LC/MS Analysis of Various Low-Molecular Weight Cationic Compounds Using HILIC Mode

Introduction

Most low-molecular weight cationic compounds are hydrophilic and hardly retained under reversed phase mode. To overcome the problem, pre-column derivertization or ion-pair reagents are often used, however they add additional steps in the analysis. The use of derivertization adds additional steps in the analysis while the use of ion-pair regent increases background level from the ion-pair reagent residues on the column and the flow-lines.

The Shodex HILICpak VC-50 2D column used in this application is packed with multi-porous polyvinyl alcohol polymers modified with carboxyl functional groups. The 2.0 mm internal diameter of the column is designed to work well with LC/MS. The method using the VC-50 2D column provides a highly sensitive analysis of various low-molecular weight cationic compounds, while requiring simpler analytical conditions than previously available methods. Figure 1 shows the schematic diagram of the packing material used in the VC-50 2D. Because of its hydrophilicity, increasing the percentage of acetonitrile in the eluent promotes HILIC mode, enhancing the retention of hydrophilic compounds. In addition, the low-molecular weight cationic compounds are retained in the column by cationic ion-exchange effects.

This technical article introduces LC/MS analysis methods using the VC-50 2D for various cationic compounds such as choline and acetylcholine, neurotransmitters, oral anti-diabetic drugs, salacinol related compounds, and amminoglycoside antibiotics.

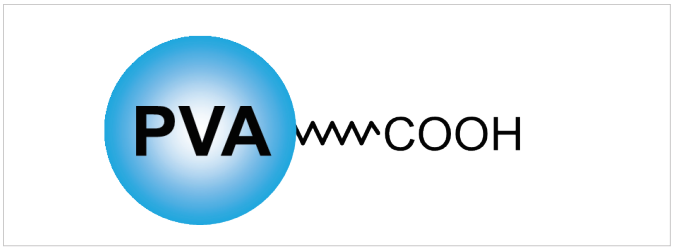


Fig. 1. Schematic diagram of VC-50 packing material

Experimental

The LC/MS system used was Shimadzu Nexera/ LCMS-8030 Plus. The column used was Shodex HILICpak VC-50 2D (2.0 mm I.D. x 150 mm; particle size 5 µm; pore size 100 Å). High pressure isocratic or linear gradient elution with formic acid and acetonitrile eluents were used for most analysis. The flow rate was set at 0.2 - 0.3 mL/min and the column temperature was set at 30 - 40 °C. An ESI was used as a means of ionization and SIM(-) or MRM(+) mode was used for the detection.

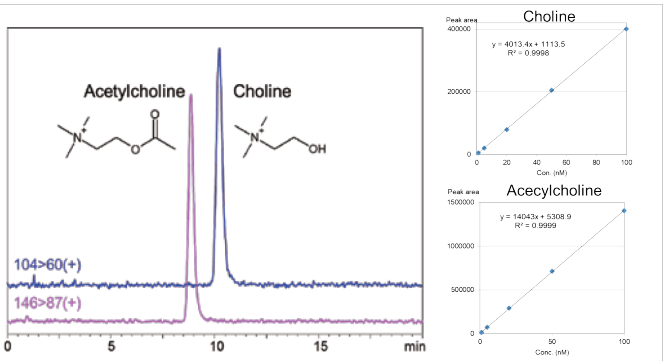
Results and Discussion

1. Analysis of Neurotransmitters

Neurotransmitters are low-molecular weight chemicals formed in neurons and transmit signals from the synapse to the target cells to excite or suppress their functions. They take major roles in many and various mental diseases. Some known neurotransmitters include monoamine type, e.g., acetylcholine and dopamine, and amino acid type, e.g., glutamine acid.

1.1 Analysis of choline and acetylcholine

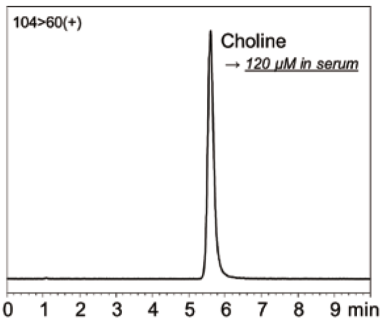
There are reports that patients with Alzheimer's disease show low levels of the brain neurotransmitter acetylcholine. Since choline is the source of acetylcholine, foods containing choline has been drawing attention for the prevention of Alzheimer's disease. Figure 2 shows the LC/MS chromatograms of choline and acetylcholine in a mixed standard. The method demonstrated its feasibility in detecting the analytes in 1-nM level.



Sample : 5 nM each (in H_2O), 5 µL
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 200 mM HCOOH aq./(B) CH_3CN = 40/60
Flow rate : 0.3 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 °C

Fig. 2. LC/MS chromatograms of choline and acetylcholine

As a next step, we quantified choline in the serum of a guinea pig. Figure 3 shows the LC/MS chromatogram of choline in the sample. The sample preparation used here was very simple - The sample was deprotonated through the addition of acetonitrile and the following centrifugation.



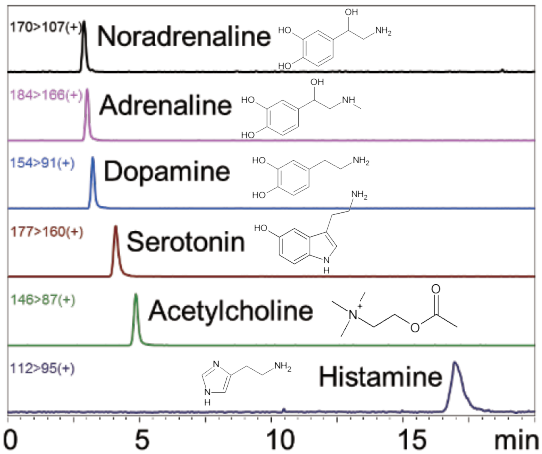
Sample preparation
1. Dilute guinea pig serum with H_2O in 10 hold.
2. Add x 3 volume of CH_3CN.
3. Centrifuge the sample.
4. Dilute the supernatant with H_2O in 10 hold.
5. Inject 20 μL.

Column : Shodex HILICpak VC-50 2D
Eluent : (A) 100 mM HCOOH aq./(B) CH_3CN = 50/50
Flow rate : 0.3 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 ºC

Fig. 3. LC/MS analysis of choline in guinea pig serum

1.2 Analysis of monoamine neurotransmitters

Figure 4 shows the LC/MS chromatograms of five monoamine neurotransmitters and acetylcholine. Histamine is a highly basic and strongly retained under isocratic method. By using a gradient method with increased formic acid ratio enabled simultaneous analysis of six compounds.

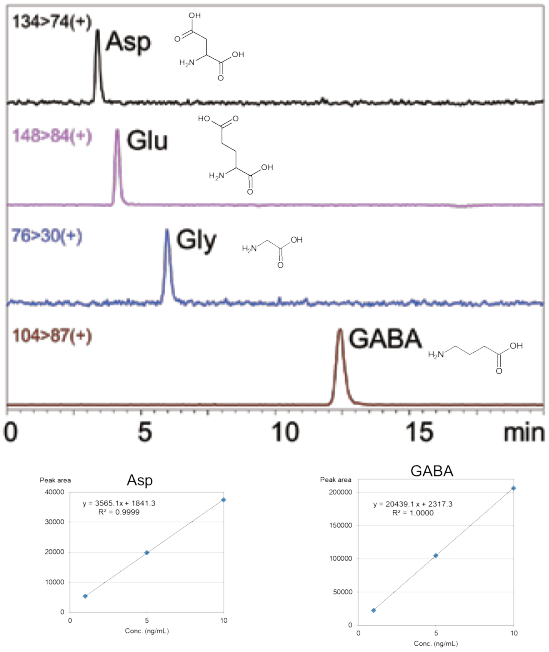


Sample : 0.1 μM each (in H_2O), 5 μL
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 200 mM HCOOH aq./(B) CH_3CN
High pressure linear gradient
60 % B (0 - 5 min) → 10 % B (6 - 20 min)
Flow rate : 0.3 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 ºC

Fig. 4. LC/MS chromatograms of five monoamine neurotransmitters and acetylcholine

1.3 Analysis of amino acid neurotransmitters

Figure 5 shows the LC/MS chromatograms of four amino acid neurotransmitters. Two acidic amino acids, aspartic and glutamic acids, tend to elute earlier than expected. This was probably because of the electrostatic repulsion effects between the amino acids and the carboxyl functional group on the surface of the packing material. Meanwhile, the retention mechanism that works between GABA (γ-amino butyric acid) and the packing material seemed to be different from that of other amino acids: Rather than HILIC mode, hydrophobic interaction is mostly responsible for the retention of GABA.

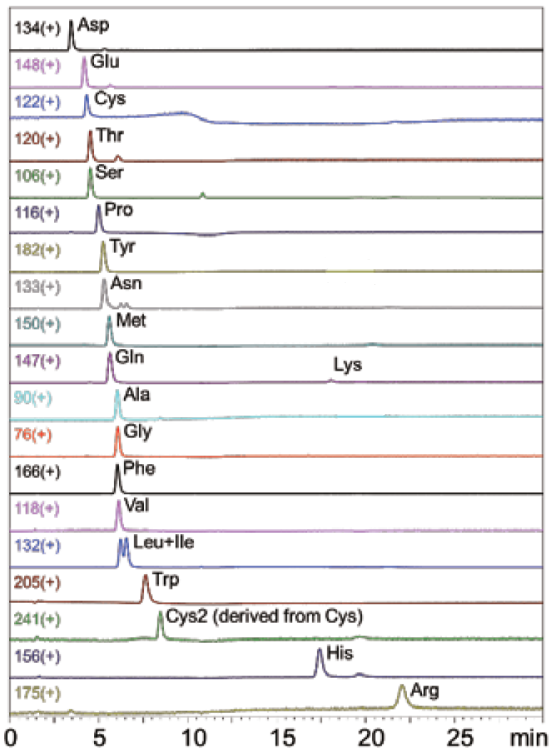


Sample : 5 nM each (in H_2O), 5 μL
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 40 mM HCOOH aq./(B) CH_3CN = 50/50
Flow rate : 0.2 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 ºC

Fig. 5. LC/MS analysis of four amino acid neurotransmitters

2. Simultaneous Analysis of 21 Amino Acids

Figure 6 shows the LC/MS chromatograms of cystine and 20 amino acids that compose proteins. The basic amino acids, histidine, lysine, and arginine, are strongly retained by cationic ion-exchange effects. Elution of these compounds required increasing the formic acid ratio in the gradient elution, which weakened the cationic ion-exchange effects. The method was also effective separating the two isomeric compounds, leucine and isoleucine.

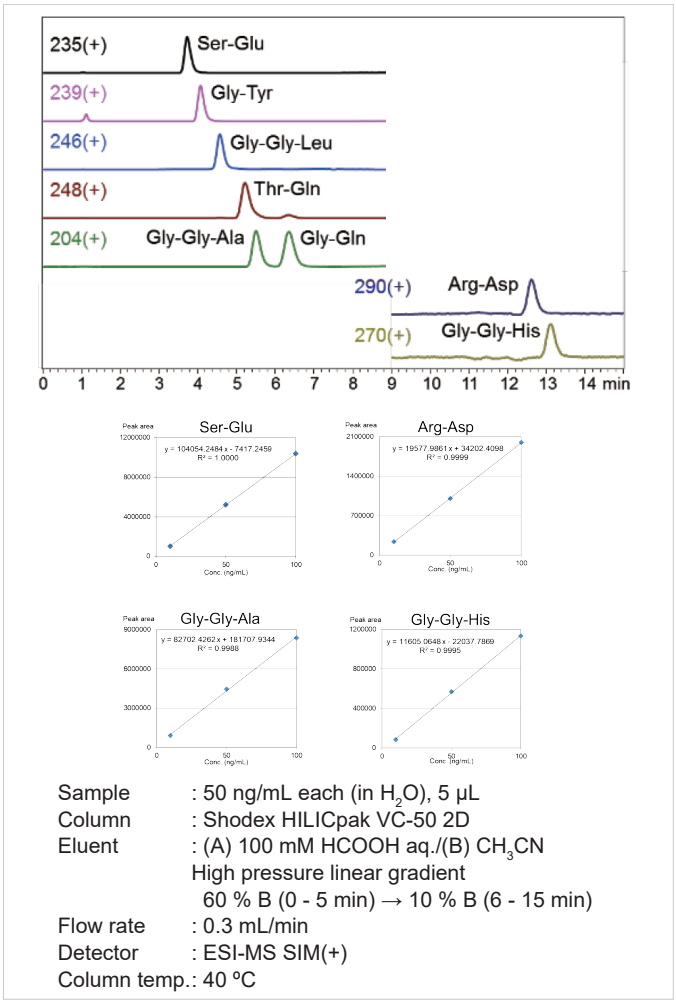


Sample : 100 ng/mL each (in H_2O), 5 µL
Column : Shodex HILICpak VC-50 2D
Eluent : H_2O/100 mM HCOOH aq./CH_3CN
Low pressure linear gradient
30/20/50 (0 min) → 0/90/10 (5 - 30 min)
Flow rate : 0.2 mL/min
Detector : ESI-MS SIM(+)
Column temp.: 30 ºC

Fig. 6. LC/MS chromatograms of 21 amino acids

3. Analysis of Low-Molecular Weight Peptides

Recently, medical, pharmaceutical, and food industries are finding potentials in functionalities of the low-molecular weight peptides. Figure 7 shows the LC/MS chromatograms of five dipeptides and three tripeptides. The results demonstrated that VC-50 2D is suitable for the analysis of low-molecular weight peptides.

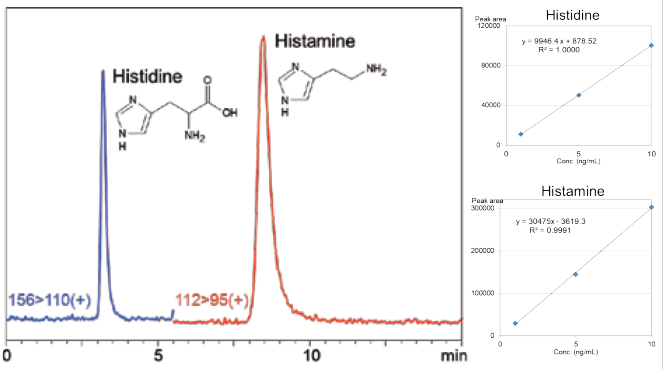


Sample : 50 ng/mL each (in H_2O), 5 µL
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 100 mM HCOOH aq./(B) CH_3CN
High pressure linear gradient
60 % B (0 - 5 min) → 10 % B (6 - 15 min)
Flow rate : 0.3 mL/min
Detector : ESI-MS SIM(+)
Column temp.: 40 ºC

Fig. 7. LC/MS chromatograms of dipeptides and tripeptides

4. Analysis of Histamine and Histidine

Figure 8 shows the LC/MS chromatograms of histamine and histidine. Histamine is known for its neurotransmission role, but it can be a cause of food poisoning. Thus, simultaneous analysis of histamine and its precursor, histidine, is sometimes required. The result shows that the VC-50 2D is applicable for the simultaneous analysis of an amino acid and an amine.

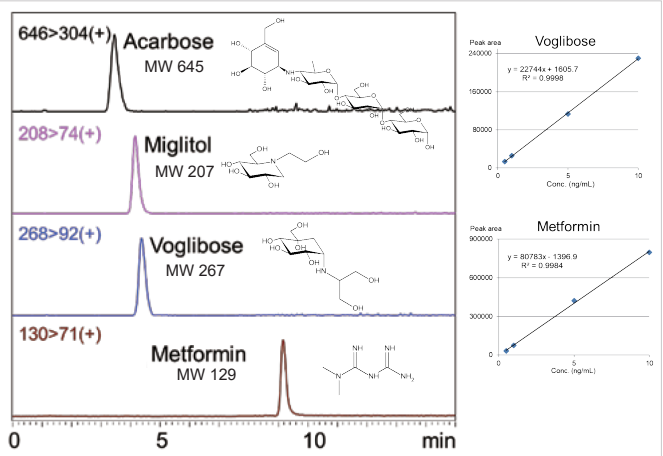


Sample : 5 ng/mL each (in H_2O), 20 µL
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 250 mM HCOOH aq./(B) CH_3CN = 70/30
Flow rate : 0.3 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 ºC

Fig. 8. LC/MS chromatograms of histamine and histidine

5. Analysis of Oral Anti-Diabetic Drugs

Figure 9 shows the LC/MS chromatograms of four oral anti-diabetic drugs. Metformin falls under the biguanide drug family and it is highly basic. By increasing formic acid ratio in the gradient elution let the metformin to elute and enabled a simultaneous analysis of metformin and three α–glucosidase inhibitor type drugs.

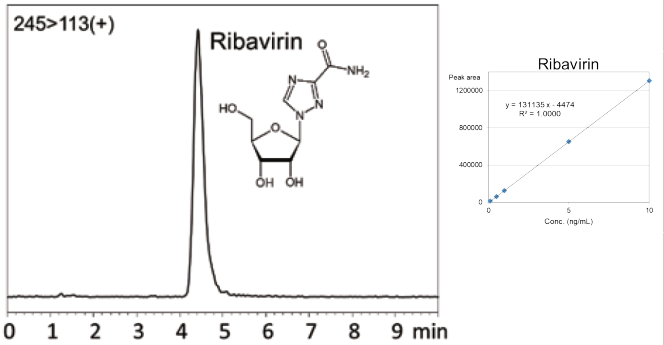


Sample : 5 ng/mL each (in H_2O), 5 µL
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 200 mM HCOOH aq./(B) CH_3CN
High pressure linear gradient
(B %) 60 (0 - 5 min) → 20 (6 - 15 min)
Flow rate : 0.3 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 ºC

Fig. 9. LC/MS chromatograms of oral anti-diabetic drugs

6. Analysis of Ribavirin

Figure 10 shows an LC/MS chromatogram of ribavirin, a nucleoside analog inhibitors of hepatitis C. Its retention by HILIC mode was increased by using 90% acetonitrile in the eluent. The high percentage of the acetonitrile also contributed to increase the ESI-MS detection sensitivity.

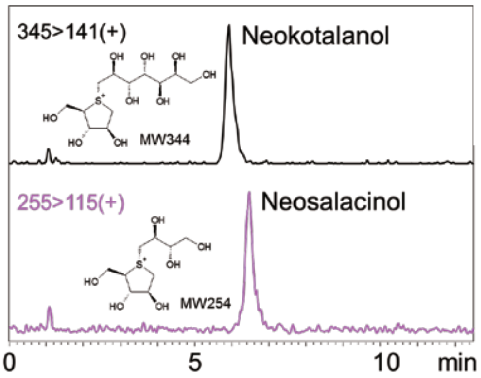


Sample : 0.5 ng/mL each (in H_2O/CH_3CN = 1/4), 5 µL
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 50 mM HCOOH aq./(B) CH_3CN = 10/90
Flow rate : 0.25 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 ºC

Fig. 10. LC/MS chromatogram of ribavirin

7. Analysis of Salacinol Related Compounds

The salacinol compounds, neokotalanol and neosalacinol, are sometimes used in functional foods, as they have blood sugar suppression effects. Figure 11 shows the LC/MS chromatograms of neokotalanol and neosalacinol in the extract of a commercial supplement. The result demonstrated that the VC-50 2D is suitable for analyzing the sulfonium-containing cationic compounds.



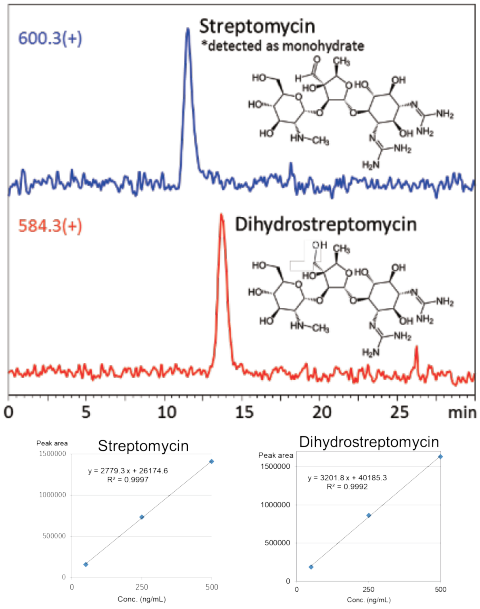
Sample preparation
1. Grind a tablet.
2. Measure 0.1 g of the sample, add 1-mL ultra-pure water.
3. Sonicate 10 min, centrifuge.
4. Add an equal portion of CH_3CN to supernatant, centrifuge.
5. Dilute supernatant with H_2O/CH_3CN = 1/1 in x 10,000 hold.
6. Inject 5 µL.

Column : Shodex HILICpak VC-50 2D
Eluent : (A) 50 mM HCOOH aq./(B) CH_3CN = 50/50
Flow rate : 0.3 mL/min
Detector : ESI-MS MRM(+)
Column temp.: 40 ºC

Fig. 11. LC/MS chromatograms of neokotalanol and neosalacinol in the extract of a commercial supplement

8. Analysis of Aminoglycoside Antibiotics

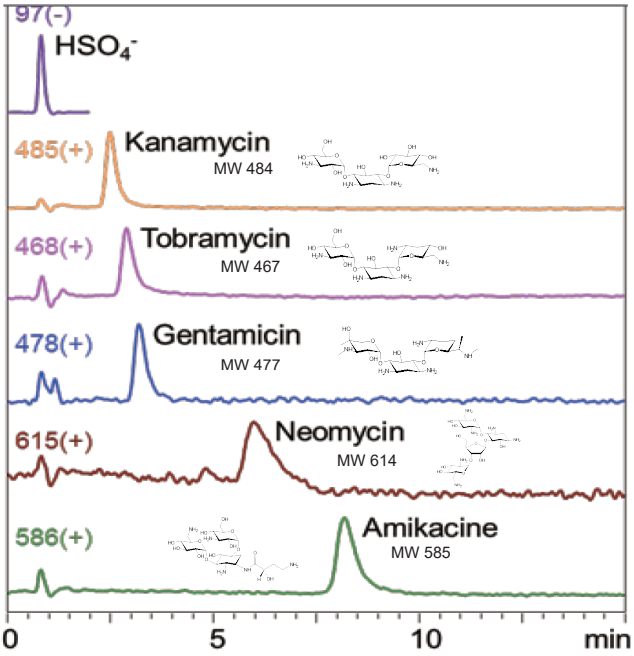
Streptomycin and its derivative, dihydrostreptomycin, are aminoglycoside antibiotics. They were analyzed under an acidic condition containing formic acid. Figure 12 shows the LC/MS chromatograms of streptomycin and dihydrostreptomycin



Sample : 50 ng/mL each (in H_2O), 20 µL
*Both are sulfates
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 250 mM HCOOH aq./(B) CH_3CN = 90/10
Flow rate : 0.3 mL/min
Detector : ESI-MS SIM(+)
Column temp.: 40 ºC

Fig. 12. LC/MS chromatograms of streptomycin and dihydrosteptomycin

On the other hand, kanamycin, tobramycin, gentamicin, neomycin, and amikacin were retained in the column even when the concentration of the formic acid was increased to 500 mM. Aminoglycoside antibiotics have more than four amino functional groups, resulting in a strongly cationic structure retaining on the column. To overcome this hurdle, we added ammonium to the eluent making it alkaline allowing the five compounds to elute. Figure 13 shows the LC/MS chromatograms of aminoglycoside antibiotics. It was assumed that using the alkaline eluent suppressed the dissociation of amino groups in the aminoglycoside molecules, and weakend the ionic interactions between the analytes and the packing material. The pH of the 1.5% ammonium water used in the experiment was about 12. Use of the basic condition was possible because of the polymer-based materials packed in the VC-50 2D.



Sample : 100 ng/mL each (in H_2O), 20 µL
*Amikacine is dehydrate, others are sulfates
Column : Shodex HILICpak VC-50 2D
Eluent : (A) 1.5 % mM NH_3 aq./(B) CH_3CN
High pressure linear gradient
30 % B (0 min) → 10 % B (5 - 15 min)
Flow rate : 0.3 mL/min
Detector : ESI-MS SIM(+/-)
Column temp.: 40 ºC

Fig. 13. LC/MS chromatograms of kanamycin, tobramycin, gentamicin, neomycin, and amikacine

Conclusions

In this technical note, the polymer-based carboxyl HILIC column, Shodex HILICpak VC-50 2D demonstrated simple LC/ESI-MS methods for the analysis of various low-molecular weight cationic compounds using formic acid/ acetonitrile eluents. The use of gradient condition adds another advantage to simultaneously analyze multiple compounds having large differences in their cationic strengths. Also, the use of alkaline condition, with an addition of ammonium to the eluent, is useful for the analysis of cationic compounds that are normally retained strongly in the column under the acidic conditions.

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