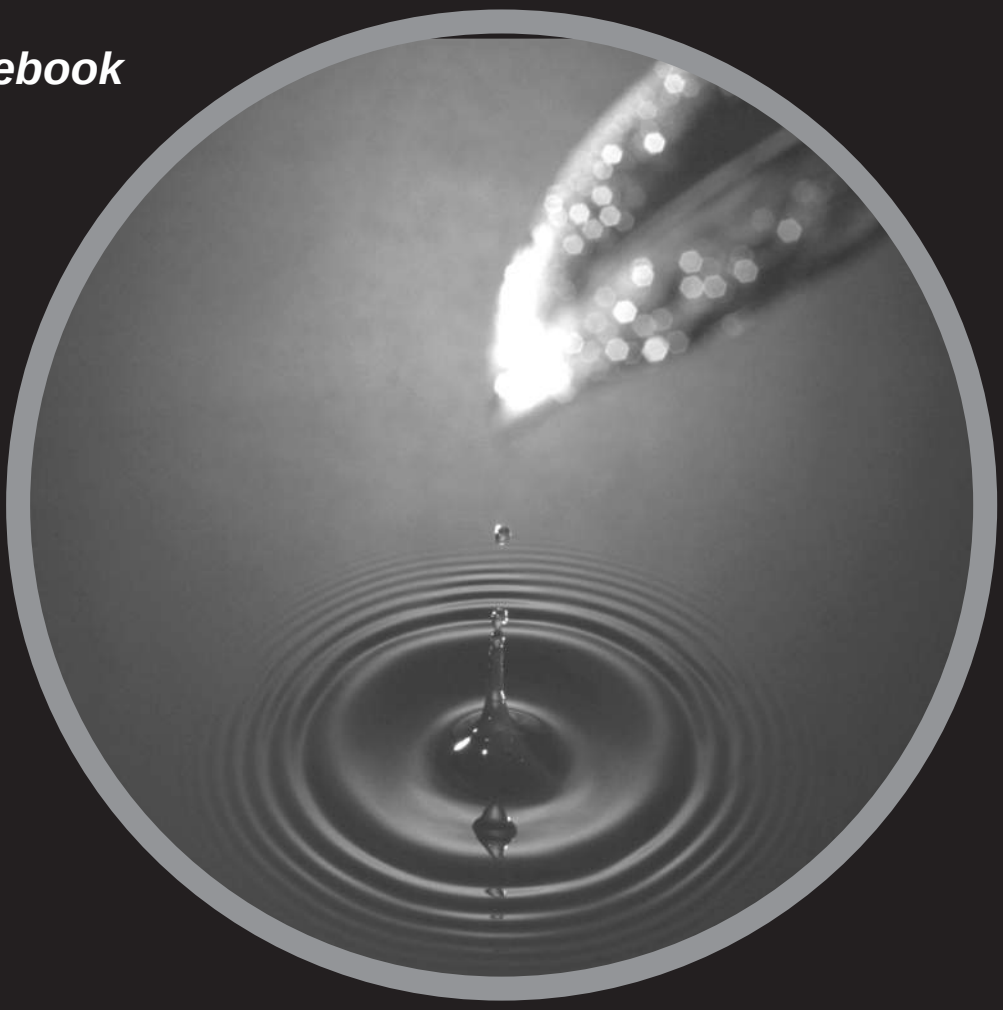
Shodex™

HPLC Columns

Shodex™ IC YS-50 columns

Simultaneous Analysis of Monovalent and Divalent Cations at a higher sensitivity

Technical notebook
No. 4



SHOWA DENKO EUROPE

Contents

1. Introduction

Ion chromatography is commonly used to assess the quality of environmental water, such as river water and ground water. Nitrogen compounds, nitrite, nitrate, and ammonium ions are key indicators of water pollution. Ammonium ions in environmental waters come from various biological activities. They are one of the main causes of acid rain. Environmental acidification is due to nitrification and overabundance of nutrients in closed water systems. Therefore it is quite important to monitor them. Cation chromatography is used to quantify ammonium ions.

The Shodex column IC YS-50 described below can be used to analyze not only common cations, such as alkali and earth ions, but also cationic low-molecular-weight compounds (e.g. alkylamines) and transition metal ions. It is suitable for cation chromatography in a wide range of applications from food and beverage, pharmaceuticals, to chemicals.

2. Advantages and Specifications

2-1. Advantages of Shodex IC YS-50

(1) Simultaneous analysis of monovalent and divalent cations with an isocratic method is possible.

This new product enables simultaneous analysis of monovalent and divalent cations at a higher sensitivity than the current product (Shodex IC YK-421). With about twice the efficiency (TPN) and about 1.3 times the resolution between sodium ion (Na^+) and ammonium ion (NH_4^+), the sensitivity for NH_4^+ in the presence of a high concentration of Na^+ has drastically improved (Na^+ : NH_4^+ = 5,000 : 1 is possible).

(2) Quantitative analysis of divalent cations is improved.

Efficiency of IC YS-50 is nearly double that of the current column, since peak shapes of divalent cations are improved, quantitative analysis of divalent cations has also improved.

(3) Many kinds of solvents can be applied as eluent.

The standard solvent for YS-50 is methanesulfonic acid aq. Other acidic solvents, like nitric acid, sulfuric acid and phosphoric acid may also be used. Operation with methanesulfonic acid means the YS-50 column can be used with both non-suppressor systems and also suppressor systems.

The polymer-based packing material tolerates a wide range of pH. The material is hardy up to 50% acetonitrile, which allows the use of many different solvents.

(4) Alkylamines can be analyzed.

Various alkylamines like methylamine, trimethylamine and ethanolamine can be analyzed with YS-50.

(5) Transition metals can be analyzed.

Transition metals, like nickel, zinc and cobalt, can be analyzed with a mixture of 6mM tartaric acid and 4mM oxalic acid aq. as eluent.

2-2. Specifications

Table 2-1 Specifications

Product Code	Product Name	Theoretical Plate Number (TP/column)	Particle Size (μm)	ID x Length (mm)
F7122000	IC YS-50	≥5,500	5	4.6 x 125
F6700530	IC YS-G	Guard Column	5	4.6 x 10

Packing Material : Poly (Vinyl Alcohol) Particle with Carboxyl Group
Housing : Stainless Steel
Usable Temp. : 20℃~60℃ (Recommended: 25℃~40℃)
Usable pH Range : 2~12
Max Flow Rate : 2.0mL/min
Max Pressure : 15MPa
Organic Modifier : ≤ 50 Volume% Acetonitrile, but no Methanol

3. Basic Features

3-1. Standard Solvent (Methanesulfonic Acid aq.)

3-1-1. Comparison of Chromatograms of YS-50 with YK-421

The recommended eluent for Shodex IC YS-50 is 4mM methanesulfonic acid. Adjusting the content of methanesulfonic acid in the eluent enables the fine tuning of the separation. (see article 3-1-4)

Figures 3-1 and 3-2 show chromatograms of six common cations with YS-50 and YK-421 respectively. The new product YS-50 shows a higher performance than the current product YK-421. The theoretical plate number (TPN) of YS-50 is about twice and the resolution factor (Rs) of Na^+/NH_4^+ is about 1.3 times than YK-421.

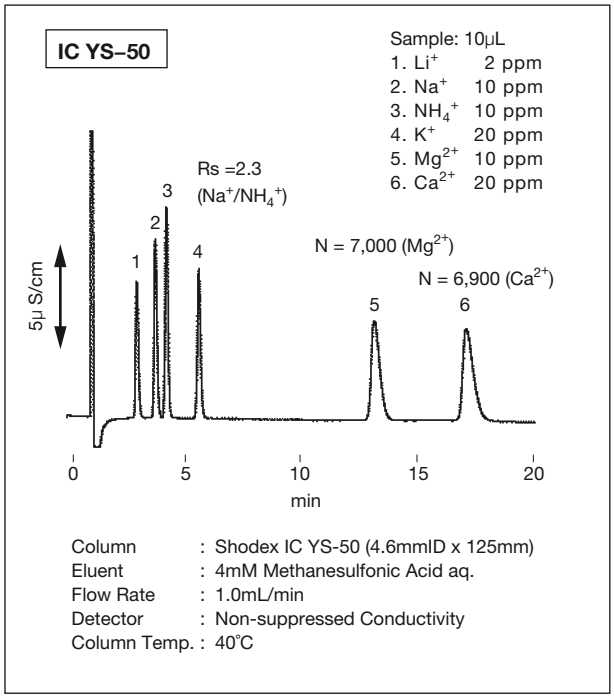


Fig. 3-1 Six Cations with YS-50

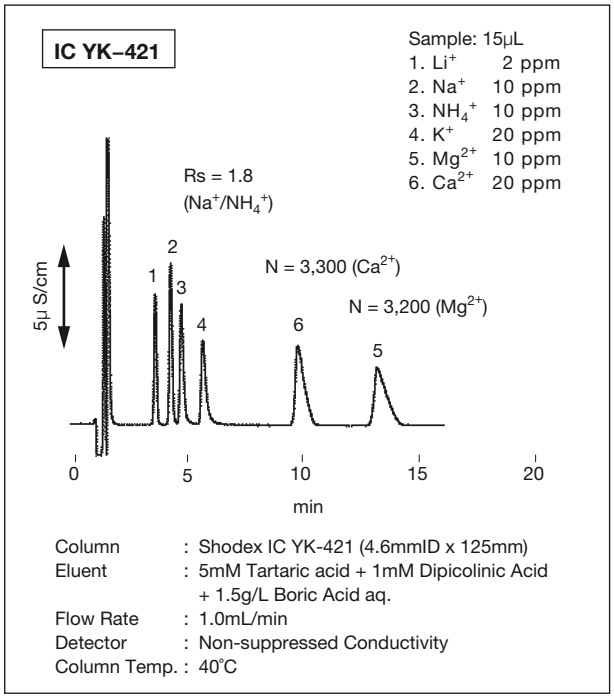


Fig. 3-2 Six Cations with YK-421

3-1-2. Quantitative Analysis

Figure 3-3 shows calibration curves for cations as determined by non-suppressor method. Each curve has excellent linearity with a correlation coefficient of 0.999 or more. This indicates the column is suitable for quantitative analysis.

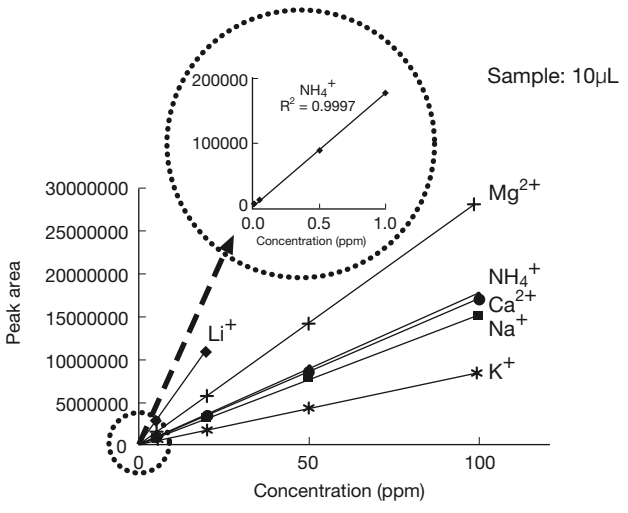


Table 3-1 R^2 value

Li^+	0.9999
Na^+	0.9998
NH_4^+	0.9997
K^+	0.9999
Mg^{2+}	0.9999
Ca^{2+}	0.9999

(1ppm~100ppm)

Column : Shodex IC YS-50 (4.6mmID x 125mm)
Eluent : 4mM Methanesulfonic Acid aq.
Flow Rate : 1.0mL/min
Detector : Non-suppressed Conductivity
Column Temp. : 40℃

Fig. 3-3 Calibration Curves

3-1-3. Separation Performance of Na^+ and NH_4^+

Due to the high resolution, even under non-suppressor conditions, it becomes possible to detect a very small quantity of NH_4^+ in a large quantity of Na^+. Na^+ and NH_4^+ can be separated and detected at the ratio of 5000 : 1 using the YS-50 column. Figure 3-4 shows the chromatogram of Na^+ and NH_4^+ with 5000 : 1 concentration ratio. Figure 3-5 shows the calibration curve of NH_4^+ in the presence of 50ppm of Na^+. The calibration curve shows good linearity in ppb level.

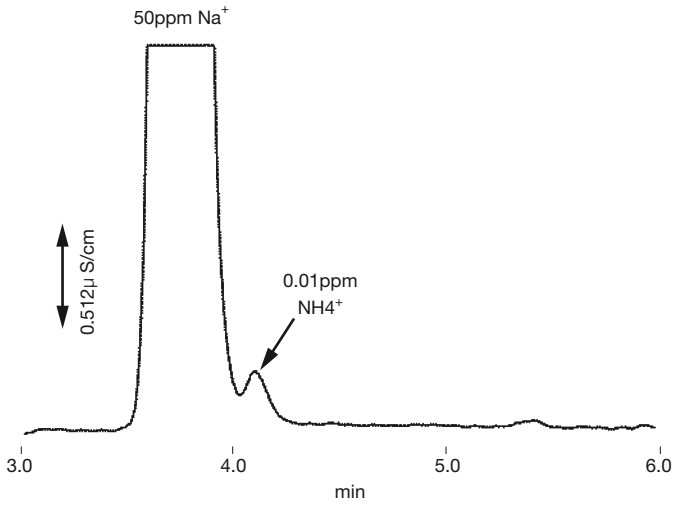
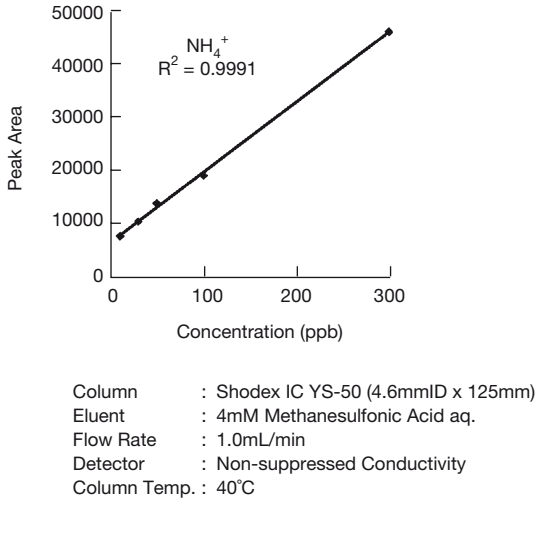


Fig. 3-4 Na^+ and NH_4^+ with 5000 : 1 Concentration Ratio

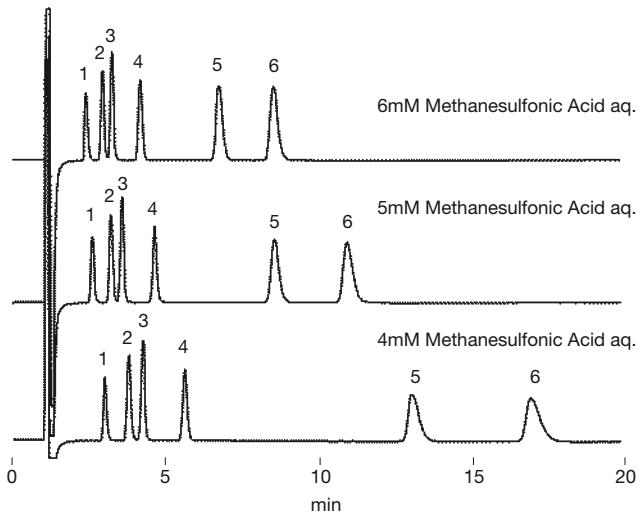


Column : Shodex IC YS-50 (4.6mmID x 125mm)
Eluent : 4mM Methanesulfonic Acid aq.
Flow Rate : 1.0mL/min
Detector : Non-suppressed Conductivity
Column Temp. : 40℃

Fig. 3-5 Calibration Curve of NH_4^+ in the Presence of 50ppm Na^+

3-1-4. Influence of Concentration of Methanesulfonic Acid

Changing the concentration of methanesulfonic acid in the eluent enables control of the ion separation and elution time. Figure 3-6 shows the chromatograms at the different concentrations of methanesulfonic acid. The higher concentration shows shorter retention time, but the eluent conductivity is higher (background level).



Sample: 10µL
1. Li^+ 2 ppm
2. Na^+ 10 ppm
3. NH_4^+ 10 ppm
4. K^+ 20 ppm
5. Mg^{2+} 10 ppm
6. Ca^{2+} 20 ppm

Column : Shodex IC YS-50 (4.6mmID x 125mm)
Eluent : Methanesulfonic Acid aq.
Flow Rate : 1.0mL/min
Detector : Non-suppressed Conductivity
Column Temp. : 40℃

Fig. 3-6 Separation Patterns with Different Concentrations of Solvent

3-1-5. Influence of Flow Rate

Changing the solvent flow rate enables control of the ion separation and elution time. Figure 3-7 shows the relation between flow rate and theoretical plate number (TPN) of K^+. Figure 3-8 shows the chromatograms of different flow rates from 0.4mL/min to 1.2mL/min. The flow rate of 1.0mL/min is recommended, because the TPN at 1.0mL/min is not significantly lower than at 0.8 mL/min and the analysis of six common cations is completed

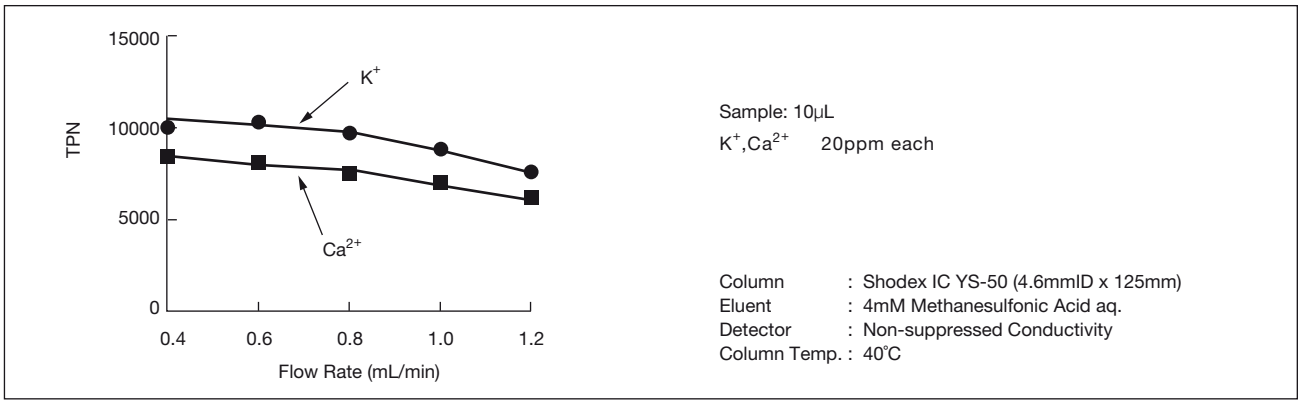


Fig. 3-7 Relation between Flow Rate and Theoretical Plate Number

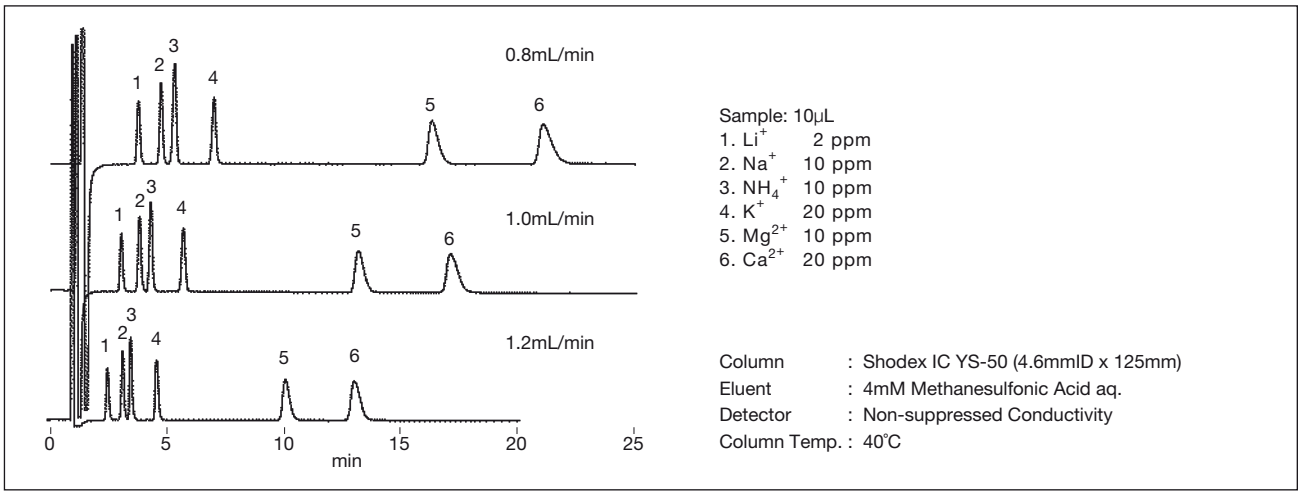


Fig. 3-8 Chromatograms at Different Flow Rates

3-1-6. Sample Condition

Figure 3-9 shows the relation of sample concentration (K^+) and TPN, and Figure 3-10 shows the relation of sample injection volume (K^+) and TPN. Sample concentrations lower than 50ppm and injection volumes less than 10µL are recommended for superior performance with YS-50.

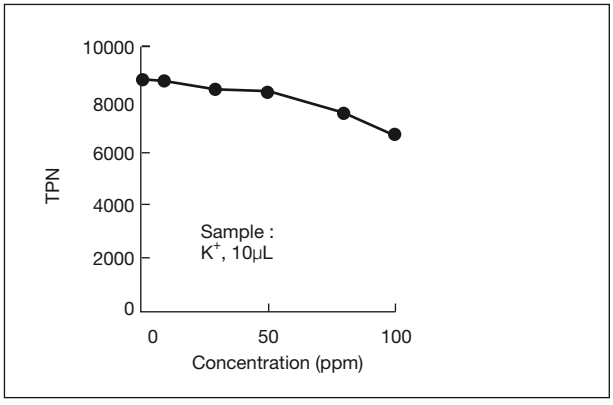


Fig. 3-9 Relation between Sample Load Amount and Theoretical Plate Number

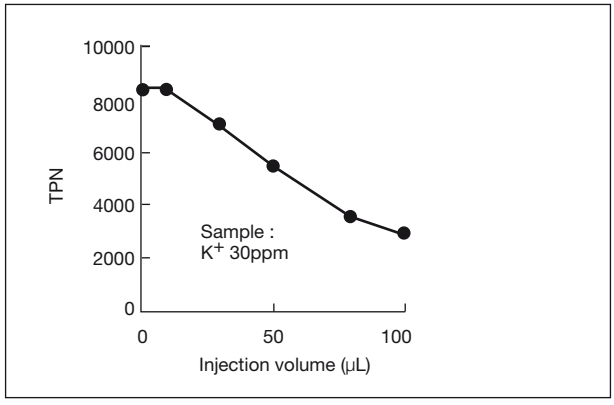


Fig. 3-10 Relation between Injection Volume and Theoretical Plate Number

3-1-7. Influence of Temperature

Figure 3-11 shows chromatograms at three different temperatures. Monovalent ions are influenced by temperature more than divalent ions.

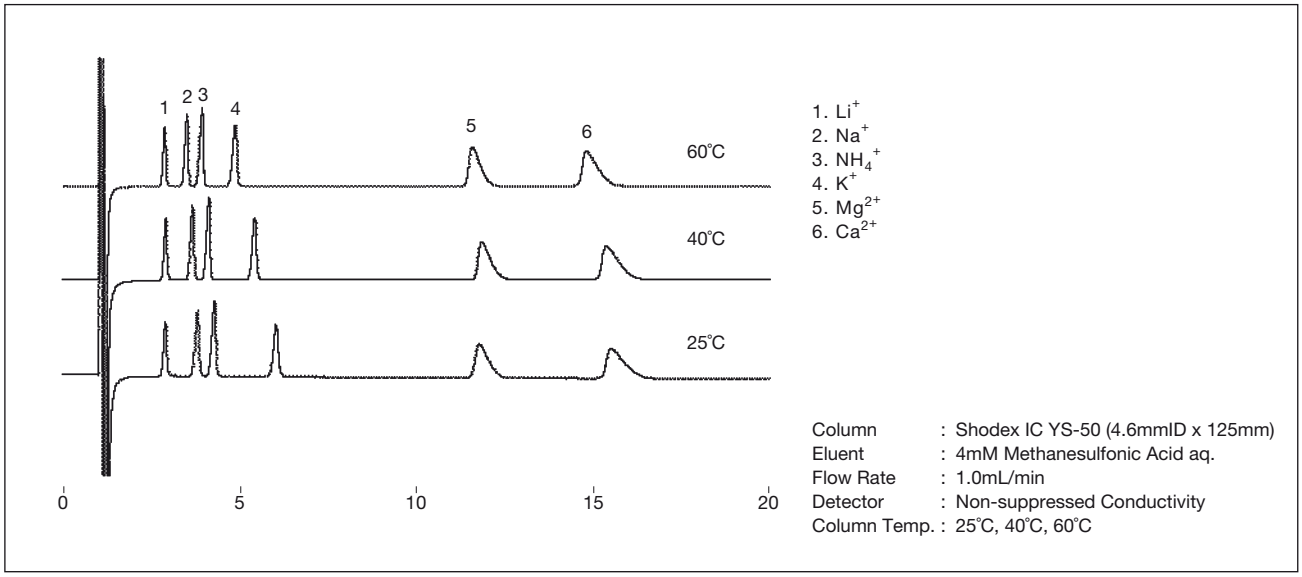


Fig. 3-11 Influence of Temperature on Separation Performance

3-1-8. Durability

Figure 3-12 shows the result of durability testing. The column was subjected to 1,000 injections and analyses of tap water. The results at the beginning and end of the test demonstrate no loss of resolution.

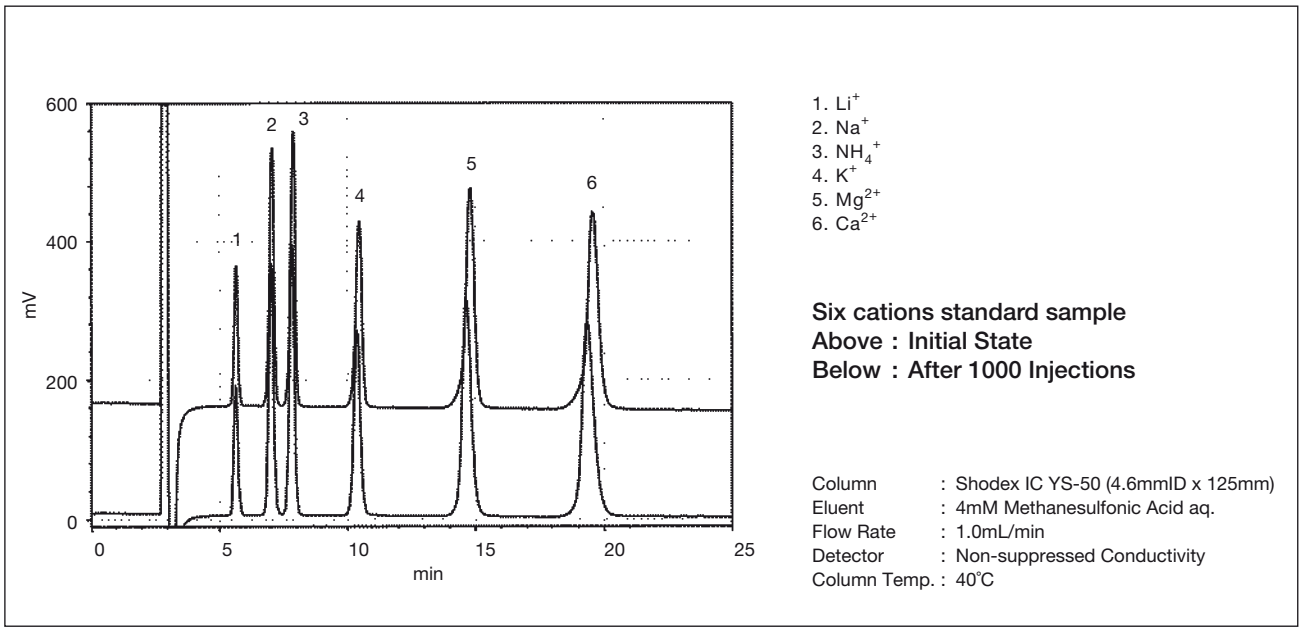
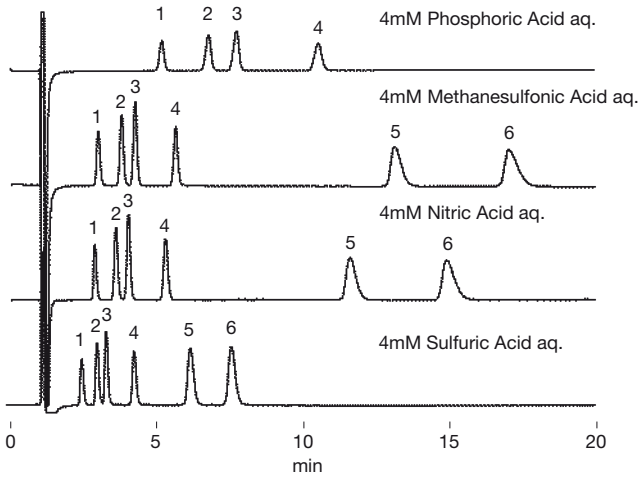


Fig. 3-12 Comparison of Column after 1000 Injections and Initial State

3-2. Selection of Solvent

The suitable acid is selected depending on the samples, analysis conditions and instruments. Fig. 3-13 shows the chromatograms with 4mM methanesulfonic acid, sulfuric acid (H_2SO_4), nitric acid (HNO_3), and phosphoric acid (H_3PO_4) as an eluent. Tables 3-2 and 3-3 show the conductivity (background level) and alpha value, respectively. Obviously, sulfuric acid has a markedly higher selectivity for divalent cations.



Sample: 10µL
1. Li^+ 2 ppm
2. Na^+ 10 ppm
3. NH_4^+ 10 ppm
4. K^+ 20 ppm
5. Mg^{2+} 10 ppm
6. Ca^{2+} 20 ppm

Column : Shodex IC YS-50 (4.6mmID x 125mm)
Flow Rate : 1.0mL/min
Detector : Non-suppressed Conductivity
Column Temp. : 40°C

Fig. 3-13. Separation Patterns with Different Solvents

Table 3-2 Conductivity of Solvents

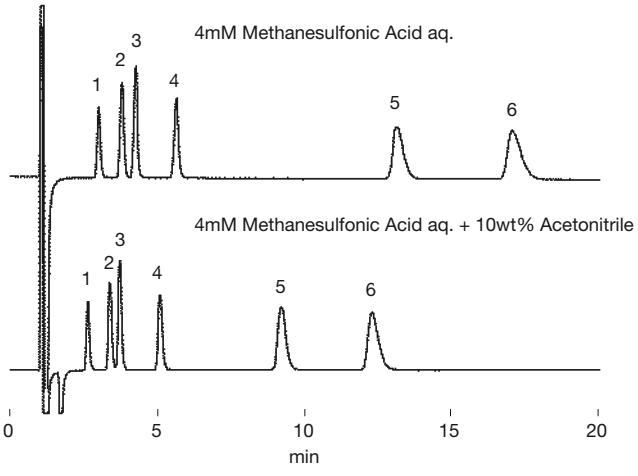
Solvent	Conductivity (µS/cm)
4mM Phosphoric Acid aq.	797
4mM Methanesulfonic Acid aq.	1,746
4mM Nitric Acid aq.	1,988
4mM Sulfuric Acid aq.	2,488

Table 3-3 α value [Rt (ion) / Rt (Li^+)]

Solvent	Li^+	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
4mM Phosphoric Acid aq.	1.0	1.4	1.6	2.3	12.6	–
4mM Methanesulfonic Acid aq.	1.0	1.4	1.6	2.3	6.0	8.0
4mM Nitric Acid aq.	1.0	1.4	1.6	2.3	3.6	4.7
4mM Sulfuric Acid aq.	1.0	1.4	1.6	2.3	5.6	7.4

3-3. Adding Organic Solvent to Eluent

Acetonitrile can be added to the eluent up to 50%. Adding acetonitrile can weaken the hydrophobic interaction between the sample and packing material, and therefore shorten the elution time of highly hydrophobic cations. Figure 3-14 shows the chromatogram of common cations with 4mM methanesulfonic acid with 10% acetonitrile added. Table 3-4 shows the list of organic solvents, for both sample solvent and eluent, which may be used with



Sample: 10µL
1. Li^+ 2 ppm
2. Na^+ 10 ppm
3. NH_4^+ 10 ppm
4. K^+ 20 ppm
5. Mg^{2+} 10 ppm
6. Ca^{2+} 20 ppm

Column : Shodex IC YS-50 (4.6mmID x 125mm)
Flow Rate : 1.0mL/min
Detector : Non-Suppressed Conductivity
Column Temp. : 40°C

Fig. 3-14 Influence of Adding Acetonitrile to Eluent

Table 3-4 Usable Organic Solvents (v/v%)

Organic Solvent	As Sample Solvent	As Eluent
Acetnitrile	0~100%	0~50%
Acetone	0~100%	0~50%
Methanol	0~100%	no
Ethanol	0~100%	no

3-4. Column Clean-up

With long time use, column performance may gradually decrease. If the cause of the performance change comes from contamination, there is a possibility that the column may be regenerated by cleaning procedures.

However, sometimes the procedure is not sufficient to regenerate the column and it is necessary to replace the column with a new one. Even if the column can be regenerated, the performance may not be equivalent to new.

Please filter the sample and eluent with a 0.45μm filter before analysis. Attaching a guard column in front of an analysis column is strongly recommended. It is the first step in prolonging column lifetime.

3-4-1. Physical Contamination

When the column pressure becomes high, there is a possibility that insoluble material is accumulated on the top of the column.

1) Remove the guard column.
2) Reverse the direction of flow through the column using the standard eluent at a flow rate equal to or less than the usual operating flow.
3) Flow to waste and not through the detector. Dislodged material might plug the detector.

3-4-2. Hydrophobic Contamination

When the sample contains hydrophobic substances, small amounts may adsorb to the packing material.

1) Wash the column with the standard eluent to which 50% acetonitrile has been added, being careful about miscibility and solubility issues. Wash the column with the equivalent of 5 column volumes at a flow rate of 0.5mL/min.
2) Replace the in-column solvent to the standard eluent.

3-4-3. Protein

When the sample contains proteins, peptides or similar material, small amounts of these substances may adsorb to the packing material.

1) Wash the column with a volume equivalent to 5 column volumes of an alkaline solution of 10mM Na_2HPO_4 aq. (pH 9~9.5), at a flow rate of 0.5mL/min.
2) Wash the column with twice the column volume of pure water.
3) Replace the in-column solvent with the standard eluent.

4. Analysis with Suppressor System

4-1. Common Cations

YS-50 may be applied to analyses using a suppressor system. Figure 4-1 shows the chromatogram of common cations using a suppressor system.

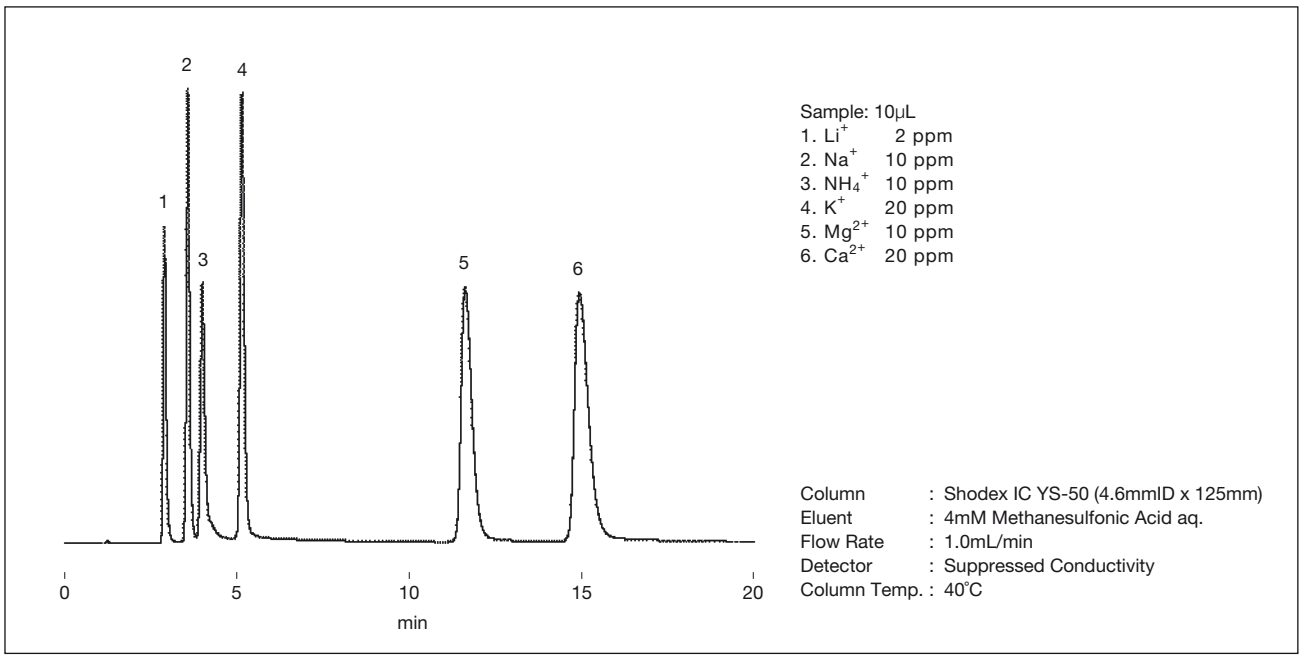


Fig. 4-1 Common Cations Analyzed with Suppressor System

4-2. NH_4^+ in an Abundance of Na^+

Figure 4-2 shows a chromatogram of Na^+ and NH_4^+ with 5000 : 1 of concentration ratio using a suppressor system. Due to the broadness of the peaks by the additional dilution in the suppressor system, the resulting separation of Na^+ and NH_4^+ is equivalent to the non-suppressor separation (refer to Fig. 3-4). However in the case of the analysis of low concentration of the sample, using a suppressor system is recommended.

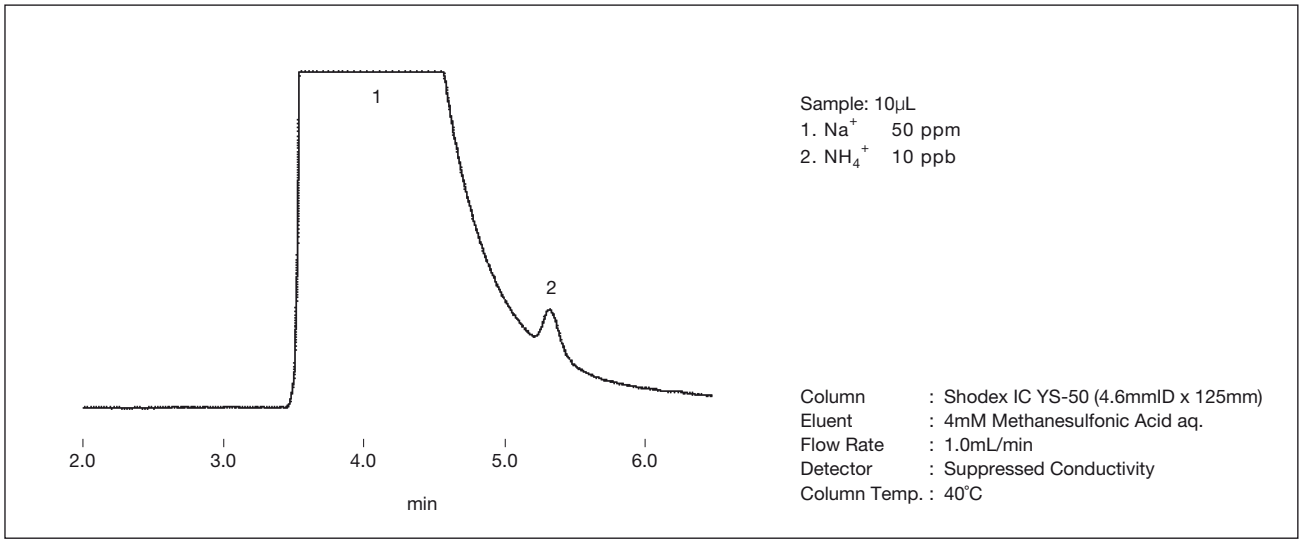


Fig. 4-2 Na^+ and NH_4^+ with 5000 : 1 Concentration Ratio (Suppressor System)

5. Applications

5-1. Alkylamines

Fig 5-1 shows the chromatogram of common cations and three alkylamines (methylamine, trimethyamine and triethylamine). Fig 5-2 shows the calibration curves of three alkylamines and table 5-1 shows the R^2 value of each amine. Alkylamines can be analyzed with common cations at the same time under a wide range of concentration. Table 5-2 shows the list of elution volumes of the other amines and common cations.

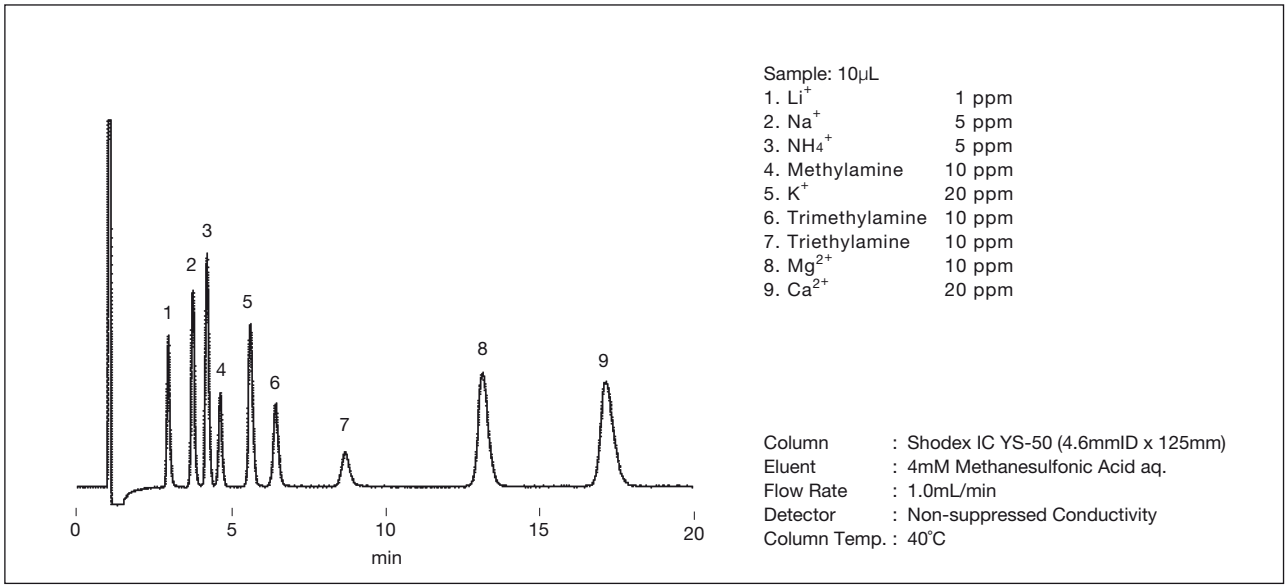


Fig. 5-1 Common Cations and Alkylamines

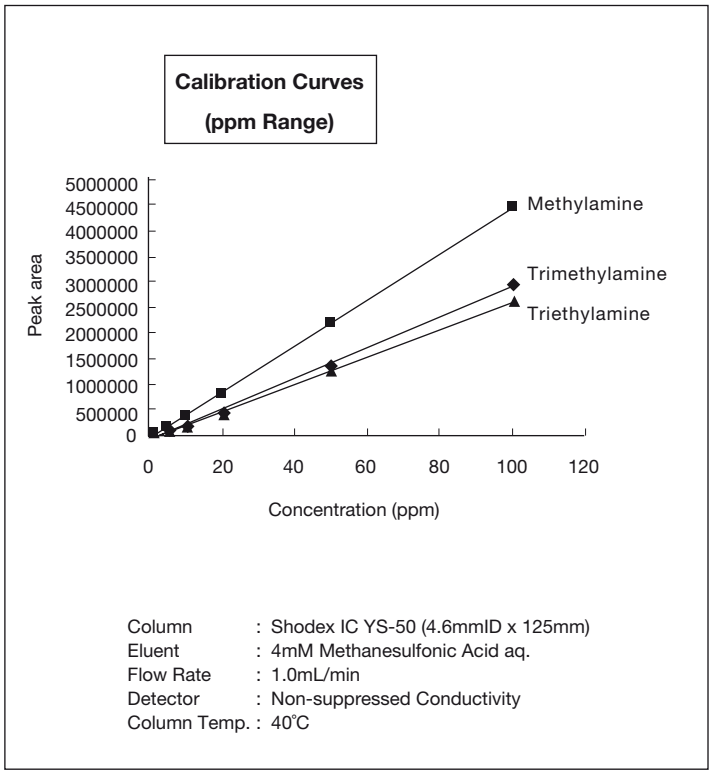


Fig. 5-2 Calibration Curves of Alkylamines

Table 5-1 Correlation Factor (R^2)

Methylamine	0.9999
Trimethylamine	0.9992
Triethylamine	0.9998

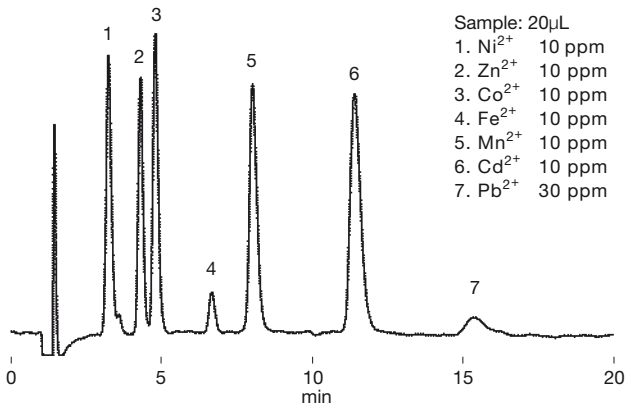
(0.1ppm~1ppm)

Table 5-2 Elution Volume of Amines and Cations

Substance	Elution vol. (mL)
Li^+	3.0
Na^+	3.8
NH_4^+	4.3
Methylamine	4.1
Trimethylamine	4.5
Triethylamine	5.4
Monoethanolamine	4.3
Diethanolamine	4.6
Triethanolamine	5.4
K^+	5.6
Mg^{2+}	13.1
Ca^{2+}	17.0

5-2. Transition Metals

Figure 5-3 shows the chromatogram of seven transition metals.
Using the mixture of 6mM tartaric acid and 4mM oxalic acid, these transition metals can be separated well.



Sample: 20μL
1. Ni^{2+} 10 ppm
2. Zn^{2+} 10 ppm
3. Co^{2+} 10 ppm
4. Fe^{2+} 10 ppm
5. Mn^{2+} 10 ppm
6. Cd^{2+} 10 ppm
7. Pb^{2+} 30 ppm

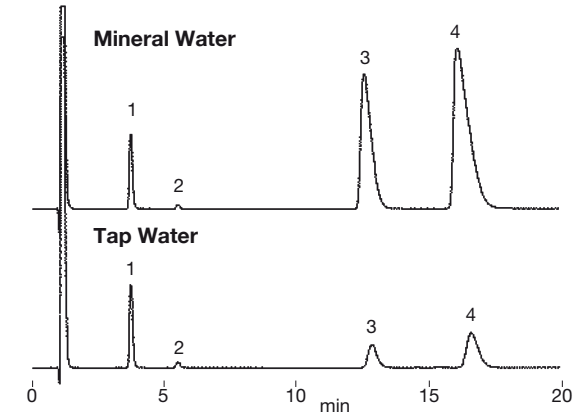
Each transition metal was dissolved with 0.1M HNO_3 at a concentration of 1,000ppm. These standard samples were further diluted with eluent to the concentrations listed.

Column : Shodex IC YS-50 (4.6mmID x 125mm)
Eluent : 6mM Tartaric Acid + 4mM Oxalic Acid aq.
Flow Rate : 1.0mL/min
Detector : Non-suppressed Conductivity
Column Temp. : 40℃

Fig. 5-3 Transition Metals

5-3. Tap Water and Mineral Water

Figure 5-4 shows the chromatograms of tap water and mineral water. The concentration of magnesium ion and calcium ion in mineral water is higher than in tap water.



Sample: 10μL

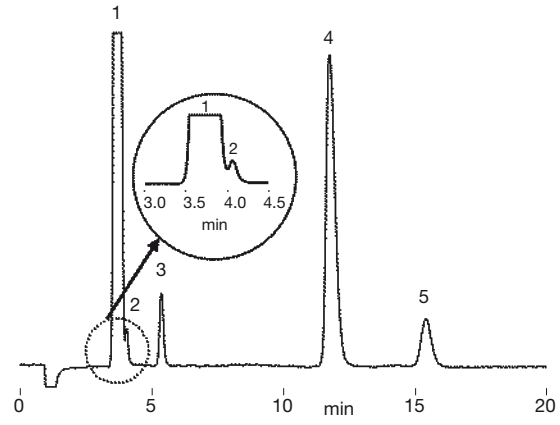
	Mineral Water	Tap Water
1. Na^+	6.6 ppm	7.3 ppm
2. K^+	0.8 ppm	1.3 ppm
3. Mg^{2+}	24.8 ppm	3.6 ppm
4. Ca^{2+}	71.9 ppm	12.0 ppm

Column : Shodex IC YS-50 (4.6mmID x 125mm)
Eluent : 4mM Methanesulfonic Acid aq.
Flow Rate : 1.0mL/min
Detector : Non-suppressed Conductivity
Column Temp. : 40℃

Fig. 5-4 Tap Water and Mineral Water

5-4. Sea Water

Figure 5-5 shows the chromatogram of 100-fold diluted sea water using a non-suppressor system. Cations are detected even though a large amount of Na^+ (98 ppm) exists.



Sample: 10μL
100-fold Diluted Sea Water
1. Na^+ 98.0 ppm
2. NH_4^+ 0.4 ppm
3. K^+ 3.6 ppm
4. Mg^{2+} 11.1 ppm
5. Ca^{2+} 3.4 ppm

Column : Shodex IC YS-50 (4.6mmID x 125mm)
Eluent : 4mM Methanesulfonic Acid aq.
Flow Rate : 1.0mL/min
Detector : Non-suppressed Conductivity
Column Temp. : 40℃

Fig. 5-5 100-fold Dilution of Sea Water

5-5. Wine

Figure 5-6 shows the chromatogram of red wine. Galactosamine is detected with cations.

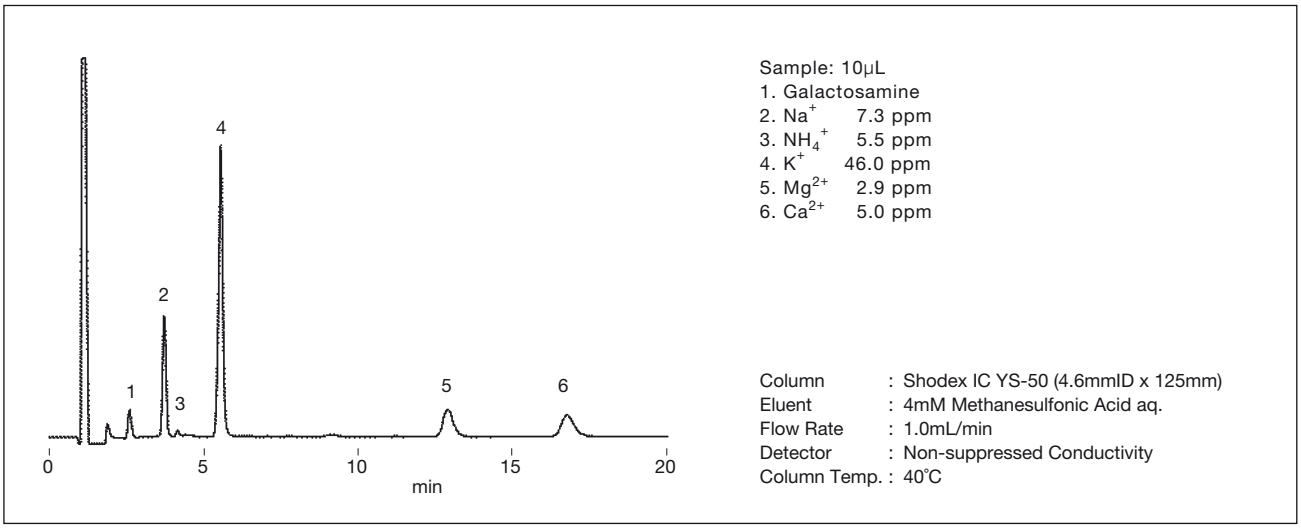


Fig. 5-6 Red Wine

5-6. Cations in Polyethylene Glycol

Cations in a solution containing hydrophilic polymers can be analyzed with YS-50. Polyethylene glycol (PEG) was used as a model compound of a hydrophilic polymer matrix, a similar situation is encountered with waste water analysis in factories of electronic devices.

Figure 5-7 shows the chromatogram of six cations in 5% PEG 2000 solution. Cation separation was excellent with no interference by PEG 2000. Table 5-4 shows the recovery of each cation was good. However, small amounts of PEG may be adsorbed by the packing material, so it should be washed away with 50% acetonitrile in eluent (see article 3-4-2).

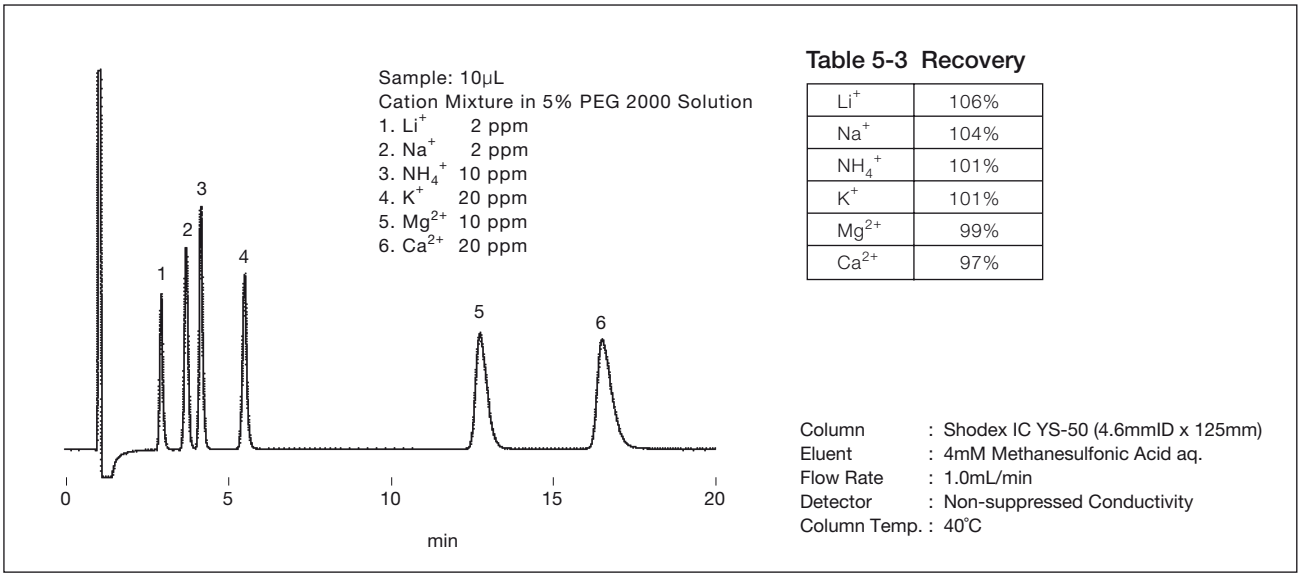


Table 5-3 Recovery

Li^+	106%
Na^+	104%
NH_4^+	101%
K^+	101%
Mg^{2+}	99%
Ca^{2+}	97%

Fig. 5-7 Common Cations in 5% PEG 2000 Solution

5-7. Cations in Albumin

Cations in a solution containing protein can be analyzed with YS-50. Albumin was used as a model compound of biological polymer matrix.

Figure 5-8 shows the chromatogram of six cations in 7% albumin solution. Cation separation was excellent with no interference by albumin. The reason for the high peak height of Na^+ is that Na^+ is originally contained in albumin. Please wash the column with the alkaline solution of 10mM Na_2HPO_4 aq. at pH9~9.5 after analyses, because small amounts of albumin may adsorb to the packing material. (see article 3-4-3)

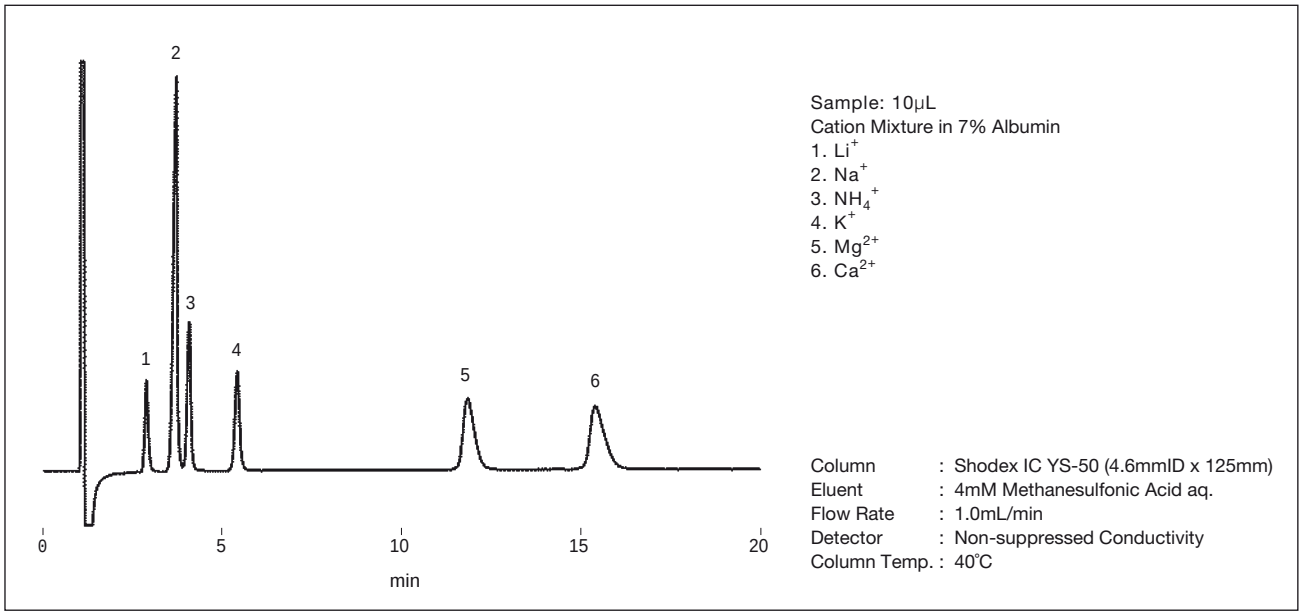


Fig. 5-8 Cations in Albumin

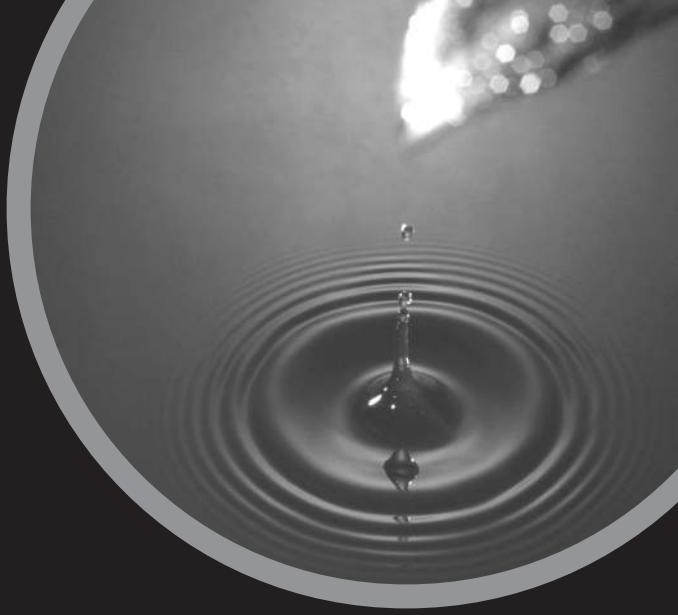
6. Conclusion

We have introduced Shodex IC YS-50 as a new column for cation analysis. It shows excellent efficiency (TPN) and good peak shapes. It can be used with a wide range of analytical conditions: different acids as eluents, addition of organic solvent to the eluent and to the sample solvent, and with both suppressor and non-suppressor systems.

Shodex IC YS-50 described above can be used to analyze not only common cations, such as alkali and alkali earth ions, but also cationic low-molecular-weight compounds (e.g. alkyl amines) and transition metal ions. It is suitable for cation chromatography in a wide range of applications from food and beverage, to pharmaceuticals, and chemicals.

Shodex™

HPLC Columns



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For any questions, requests or technical support you can contact us at:
info@shodex.de +49(0)8993996237

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Manufactured by

SHOWA DENKO K.K.

Shodex offices

(HEADQUARTERS)
Showa Denko K.K
Shodex (Separation & HPLC) Group
23F Muza Kawasaki Central Tower, 1310, Omiya-cho, Saiwai-ku, Kawasaki, Kanagawa 212-0014 JAPAN
Tel: +81(0)445201380 Fax: +81(0)445201383 E-mail: sdk_shodex@sdk.co.jp Web: www.shodex.com

(NORTH & LATIN AMERICA)
Showa Denko America, Inc.
420 Lexington Avenue, Suite 2850, New York, NY 10170 USA
Tel: +12123700033 Fax: +12123704566 E-mail: support@shodex.net Web: www.shodex.net

(EUROPE, MIDDLE EAST & AFRICA)
Showa Denko Europe GmbH
Konrad-Zuse-Platz 3, 81829 Munich, GERMANY
Tel: +49(0)8993996234 Fax: +49(0)899399627734 E-mail: info@shodex.de Web: www.shodex.de

(CHINA)
Shodex China Co., Ltd.
18F, Wang Wang Building, No. 211 Shi Men Yi Road, Jing An, Shanghai, 200041, CHINA
Tel: +86(0)2162176111 Fax: +86(0)2162179879 E-mail: support@shodexchina.com
Web: www.shodex.com/index_ch.html

(JAPAN)
Shoko Co., Ltd.
4-1, Shibakohen 2-chome, Minato-ku, Tokyo, 105-8432, JAPAN
Tel: +81(0)334595104 Fax: +81(0)334595081 E-mail: shodex.tokyo@shoko.co.jp
Web: www.shodex.com

(KOREA)
Shoko Korea Co., Ltd
322, Chungjeong Rizion, 465, Chungjeongno 3-ga, Seodaemun-gu, Seoul 120-013, KOREA
Tel: +82(0)27845111 Fax: +82(0)27845125 E-mail: shoko.korea@shokokorea.com
Web: www.shodex.com/index_kr.html