Dual-column Switching Ion Chromatography for the Simultaneous Determination of Inorganic Cations and Anions (particularly Thiocyanate) in Human Urine and Saliva Samples to Identify Smokers Types

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Abstract. An ion chromatography (IC) have been developed for the simultaneous determination of inorganic cations (Na+, NH4+, K+, Mg2+, and Ca2+) and inorganic anions (PO43−, Cl−, NO3−, and SCN−) using a single pump, a single eluent and a single detector. The method is based on column switching means allows anions and/or cations could be determined in a single analysis system. While one ion-exchange column is being operated, the other ion-exchange column is being conditioned, i.e., the columns are always ready for analysis at any time. When the combination of 10 mM tartaric acid was used as the eluent, and operated at eluent flow rate of 0.5 mL/min, five cations and four anions could be separated on the cation-exchange column and the anion-exchange column, respectively. The separation of cations was completed within 15 min, whereas the separation of anions was completed within 40 min. The detection limits was calculated at S/N=3 were 3.10−12.14 ppb (µg/L) for the cations and 16.00−88.10 ppb for the anions. The relative standard deviations of the all ions were less than 4.81%, 6.30%, and 6.22% for peak height, peak area, and retention time, respectively. The developed technique was successfully applied to the simultaneous determination of inorganic cations and anions in human saliva and urine samples.

Keywords: Ion Chromatography, simultaneous, inorganic cations and anions
1. Introduction

Since it was first introduced by Small et al. in 1975, IC has become a reliable analytical technique for determining cations and anions various samples until ppb level of concentration or more. IC has been widely applied in agriculture, food, environmental, industrial, medicine [1−4], however, in the field of health is still not much application, and that is why it needs to be given more attention.

Dual-column switching ion chromatography (DSIC) means that the separation of cations and/or the separation of anions can be done at different times in a single of analysis systems. The flow can be switched from the cation-exchange to the anion-exchange column by simply rotating the direction of the switching valve.

Mostly, cations and anions are separated separately and use different eluents. So, the analysis time will be longer to get completed data of the cations and anions.

A number of articles related to the separation of cations and anions in a single analysis system and using different packing material columns, it can be divided into 2 types of methods, namely (1) the cation-exchange column and the anion-exchange column are arranged in series and/or parallel [5], and (2) the both ion-exchange columns are also arranged in series and connected via the switching valve [6−12]. For both methods, a suitable eluent should be chosen for the simultaneous separation of cations and anions. But, it should be noted that without a switching valve, these (cations and anions) peaks sometimes overlap and the method is not applicable widely. Otherwise, the method using a switching valve, peak overlap can be avoided.

Urine and saliva may contain many ions, elements, compounds that can provide information of health and clinical conditions of a person, as well as to identify smoker type purposes. So, it is important to know qualitatively and quantitatively of the ions, elements and compounds contained in these samples. And new approaches in development of IC technique for the determination of these ions in this field always get priority and attention [13−19].

In this study, discuss 1) the improvement of IC based on column switching to determine cations and anions with a single eluent in single analysis system, 2) the improved IC then applied to identify the type of smokers (heavy smoker, moderate smoker, light smoker, passive smoker, and non-smoker) by determining the concentration of inorganic cations and anions in their urine and saliva samples. However, prior to that, SCN$^-$ ion is the main target of ion should be determined, while the other inorganic cations (Na$^+$, NH$_4^+$, K$^+$, Mg$^{2+}$, and Ca$^{2+}$) and inorganic anions (PO$_4^{3-}$, Cl$^-$, and NO$_3^-$) were also examined.

2. Materials and Methods

2.1 IC instrument

The experimental apparatus was assembled from a PU-2080i plus HPLC pump (Jasco, Tokyo, Japan), a model 5095 loop injector (Rheodyne, Cotati, CA, USA) with an injection-loop volume of 20 µl, a CM-8020 conductivity detector (Tosoh, Tokyo, Japan), a Shodex IC YK-421 column (150×4.6 mm I.D.), a TSK$_{gel}$ IC-Anion-SW column (50×4.6 mm I.D.), a Model 7610-600 10-port switching valve (Rheodyne) and a Computer Aided Chromatography data processor (Nippon Filcon, Tokyo, Japan). The two separation columns were connected in series via the 10-port switching valve. The flow system allows us to select the position of the switching valve for peak separation. The diagram of the apparatus employed in this study is illustrated in Fig. 1.
2.2 Chemicals
Analytical reagent grade chemicals were purchased from Nacalai Tesque (Kyoto, Japan), unless otherwise noted. Standard solutions were prepared by using deionized water. The deionized water throughout this study was prepared in the laboratory using a GS-590 water distillation system (Advantec, Tokyo, Japan). Stock standard solutions for anions and cations containing a mixture of interest ions were prepared. The details of analysis condition, as described in Table 1. The standard solution of cations was prepared by dissolving 1.0 mM of each NaCl, NH₄Cl, KCl, MgCl₂, and CaCl₂, whereas the standard solution of anions was prepared by dissolving 1.0 mM of each NaHPO₄, NaCl, NaNO₃, and NaSCN. All standard solutions were stored in polyethylene containers and kept under refrigeration at 4°C. Tartaric acid was obtained from Wako (Osaka, Japan), and used for the eluent. The eluent was prepared before use.

Figure 1. Flow scheme of the instruments and the positions of the switching valve and the both columns.
Table 1. Analysis Conditions

| Parameter       | Analysis conditions                  |
|-----------------|--------------------------------------|
| Eluent          | 10 mM Tartaric acid                  |
| Analytical Columns | Shodex IC YK-421  
|                  | TSKgel IC-Anion-SW  
| Detector type   | Conductivity                         |
| Flow rate of eluent | 0.5 mL/min                           |
| Total pressure  | 6.5 MPa                              |
| Injector volume | 20 µL                                |

The pH of tartaric acid eluent was measured with a Toa Electronics Ltd. (Tokyo, Japan) IM-20E ion meter with a glass electrode.

2.3 Dual-column switching work mechanism

Figure 1 shows the flow scheme of the system, the position of ion-exchange columns, injection valve, and 10-port switching valve. The anion-exchange and cation-exchange columns were connected serially via 10-port switching valve to allow separation being carried out in either column. The injection valve is placed between the ion-exchange columns. The separation system performed according to the procedure below:

Position A: for separation of cations refers to the switching valve for the separation of cations and the eluent passes through both anion-exchange and cation exchange columns until a straight baseline are obtained. A 20µL volume of the sample solution was then injected to the IC system. The analytes will reach the cation-exchange column and then the cations will be separated. While the separation of cations on the cation-exchange column is carried out, the anion-exchange column is being conditioned with the eluent. Therefore, the anion-exchange column is ready for use at anytime.

Position B: for separation of anions refers to the switching valve for the separation of anions. A 20µL volume of the sample solution was then injected to the system. The analytes will reach the anion-exchange column and then the anions will be separated. While the separation of anions on the anion-exchange column is carried out, the cation exchange column is being conditioned with the eluent. Therefore, the cation-exchange column is ready for use at anytime.

Eluent. The eluent used for the determination of anions and cations was 10 mM tartaric acid. The pump was operated at a flow rate of 0.5 ml/min to keep the inlet pressure lower than 7.0 MPa.

2.4 Preparation of urine and saliva samples

Urine sample was just diluted with deionized water for 5 times and then filtered with a 0.45-µm membrane filter, while the saliva sample was also diluted 5 times with deionized water, and were centrifuged at 2000 rpm for 5 min, followed by filtration with a 0.45-µm membrane filter prior injection. All samples were then stored in a refrigerator at 4°C.

3. Results and discussion

3.1 Effect of tartaric acid concentrations

In this study, tartaric acid was used as the eluent to determine both cations and anions. By considering of monovalent ions (Na⁺, NH₄⁺, K⁺, Cl⁻, NO₃⁻, and SCN⁻), divalent ions (Mg²⁺ and Ca²⁺) as well as
trivalent ion (PO$_4^{3-}$), the difference of eluent concentration should be given more attention. In IC, especially non-suppressed systems, weak acids such as tartaric acid are often used as eluents to separate the inorganic cations and anions. The eluent used, must have a strong affinity for ion exchange resins, so that the separations can be more effective. Tartaric acid has a carboxyl group which can help form a complex compound between ions and eluent.

The effect of the difference of eluent concentration on the retention time of inorganic cation and anions were observed in this study. With the five different eluent concentrations were used in range of 5-15 mM, the retention time for these ions increased as the eluent concentration decreased, as in Figure 2. The retention behavior is influenced by hydrophobic absorption of the ion-exchange (polymer and silica) resins and the pH of eluent.

![Figure 2](image)

**Figure 2.** Effect of tartaric acid concentration in the eluent on retention time of cations (A) and anions (B). Eluent: 10 mM tartaric acid. Columns: Shodex IC YK-421 (150 x 4.6 mm I.D.) and TSKgel IC-Anion-SW (50 x 4.6 mm I.D.). Eluent flow-rate: 0.5 mL/min. Column temperature: 35°C. Injection volume: 20 µL. Lines for cations: 1=Na$^+$; 2=NH$_4^+$; 3=K$^+$; 4=Mg$^{2+}$; 5=Ca$^{2+}$. Lines for anions: 1=PO$_4^{3-}$; 2=NO$_3^-$; 3=Cl$^-$; 4=SCN$^-$.
3.2 Determination of inorganic cations using standard samples

Figure 3 shows the determination of cations using standard sample. Based on cation-exchange mechanism, five cations (Na\(^{+}\), NH\(_{4}\)^{+}, K\(^{+}\), Mg\(^{2+}\), and Ca\(^{2+}\)) could be eluted on the stationary phase within 15 min. The five cations determined in those order by different of their ion size and charge and their interaction with stationary phase.

As in the chromatogram, all cations appeared as negative peaks. By this result was due to the difference in the conductivity between the analyte ions contained in the samples and the driving eluent ion contained in the tartaric acid.

3.3 Determination of inorganic anions using standard samples

Figure 4 shows the determination of anions using standard sample and 10 mM tartaric acid was used as the eluent. Based on anion-exchange mechanism, four anions (PO\(_{4}\)^{3−}, Cl\(^{−}\), NO\(_{3}\)^{−}, and SCN\(^{−}\)) commonly contained in urine and saliva samples were well eluted in within 40 min. The order of these anions was occurred by different of their ion size and charge and interaction with stationary phase.

All anion peaks were appeared as positive peaks. By this result was due to the difference in the conductivity between the analyte ions and the driving eluent ion. Compare to cation separation, anion separation took longer time. And it is expected that due to the different type of resins from the both columns.

![Figure 3](image-url)

**Figure 3.** The representative chromatogram of inorganic cations using standard sample. Eluent: 10 mM tartaric acid. Column: Shodex IC YK-421 (150 mm x 4.6 mm I.D.). Eluent flow-rate: 0.5 mL/min. Column temperature: 35°C. Injection volume: 20 µL. Analytes (concentration, 1.0 mM each): 1=Na\(^{+}\), 2=NH\(_{4}\)^{+}, 3=K\(^{+}\), 4=Mg\(^{2+}\), and 5=Ca\(^{2+}\).
Figure 4. The representative chromatogram of inorganic anions using standard sample. Eluent: 10 mM tartaric acid. Column: IC-Anion-SW (50 mm x 4.6 mm I.D.). Eluent flow-rate: 0.5 mL/min. Column temperature: 35°C. Injection volume: 20 µL. Analytes (concentration, 1.0 mM each): 1=PO$_4^{3-}$, 2=Cl$^-$, 3=NO$_3^-$, and 4=SCN$^-$.  

3.4 Validation of the system

Table 2 shows the linear relationships between the peak height of each ions and molar concentration were observed up to 1 mM with the correlation coefficients, $R^2 \geq 0.99$ for all anions and cations, and indicated as a good linearity.

The detection limit (LODs) for 20 µl injection volume of sample was calculated at a signal-to-noise ratio (S/N) of 3:1. As in Table 2, the LODs obtained were 3.10-12.14 ppb (µg/l) for cations and 16.00-88.10 ppb for anions.

Table 2. Summarized data for the detection limits, correlation coefficient, and retention time of ions under the optimum operating conditions, as in Figures 3 and 4

| Analytes | Detection Limit/ ppb | Correlation coefficient ($r^2$) | Retention times ($t_R$)/ min |
|----------|----------------------|-------------------------------|----------------------------|
| **Cations** |                    |                               |                           |
| Na$^+$   | 3.10                | 0.9956                        | 5.51                      |
| NH$_4^+$ | 3.99                | 0.9921                        | 6.01                      |
| K$^+$    | 12.14               | 0.9935                        | 7.18                      |
| Mg$^{2+}$| 4.87                | 0.9918                        | 11.22                     |
| Ca$^{2+}$| 10.80               | 0.9954                        | 12.58                     |
| **Anions** |                   |                               |                           |
| PO$_4^{3-}$| 88.10              | 0.9994                        | 6.30                      |
| Cl$^-$   | 18.12               | 0.9971                        | 12.02                     |
| NO$_3^-$ | 83.11               | 0.9963                        | 17.07                     |
| SCN$^-$  | 16.00               | 0.9998                        | 34.55                     |
Table 3 shows the reproducibility of peak heights, peak areas, and retention times under the optimum condition in Figures 3 and 4. The relative standard deviations (RSDs, %) of these ions were less than 4.81% for peak height, less than 6.30% for peak area, and less than 6.22% for retention time, respectively.

**Table 3. RSD of ions under the optimum operating conditions, as in Figures 3 and 4**

| Analytes | RSD (%) | n=5<br>a |
| --- | --- | --- |
| | Peak height | Peak area | Retention time |
| **Cations** | | | |
| Na⁺ | 2.72 | 2.65 | 3.49 |
| NH₄⁺ | 3.28 | 2.79 | 3.37 |
| K⁺ | 3.79 | 3.25 | 3.58 |
| Mg²⁺ | 4.43 | 3.29 | 4.56 |
| Ca²⁺ | 4.36 | 4.81 | 4.73 |
| **Anions** | | | |
| PO₄³⁻ | 3.59 | 3.45 | 3.49 |
| Cl⁻ | 4.68 | 4.79 | 4.45 |
| NO₃⁻ | 4.59 | 5.43 | 4.06 |
| SCN⁻ | 4.81 | 6.30 | 6.22 |

a*n* = number of measurements

3.5 Application to urine and saliva samples
Good chromatograms for inorganic cations in both heavy smoker’s urine and saliva sample were achieved, as in Figure 5.
Figure 5. Determination of inorganic cations in heavy smoker’s urine (A) and saliva (B) samples with 5-times of dilution. Ions: 1=Na\(^+\), 2=NH\(_4\)\(^+\), 3=K\(^+\), 4=Mg\(^{2+}\), and 5=Ca\(^{2+}\). Other chromatographic conditions, as in Figure 3

The ammonium ion found as much more concentration, especially in saliva samples. Since ammonium is one of compounds contained in cigarettes, that can influence the physically and chemically composition of human physiological fluids such as urine and saliva.

The concentration of sodium, ammonium, potassium, magnesium, and calcium ions for heavy smoker’s urine were determined to be 3.45, 5.02, 3.03, 0.18, and 0.42 mM, respectively, whereas for heavy smoker’s saliva sample, were determined to be 1.12, 5.19, 1.24, 0.16, and 0.48 mM, respectively, as listed in Table 4.

Figure 6. Determination of inorganic cations in non-smoker’s urine (A) and saliva (B) samples with 5-times of dilution. Ions: 1=Na\(^+\), 2=NH\(_4\)\(^+\), 3=K\(^+\), 4=Mg\(^{2+}\), and 5=Ca\(^{2+}\). Other chromatographic conditions, as in Figure 3

Figure 6 shows good determination for inorganic cations in both non-smoker’s urine and saliva samples. An uneven baseline occurred when the saliva sample was injected to the IC system. It might
be due to analyte chemical composition. However, it should be noted that the results did not influence. Table 4, summarizes the concentrations of cations for all smoker’s urine and saliva samples, and the composition of cations contained both types of samples, as shown in Figure 7. Moreover, the obtained results also show the concentrations of these cations in moderate smoker, light smoker, and passive smoker for urine and saliva, respectively.

| Smoker types       | Cations (mM) | Sample types | Na\(^+\) | NH\(_4\)\(^+\) | K\(^+\) | Mg\(^{2+}\) | Ca\(^{2+}\) |
|-------------------|--------------|--------------|----------|----------------|--------|------------|------------|
| Heavy smoker      |              | Urine        | 3.45     | 5.02           | 3.03   | 0.18       | 0.44       |
|                   |              | Saliva       | 1.12     | 5.19           | 1.24   | 0.16       | 0.48       |
| Moderate smoker   |              | Urine        | 3.22     | 4.64           | 3.10   | 0.20       | 0.52       |
|                   |              | Saliva       | 1.62     | 4.66           | 1.22   | 0.22       | 0.42       |
| Light smoker      |              | Urine        | 2.53     | 4.02           | 4.28   | 0.26       | 0.52       |
|                   |              | Saliva       | 2.39     | 4.00           | 0.82   | 0.19       | 0.68       |
| Passive smoker    |              | Urine        | 2.40     | 2.61           | 4.54   | 0.19       | 0.60       |
|                   |              | Saliva       | 1.98     | 4.1            | 0.65   | 0.30       | 0.42       |
| Non-smoker        |              | Urine        | 0.53     | 0.12           | 6.50   | 0.22       | 0.64       |
|                   |              | Saliva       | 1.55     | 3.22           | 0.26   | 0.34       | 0.38       |

**Figure 7.** Composition of inorganic cations contained in smoker’s urine and saliva samples. Other chromatographic conditions, as in Figure 3.

Good chromatograms for inorganic anions in both heavy smoker’s urine and saliva samples as showed in Figure 8, and for non-smoker’s samples as showed in Figure 9. From Figure 8, the four anions (particularly thiocyanate ion) commonly contained in these types of samples could be determined under this optimum conditions. The determination of SCN\(^-\) ion in urine and saliva samples is a great interest to monitor cyanide exposure from tobacco smoke. Therefore, in this work, SCN\(^-\) ion is the main target ion to be determined, and it can be evaluated in the zoomed chromatograms. Table 5
summarized the concentration of these anions. When the heavy smoker’s urine sample was injected to
the IC instrument, an uneven baseline occurred. It might be due to the chemically compositions
contained in the samples. Therefore, the concentration of SCN\(^-\) ion could be calculated.

**Figure 8.** Determination of inorganic anions in heavy smoker’s urine (A) and saliva (B) samples with
5-times of dilution. Ions: 1=PO\(_4\)^{3-}, 2=Cl\(^-\), 3=NO\(_3\)^{-}, and 4=SCN\(^-\). Other chromatographic conditions, as
in Figure 4.

**Figure 9.** Determination of anions in non-smoker’s urine (A) and saliva (B) samples with 5-times of
dilution. Ions: 1=PO\(_4\)^{3-}, 2=Cl\(^-\), and 3=NO\(_3\)^{-}. Other conditions, as in Figure 4.
Figure 10. Composition of inorganic anions contained in smoker’s urine and saliva samples. Other chromatographic conditions, as in Figure 4.

Figure 9 illustrates the chromatograms of anions for all non-smoker’s urine and saliva samples. All anions could be detected except SCN$^-$ ion. It assumed that the concentration of SCN$^-$ was lower than the detection limit, so that it could not be calculated.

Table 5, summarizes the concentrations of anions for all smoker’s urine and saliva samples, and the composition of anions contained in both type of samples, as shown in Figure 10. Moreover, the obtained results also show the concentrations of these anions contained in moderate smoker, light smoker, and passive smoker for urine and saliva samples, respectively.

Table 5. Determination results for inorganic anions in urine and saliva samples (n=5)

| Smoker types     | Sample types | Anions (mM) |
|------------------|--------------|-------------|
|                  |              | PO$_4^{3-}$ | Cl$^-$   | NO$_3^-$ | SCN$^-$ |
| Heavy smoker     | o Urine      | 4.82        | 0.32     | 0.11     | 0.09    |
|                  | o Saliva     | 6.20        | 3.52     | 0.21     | 0.10    |
| Moderate smoker  | o Urine      | 4.98        | 0.80     | 0.08     | 0.06    |
|                  | o Saliva     | 6.04        | 3.92     | 0.24     | 0.06    |
| Light smoker     | o Urine      | 5.25        | 2.24     | 0.10     | 0.03    |
|                  | o Saliva     | 5.82        | 4.02     | 0.22     | 0.04    |
| Passive smoker   | o Urine      | 5.11        | 2.96     | 0.12     | 0.02    |
|                  | o Saliva     | 4.86        | 4.90     | ND       | ND      |
| Non-smoker       | o Urine      | 6.50        | 3.10     | 0.28     | ND      |
|                  | o Saliva     | 4.62        | 6.41     | ND       | ND      |

Note: ND=not detected
4. Conclusions
The most interesting aspect of this dual-column switching technique is the fact that cation-exchange column and anion-exchange columns were connected serially via 10-port switching valve, a single pump, and a single detector for the determination of five important cations (Na\(^{+}\), NH\(_4\)^{+}, K\(^{+}\), Mg\(^{2+}\), and Ca\(^{2+}\)) and the four anions (PO\(_4\)^{3-}, Cl\(^{-}\), NO\(_3\)^{-}, and particularly SCN\(^{-}\)) in smoker’s urine and saliva samples. The eluent of 10 mM tartaric acid showed good performance in term of selectivity and good peaks shapes for the determination of the above cations and anions. The described technique offered simple and convenient operation, and could be applied in physiological fluid such urine and saliva samples.

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