



LC/MS analysis of choline and acetylcholine in living organisms with using polymer-based cation IC column

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Abstract

Choline is a water-soluble essential nutrient and is a component of phospholipids that are a major constituent of cell membranes. Choline is a precursor of the neurotransmitter acetylcholine found in living organisms. Their presence is related to several life phenomena and diseases, and it is important to be able to measure the ratio of these molecules.

Typically, reverse-phase chromatography is used for analyzing choline and acetylcholine. Due to their hydrophilic nature, an ion-pair reagent is utilized for the mobile phase. A post-separation affinity column modified with choline oxidase / acetylcholine esterase, as well as, an electrochemical detector for monitoring hydrogen peroxide are needed to enable highly sensitive analysis. In order to simplify the analysis, a new LC/ESI-MS method was studied.

We have found that an ion chromatography column, Shodex IC YS-50, with nitric acid aqueous solution / acetonitrile as eluent is useful for this analysis. Peak shapes of choline and acetylcholine were sharp with baseline separation, and the calibration curves were linear. Both detection limits were 0.005 μM.

Moreover, it has been demonstrated that this method is applicable for various amine or amino acid neurotransmitters.

Our described LC/MS method should be more facile with higher selectivity than the previous method.

Shodex IC YS-50

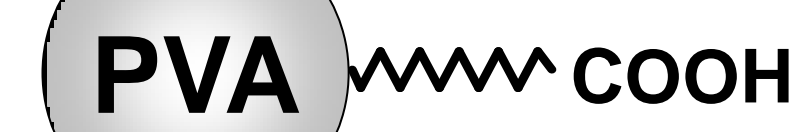
Column for Ion Chromatography (Cation Analysis)

Product specifications and Line up

Packing material : Poly vinyl alcohol particle with carboxyl group
Housing : Stainless steel
Usable temp. : 20~60 °C (recommend 25~40 °C)
Usable pH range : 2~12
Max flow rate : 2.0 mL/min
Max pressure : 15 MPa
Organic modifier : ≤50 volume% Acetonitrile, but no Methanol

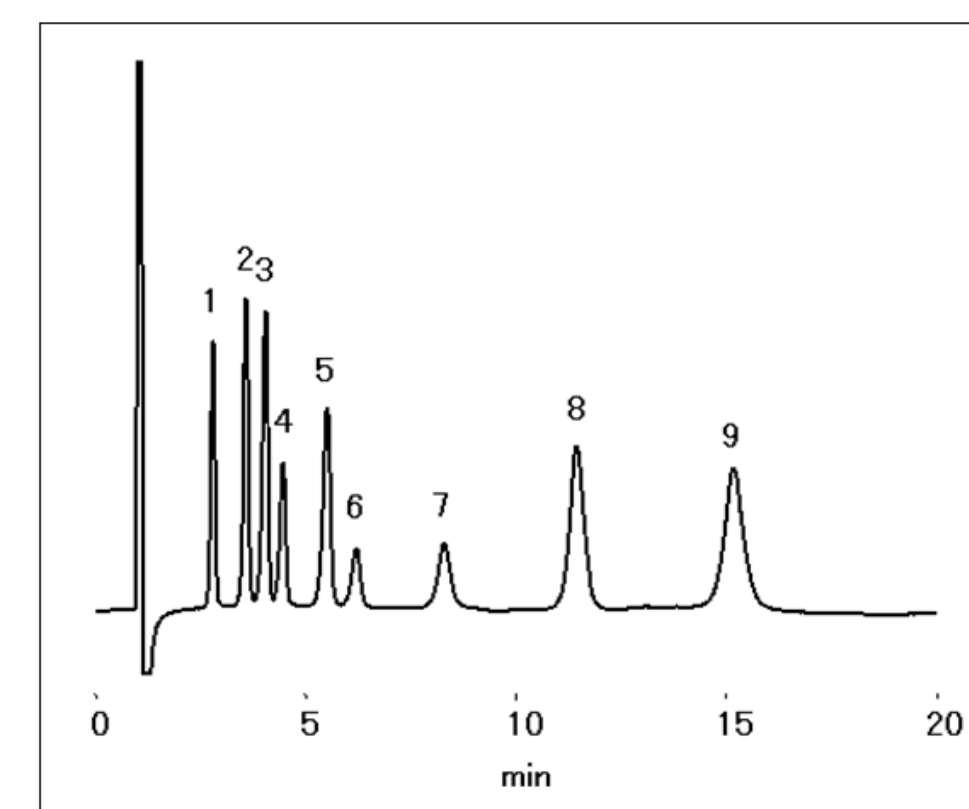
Polymer based

Wide pH range



Product name	Plate Number (TP/Column)	Particle Size (μm)	Column Size I.D. x Length (mm)
IC YS-50	≥ 3,500	5	4.6 x 125
IC YS-G	(guard column)	5	4.6 x 10

Chromatogram of various cations



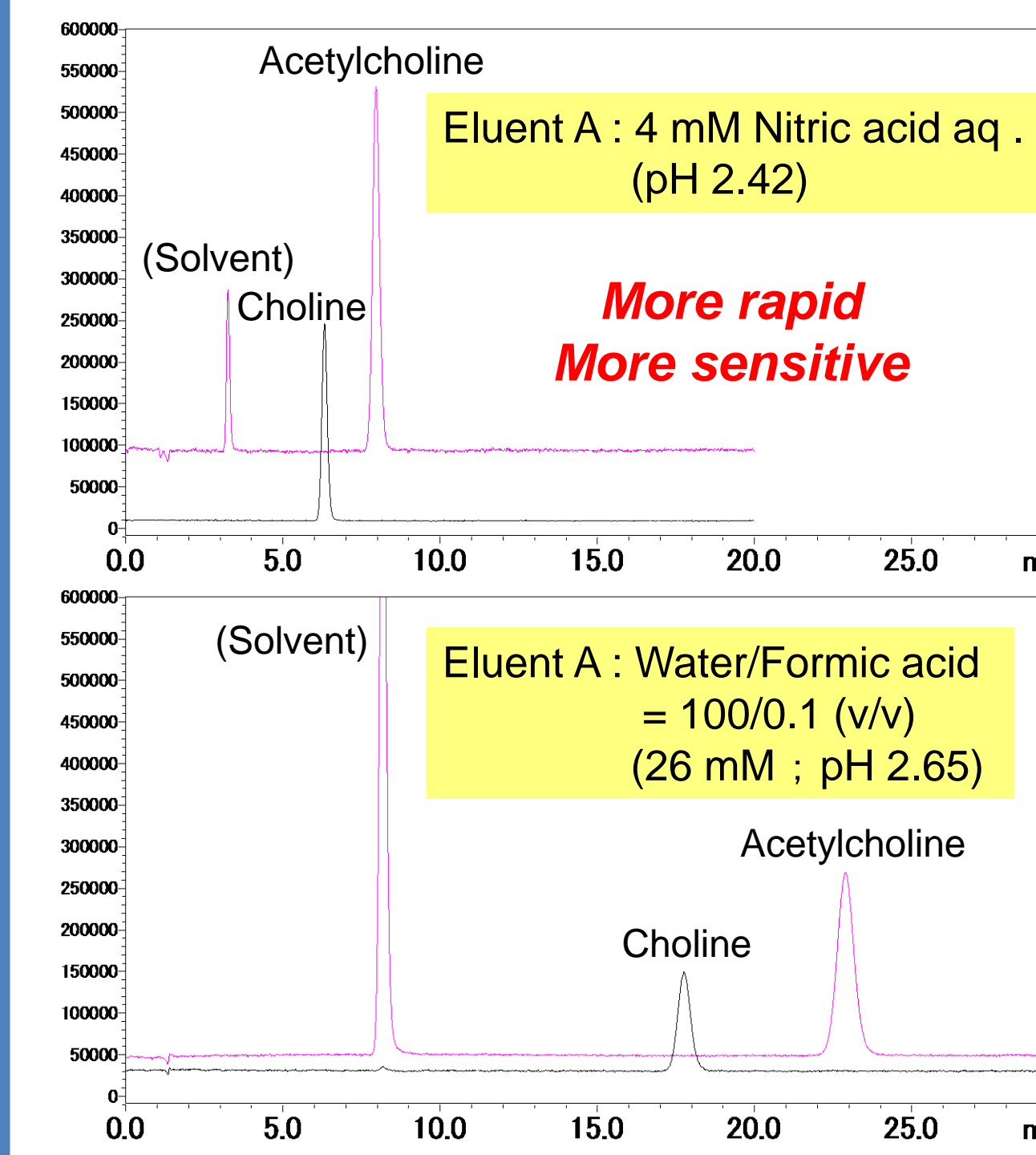
Sample : 10μL
1. Li⁺ 2mg/L
2. Na⁺ 10mg/L
3. NH₄⁺ 10mg/L
4. Methylamine 10mg/L
5. K⁺ 20mg/L
6. Trimethylamine, TMA 20mg/L
7. Triethylamine, TEA 20mg/L
8. Mg²⁺ 10mg/L
9. Ca²⁺ 20mg/L

Column : Shodex IC YS-50
Eluent : 4 mM Methanesulfonic acid aq.
Flow rate : 1.0 mL/min
Detector : Non-suppressed conductivity
Column temp. : 40 °C

- This column is applicable for not only inorganic cations but also organic amines.
- It is necessary to use volatile acid for LC/MS.

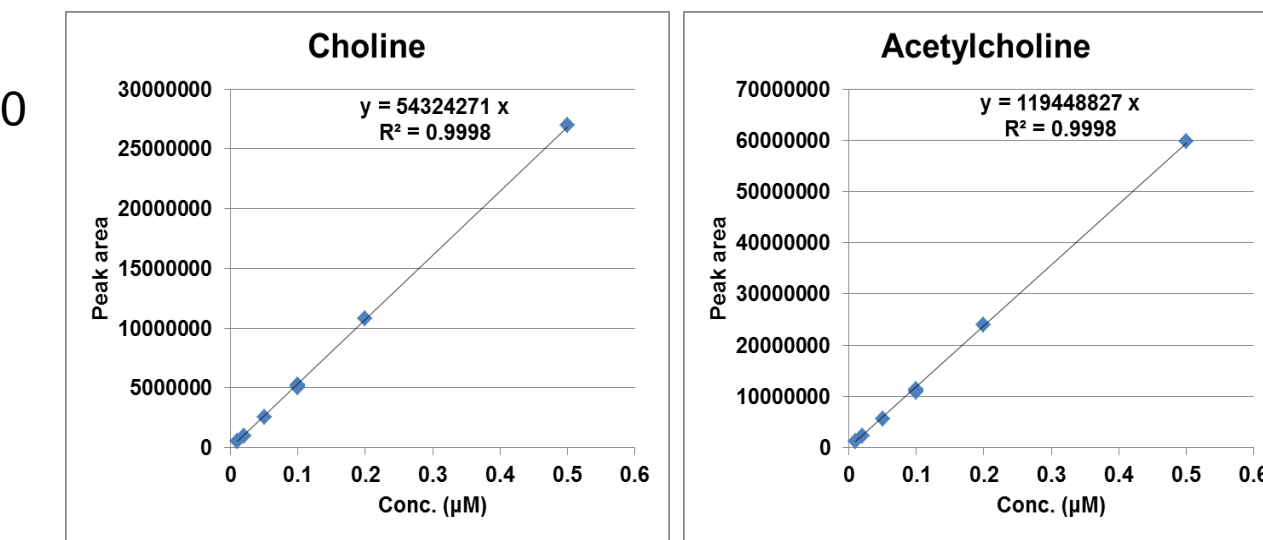
[Result 1] Analysis of choline and acetylcholine

Chromatograms



Sample : 1 μM each (in water), 20 μL
Instrument : Shimadzu Nexera / LCMS-8030
Column : Shodex IC YS-50
Eluent : (A) shown in data
(B) Acetonitrile
Isocratic ; (B%) 30%
Flow rate : 1.0 mL/min
Detector : ESI-MS (SIM)
Choline ; m/z104(+)
Acetylcholine ; m/z146(+)
Column temp. : 30 °C

Calibration curves

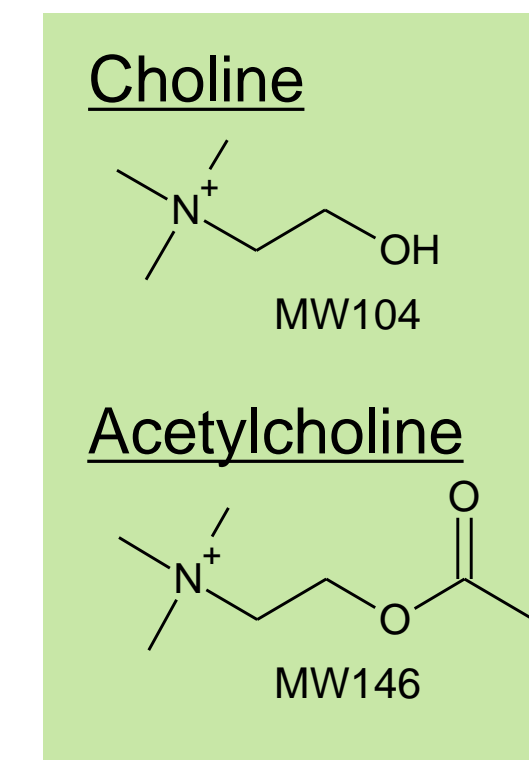


- High linearity
- High sensitivity (both 0.005 μM detectable)

Recovery rates for serum of guinea pig

	Recovery rates (%)	Standard amount spiked to serum
Choline	110	1 μM
Acetylcholine	103	0.1 μM

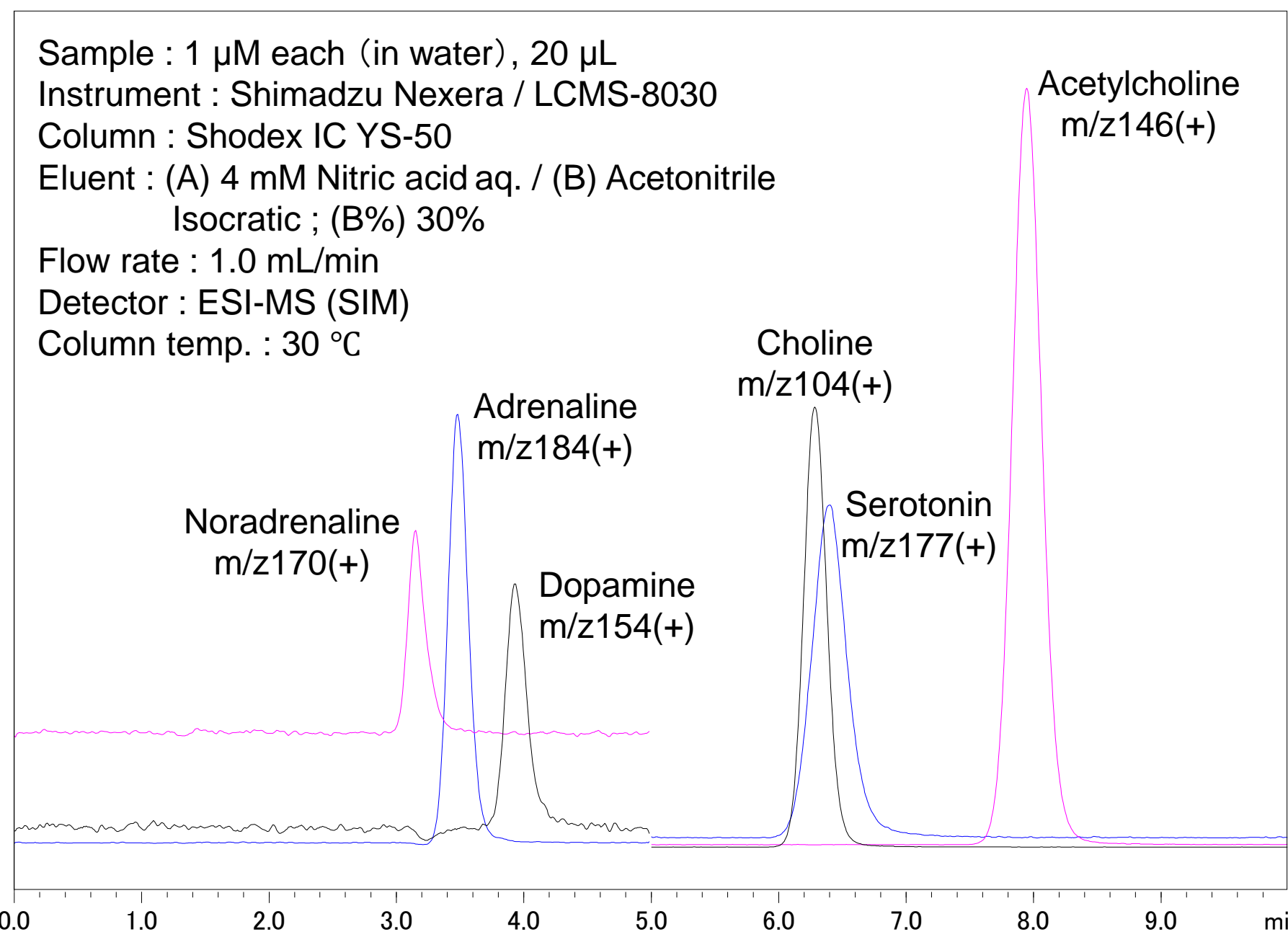
*The serum samples were analyzed after the deproteination by the addition of acetonitrile.
*The serum of guinea pig originally contained about 14 μM Choline.
*Since the serum contained cholinesterase, it was deactivated by heating before standard was spiked.



- Choline and acetylcholine were separated by cation exchange mode with hydrophobic interaction using mobile phase containing nitric acid.
- Addition of acetonitrile to mobile phase enables high sensitive analysis using LC/MS.
- This method is more simple and more sensitive than HPLC-ECD method.

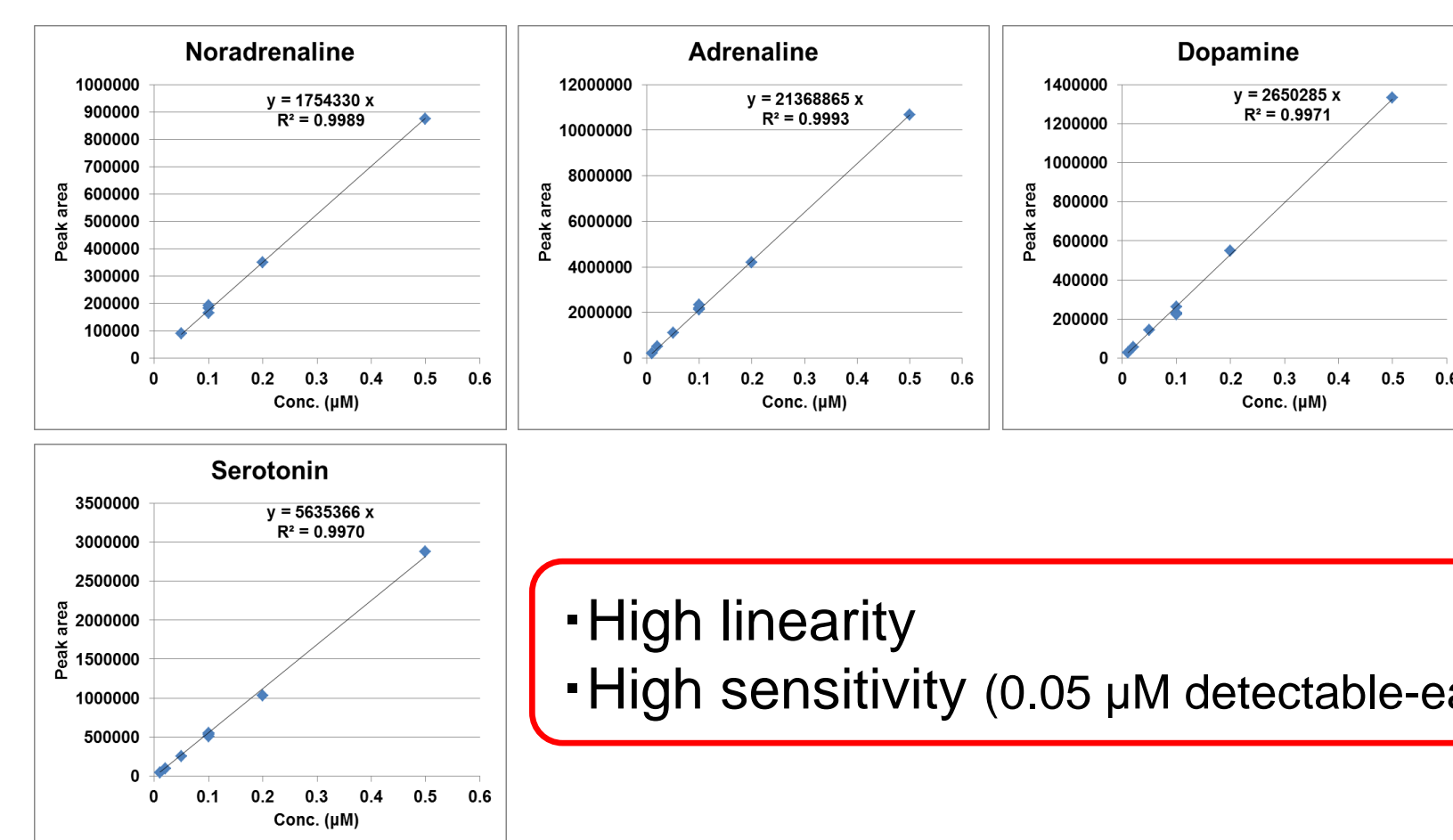
[Result 2] Simultaneous analysis of various amine neurotransmitters

Chromatograms (4 mM Nitric acid as Eluent A)



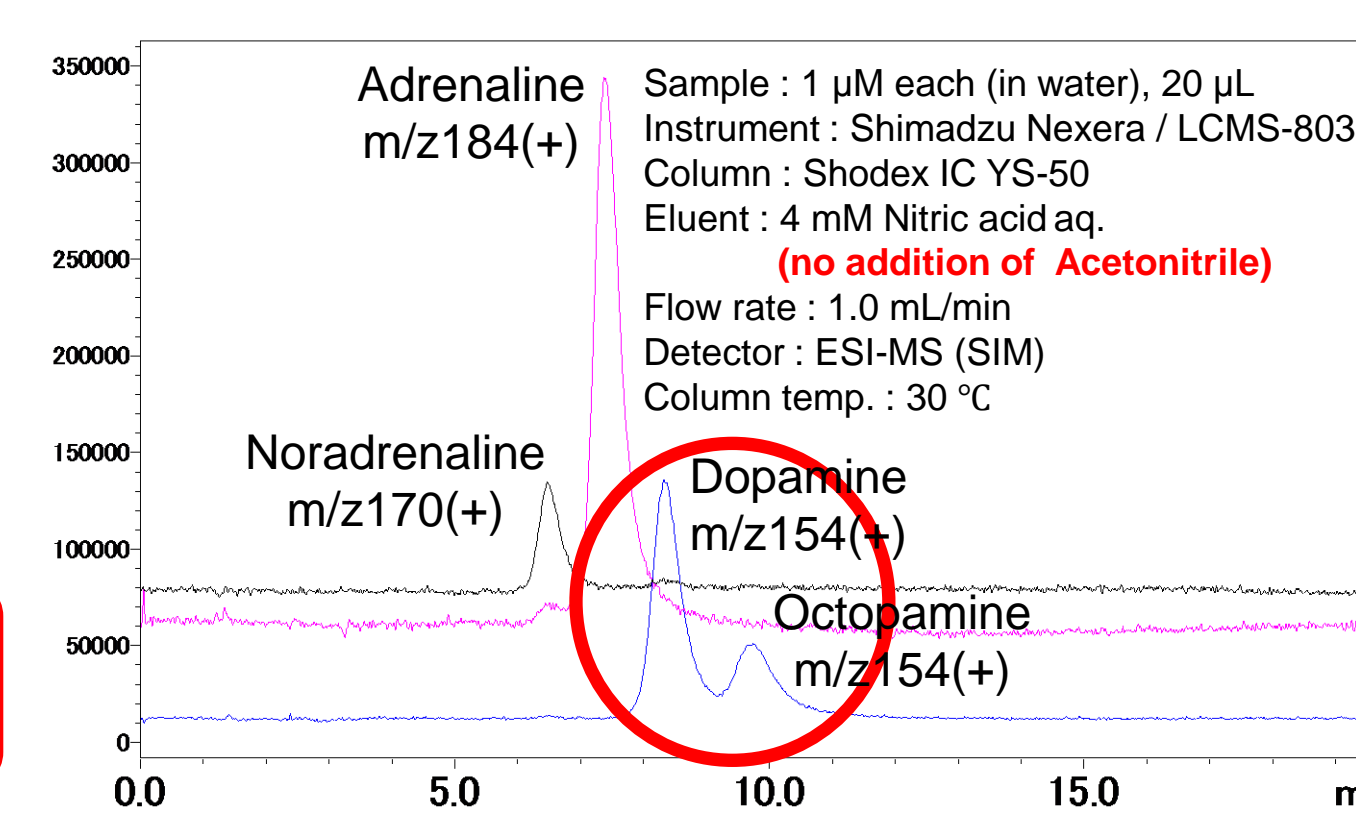
Sample : 1 μM each (in water), 20 μL
Instrument : Shimadzu Nexera / LCMS-8030
Column : Shodex IC YS-50
Eluent : (A) 4 mM Nitric acid aq. / (B) Acetonitrile
Isocratic ; (B%) 30%
Flow rate : 1.0 mL/min
Detector : ESI-MS (SIM)
Column temp. : 30 °C

Calibration curves

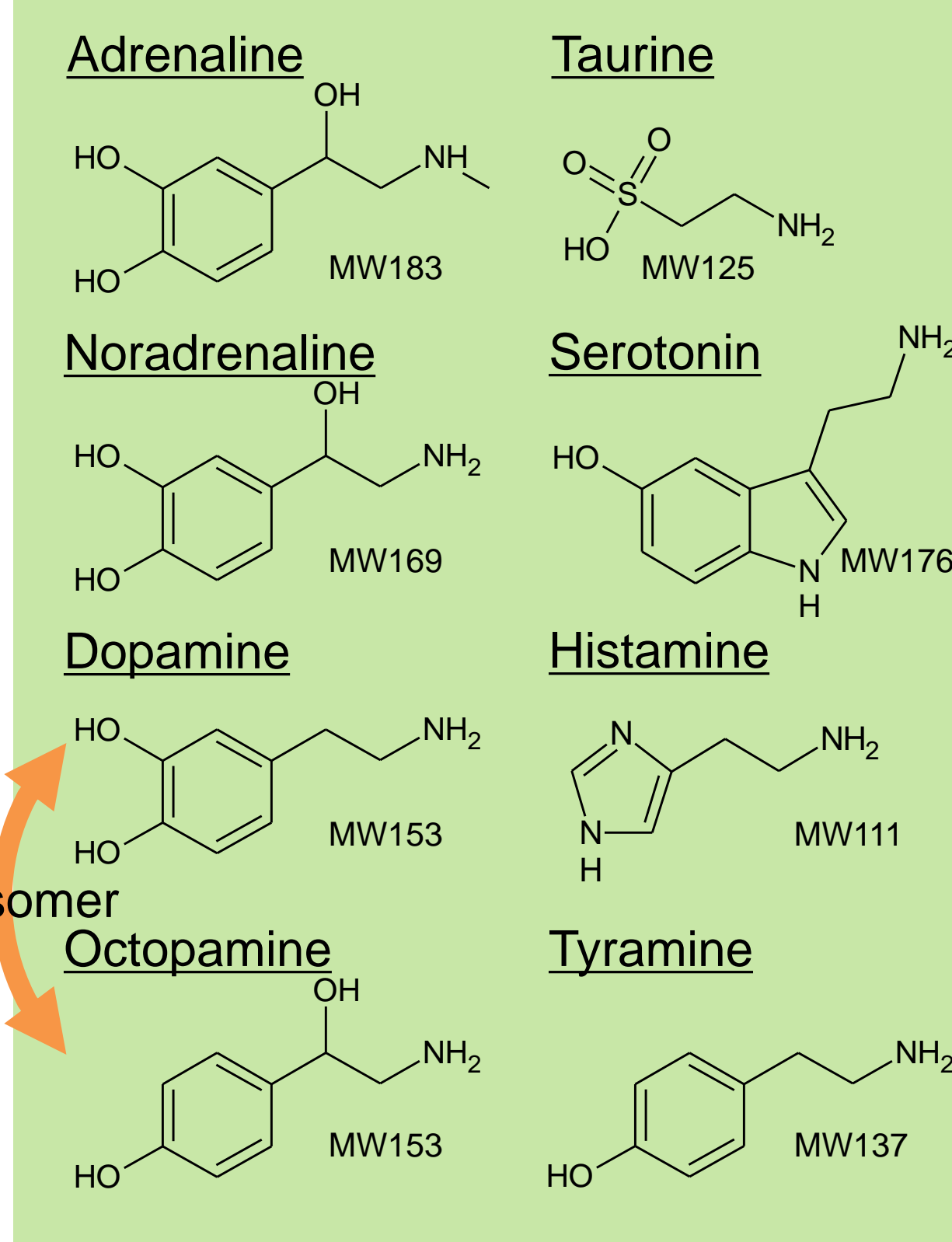


- High linearity
- High sensitivity (0.05 μM detectable-each)

Separation of dopamine and octopamine

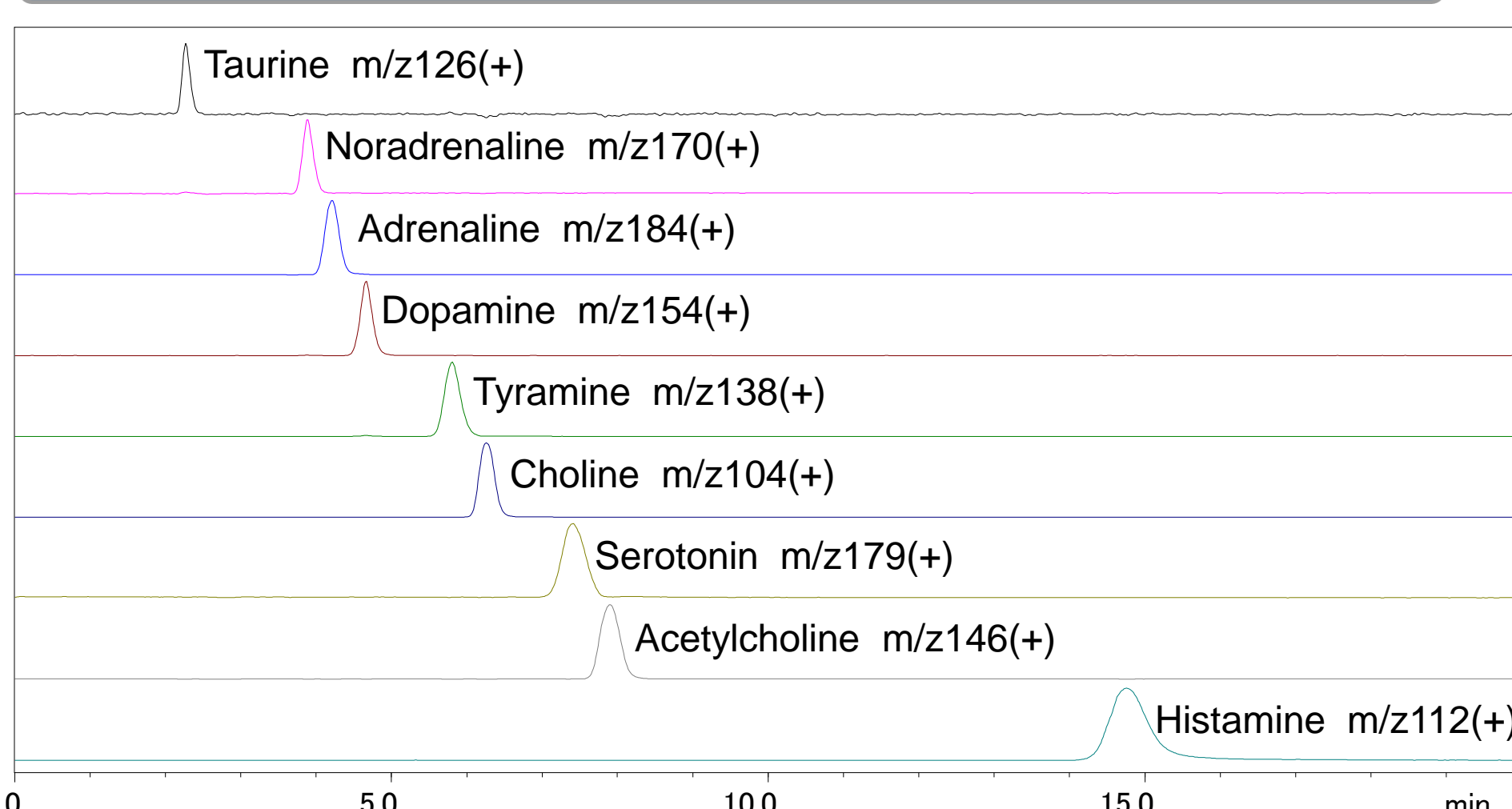


Sample : 1 μM each (in water), 20 μL
Instrument : Shimadzu Nexera / LCMS-8030
Column : Shodex IC YS-50
Eluent : 4 mM Nitric acid aq.
(no addition of Acetonitrile)
Flow rate : 1.0 mL/min
Detector : ESI-MS (SIM)
Column temp. : 30 °C



- Good separation was obtained.
- Choline and serotonin were not separated.
- The separation of dopamine and octopamine was improved without addition of acetonitrile to eluent.

Chromatograms (10 mM Nitric acid as Eluent A)



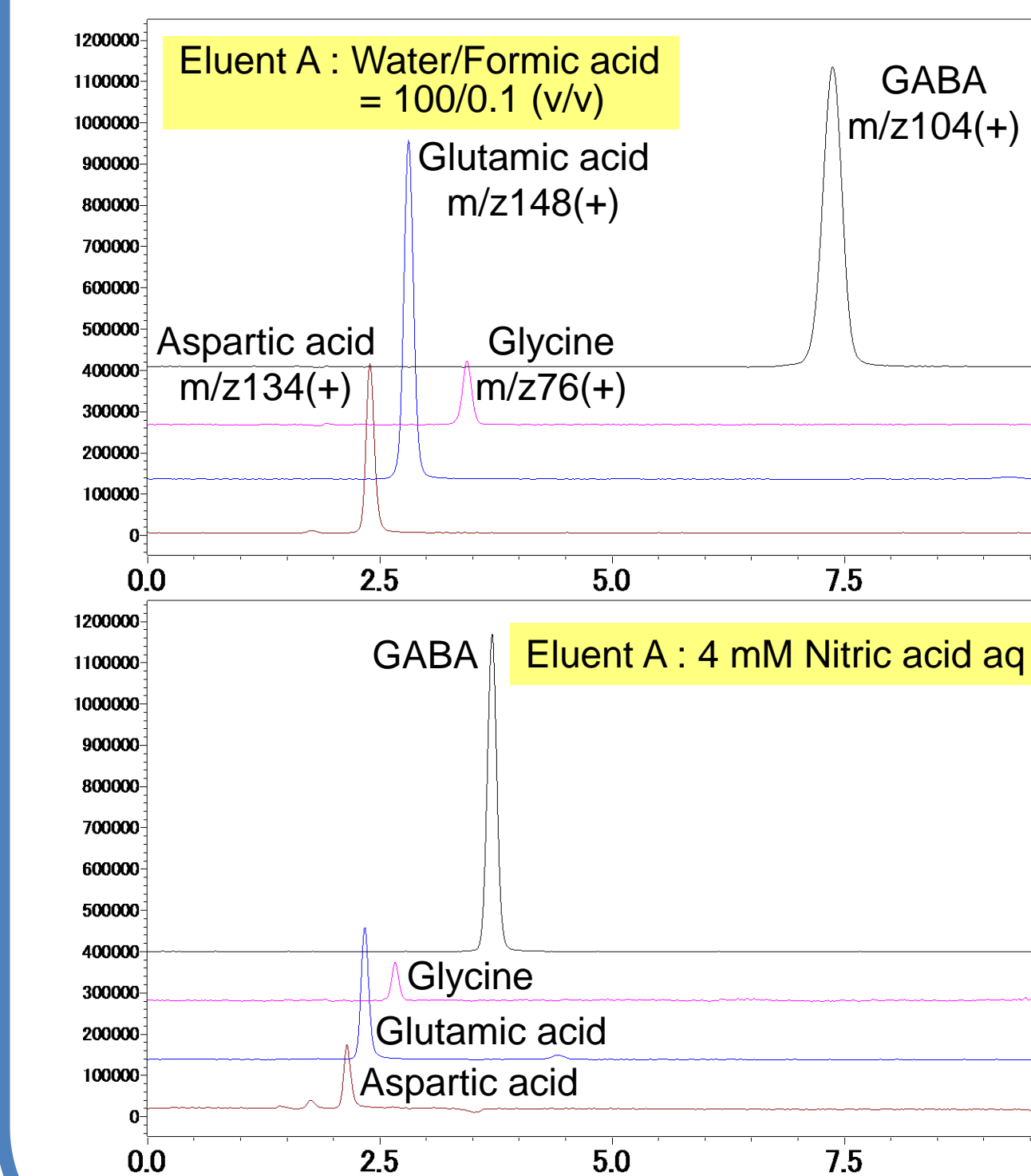
Sample : 1 μM each (in water), 20 μL
Instrument : Shimadzu Nexera / LCMS-8030
Column : Shodex IC YS-50
Eluent : (A) 10 mM Nitric acid aq. (pH 2.12) / (B) Acetonitrile
Isocratic ; (B%) 25%
Flow rate : 0.5 mL/min
Detector : ESI-MS (SIM)
Column temp. : 30 °C

Lower flow rate

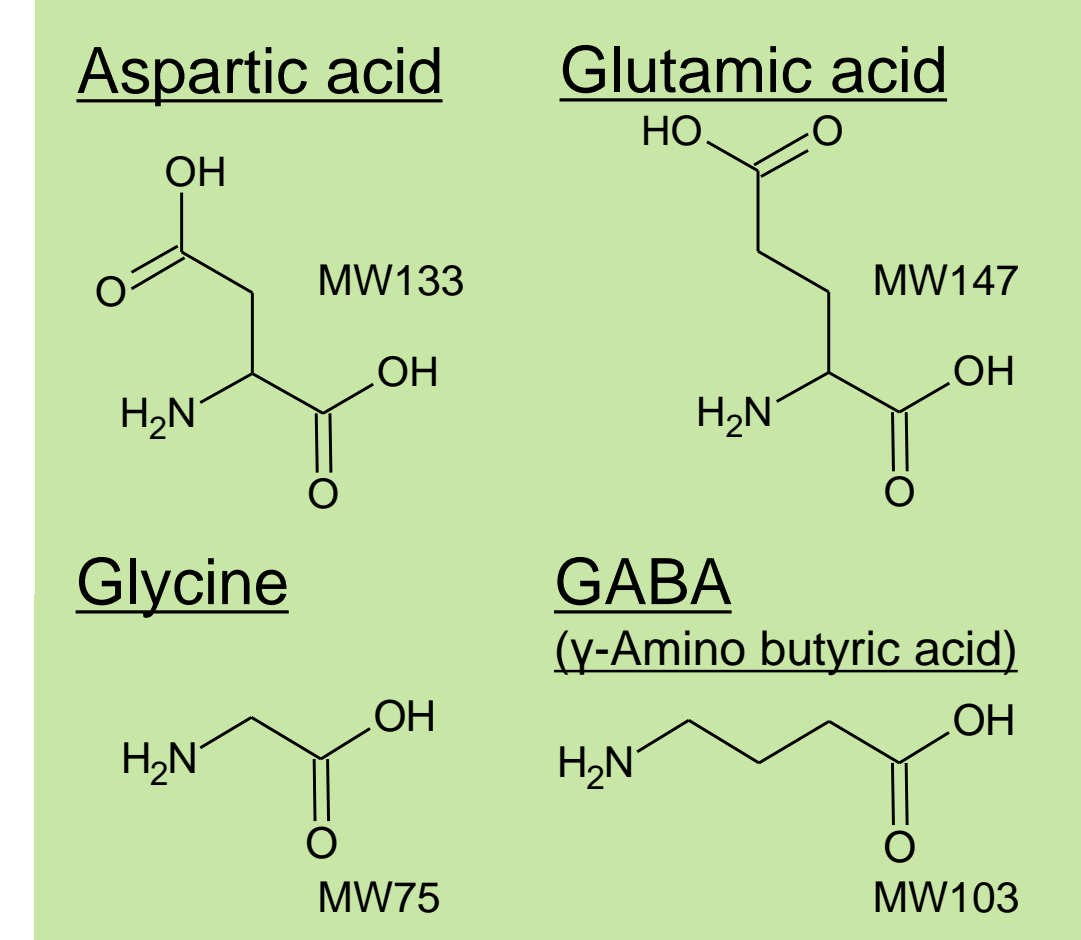
- Excellent separation was obtained.
- Lower flow rate is more effective for the sensitivity of ESI-MS detection.
- Taurine and histamine can be also separated with other amine neurotransmitters.

[Result 3] Analysis of amino acid neurotransmitters

Chromatograms



Sample : 100 ng/mL each (in water), 20 μL
Instrument : Shimadzu Nexera / LCMS-8030
Column : Shodex IC YS-50
Eluent : (A) shown in data
(B) Acetonitrile
Isocratic ; (B%) 20%
Flow rate : 0.8 mL/min
Detector : ESI-MS (SIM)
Column temp. : 30 °C



More sensitive

More rapid

- YS-50 is also useful for the analysis of amino acid neurotransmitters.
- As the acid added to eluent, formic acid is more suitable than nitric acid in terms of sensitivity.

Conclusion

Shodex IC YS-50, a polymer-based ion chromatography column coupled with ESI-MS is very feasible for the analysis of choline, acetylcholine, and other various neurotransmitters at low levels of concentration.

The presented method using the YS-50 column provides a simple and rapid analysis of neurotransmitters, and the eluent conditions used for ion chromatography are also applicable for ESI-MS measurements.