

PROCEEDING

The 1st International Conference On
Engineering Technology Innovation
For Archipelago

(ICETIA-2016)

"Green Technology For A Better Future"



Faculty Of Engineering
Universitas Khairun



PPLP
MALUT

Greetings from Dean of Engineering Faculty

Bismillahirrahmanirrahim,
Assalamu Alaykum Warahmatullahi Wabarakatuh.
Gratitude Praise to Allah SWT, who given us a guidance and blessing.

Faculty of Engineering is one of eight faculties at Khairun University. Currently, Faculty of Engineering has entered its 15th years. Recently, Faculty of Engineering has six study programs. They are Civil Engineering, Mechanical Engineering, Electrical Engineering, Architectural Engineering, Informatics Engineering, and the last is Mining Engineering. We have 84 lecturers, 15 skilled staffs, and around 1,600 registered students.

I thank to you all honorable keynote speakers from Malaysia, Japan International Cooperation Agency (JICA), Timor Leste, and private sector. Participants to the conference mostly come from Eastern Indonesia. To all participants, we welcome you to Ternate and join this conference.

The title for this conference is “The 1st International Conference on Engineering, and Technology Innovation for Archipelago (ICETIA 2016).” The main theme is “Green Technology for a Better Future.” It lasts for two days on October 27-28, 2016. The conference will be held annually.

The main purpose of this activity is to collect and disseminate recent innovation in engineering, science, and green technology to be applied in archipelago region. The conference meets university academicians and researchers, industries, and government, both local and regional in Eastern Part of Indonesia.

In line with the theme of this conference, we hope we can explore and expand all of our potencies. The conference of course enriches the capacity of stakeholders in engineering, science, and technology. The steering committee and organizing committee gave special thanks to Khairun University Rector, Prof. Dr. Husen Alting, S.H., M.H. and all Vice Rectors who are always supporting our activities. Last but not at least, I, on behalf of Engineering Faculty Khairun University, thanks to all of our generous sponsors succeeding this conference.

Ternate, October, 27, 2016

Selamat datang...!
Welcome...!
歡迎
Bem-vindo...!

Ir. Ahmad Seng, M.Eng.
Dean of Engineering Faculty
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UV Detection Ion Chromatographic of Thiocyanate and other Inorganic Anions in Urine and Saliva Samples for the Identification of Smokers and Non-smokers

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Abstract—A simple and practical UV detection ion chromatography (IC) method has been developed for the identification of smokers and non-smokers by determining the concentration of thiocyanate ion (SCN^-), and a number of other inorganic anions, such as $\text{S}_2\text{O}_3^{2-}$, $\text{C}_2\text{O}_4^{2-}$, Γ , IO_3^- , BrO_3^- , NO_2^- , Br^- and NO_3^- in human physiological fluid samples such as urine and saliva. The method performed sodium hydrogen carbonate as the eluent and combining with IC-Anion PWxl PEEK separation column in all determinations. The determination of SCN^- and other anions are carried out in ultraviolet detection ($\lambda=210$ nm). Good chromatograms were achieved and all target anions were eluted within 45 min. The detection limits were calculated at $S/N=3$ were 0.11–1.75 ppm (mg/L). The repeatability values were below 4.82, 4.84, and 4.87% for peak height, peak area and retention time, respectively. The developed method was successfully applied to the determination of all targeted anions especially thiocyanate ion (SCN^-) in smokers and non-smokers urine and saliva samples. The samples were obtained from healthy adult smoker and non-smoker volunteers.

Keywords—ion chromatography; UV detection; thiocyanate ion; inorganic anions; urine; saliva

I. INTRODUCTION

In clinical chemistry, the identification of organic compound and inorganic ions in human physiological fluids such as urine, saliva, sweat, serum, blood, etc., can provide information of someone's clinical conditions. Quantitative data content of ions (cations and/or anions) will become more important when diagnosing a disease as well as therapy. The inorganic anions such as thiocyanate ion (SCN^-), oxalate ion ($\text{C}_2\text{O}_4^{2-}$), thiosulfate ion ($\text{S}_2\text{O}_3^{2-}$), iodide ion (Γ), and a number of other inorganic anions such as IO_3^- , BrO_3^- , NO_2^- , Br^- , and NO_3^- can be determined using IC method [1-6].

Several methods have been developed to determine the concentration of the above anions such as colorimetric [7] and spectrometric [8] methods. However, these methods still require extra work and need long time in their analysis.

A versatile application of IC is in the determination of inorganic anions especially thiocyanate ion (SCN^-) in urine and saliva samples [4-6]. The determination of this ion has become very important ion to identify the clinical condition

and the types of smokers and non-smokers. However, compared to other human physiological fluids, this ion normally found much more in urine and saliva samples. Hence, the pre-treatment of these samples and selective method should be given attention due to the ions interference contained in the samples.

Since its introduction in 1975 by Small et al. [9], IC has become a super power analytical method for the determination of ionic species present in various samples with a high level of accuracy. In addition, this technique also offers a high speed of analysis, selectivity of ions, and the convenience of preparing a sample means the number of required samples is relatively small at each sample injection. By the number of these advantages, then the IC method is to be practical and suitable for routine analysis purposes [10-18].

In this study, the aims are to identify the type of smokers and non-smokers by determining the concentration of anions in urine and saliva samples. However, prior to that, a simple and practical detection method in IC was developed in order to determine the above samples. SCN^- ion is the main ion target should be determined and the other inorganic anions such as IO_3^- , BrO_3^- , NO_2^- , Br^- , NO_3^- , $\text{C}_2\text{O}_4^{2-}$, $\text{S}_2\text{O}_3^{2-}$, and Γ were also examined.

II. EXPERIMENTAL

A. IC System

The ion chromatograph consisted of a 880-PU HPLC pump (Jasco, Tokyo, Japan), a Rheodyne 5095 injector equipped with a 20- μl sample loop (Cotati, CA, USA), a UV-970 detector (Jasco, Tokyo, Japan), and a CDS plus Ver 5.0 chromatography data system (Tokyo, Japan). The arrangement of IC instrument is described in Figure 1. The column employed for anion separation was TSKgel IC-Anion-PW_{XL} PEEK (75 mm \times 4.6 mm I.D.) column.

B. Chemical Standards

The standard solution of anions was prepared by dissolving NaIO_3 , NaBrO_3 , NaNO_2 , NaBr , NaNO_3 , NaSCN , NaI , $\text{Na}_2\text{C}_2\text{O}_4$, and $\text{Na}_2\text{S}_2\text{O}_3$. The standard solutions were prepared

containing each anion at different concentration (in mM): NaIO₃ (0.4), NaBrO₃ (0.6), NaNO₂ (0.4), NaBr (0.5), NaNO₃ (0.25), NaSCN (0.6), NaI (0.6), Na₂C₂O₄ (0.8), and Na₂S₂O₃ (0.3). All standards were prepared with freshly deionized water for about 15 min in ultrasonic bath. All standard solutions are stored in polyethylene containers and kept under refrigeration at 4°C.

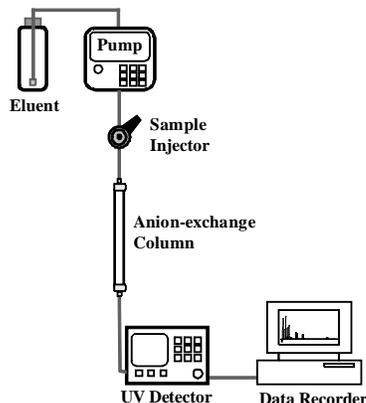


Fig. 1 Basic components of an IC instrument

C. Eluent

Several eluents are used for the simultaneous determination of inorganic anions using standard and all real samples such as NaCl, KCl, NaHCO₃, NH₄HCO₃, Na₂CO₃, and some aromatic carboxylic acids such as trimellitic acid, pyromellitic acid, phthalic acid, and benzoic acid. Eluents were prepared with freshly deionized water for about 15 min in ultrasonic baths.

D. Preparation of Urine and Saliva Samples

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

III. RESULTS AND DISCUSSION

A. Selection of Eluents

In this work, several kinds of inorganic compounds and organic acids containing carboxyl groups were examined as the eluent for the separation of common inorganic anions (IO₃⁻, BrO₃⁻, NO₂⁻, Br⁻, NO₃⁻, C₂O₄²⁻, S₂O₃²⁻, I⁻ and SCN⁻). Organic acids examined involved trimellitic acid, phthalic acid, benzoic acid, and pyromellitic acid containing multiple carboxyl groups, while the inorganic compound eluents were sodium hydrogen carbonate, ammonium hydrogen carbonate, sodium chloride and potassium chloride. The performance of these eluents were compared in terms of the retention times, peak shapes and selectivity of the anions. Among the above mentioned eluents, it was found that sodium hydrogen

carbonate eluent alone achieved complete separation of the nine anions of interest. It should also be noted that only NaHCO₃ eluent gave better peak shapes and good selectivity. In addition, a TSKgel IC-Anion-PW_{XL} PEEK (75 mm×4.6 mm I.D.) was selected as the polymer-based anion-exchange column.

B. Effects of Sodium Hydrogen Carbonate (NaHCO₃) Concentration on Retention Time

In order to survey the optimum concentration of the eluent, various concentrations containing NaHCO₃ was used to check the effect of determination of anions on retention time. The standard samples for anions were anion-exchange column, where the concentration of NaHCO₃ was in the range of 15–30 mM. Figure 2 shows the chromatograms of the nine anions at different eluent concentrations. The eluent concentration increased as retention time values anions (IO₃⁻, BrO₃⁻, NO₂⁻, Br⁻, NO₃⁻, C₂O₄²⁻, S₂O₃²⁻, I⁻, and SCN⁻) decreased.

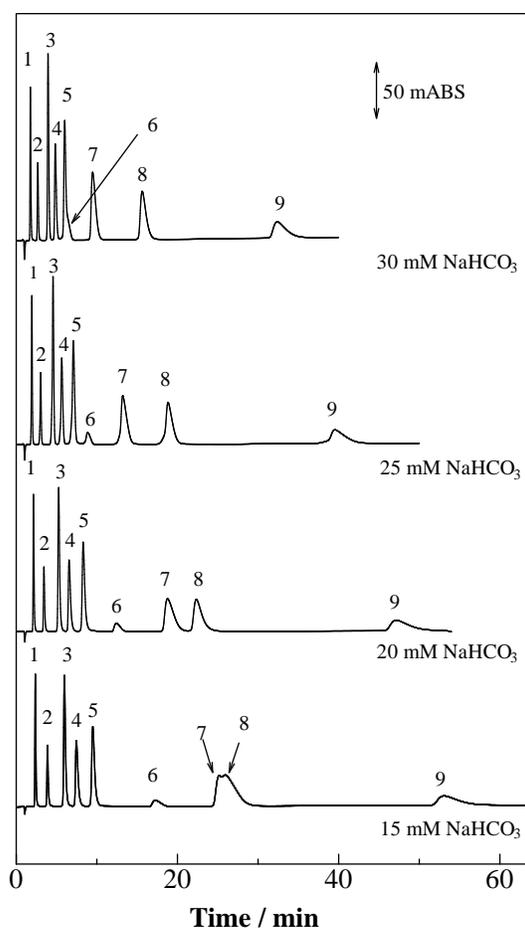


Fig. 2. The differences of chromatographic profiles on retention time for inorganic anions by changing the concentration of eluent. Eluent: 15-30 mM NaHCO₃. Column: TSKgel IC-Anion PW_{XL} PEEK (75 mm x 4.6 mm I.D.). Eluent flow-rate: 0.8 mL/min. Column temperature: 35°C. Injection volume: 20 μ L. Analytes: 1=IO₃⁻; 2=BrO₃⁻; 3=NO₂⁻; 4=Br⁻; 5=NO₃⁻; 6=C₂O₄²⁻; 7=S₂O₃²⁻; 8=I⁻; 9=SCN⁻.

When the eluent concentration was less than 25 mM, the divalent anion ($C_2O_4^{2-}$) overlapped with NO_3^- . And when the eluent concentration was higher than 25 mM, the anions $S_2O_3^{2-}$ and I^- overlapped each other and the completed separation was longer. Considering of the above results, in this method, 25 mM of the eluent concentration was used in subsequent experiments.

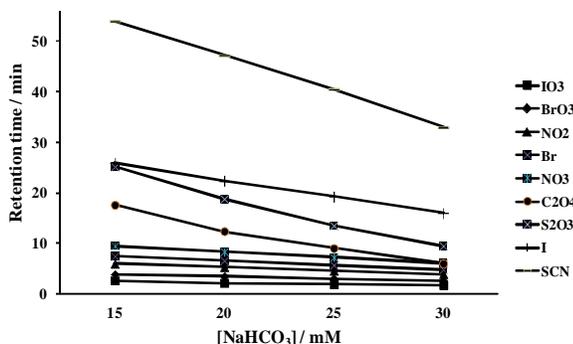


Fig. 3. Effect of sodium hydrogen carbonate concentration in the eluent on retention times of inorganic anions. Eluent: 15–30 mM $NaHCO_3$. Other operating conditions, as in Figure 2

The elution order at the optimized concentration of eluent was $IO_3^- < BrO_3^- < NO_2^- < Br^- < NO_3^- < C_2O_4^{2-} < S_2O_3^{2-} < I^- < SCN^-$. Figure 3 shows the relationship between the concentration of sodium hydrogen carbonate as the eluent on the retention times (t_R) of anions using anion-exchange column commercially available. From Figure 3, the t_R values of anions decreased with increasing the concentration of sodium hydrogen carbonate.

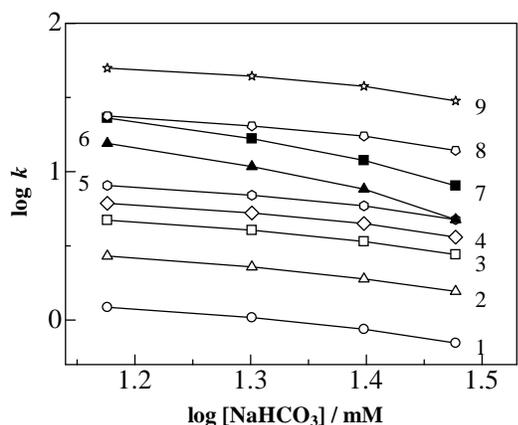


Fig. 4. The logarithm of the retention factor versus the logarithm of the sodium hydrogen carbonate concentration for inorganic anions. Eluent: 15–30 mM $NaHCO_3$. Injection volume: 20 μ l. Plot lines: 1= IO_3^- ; 2= BrO_3^- ; 3= NO_2^- ; 4= Br^- ; 5= NO_3^- ; 6= $C_2O_4^{2-}$; 7= $S_2O_3^{2-}$; 8= I^- ; 9= SCN^- . The other operating conditions, as in Figure 2

Linear relationship between the logarithm of the retention factor ($\log k$) of inorganic anions and the logarithm of the eluent concentrations ($\text{Log} [\text{Eluent}]$) were observed, as plotted

in Figure 4. This reveals that the analyte anions are retained on the stationary phase based on ion exchange mechanism. Theoretically, the slope values are the ratio of the analyte ionic valency and the eluent ionic valency. Figure 3 shows the slopes for the analyte anions were $-0.788, -0.783, -0.759, -0.741, -0.748, -1.665, -1.503, -0.752,$ and -0.713 for IO_3^- , BrO_3^- , NO_2^- , Br^- , NO_3^- , $C_2O_4^{2-}$, $S_2O_3^{2-}$, I^- , and SCN^- , respectively. It was found that the average valence of sodium hydrogen carbonate eluent was about 1.30. From this, we assumed that the eluent cations is divalent, the slope value should be -1 and -2 for the monovalent and divalent analyte anions, respectively.

The above slight deviation from the expected values may be due to the fact the dissociation of sodium hydrogen carbonate is not perfect.

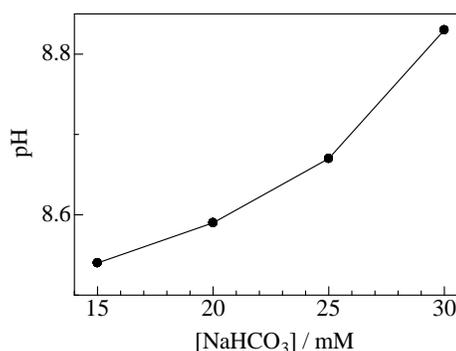


Fig. 5. pH of sodium hydrogen carbonate eluent at different concentration

Figure 5 shows that the pH gradually increases with increasing eluent concentration. The pH of 25 mM of sodium hydrogen carbonate is 8.68. The formation of complex between divalent anions and sodium hydrogen carbonate may be another possible reason.

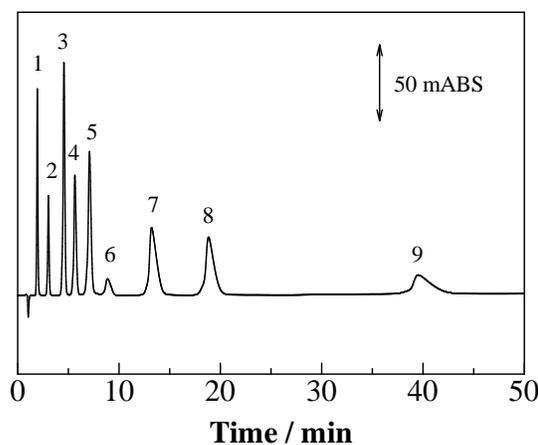


Fig. 6. Separation of inorganic anions using standard sample. Eluent: 25 mM $NaHCO_3$. Analyte (concentration in mM): 1= IO_3^- (0.4), 2= BrO_3^- (0.6), 3= NO_2^- (0.4), 4= Br^- (0.5), 5= NO_3^- (0.25), 6= $C_2O_4^{2-}$ (0.8), 7= $S_2O_3^{2-}$ (0.3), 8= I^- (0.6), 9= SCN^- (0.6). Other operating conditions, as in Figure 2

C. Determination of Inorganic Anions using Standard Samples

In the chromatogram, as in Figure 5, the nine anions (IO_3^- , BrO_3^- , NO_2^- , Br^- , NO_3^- , $\text{C}_2\text{O}_4^{2-}$, $\text{S}_2\text{O}_3^{2-}$, Γ , and SCN^-) completely eluted within 45 min. At lower concentration, the separation took longer time and some peaks were overlapped (e.g., 15 mM NaHCO_3) as described in Figure 1, while at higher concentration, the shorter analysis time was achieved but some peaks were also overlapped (e.g., 30 mM NaHCO_3). However, it should be noted that the chromatogram shows the baseline of all ions were achieved under this optimum condition.

D. Validation of the Developed Method

The detection limits of the present method were determined by injecting 20- μL volume of the sample standard solution and were calculated at $S/N=3$. The results are shown in Table 1. The detection limits obtained by the method were 0.03–1.75 ppm (mg/L). Sub-ppm level of detection will be feasible using this proposed method for the determination of inorganic anions in real samples.

Linear relationships between the peak heights and the analyte concentrations were found experimentally for all inorganic anions, as shown in Table 1. All the calibration curve of the anions showed good linear correlations. From the Table, the correlation coefficients $r^2 > 0.99$ proved good linearity of the present method.

TABLE 1. SUMMARIZED DATA FOR THE DETECTION LIMITS (LOD) AT $S/N=3$, CORRELATION COEFFICIENT, AND RETENTION TIME OF ANIONS OBTAINED UNDER THE OPTIMUM OPERATING CONDITIONS, AS IN FIGURE 5

Anions	LOD ($S/N=3$)		Correlation coefficient (r^2)	Retention times (t_R), min
	μM	ppm		
IO_3^-	0.62	0.12	0.9971	2.01
BrO_3^-	1.90	0.24	0.9956	3.03
NO_2^-	0.55	0.03	0.9921	4.59
Br^-	1.37	0.11	0.9935	5.58
NO_3^-	0.56	0.03	0.9918	7.16
$\text{C}_2\text{O}_4^{2-}$	15.60	1.75	0.9954	8.56
$\text{S}_2\text{O}_3^{2-}$	1.46	0.13	0.9963	13.21
Γ	3.47	0.44	0.9994	18.59
SCN^-	10.96	0.64	0.9998	39.49

Table 2 shows the repeatability of the signals (peak heights, peak areas, and retention times) for five successive measurements under the optimum conditions, as in Figure 5. The repeatability values were below 4.82, 4.84, and 4.87% for peak height, peak area and retention time, respectively.

E. Application to Urine and Saliva Samples

The method was applied to the determination of UV-absorbing anions contained in urine and saliva samples. The both samples were obtained from healthy smoker and non-smoker adult males volunteer donors. The smoker volunteers smoked more than 10 cigarettes per day, while the non-smoker volunteers well known had never smoked or had never been a daily smoker.

To avoid accidental contamination of the samples, it then prepared according to following procedures. A saliva sample was diluted 5 times with deionized water, and centrifuged at 2000 rpm for 5 min, followed by filtration with a 0.45- μm membrane filter. While urine sample was just diluted 5-times with deionized water and then filtered with a 0.45- μm membrane filter prior injection. All samples were stored in a refrigerator at 4°C.

TABLE 2. RELATIVE STANDARD DEVIATIONS (RSD) OF ANIONS UNDER THE OPTIMUM OPERATING CONDITIONS, AS IN FIGURE. 5

Anions	RSD (%), $n=5^a$		
	Peak Height	Peak area	Retention time
IO_3^-	2.34	2.16	2.59
BrO_3^-	2.78	2.89	2.82
NO_2^-	2.92	2.72	2.92
Br^-	3.16	2.98	2.74
NO_3^-	2.39	3.06	3.09
$\text{C}_2\text{O}_4^{2-}$	4.82	3.93	3.96
$\text{S}_2\text{O}_3^{2-}$	3.94	2.15	3.78
Γ	3.80	3.98	4.87
SCN^-	4.22	4.84	4.05

^an=number of measurements

Good chromatograms for inorganic anions in urine sample were achieved, as in Figure 6. Iodide and thiocyanate ions were achieved with good separation, although other inorganic anions were overlapped. The concentration of nitrate, iodide and thiocyanate ions for smoker were determined to be 0.059, 0.023, and 0.057 mM, respectively, whereas for non-smoker urine sample, the concentration of nitrate and thiosulfate ions were determined to be 0.082 and 0.009 mM, respectively.

Good chromatograms for inorganic anions in saliva sample were achieved, as in Figure 7. Iodide and thiocyanate ions concentration were obtained with good separation. The concentration of nitrate, iodide, and thiocyanate ions for smoker saliva sample were determined to be 0.035, 0.076, and 0.097 mM, respectively, whereas for non-smoker sample, nitrate and thiocyanate ions were determined to be 0.031 and 0.085 mM, respectively.

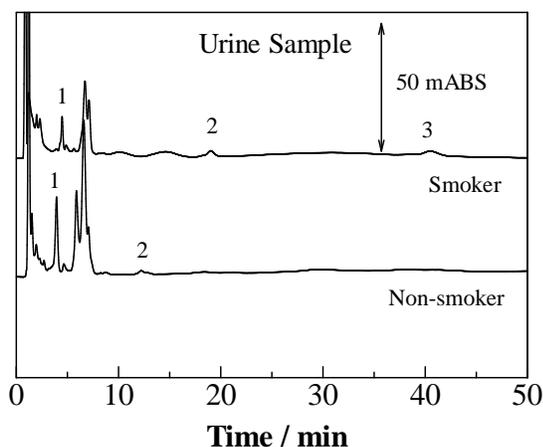


Fig. 6 Separation of UV-absorption spectrum of inorganic anions in smoker and non-smoker urine samples with 5-times of dilution. Smokers, analytes: 1= NO_3^- , 2= I^- , and 3= SCN^- , and non-smokers, analytes: 1= NO_3^- , 2= $\text{S}_2\text{O}_3^{2-}$. Other chromatographic conditions, as in Figure 5

It can be seen that commonly the concentration of thiocyanate ion and other inorganic anions in saliva sample was higher compared to urine sample. We assumed that natural saliva is easier to be interfered by foreign ions such as the ions from cigarette smoke.

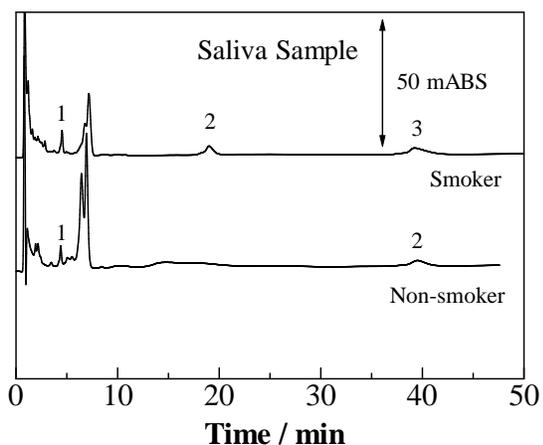


Fig. 7. Separation of UV-absorption spectrum of anions in smoker and non-smoker saliva samples with 5-times of dilution. Smokers, analytes: 1= NO_3^- , 2= I^- , and 3= SCN^- , and non-smokers, analytes: 1= NO_3^- and 2= SCN^- . Other chromatographic conditions, as in Figure 5

F. Conclusions

The present developed method using indirect UV-absorption spectra was achieved by combination of a TSKgel IC-Anion-PWxl column and eluent of sodium hydrogen carbonate. In the indirect UV mode, it was possible to detect nine anions on the polymer based anion-exchange column, particularly thiocyanate ion. Although, a drawback of this

technique should be improved in detection selectivity and sensitivity, especially IO_3^- , BrO_3^- , NO_2^- , and Br^- ions due to the ions interference in the real samples when the samples were injected to the system. However, it should be noted that the present work promises a simple and practical method and it can be used for the routine analysis. Moreover, this developed method can be widely applied to identify the type of smokers and non-smokers by determining the targeted ions contained in the samples.

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