columns was characterized by an SU-3500 scanning electron microscope (Hitachi, Tokyo, Japan).

**Preparation of amino-functionalized silica hybrid monolithic columns**

The fused silica capillary was pre-treated by washing with 1 M NaOH, deionized water, 1 M HCl, and then deionized water for 30 min, respectively. After that, the capillary was dried by nitrogen gas at room temperature for another 30 min. Several types of amino-functionalized silica hybrid monolithic columns were prepared by mixing the porogens, i.e., CTAB, water, ethanol, PEG, urea, and acetic acid with two types of tetraalkoxysilanes such as TMOS or TEOS and amino-functionalized trialkoxysilanes (as shown in Figure 1) such as APTES, AEAPTMS, and PAPTMS at 0 °C. After that, the solutions were manually injected into pre-treated fused-silica capillaries and reacted at several temperatures for 24 h. The monolithic columns formed were flushed with acetone and water to remove the porogen and other residuals. For use under the ion-exchange mode, the monoliths were protonated with 50 mM HCl prior to use.

![Figure 1. Structures of amino-functionalized silica precursors employed in this study.](image)

**Results and Discussion**

**Preparation of amino-functionalized silica hybrid monolithic columns**

**Preparation of Si-APTES hybrid monolithic column**

The fabrication of Si-APTES hybrid monolithic column was carried out by using CTAB, water, and ethanol as the porogen [13,25,34] as listed in Supplementary Materials Table S1. Basic condition was chosen for this preparation condition (pH 8). Under acid catalysis, the initial step of hydrolysis, the conversion of the precursor molecules into the triethoxysilanol proceeds rapidly, and the gelation takes no time to occur [35]. The existence of water in the mixture can also act as a catalyst and is necessary to complete the hydrolysis of the alkxy groups of the monomer [27]. When no water was added into the solution, the gelation process did not occur both inside the capillaries as well as sample vials. The homogeneous monolith was achieved by adding 15% of water into the solution. However, increasing the amount of water into the solution led to faster gelation time due to increased reactant dilution, and the solution could not be filled into the capillaries.
The amount of APTES also affects the gelation time. By increasing the amount of APTES and keeping the amount of TEOS, longer gelation time was observed. Therefore, it is favorable for the monolith condition. Increasing the amount of APTES to 0.08 g, the number of a theoretical plate for Br increased. However, by increasing the amount of APTES to 0.12 g led to many unreacted of APTES because the solution is already saturated and the theoretical plate number was also decreased.

Reaction temperature also affected the formation of Si-APTES hybrid monolithic columns. The monoliths were prepared at different reaction temperatures, ranging from 30-60 °C (columns F, H, I, and J). At 30 °C (i.e. column F) monolith was detached from the capillary, on the other hand, the monoliths were firmly attached to the wall of capillaries at 40 and 50 °C (i.e. column H and I), while a very dense monolith was observed when prepared at 60 °C (i.e. column J).

Besides temperature, reaction time also has been investigated in the formation of Si-APTES hybrid monolithic columns. Several monoliths were prepared at different reaction times i.e. 8, 12 and 36 h, denoted as columns K, L, and M, respectively. The monolith was severely detached from the capillary at 12 h reaction time (column L), while at 8 h reaction time (column K), the monolith was not formed due to the insufficient polymerization time. The permeability of the monolithic column decreased at preparation time that conducted at 36 h (column M) due to the very high back pressure of the monolithic column that reached 5.0 MPa for 3 μL/min. These results indicated that the ideal time of the preparation of Si-APTES hybrid monolithic column was 24 h. Based on these results, column F was chosen for the following experiments. Scanning electron microscopy (SEM) images of column F are shown in Figure 2(a) and (b). Elemental analysis of column F showed 4.31, 15.2 and 4.48% of C, H, and N, respectively.

Preparation of Si-AEAPTMS hybrid monolithic column

The preparation of Si-AEAPTMS hybrid monolithic column was carried out by one-pot reaction using PEG, water, and ethanol as the porogen. TEOS was used as tetraalkoxysilane precursors in place of TMOS which has lower reactivity and thus slows the gelation time. CTAB acts as a macromolecular template in the formation of a sol-gel monolith. When CTAB was used as porogen, a homogeneous gel with no pores was formed, as can be seen from the SEM picture Figure 2(c). By changing CTAB with PEG (Mw = 10000) and keep ethanol and water, an interconnected monolith-form with pores can be seen from the morphology of the monolith. PEG is more hydrophilic than CTAB, thus the resulting PEG is more soluble and more biocompatible to react with TEOS, which is also a polar compound. However, this column was shrunk after two times of use for separation. Another type of porogens composition and reaction condition also has been investigated to make a good monolith, i.e., methanol, dimethylsulfoxide, and acetic acid (acidic condition). However, the gelation process did not occur both inside of the capillaries as well as in the sample vials. Therefore, the best porogen and solvent condition so far are PEG, water, and ethanol. The variation of temperatures also has been investigated, ranging from 40, 50, and 60 °C (i.e. column O, O2, and O3). Unfortunately, the same phenomenon occurred for all of these columns. The baseline separation was achieved, but the monolith shrunk after 2 times of sample injection. The separation could be achieved due to the presence of N in the Si-AEAPTMS hybrid monolithic column as shown in the elemental analysis data. The percentage of C, H, and N were 4.74, 20.8 and 5.04%, respectively. One of the reasons for the shrinkage may be the incompatibility or the uncomplete hydrolysis process between TEOS (ethyl group) and AEAPTMS (methyl). The exact reason for this is still under investigation.

Preparation of Si-PAPTMS hybrid monolithic column

Si-PAPTMS hybrid monolithic column was prepared by single-step reaction, TMOS and PAPTMS were mixed with PEG, urea, and acetic acid. Referring to Si-APTES hybrid monolithic column, the mixture was prepared under basic condition. However, no gelation was formed both inside the capillaries and the sample vials. These results can be explained by the absence of NH2-group in PAPTMS, which could act as its own catalyst in the basic condition. For this reason, the acidic condition was used for the preparation of Si-PAPTMS hybrid monolithic column [30].

The reaction temperature was investigated by varying the temperature ranging from 40-80 °C. A homogeneous gel form with no through pores was observed at the preparation temperature 40 °C. It is considered that at low temperature, the condensation process still could not be finished. Thus, this resulted to a gel-like monolith. At 50 and 60 °C, the structure of the monolith was slightly formed. However, there
is some agglomeration of the particles in the monoliths, which led to unsatisfactory separation of ions in the monoliths. An inter-connected homogeneous monolith network was observed at 70 °C. The separation of anions for 70 °C also gives a good separation. Increasing the temperature to 80°C leads to a compact monolith as the back pressure of the monolith reaches 2.7 MPa at 80°C. Therefore, the preparation temperature at 70°C was used for further experiments.

The ratio of TMOS and PAPTMS was also investigated by increasing the amount of PAPTMS from 0.04, 0.08, 0.12 to 0.16 (i.e. column W2 to W4). The back pressure of the monolith became higher when the amount of PAPTMS in the mixture was increased, and nonhomogeneous mixture was observed in the sample vials. Based on these results, column W was chosen for the following experiments. Elemental analysis of column W showed 4.68, 28.3 and 2.60% of C, H, and N, respectively, proving the presence of N in the Si-PAPTMS hybrid monolithic column.

In order to evaluate the retention behavior of the three prepared hybrid monolithic columns, the common inorganic anions such as iodate, bromate, nitrite, bromide, nitrate, iodide, and thiocyanate were used as analytes. Figure 3 shows the result of retention time of anions in the Si-hybrid monolithic columns. For the Si-APTES hybrid monolithic column, anion samples were slightly separated, even though some peaks were overlapped and not baseline separated. From this result it was suggested that anion exchange capacity or protonation of the monolith is not enough to separate the analytes. However, an unusual elution order was observed in the separation of anions. In order to understand the reasons behind it, the separation of anions on the other two hybrid monolithic columns were also investigated. Si-AEAPTMS hybrid monolithic column is expected to retain the analyte anions by electrostatic interaction due to the presence of secondary amine in the stationary phase. As expected, Si-AEAPTMS hybrid monolithic column could retain all analytes, and Br and NO3 were overlapped each other. However, this monolith always shrank after two times of injection. As explained before, the reasons for it may be the incompatibility or the incomplete hydrolysis process between TEOS (ethyl group) and AEAPTMS (methyl group). Therefore, the investigation of Si-AEAPTMS could not be carried out in further experiments. Si-PAPTMS hybrid monolithic column also has secondary amine in the stationary phase. All analytes could be retained, and the elution order of samples was the same as one obtained by common ion exchange chromatography. From these results, it can be concluded that primary amine in the stationary phase affects the elution order of the anions. Strong electrostatic interaction could give the same elution order as typical ion exchange chromatography. The other possibility is the separation mechanism that occurred in the Si-APTES hybrid monolithic column is not purely based on the ion exchange mechanism. Since Si-APTES hybrid monolithic column is more hydrophilic than Si-AEAPTMS and Si-PAPTMS, the hydrophilic anions, i.e., IO3 retain more strongly than...
I- and SCN-. Nevertheless, the resolution of two third of the peaks is still below 1.5 (Supplementary Materials, Table S2). A resolution of at least 0.6 is needed to observe if there is a “valley” (i.e., separation) between two peaks, and a resolution of 1.5 is needed for baseline-separation [36].

Figure 3. Retention behavior of inorganic anions on amino-functionalized silica hybrid monolithic columns. Column: Si-APTES hybrid, Si-AEAPTS hybrid, and Si PAPTMS hybrid (100 x 0.32 mm I.D.); eluent: 50 mM NaCl; analytes: 0.5 mM each of 1:IO$_3^-$, 2:BrO$_3^-$, 3:NO$_2^-$, 4:Br$_2^-$, 5:NO$_3^-$, 6:I-, 7:SCN$^-; injection volume: 0.2 µL; wavelength of UV detection: 210 nm; flow-rate: 3 µL/min.

Effect of the eluent concentration on retention behavior of inorganic anions on amino-functionalized silica hybrid monolithic columns

The retention behaviors of inorganic anions on Si-APTES hybrid monolith with various eluents were also investigated (Supplementary material Figure S2). Retention times of all analytes became longer as 2 mM of hydrochloric acid was added into the eluent. This is because, in acidic conditions, protonation gives positive charges to the primary amine groups on the stationary phase, and it attracts analytes sample more strongly. The retention of all analytes decreases by using 25 mM of sodium sulfate in 2 mM of sulfuric acid as eluent. This happens because double-charge of SO$_4^{2-}$ retain more strongly on the positive charge of the stationary phase, and the competition between analyte anions and negative charges of eluent occurred. Thus, this makes analyte anions retained more weakly. Another type of salt, i.e., sodium dihydrogen phosphate in phosphoric acid, was also used as eluent (pH 3.5). The retention of all analytes decreased compared to those in sodium chloride but increased compared to those in sodium sulfate. The reason for it because the pH of phosphate eluent is the weakest between these three eluents. Therefore, it makes the electrostatic interaction is weak, leading to fast retention. Besides, H$_2$PO$_4^-$ is only singly charged, which makes it less strong compared to SO$_4^{2-}$. Thus, resulting in increased retention time. The retention behaviors of inorganic anions on the Si-PAPTMS hybrid monolithic column were also investigated (Supplementary material Figure S3). The same trend with Si-APTES hybrid monolithic column was observed. The addition of hydrochloric acid into the eluent increases the retention time of analytes. It was also found that by using sodium dihydrogen phosphate in phosphoric acid as eluent, the retention times of all analytes were shorter compared to sodium chloride but longer compared to sodium sulfate.

Effect of the eluent concentration on retention behavior of inorganic anions on amino-functionalized silica hybrid monolithic columns

The retention time of all samples decreased with increasing the eluent concentration for both of Si-APTES and Si-PAPTMS hybrid monolithic columns (Supplementary material Figure S4). These results indicate that the separation mechanism is based on ion exchange mode. Figure 4 shows the logarithm of retention factor (k) of analyte versus the logarithm of the eluent (NaCl) concentration of Si-APTES and Si-PAPTMS hybrid monolithic columns. The slope values of all anion should be -1, to prove the separation mechanism is purely based on ion exchange mode. The slopes obtained in Figure 4(a) were -1.34, -1.14, -0.98, -0.90, -1.10, -0.89, and -0.98 for IO$_3^-$, BrO$_3^-$, NO$_2^-$, Br$_2^-$, NO$_3^-$, I-, SCN$^-$ respectively. The slopes obtained in Figure 4(b) were -0.98, -0.95, -0.84, -0.77, -0.75, and -0.69 for IO$_3^-$, BrO$_3^-$, NO$_2^-$, NO$_3^-$, I-, SCN$^-$ respectively. Based on these results, it is confirmed that not only ion exchange mechanism but also other retention mechanism had occurred in the separation of the anions. There is a possibility that hydrophilic interaction occurs in the Si-APTES hybrid monolithic column and hydrophobic interaction occurs in the Si-PAPTMS hybrid monolithic column.

Effect of the flow-rate on retention behavior of inorganic anions on amino-functionalized silica hybrid monolithic columns

The effect of flow-rate on the retention of inorganic anions on Si-APTES and Si-PAPTMS hybrid monolithic columns was investigated by varying the flow-rate of the eluent (Supplementary material Figure S5). As the flow-rate of the eluent in-
Figure 4. Plotting of logarithm of the retention factor \(k\) versus logarithm of the NaCl concentration (a) Si-APTES hybrid monolithic column, (b) Si-PAPTMS hybrid monolithic column.

Increases, the retention time of all analytes decreases. The back pressures of the hybrid monolithic columns were monitored for the flow-rate ranging from 2 to 10 \(\mu\)L/min. Figure 5(a) shows the effect of the flow-rate on the column backpressure. The pressure was 1.8 MPa and 1.6 MPa at flow-rate 10 \(\mu\)L/min for Si-APTES and Si-PAPTMS, respectively. The low backpressure could be achieved because of the presence of the macropores on the monolith structure. These results show that Si-APTES and Si-PAPTMS hybrid monolithic columns could be operated at a high flow-rate with low backpressure.

The effect of height equivalent to a theoretical plate (HETP) at a different flow-rate of the mobile phase was investigated to know the efficiency of the Si-APTES and Si-PAPTMS hybrid monolithic columns. As can be seen in Figure 5(b), HETP increases with increasing flow-rate. The lowest plate height was about 0.15 mm at a flow-rate 3 \(\mu\)L/min and 0.11 mm at a flow-rate 4 \(\mu\)L/min for bromide using Si-APTES and Si-PAPTMS hybrid monolithic columns, respectively. The efficiency of these columns was relatively low compared to other anion exchange columns [9, 37]. The van Deemter curve for Si-PAPTMS is significantly flatter than Si-APTES hybrid monolithic column. It was observed that no decrease in column performance when the flow-rate was higher than 4 \(\mu\)L/min. This result means that Si-PAPTMS hybrid monolithic column is more suitable to be used for rapid separation of inorganic anions.

Figure 5. (a) Plotting of backpressure versus flow-rate on Si-APTES and Si-PAPTMS hybrid monolithic columns (b) van Deemter plot of Si-APTES and Si-PAPTMS hybrid monolithic columns.

\(\bullet\) = Si-APTES, \(\Delta\) = Si-PAPTMS

Repeatability of amino-functionalized silica hybrid monolithic columns

The repeatability of Si-APTES hybrid monolithic column was investigated under the optimum conditions. The repeatability was determined by calculating the relative standard deviation (RSD). RSD values for run-to-run \((n = 5)\) of the retention time, peak height, and peak area were found less than 5\%, 6\%, and 8\%, respectively. Even though the RSD value of peak area was found a little high, but it still considered as a good RSD value. These results indicated satisfactory repeatability of Si-APTES hybrid monolithic column for separation of inorganic
anions. The repeatability of Si-PAPTMS hybrid monolithic column was also investigated. RSD values for run-to-run (n=5) of the retention time, peak height, and peak area were found less than 2%, 6%, and 7%, respectively. These results indicated that Si-PAPTMS hybrid monolithic column is more accurate for the separation of inorganic anions compared to Si-APTES hybrid monolithic column.

Retention behavior of polar compounds on Si-APTES hybrid monolithic column
The presence of amine in the Si-APTES hybrid monolithic column could also be used for the separation of some HILIC samples, i.e., nucleobases or nucleosides. The separation of 5 polar compounds on the Si-APTES hybrid monolithic column with 90% of acetonitrile as eluent was shown in supplementary material Figure S5. It was observed that some peaks were overlapped. In addition, the effect of acetonitrile concentration was investigated, ranging from 90% to 98% in the eluent. The retention time of all analytes is increased with the increase of ACN concentration, which showed the common mechanism for HILIC. However, further investigation is needed for better separation.

Conclusions
Si-APTES and Si-PAPTMS hybrid monolithic columns were successfully prepared by the “single-step one-pot” approach under various conditions. The hybrid monolithic columns were evaluated for the separation of the inorganic anions (IO₃⁻, BrO₃⁻, NO₂⁻, Br⁻, NO₃⁻, I⁻, SCN⁻) by using NaCl as the eluent. It was also found that increasing the concentration of the mobile phase causes the decreasing of the retention time of the inorganic anions. Si-PAPTMS hybrid monolithic column shows better efficiency and lower backpressure compared to Si-APTES hybrid monolithic column and favorable to operate at a high flow-rate. The run-to-run repeatability of Si-APTES and Si-PAPTMS hybrid monolithic columns were satisfactory with the relative standard deviations run-to-run (n = 5) less than 8% for all the analyte anions. Si-APTES hybrid monolithic column also showed some separation of polar compounds under HILIC mode.

Conflict of Interest
The authors declare that there is no conflict of interest of whatsoever case.

Supplementary Materials
The following supplementary materials are available online at http://journal.hki.or.id/JICS

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