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Amino acids and metabolites/derivatives analysis without derivatization using a novel mixed-mode column

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INTRODUCTION

Amino acids and derived metabolites/derivatives are usually difficult to analyze without derivatization procedure due to poor retention on conventional reversed-phase columns.

Also it is difficult to use non-volatile ion-pairing reagent for LC-MS. Therefore, it is important to break-through this difficult separation issue by creating a new stationary phase for underivatized polar compounds.

We have focused attention on ion-exchange (IEX) mode due to the compounds structure which has positive charge under acidic conditions, and we have succeeded to develop a specialized amino analysis column for LC-MS using normal-phase(NP) and IEX mixed mode.

Also we found that this mixed-mode column can analyze not only amino acids but also derived metabolites.

METHOD

We used a NP+IEX column, Intrada Amino Acid (Imtakt Corp.), to analyze amino acids and metabolites/derivatives using LCMS-2020 (Shimadzu Corp.).

RESULTS AND DISCUSSION

We found that not only amino acids but also polar metabolites and derivatives were directly analyzed using developed columns with various column dimensions. We got good results about the following analytical subjects:

Fig. 5	20 free amino acids	Serum amino acids
Fig. 6, 7	Trp, Tyr, Glu, Gly	Neurotransmitters
Fig. 8	Glu	GABA Isomers
Fig. 9	Phe, Tyr, Trp	Biogenic Amines
Fig.10	His, Orn, Lys, Arg	Biogenic Amines
Fig.11	Tyr	Parkinson's Disease
Fig.12	Lys, Met	Carnitine Biosynthesis
Fig.13	His	Skeletal Muscle Breakdown
Fig.14	Met	Hyperhomocysteinemia
Fig.15	Met	SAM (S-Adenosyl methionine)
Fig.16	Trp	Kynurenine Pathway

CONCLUSION

This novel separation column will be a powerful tool for amino acids, metabolites and derivatives LC-MS analysis in many biochemistry applications.

1



Amino acid charge

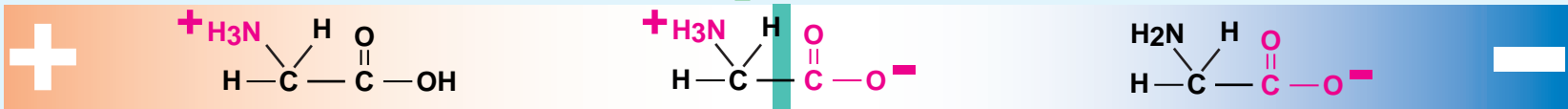
pI: Isoelectric Point

Neutral Side Chain

glycine (Gly / G)

Positive Charge

pI 6



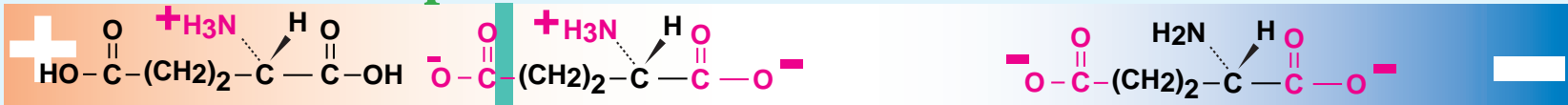
Low pH

High pH

Acidic Amino Acid

L-glutamic acid (Glu / E)

pI 3.2



Basic Amino Acid

L-lysine (Lys / K)

pI 9.8

塩基性アミノ酸



2

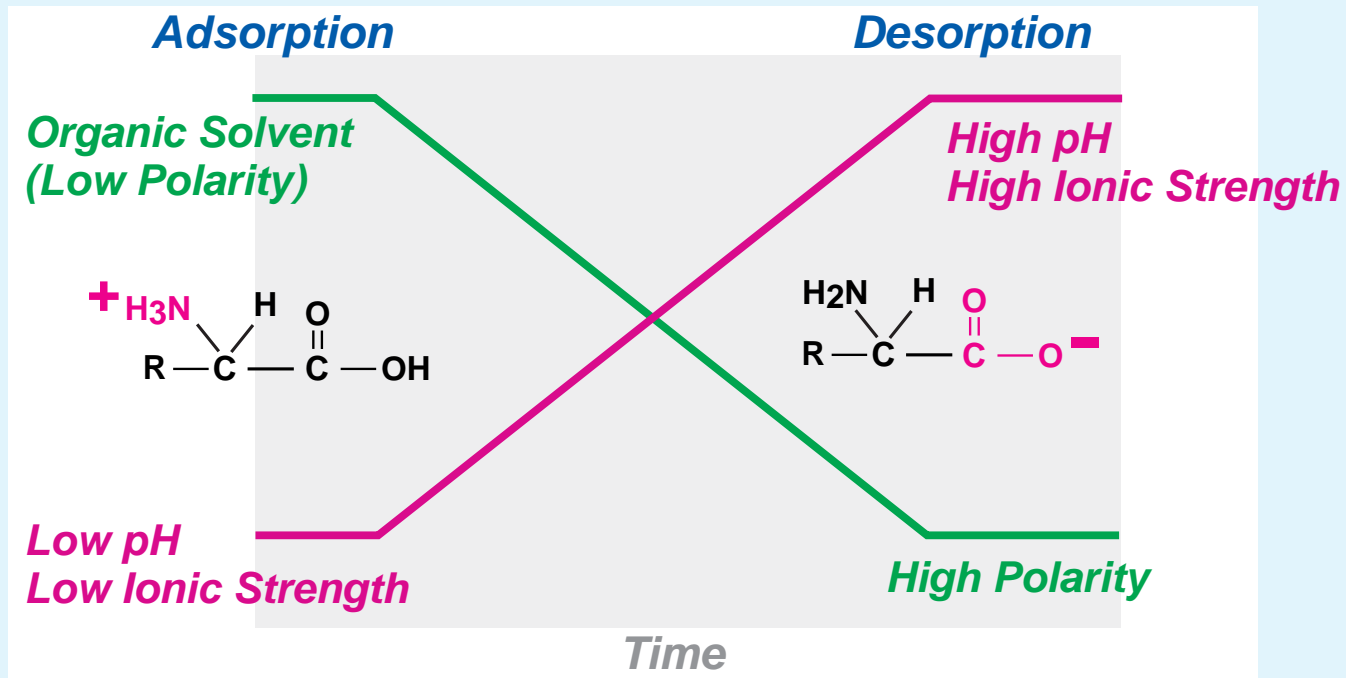


Amino Acid Analysis Column for LC-MS

 **Imtakt**

Intrada Amino Acid

*Mixed-mode
Normal Phase + Ion Exchange*



LC-MS analysis for 55 Amino Acids

Intrada Amino Acid, 50 x 3 mm

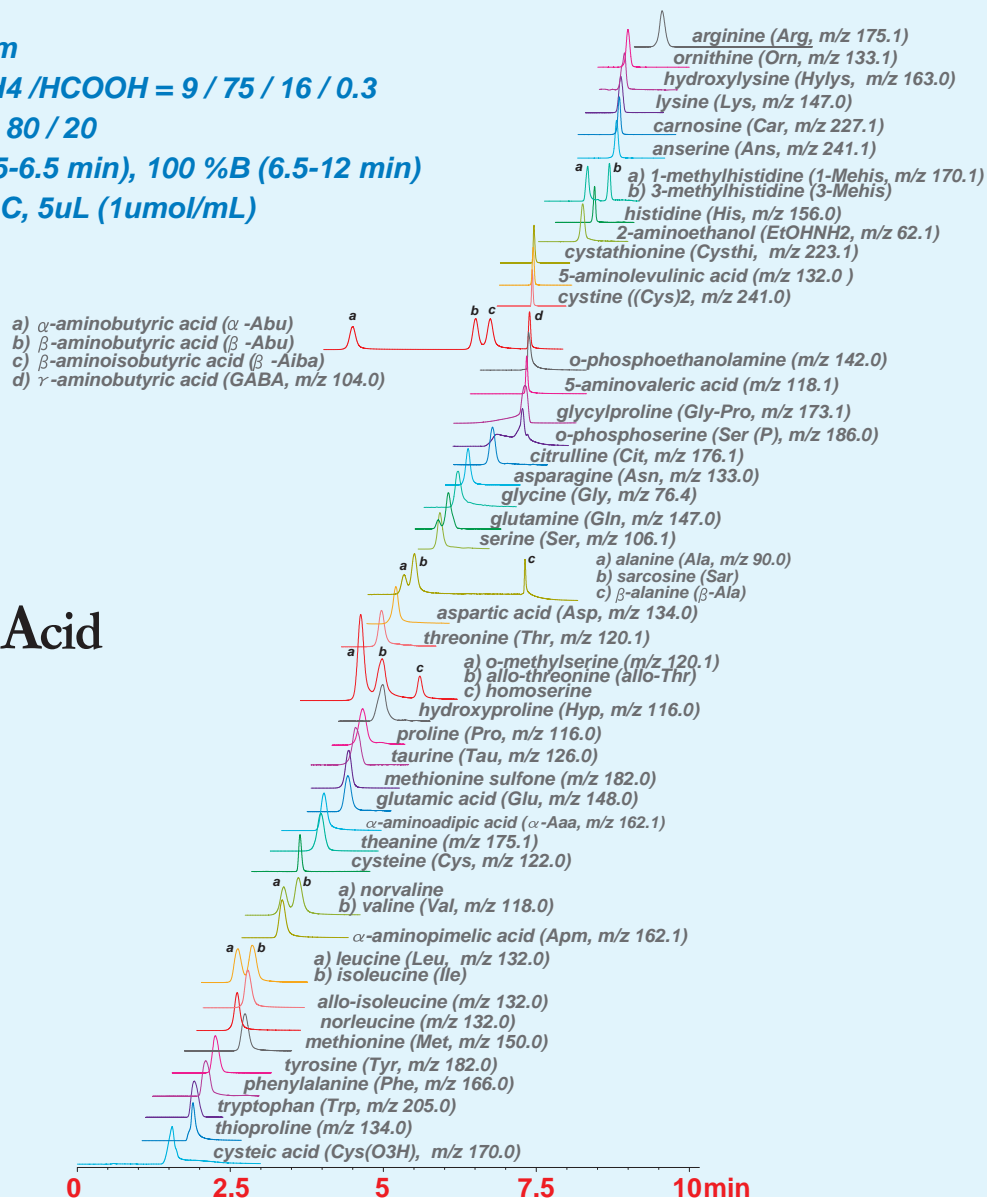
A: ACN / THF / 25mM HCOONH₄ / HCOOH = 9 / 75 / 16 / 0.3

B: 100mM HCOONH₄ / ACN = 80 / 20

0 %B (0-2.5 min), 0-17 %B (2.5-6.5 min), 100 %B (6.5-12 min)

0.6 mL/min (10 MPa), 35 deg.C, 5uL (1umol/mL)

ESI (SIM, Positive)

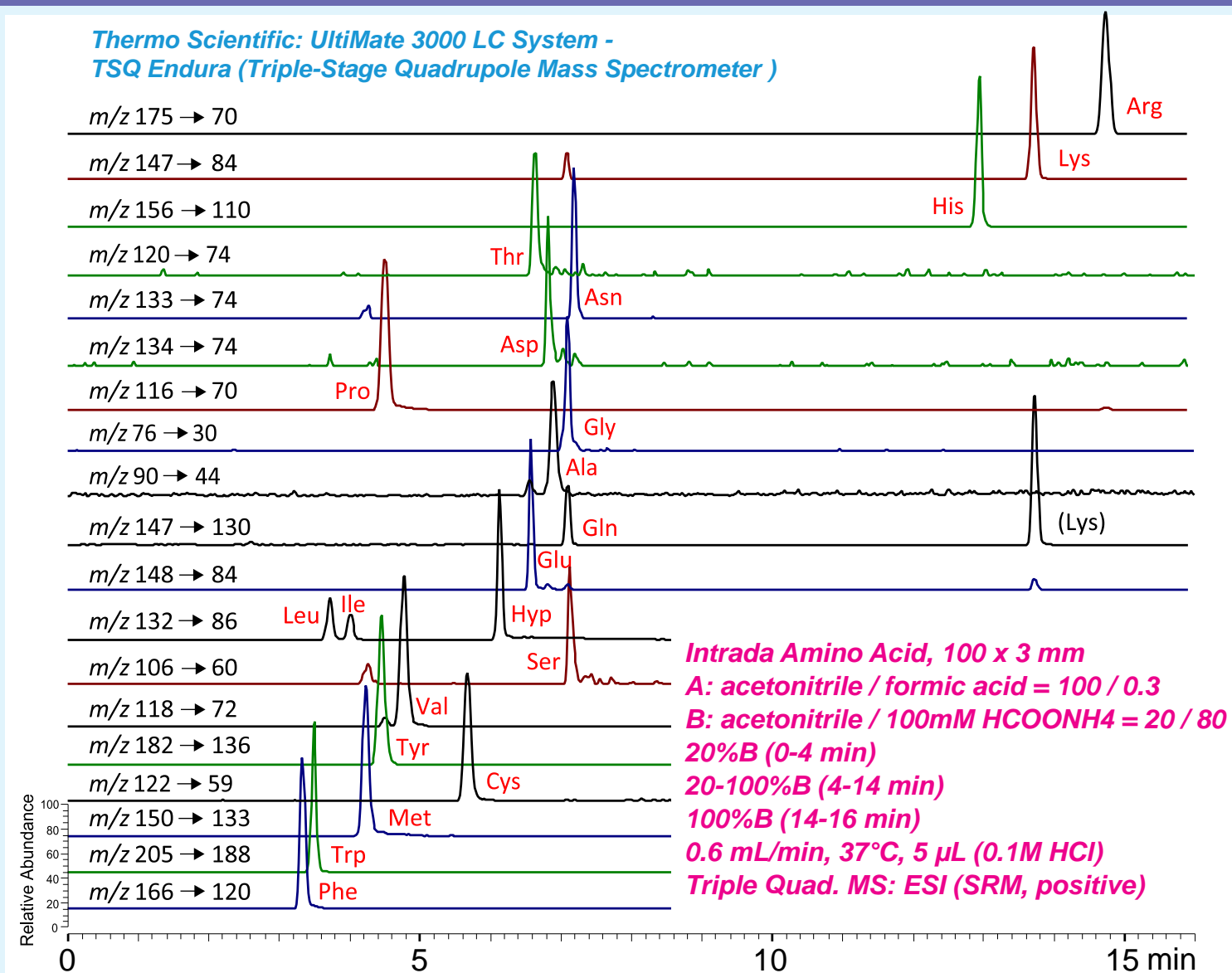


Intrada Amino Acid

4



Amino Acid Analysis on LC-MS/MS



Courtesy of Dr. Kuniko MITAMURA, KINDAI UNIVERSITY, JAPAN

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Amino acids in human serum (SSA)

Human Serum (commercial reagent)

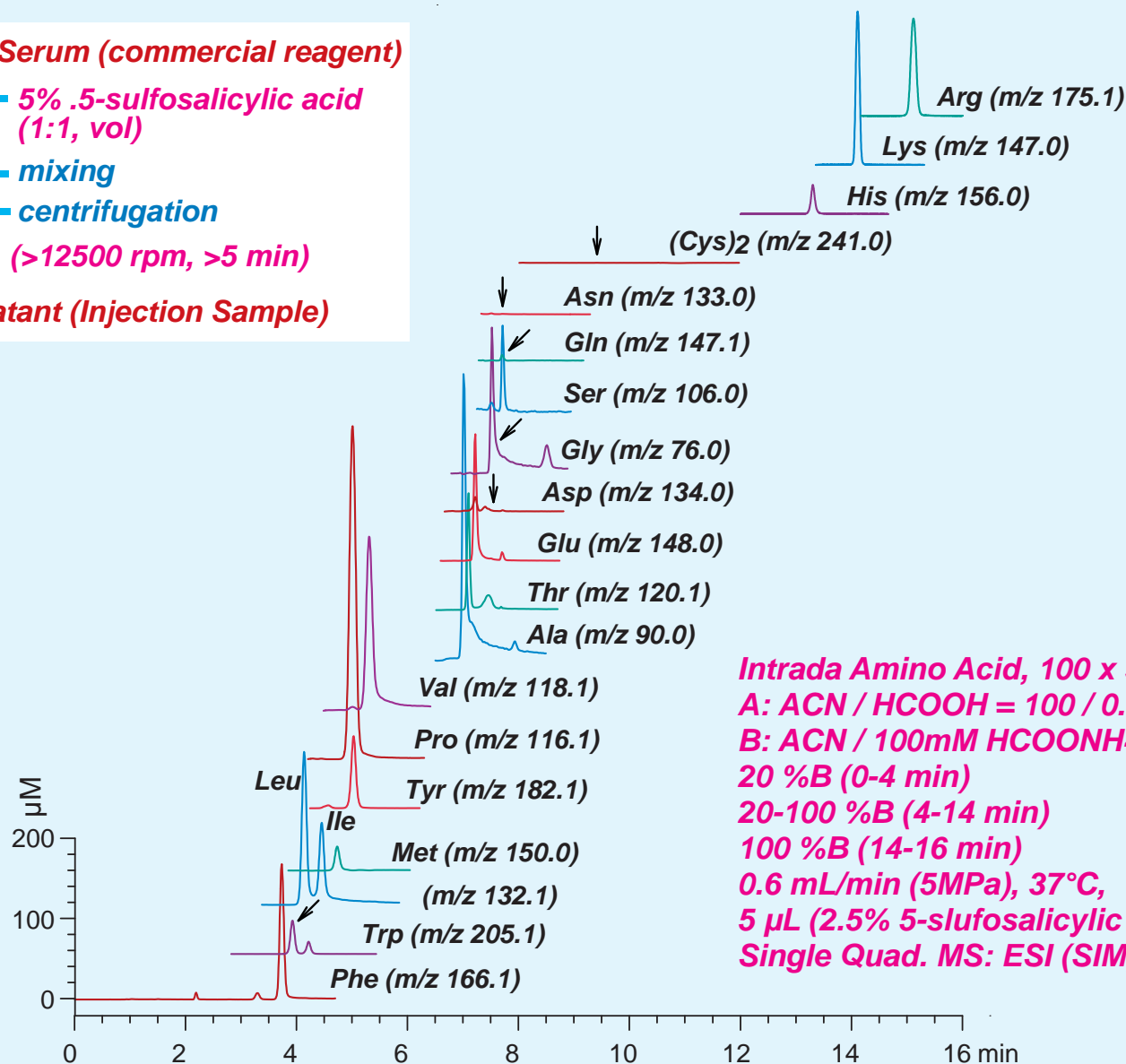
← 5% 5-sulfosalicylic acid
(1:1, vol)

← mixing

← centrifugation

(>12500 rpm, >5 min)

Supernatant (Injection Sample)



Intrada Amino Acid, 100 x 3 mm

A: ACN / HCOOH = 100 / 0.3

B: ACN / 100mM HCOONH4 = 20 / 80

20 %B (0-4 min)

20-100 %B (4-14 min)

100 %B (14-16 min)

0.6 mL/min (5MPa), 37°C,

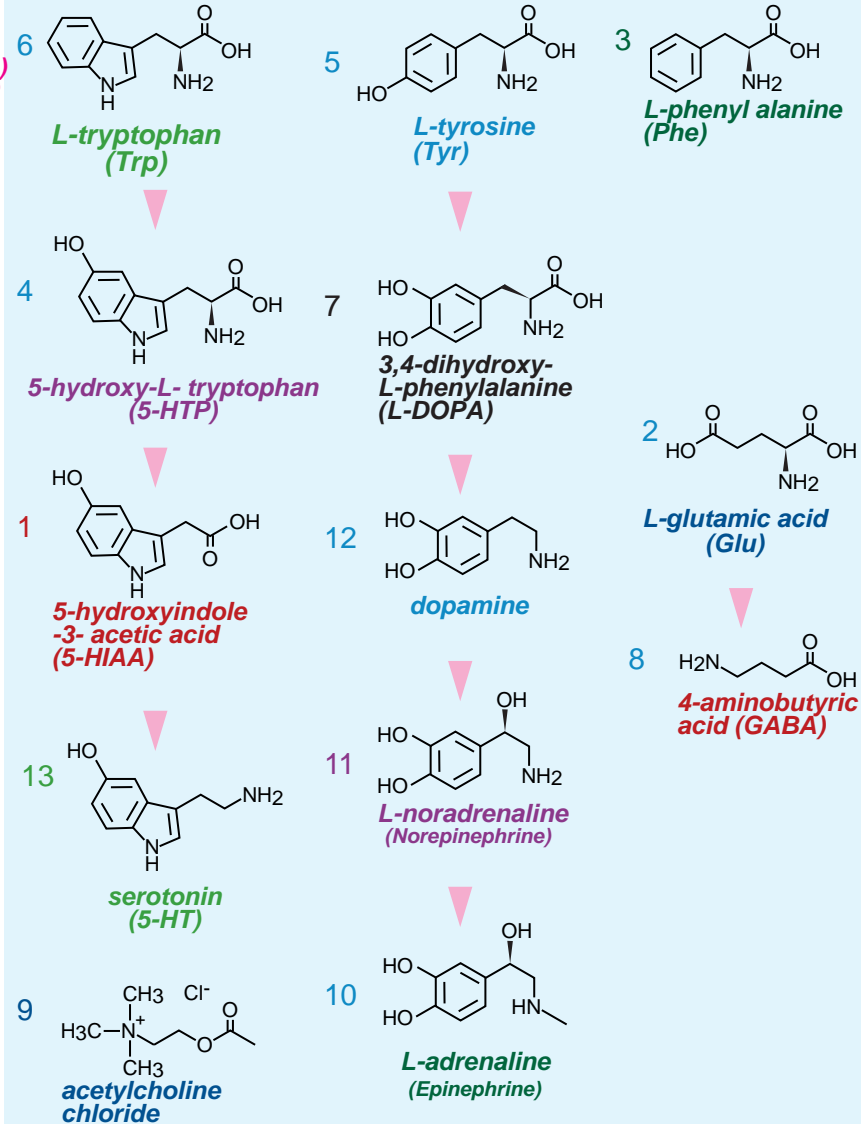
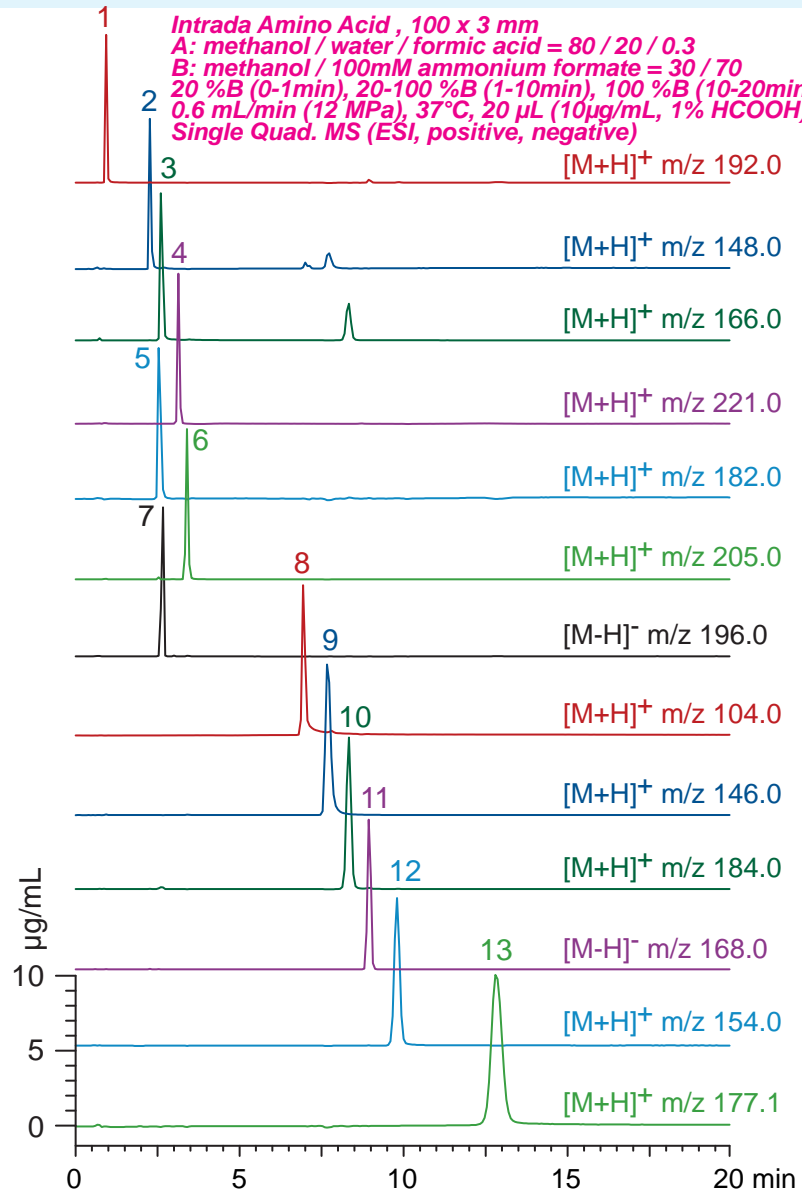
5 μL (2.5% 5-sulfosalicylic acid)

Single Quad. MS: ESI (SIM, positive)

6



Phe, Trp, Tyr, Glu, Gly - Neurotransmitters





LC-MS Analysis for amino acids and catecholamines in rat pituitary

Determination of Neuronal Peptides and Catecholamines and Effects of Diethylstilbestrol on Male Rat Pituitary

Naoyuki Maeda^{1,2}, Emi Tanaka¹, Kanae Masu¹, Kanako Okumura², Yuki Ikeda², Taku Miyasho², Satoko Haeno² and Hiroshi Yokota².

1) Japan Meat Science & Technology Institute

2) Rakuno Gakuen University

ACMS2013 (61st), Sept 10, 2013, Tsukuba, Japan

The separation was achieved using an Intra Amino Acid

(100 x 3 mm 3 μ m particle size, Imtakt)

(A) CH₃CN / THF / 25mM HCOONH₄ / HCOOH=10 / 80 / 10 / 0.4

(B) CH₃CN/100mM HCOONH₄ = 20/80

0 %B (0-1 min)

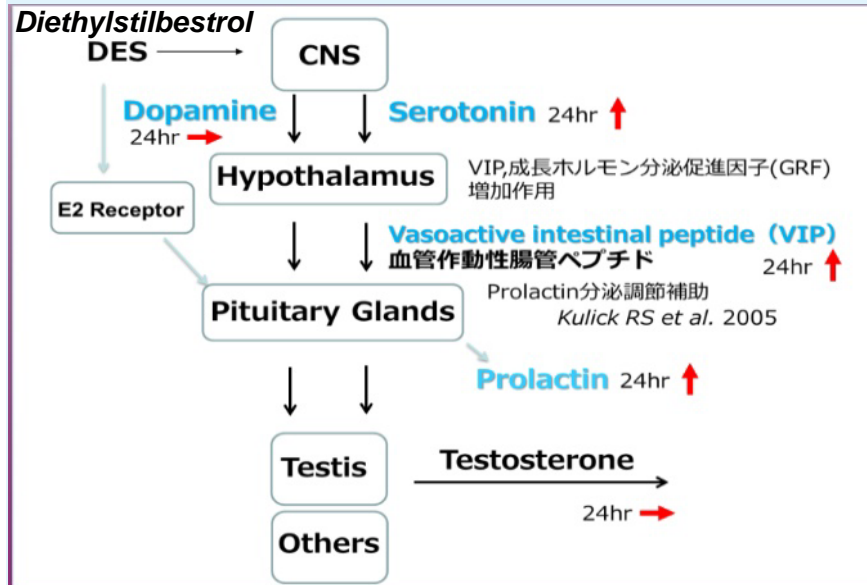
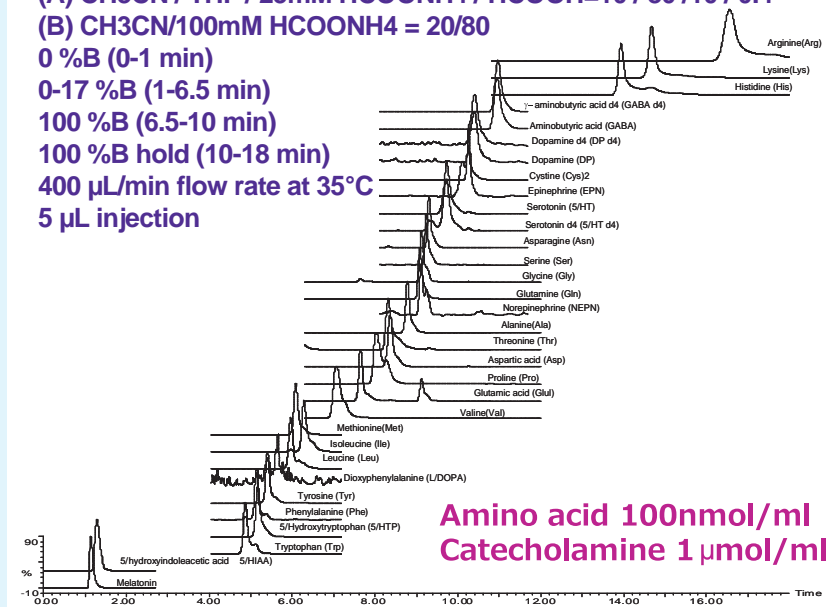
0-17 %B (1-6.5 min)

100 %B (6.5-10 min)

100 %B hold (10-18 min)

400 μ L/min flow rate at 35°C

5 μ L injection



Determination procedure for neuronal peptides and catecholamines were developed by MS analysis.

The regulatory factors for prolactin induction mediated by diethylstilbestrol were analyzed by LC-MS analysis using the selected reaction monitoring (SRM). Dopamine suppressing prolactin secretion did not decreased, on the other hand, vasoactive intestinal peptide (VIP) which mediates the acute release of prolactin was increased in the pituitary glands of the DES- treated rats.

Simultaneous Determination of the Neurotransmitters and Free Amino Acids in Rat Organs by LC-MS Analysis

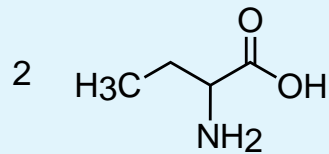
Naoyuki Maeda, Michiko Sato, Satoko Haeno and Hiroshi Yokota

Science Journal ROH, March, 201

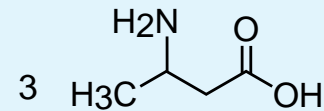
8



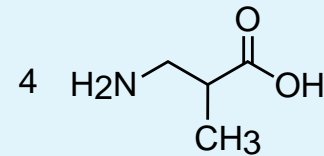
Glu - GABA Isomers



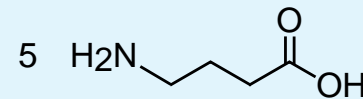
2-aminobutyric acid
(*alpha*-aminobutyric acid)



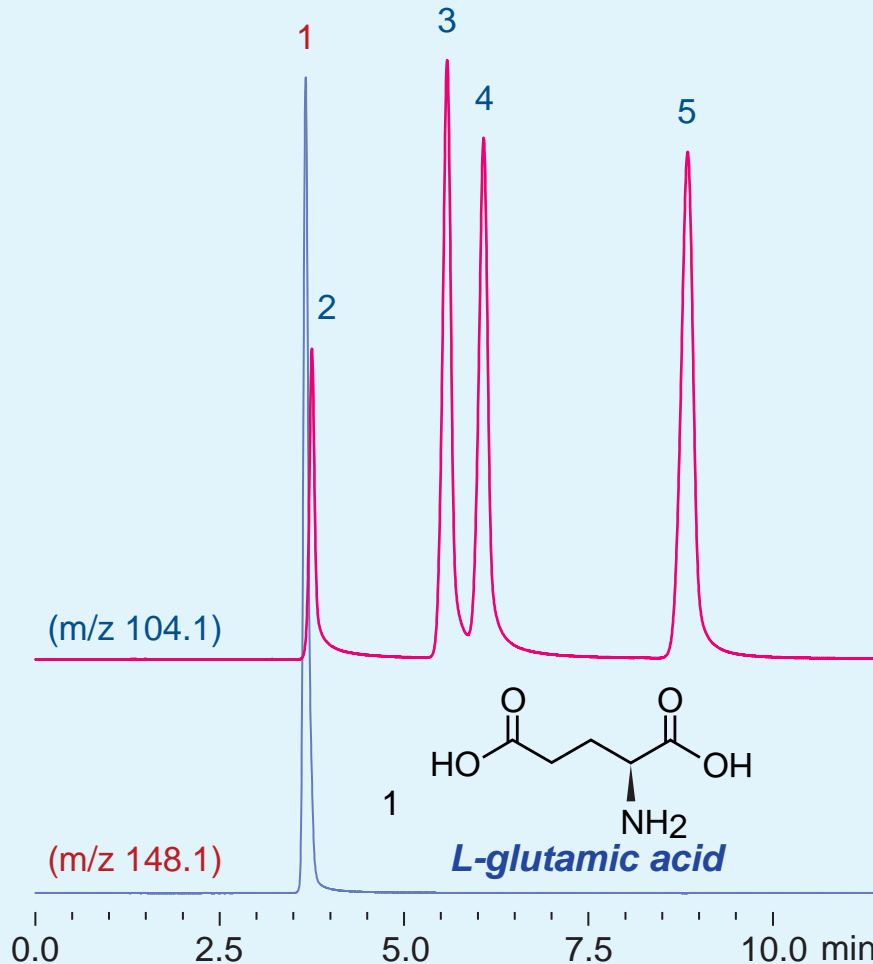
3-aminobutyric acid
(*beta*-aminobutyric acid)



3-aminoisobutyric acid
(*beta*-aminoisobutyric acid)



4-aminobutyric acid
(**GABA**,
gamma-aminobutyric acid)



150 x 3 mm

Intrada Amino Acid, 150 x 3 mm

A: ACN /HCOOH = 100 / 0.3

B: 100mM HCOONH₄

30-35 %B (0-12 min)

100 %B (12-15min)

0.5 mL/min (6MPa)

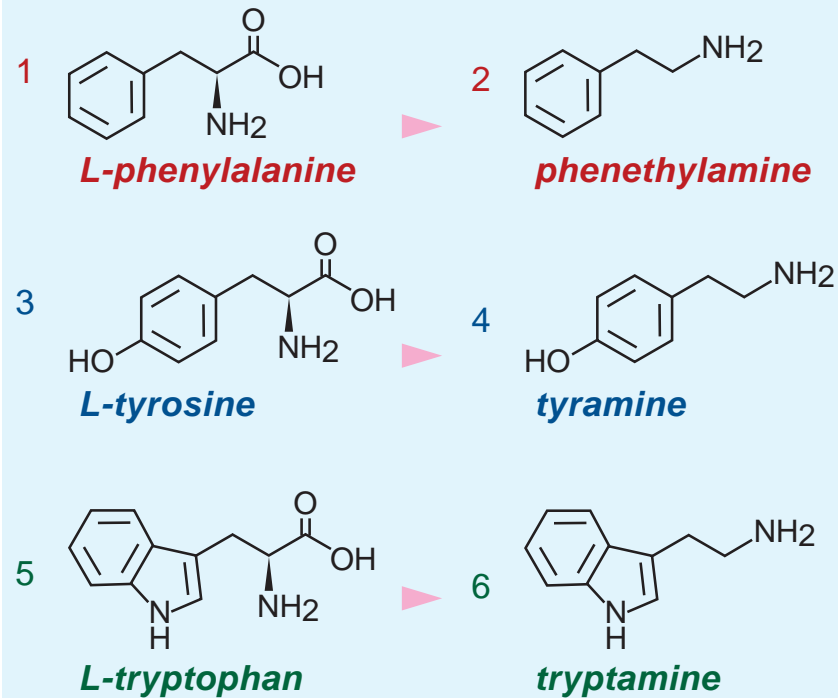
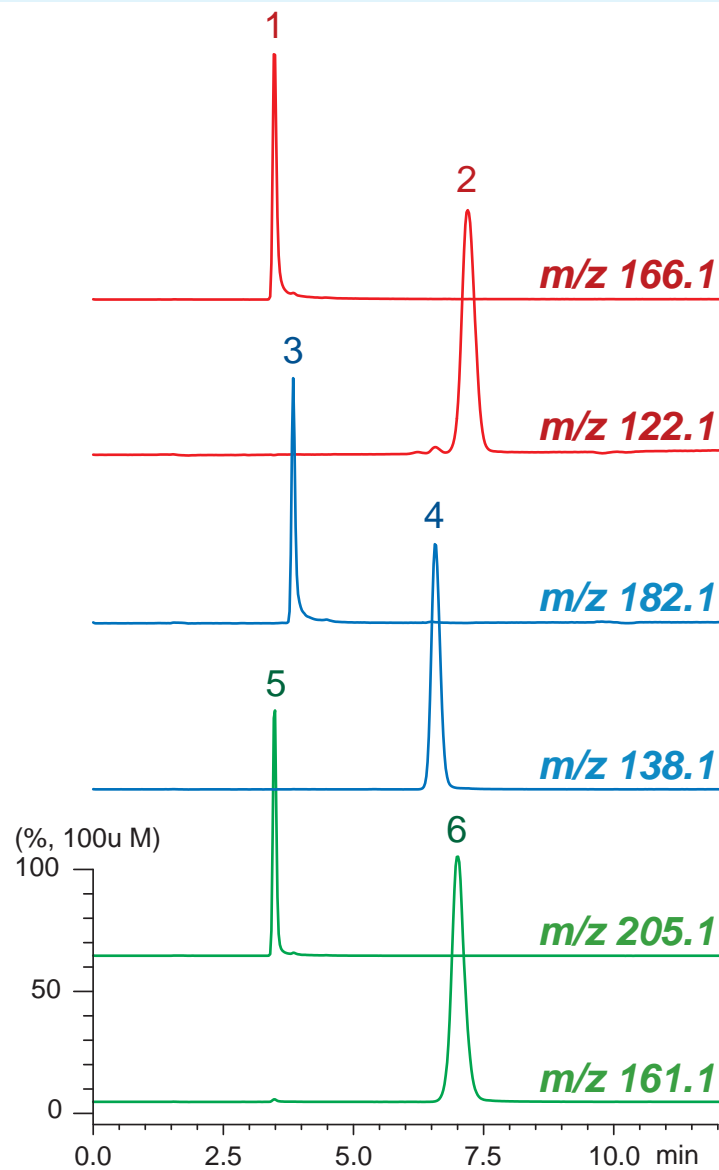
60 deg.C, 5 uL (1mM, 0.1N HCl)

ESI (SIM, positive)

9



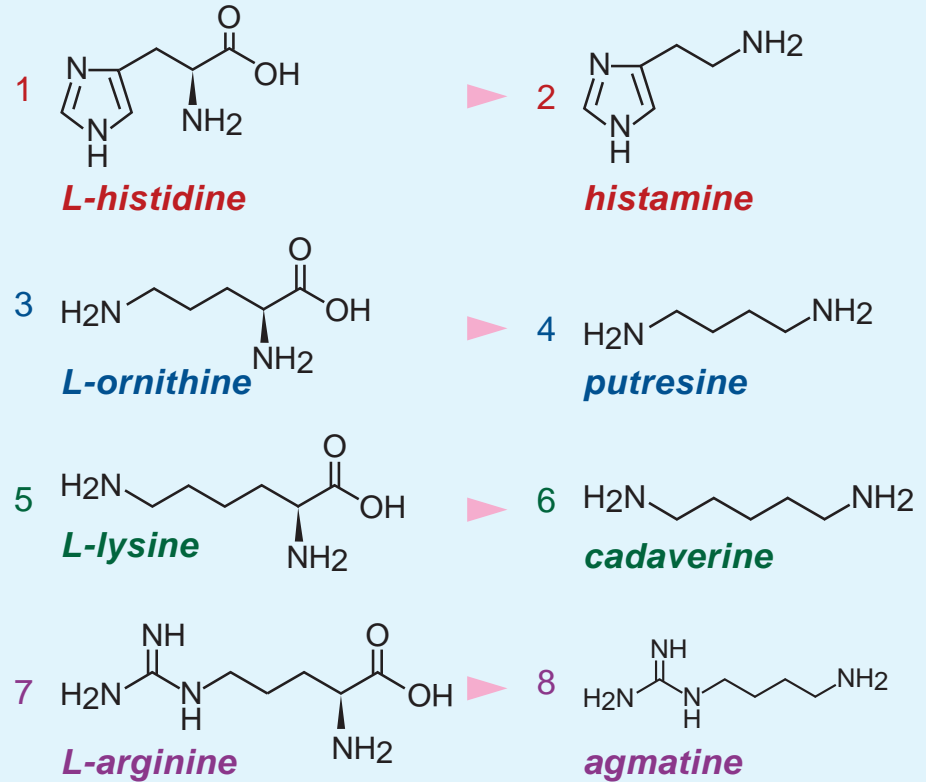
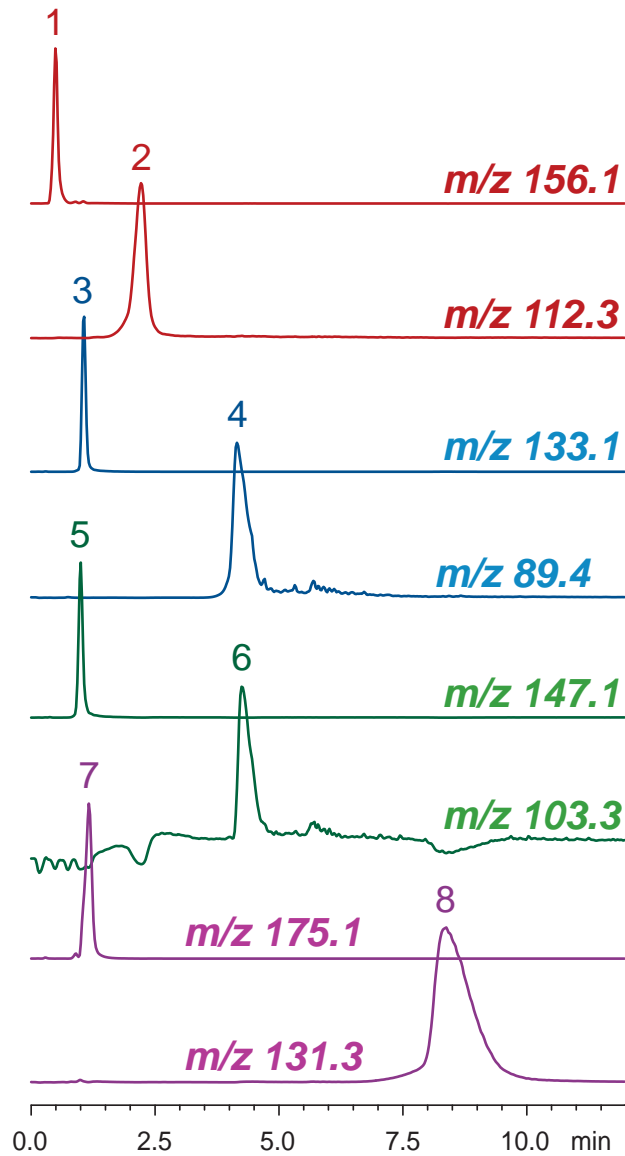
Phe, Tyr, Trp - Biogenic Amines



Intrada Amino Acid, 100 x 3 mm
A: acetonitrile / formic acid = 100 / 0.1
B: acetonitrile / 100 mM ammonium formate = 20 / 80
25-100 %B (0-10min), 100 %B (10-12min)
0.4 mL/min (4 MPa), 37°C
5uL (100nmol/mL, 0.1N HCl)
Single Quad. MS (ESI, positive)



His, Orn, Lys, Arg - Biogenic Amines



Intrada Amino Acid, 10 x 3 mm

A: methanol / formic acid = 100 / 0.1

B: 180 mM ammonium formate

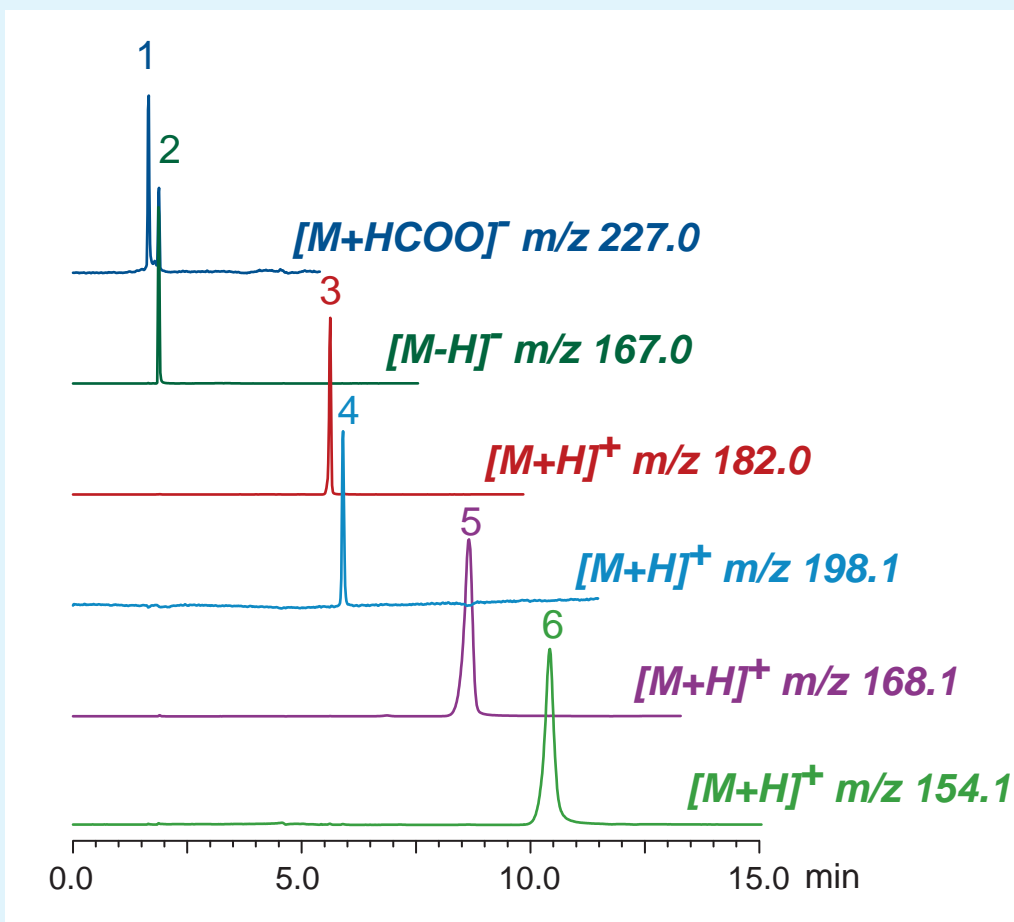
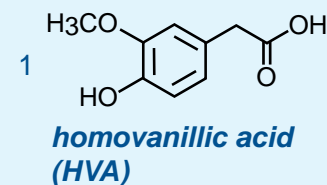
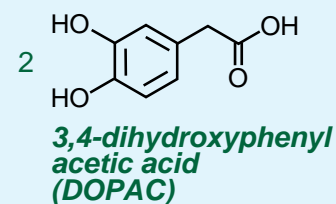
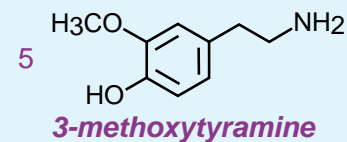
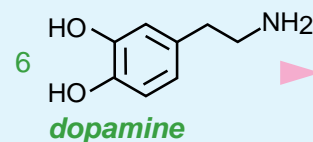
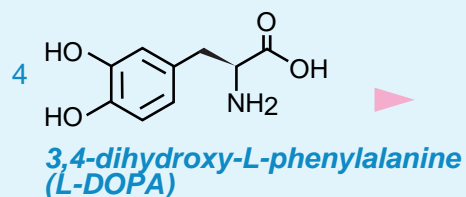
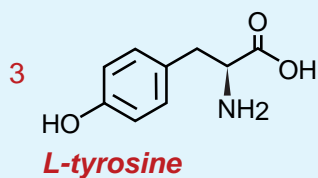
40-100 %B (0-2min), 100 %B (2-12min)

0.4 mL/min (3 MPa), 37°C, 15µL (500nmol/mL, 0.1N HCl)

Single Quad. MS (ESI, positive)



Tyr - Parkinson's Disease

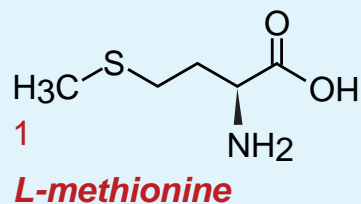


Intrada Amino Acid, 150 x 3 mm
A: acetonitrile / formic acid = 100 / 0.3
B: 100mM ammonium formate
10-100 %B (0-10min), 100 %B (10-15min)
0.5 mL/min (6 MPa), 37°C
20 μ L (10 μ g/mL, 1% HCOOH)
Single Quad. MS (ESI)

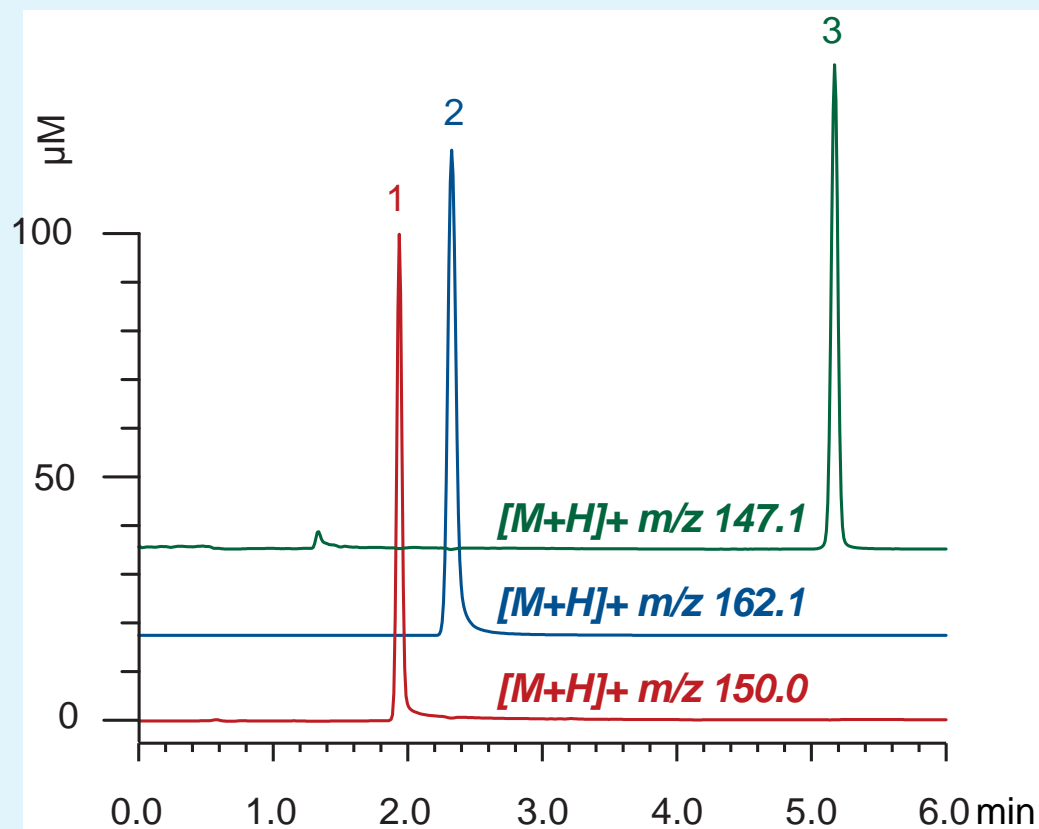
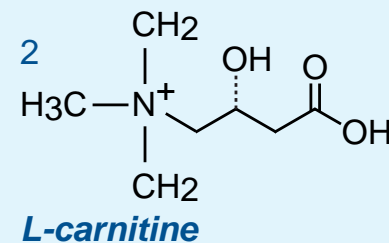
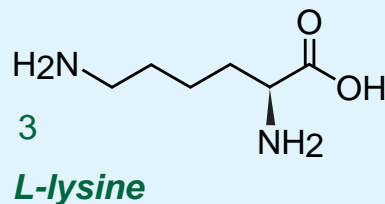
12



Lys, Met - Carnitine Biosynthesis



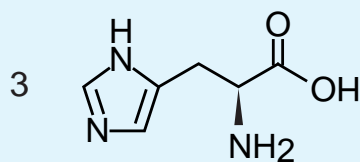
+



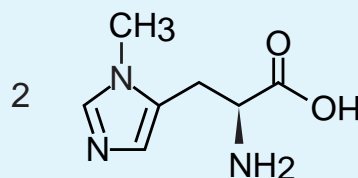
Intrada Amino Acid, 50 x 3 mm
A: acetonitrile / formic acid = 100 / 0.1
B: 100mM ammonium formate
15-100 %B (0-6min), 15 %B (6-10min)
0.6 mL/min (3 MPa), 37°C
5 μL (100nmol/mL, 1% HCOOH)
Single Quad. MS (ESI, positive)

13

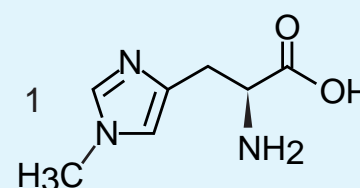
His - Skeletal Muscle Breakdown



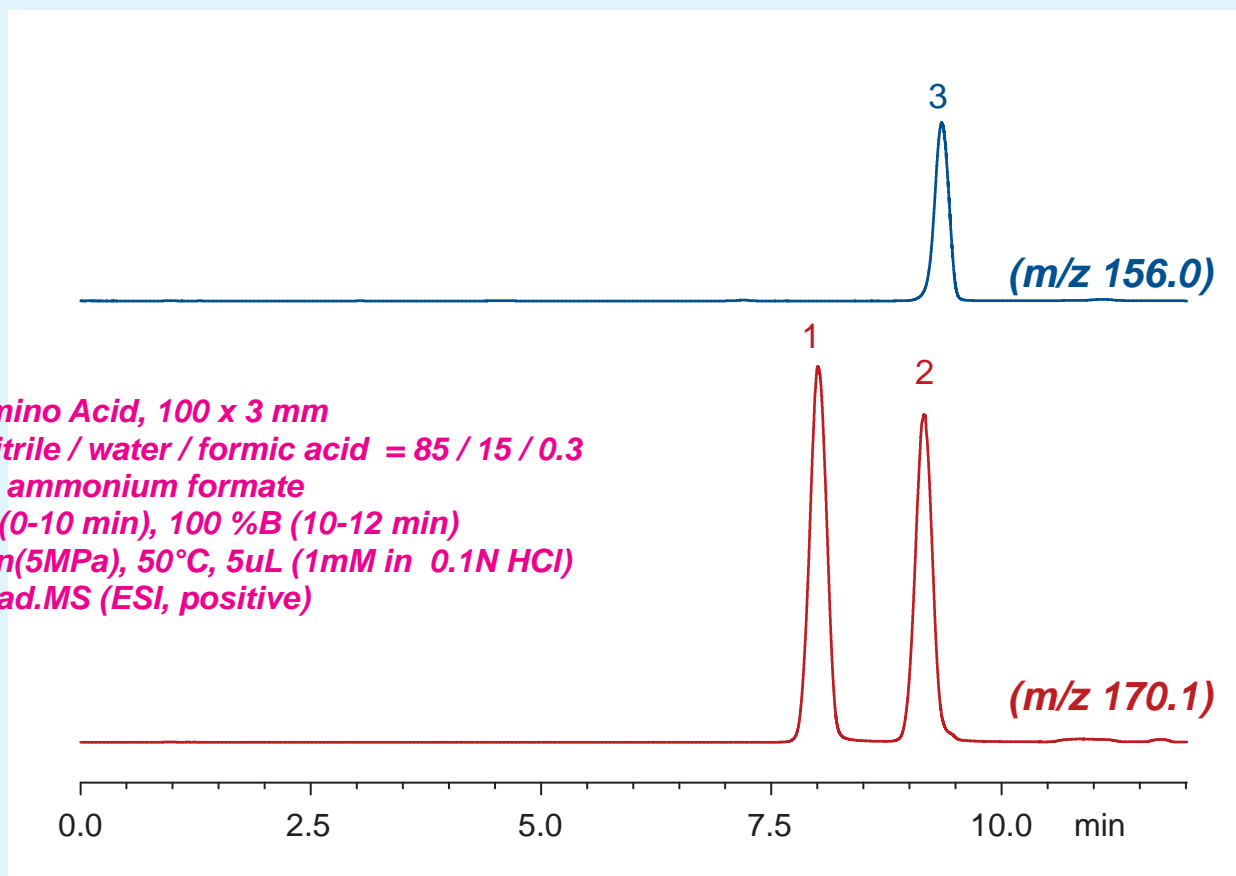
L-histidine



3-methyl-L-histidine



1-methyl-L-histidine

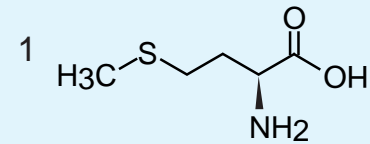
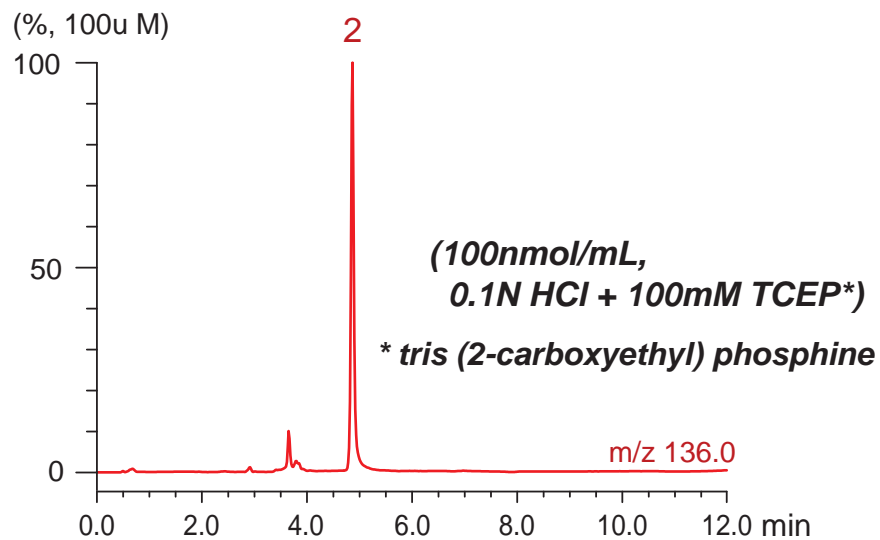
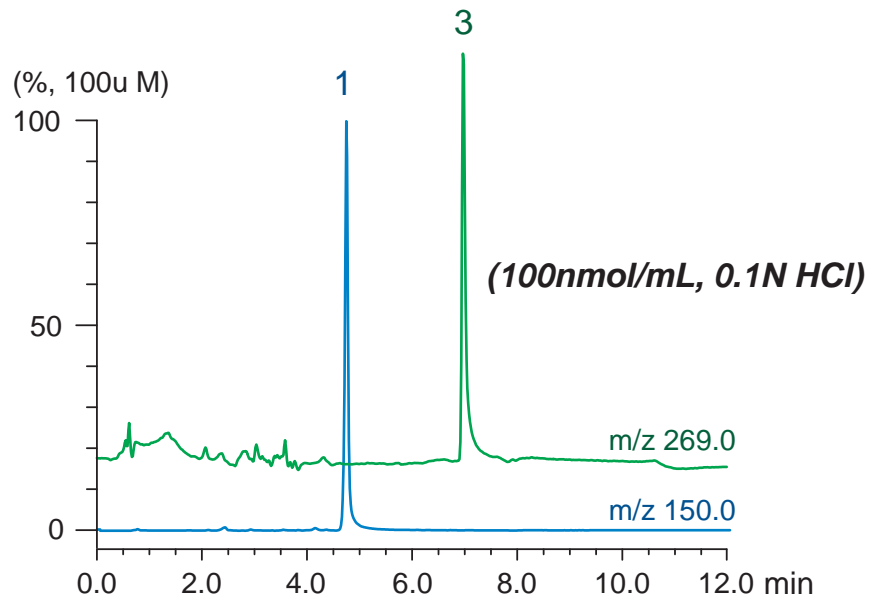


Intrada Amino Acid, 100 x 3 mm
A: acetonitrile / water / formic acid = 85 / 15 / 0.3
B: 100mM ammonium formate
55-60 %B (0-10 min), 100 %B (10-12 min)
0.4 mL/min(5MPa), 50°C, 5uL (1mM in 0.1N HCl)
Single Quad.MS (ESI, positive)

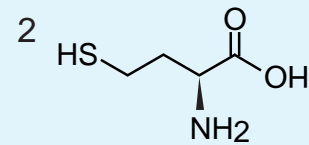
14



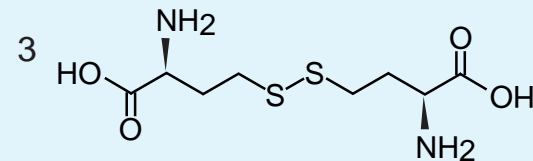
Met - Hyperhomocysteinemia



L-methionine



L-homocysteine

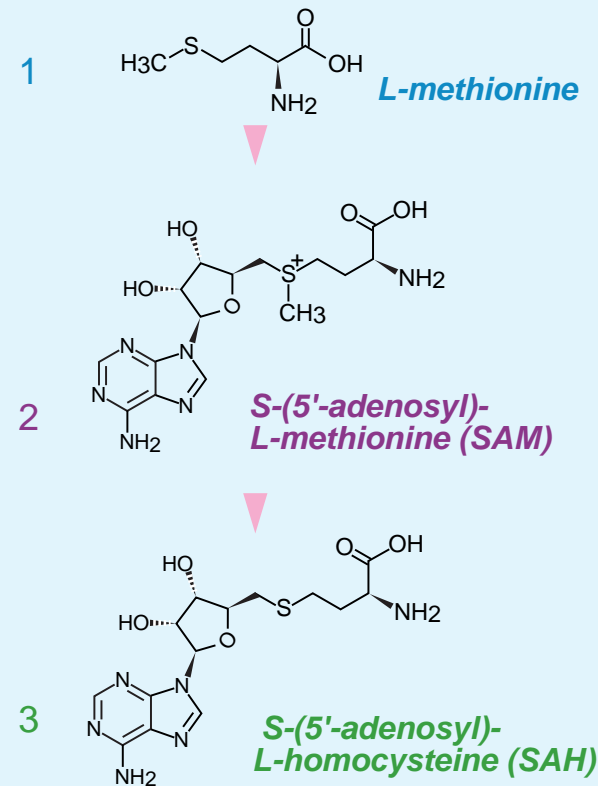
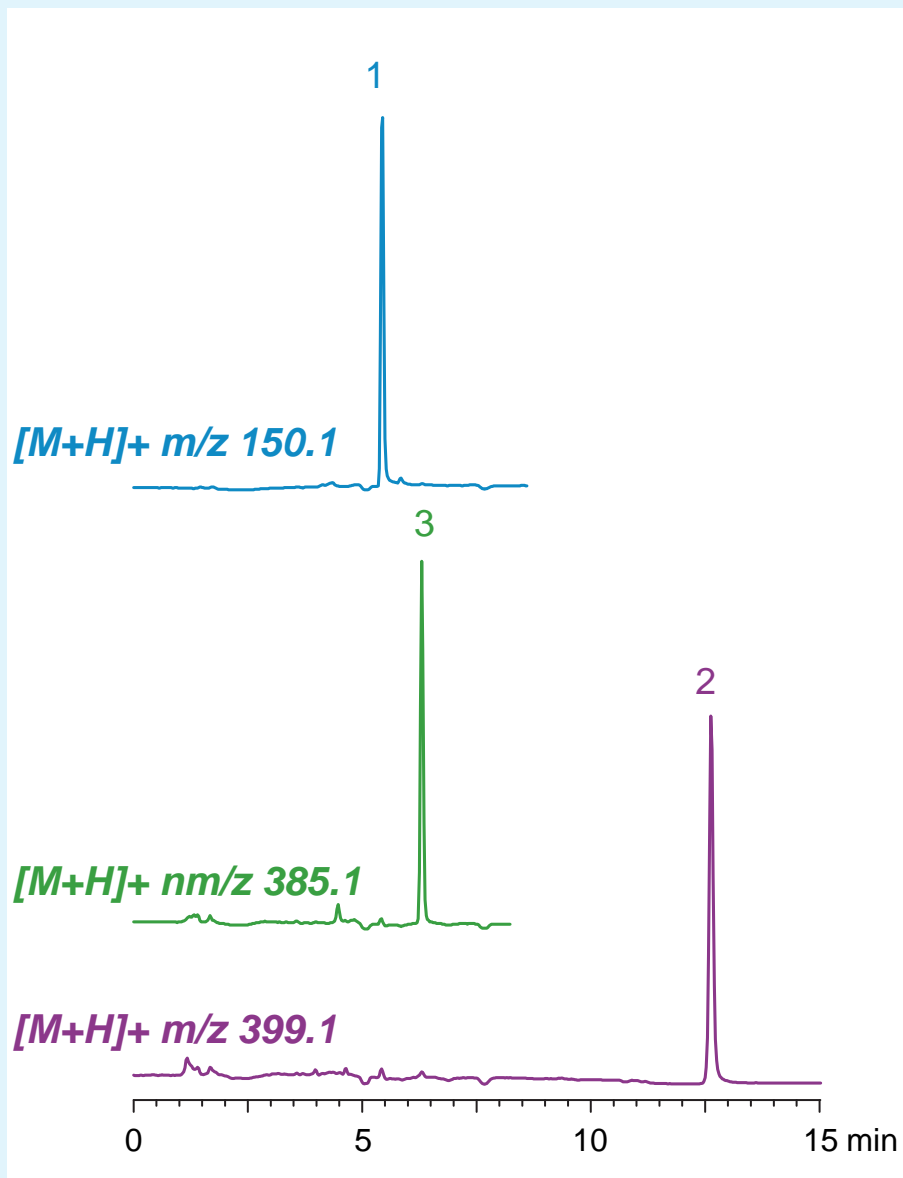


L-homocystine

Intrada Amino Acid, 50 x 3 mm
A: acetonitrile / formic acid = 100 / 0.3
B: 100mM ammonium formate
0-60 %B (0-10 min), 100 %B (10-12 min)
0.6 mL/min(3 MPa), 37°C, 5uL (0.1N HCl)
Single Quad. MS (ESI, positive)

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Met - SAM, SAH



Intrada Amino Acid , 100 x 3 mm

A: acetonitrile / formic acid = 100 / 0.3

B: acetonitrile / 100mM HCOONH₄ = 20 / 80

10-100 %B (0-10min), 100 %B (10-15min)

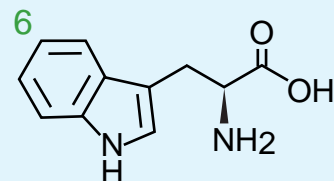
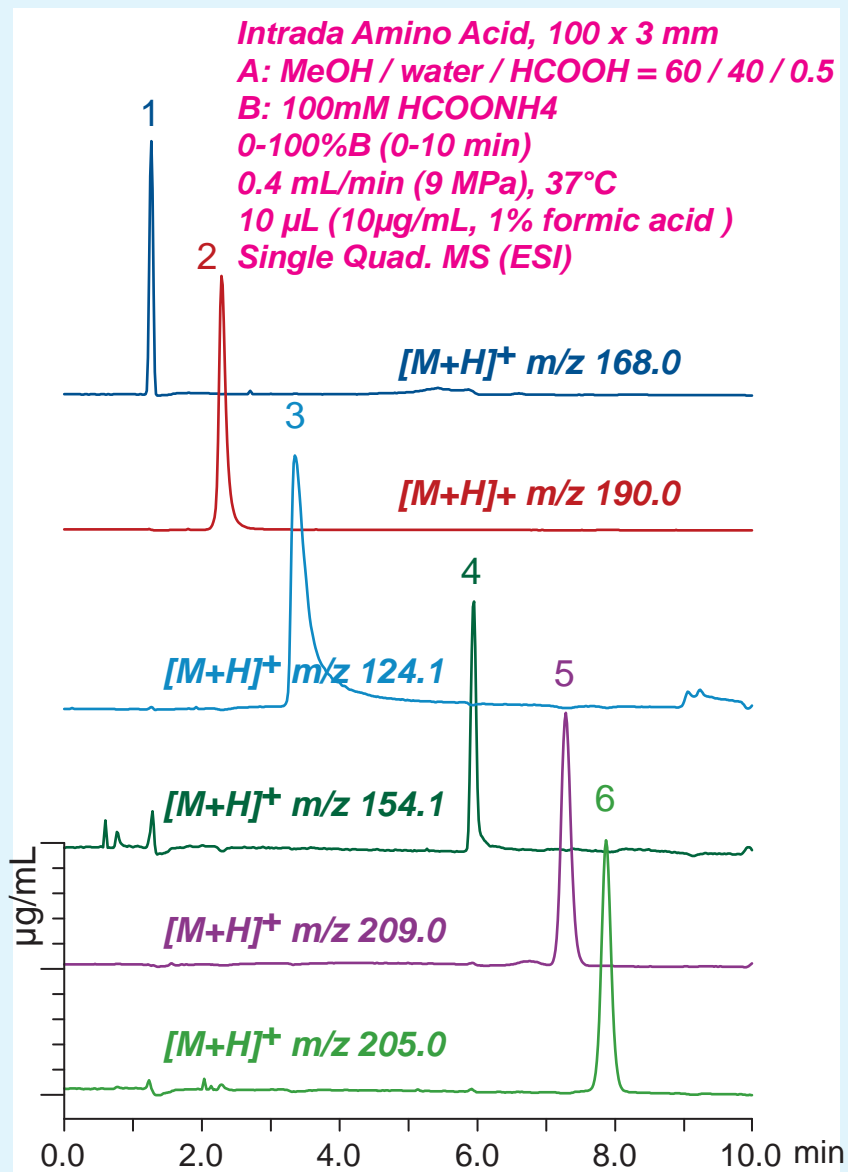
0.4 mL/min (3 MPa), 37°C

10 μ L (5 μ g/mL, 2% HCOOH)

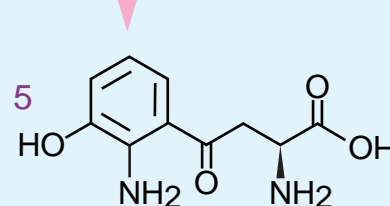
Single Quad. MS (ESI, positive)

16

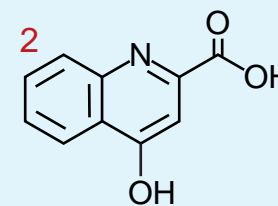
Trp - Kynurenine metabolism pathway



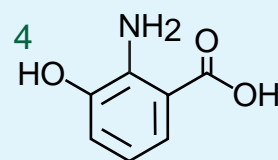
L-tryptophan



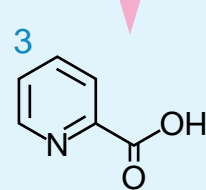
L-kynurenine



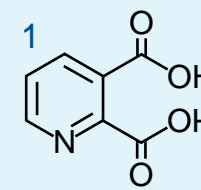
kynurenic acid



3-hydroxyanthranilic acid



picolinic acid



quinolinic acid

1min High Throughput Analysis

Intrada Amino Acid, 10 x 2 mm

A: ACN /HCOOH = 100 / 0.1

B: 100mM HCOONH₄

15-100 %B (0-0.8 min), 100 %B (0.8-1.0 min)

0.4 mL/min (1.6 MPa), 35deg.C, 1 uL (0.1N HCl)

ESI (SIM, positive)

10 x 2 mm

