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Development of a novel amino acids analysis column for LC-MS without derivatization

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OVERVIEW

We have developed a novel amino acid separation column for LC-MS without derivertization.

- Mixed-mode stationary pahse with NP+IEX
- Achieved to separate and detect or non-label 55 amino acids
- Simple gradient separation with two mobile phases
- High-throughput separation within 1 to 10 min
- Successful separation for isomers (Leu/Ile, GABA etc.)
- Good reproducibility and linearity
- Applicable to dipeptides analysis
- Extensibility to various amino acid related compounds

This novel HPLC method will be a powerful tool for amino acid LC-MS analysis in many different biochemistry applications.

INTRODUCTION

There are four established methods for analyzing amino acids: prelabeled, post-labeled, ion-pairing reversed-phase, and normal-phase, but each of these methods has disadvantages.
The pre-labeled method has problems with derivitization efficiency and cost, while the post-labeled method is usually not compatible with LC-MS due to non-volatile mobile phases. The ion-pairing reversed-phase method has difficulty separating polar amino acids; on the other hand, the normal-phase mode has problems separating all the compounds, especially the Leu and Ile isomers.
We have developed a novel amino acid separation column for LC-MS(/MS) which can separate all 20 amino acids in protein using a mixed-mode stationary phase structure. We have also estimated separation and detection characteristics using LC-MS instruments.

METHOD

We used a novel amino acid analysis column for LC-MS. This stationary phase consists of 3um silica particles modified with ion ligands which provide IEX and normal phase mixed-mode separation.

LC-MS instrument:

A mass spectrometer, LCMS-2020 (Single Quad) with LC-30A binary pumps (Shimadzu Corporation, Kyoto)

Reagents:

All of reagents including standard amino acids and bovine serum were purchased as commercial reagents.

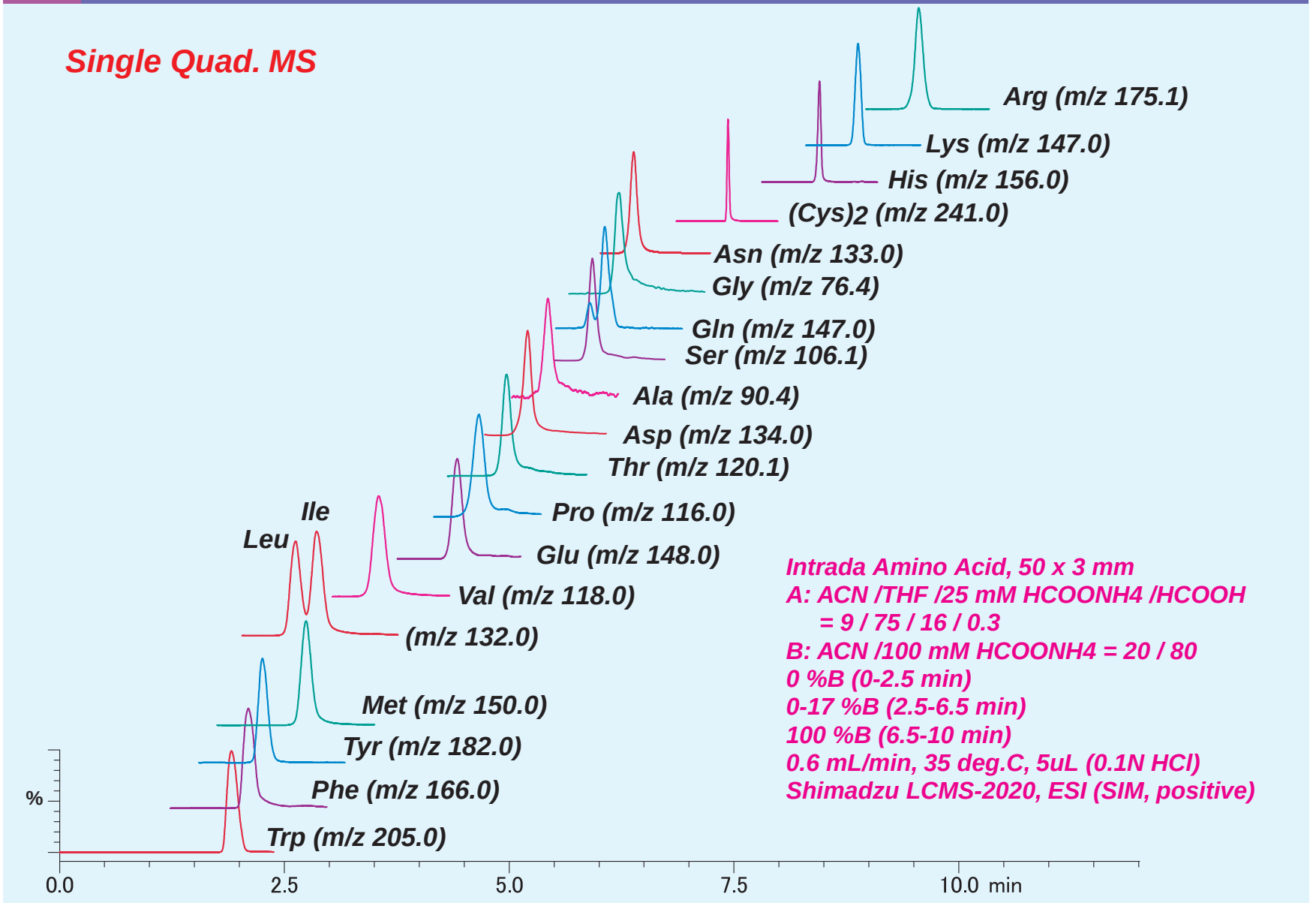
RESULTS AND DISCUSSION

Described in each figure.

CONCLUSION

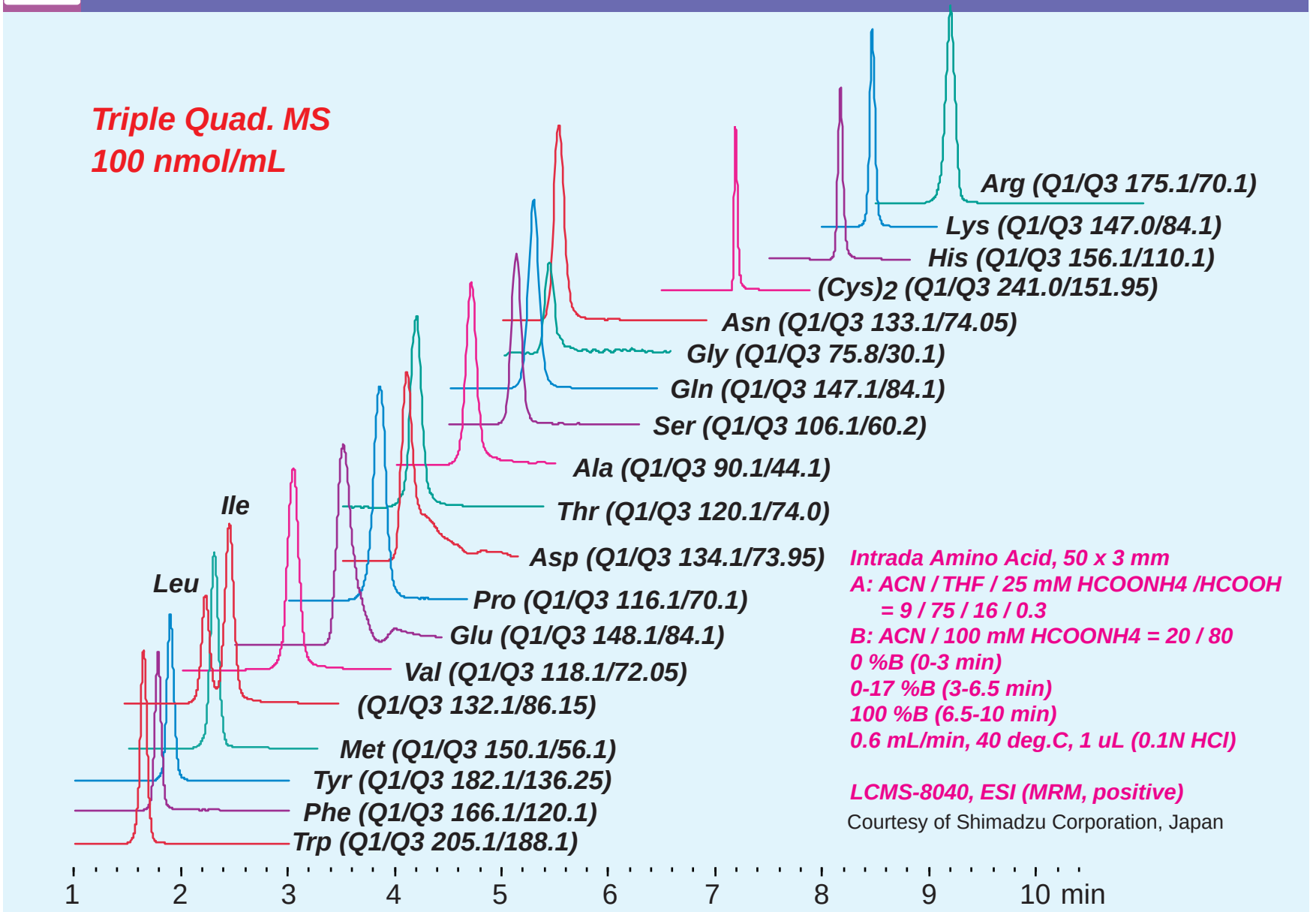
This novel HPLC method will be a powerful tool for amino acid LC-MS analysis in many different biochemistry applications.

1 LC-MS analysis of 20 amino acids



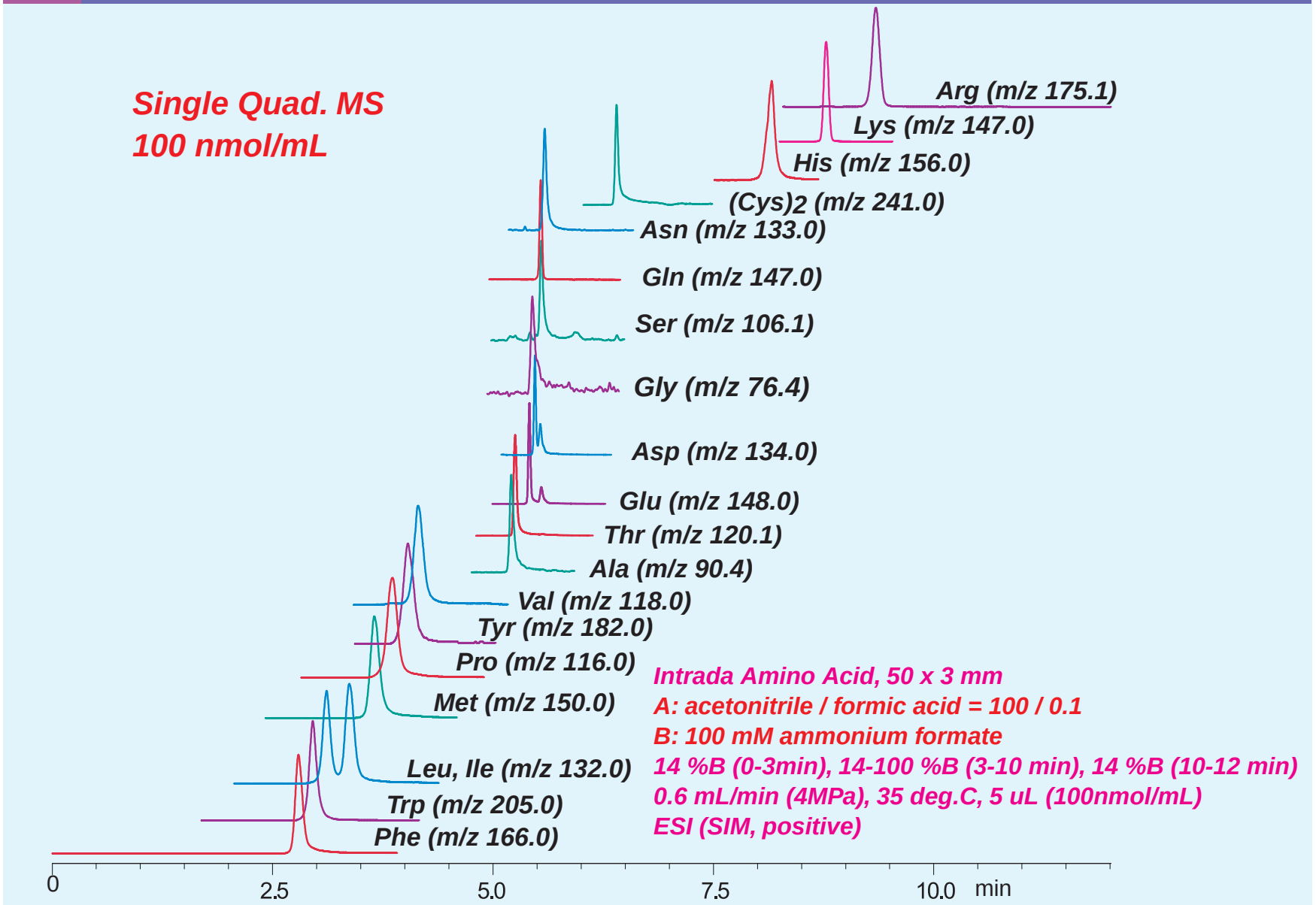
20 standard amino acids of protein were separated and detected on LC-MS (Single Quad). Leucine and Isoleucine isomers were also separated in several minutes.

2 LC-MS/MS (MRM) Analysis



20 standard amino acids of protein were separated and detected on triple quad. (MRM) with excellent senditivity.

3 Simple Gradient Conditions

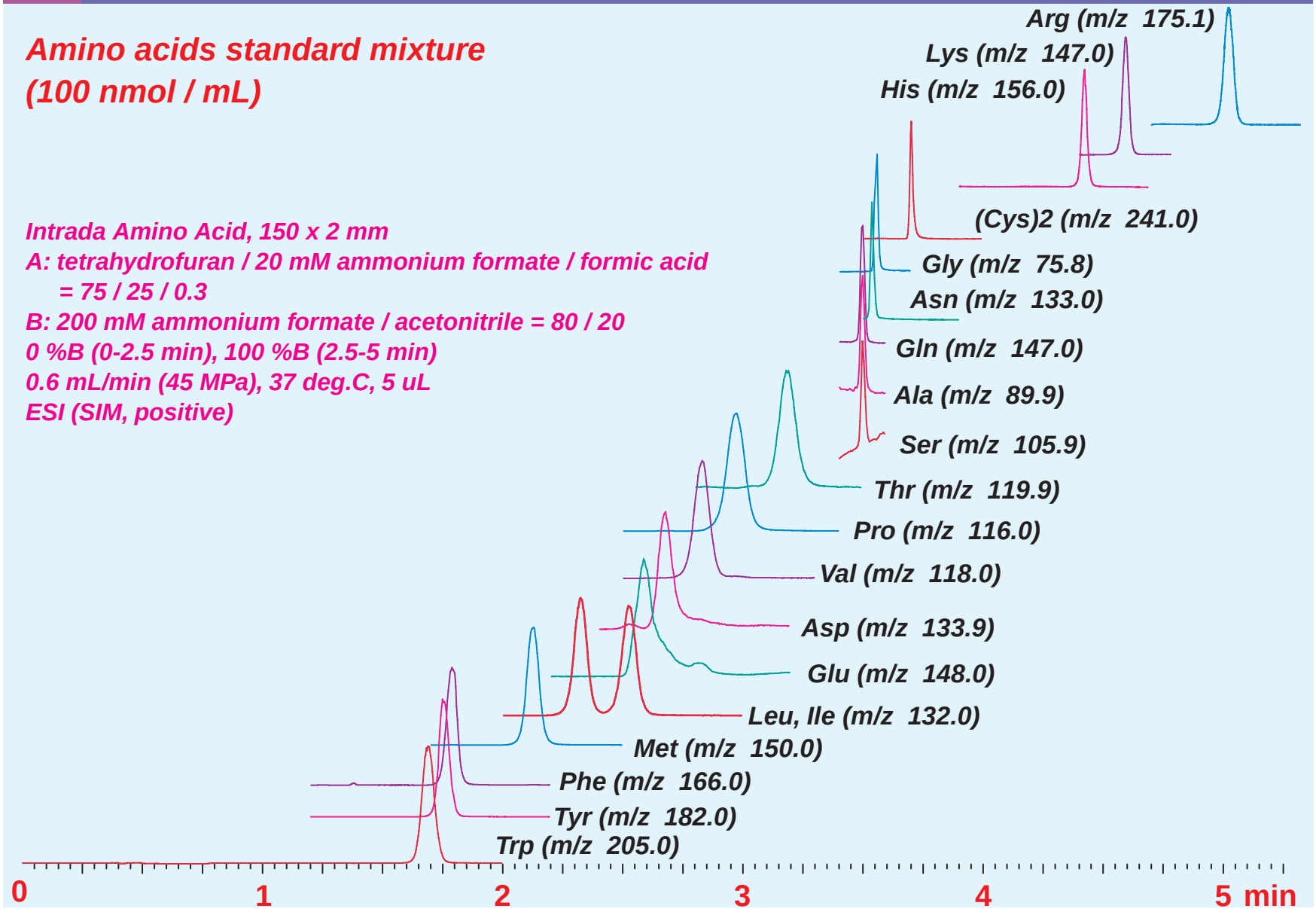


Simple gradient elution condition was also successful for standard amino acids separation in 10min with low pressure.

4 5min High Throughput Analysis

Amino acids standard mixture
(100 nmol / mL)

Intrada Amino Acid, 150 x 2 mm
A: tetrahydrofuran / 20 mM ammonium formate / formic acid
= 75 / 25 / 0.3
B: 200 mM ammonium formate / acetonitrile = 80 / 20
0 %B (0-2.5 min), 100 %B (2.5-5 min)
0.6 mL/min (45 MPa), 37 deg.C, 5 uL
ESI (SIM, positive)

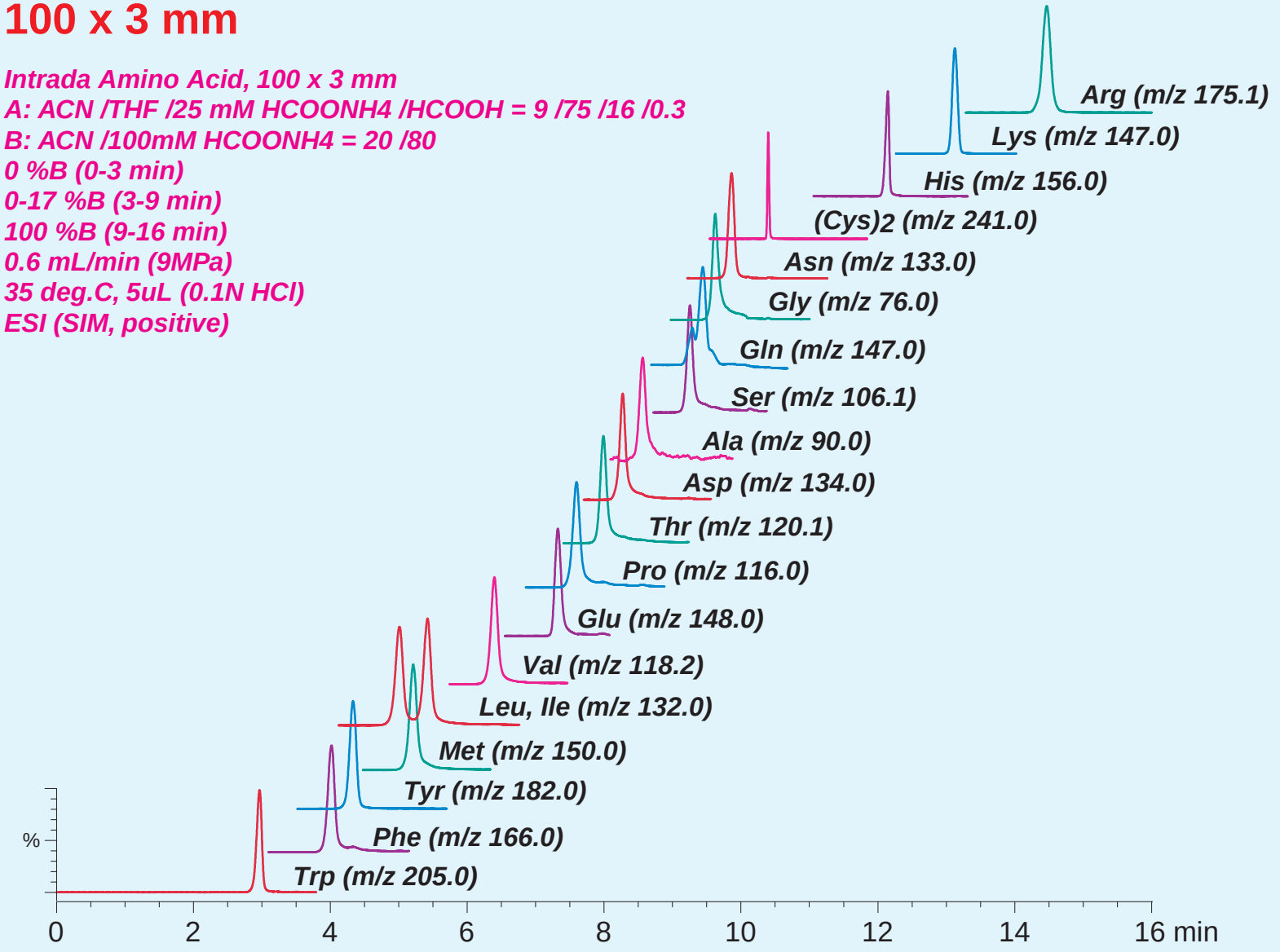


High throuput analysis using UHPLC system was achieved to analyze 20 amino acids within 5min.

5 Accurate method with 100 x 3 mm

100 x 3 mm

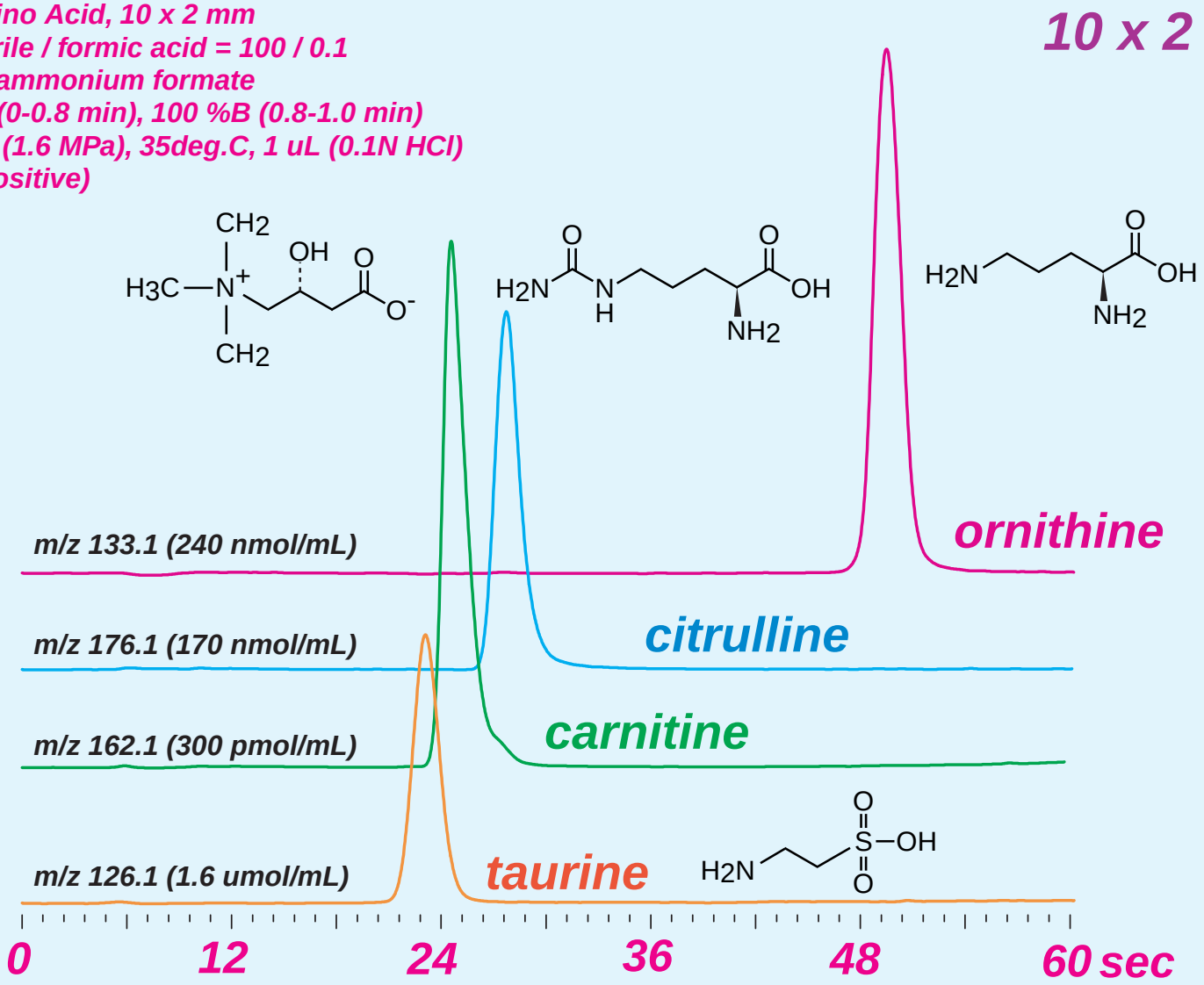
Intrada Amino Acid, 100 x 3 mm
A: ACN /THF /25 mM HCOONH4 /HCOOH = 9 /75 /16 /0.3
B: ACN /100mM HCOONH4 = 20 /80
0 %B (0-3 min)
0-17 %B (3-9 min)
100 %B (9-16 min)
0.6 mL/min (9MPa)
35 deg.C, 5uL (0.1N HCl)
ESI (SIM, positive)



100 x 3 mm column provided more accurate analysis for 20 standard amino acids with better peak shape and separation.

6 One-Minute High Throughput Analysis

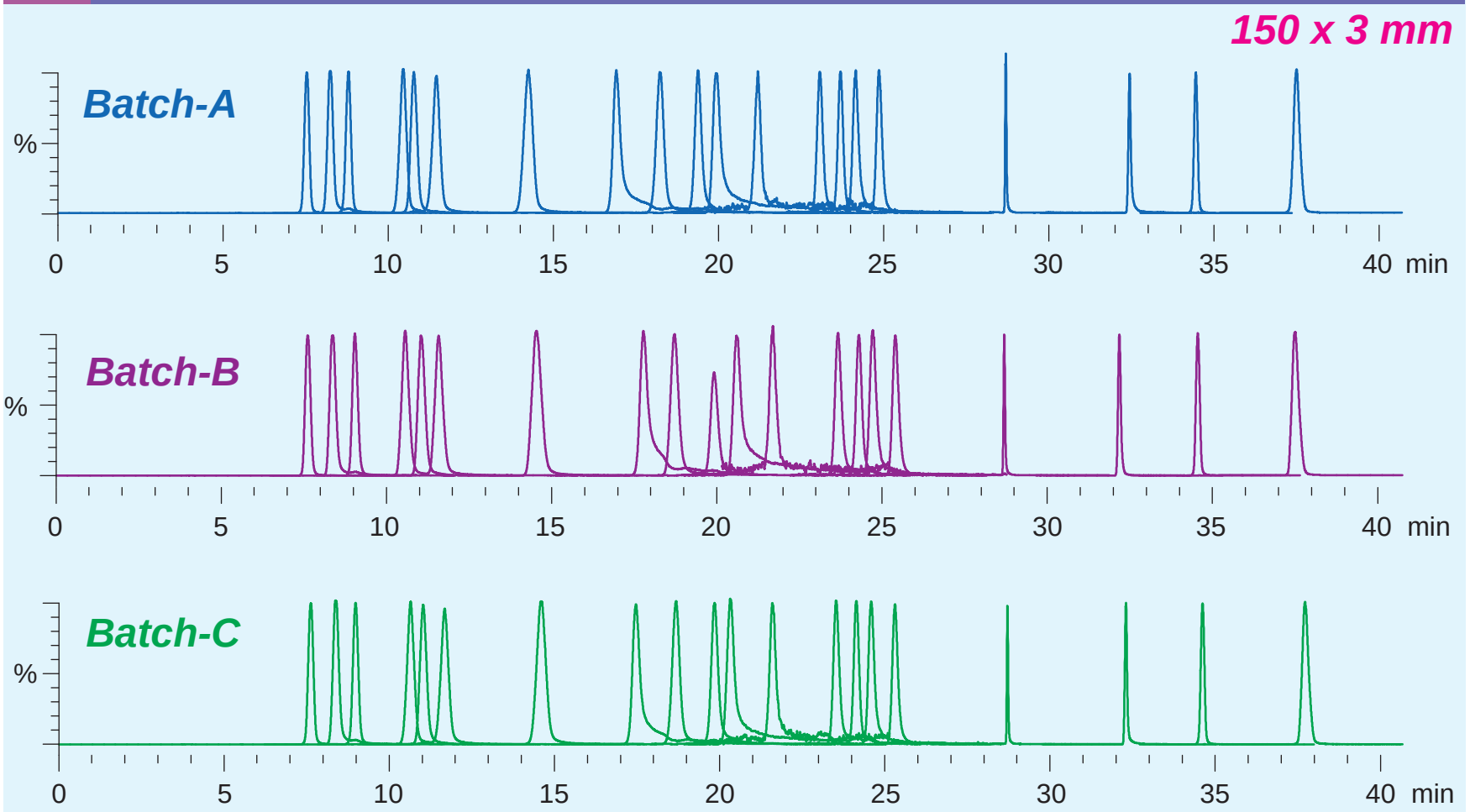
Intrada Amino Acid, 10 x 2 mm
A: acetonitrile / formic acid = 100 / 0.1
B: 100 mM ammonium formate
15-100 %B (0-0.8 min), 100 %B (0.8-1.0 min)
0.4 mL/min (1.6 MPa), 35deg.C, 1 uL (0.1N HCl)
ESI (SIM, positive)



Amino acid related compounds were analyzed by one-munite high throuhput separation with 10mm length small column.

7

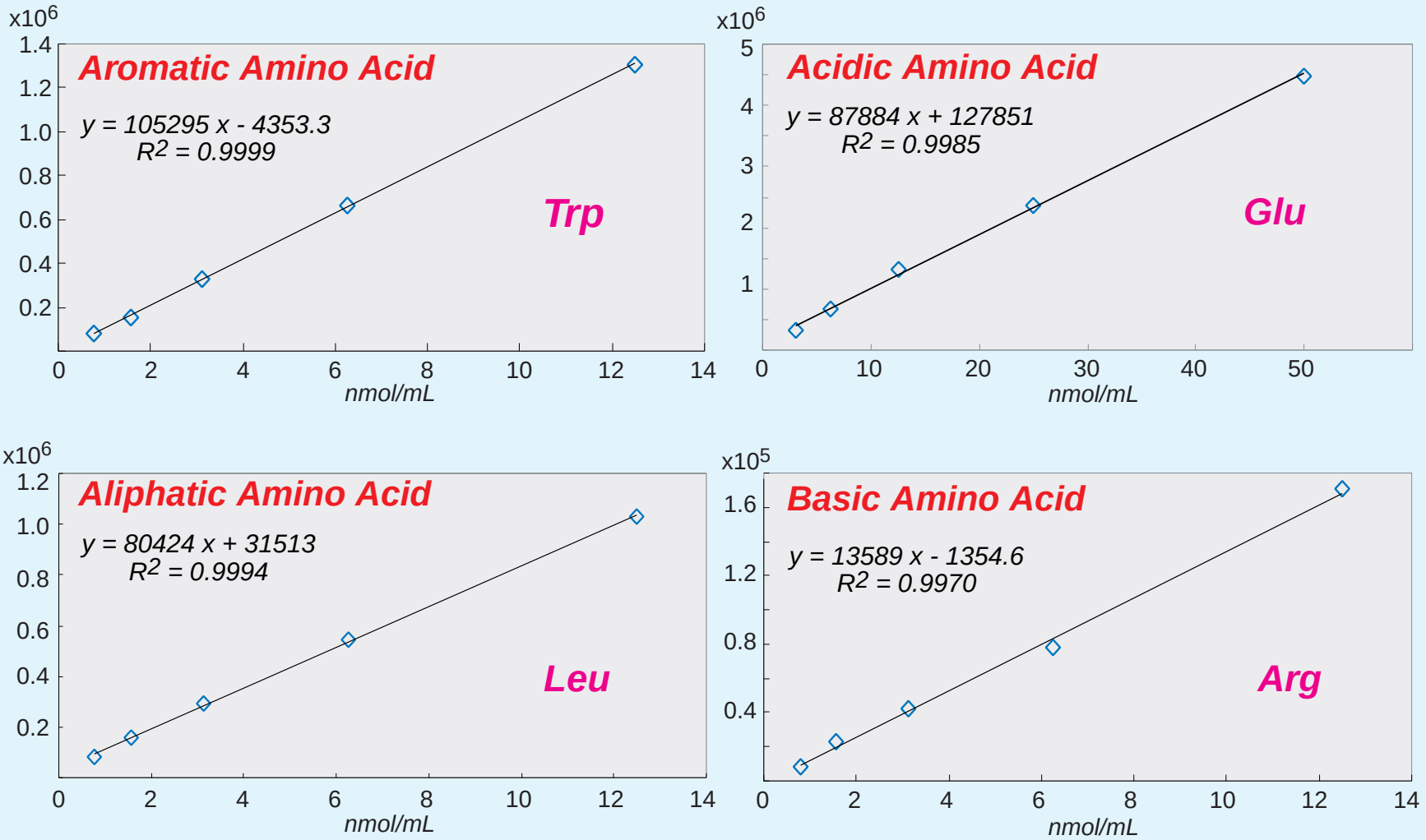
Batch-to-Batch Reproducibility (20 AA)



Intrada Amino Acid, 150 x 3 mm
A: ACN /THF /25mM HCOONH4 /HCOOH = 9 / 75 / 16 / 0.3 (v/v/v/v)
B: ACN /100mM HCOONH4 = 20 / 80 (v/v)
0 %B (0-12 min), 0-17 %B (12-26 min), 100 %B (26-45 min)
0.4 mL/min (7MPa), 35 deg.C, 1.2 uL (1 umol/mL in 0.1N HCl), ESI, positive

Amino acid analysis packing materials were showed excellent batch-to-batch reproducibility.

Linearity



Intrada Amino Acid, 50 x 3 mm, LCMS-2020 (single quad.), ESI (SIM, positive)

Typical standard (aromatic, aliphatic, acidic and basic) amino acids showed good results for linearity and sensitivity.

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Repeatability

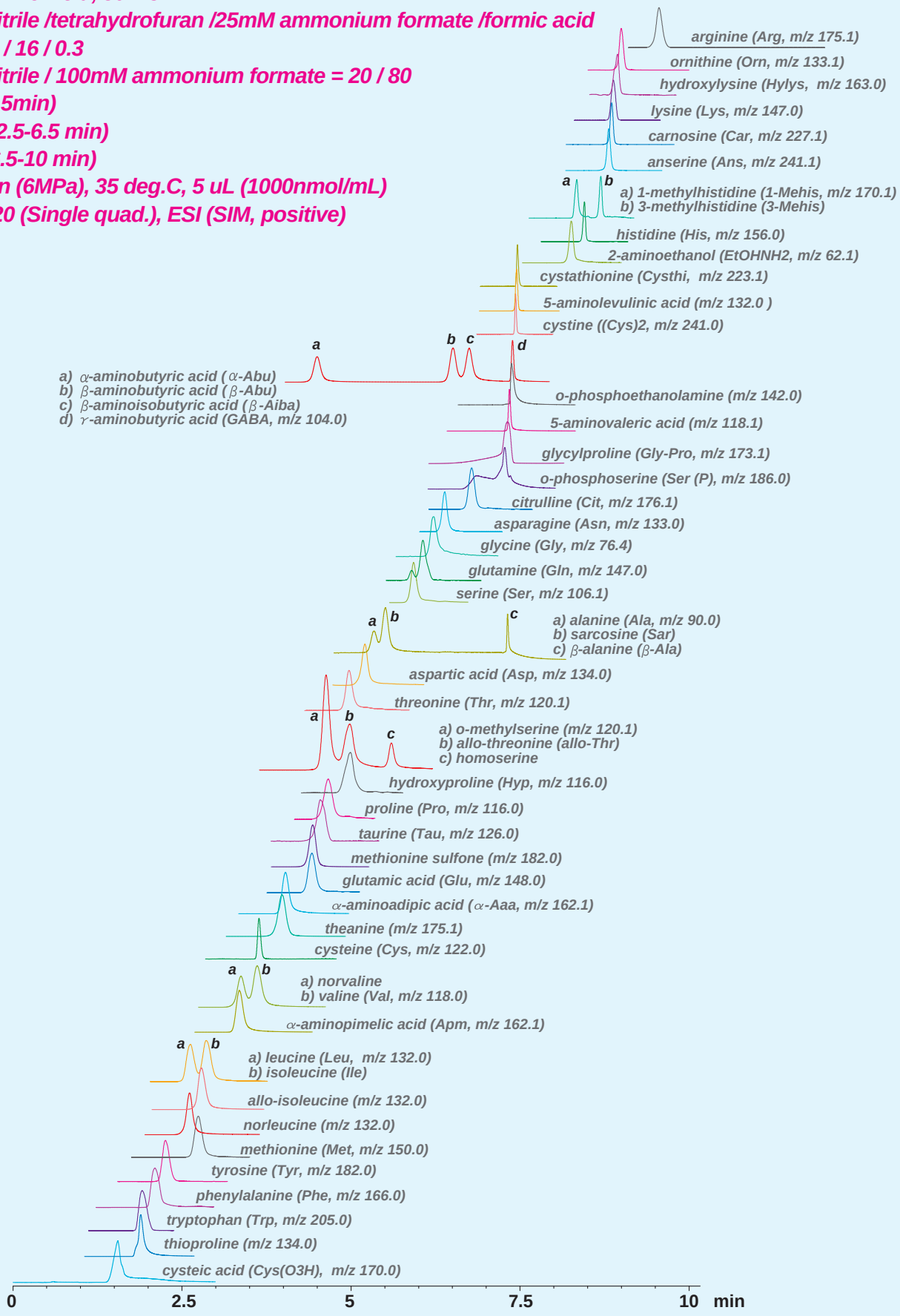
Amino Acid	%RSD(t_R)	%RSD(Area)
Trp	0.48	0.82
Phe	0.49	0.97
Tyr	0.70	1.09
Met	0.45	1.21
Leu	0.43	2.33
Ile	0.39	3.11
Val	0.41	2.26
Glu	0.40	1.71
Pro	0.34	2.08
Asp	0.28	2.60
Thr	0.38	2.39
Ala	0.24	3.10
Ser	0.22	2.71
Gln	0.22	3.21
Gly	0.21	2.92
Asn	0.20	2.35
(Cys)2	0.04	4.14
His	0.08	4.30
Lys	0.08	4.85
Arg	0.11	4.11

Intrada Amino Acid, 50 x 3 mm
A: ACN /THF /25mM HCOONH4 /HCOOH = 9 /75 /16 /0.3
B: ACN /100 mM HCOONH4 = 80 / 20
0 %B (0-2.5 min)
0-17 %B (2.5-6.5 min)
100 %B (6.5-10 min)
0.6 mL/min
35 deg.C
5uL (500nmol/mL)
ESI, Positive
n = 15
ISTD: theanine

Amino acid analysis column showed excellent repeatability in retention and acceptable for peak area.

10 55 Amino acids (standard)

Intrada Amino Acid, 50 x 3 mm
A: acetonitrile /tetrahydrofuran /25mM ammonium formate /formic acid = 9 / 75 / 16 / 0.3
B: acetonitrile / 100mM ammonium formate = 20 / 80
0 %B (0-2.5min)
0-17 %B (2.5-6.5 min)
100 %B (6.5-10 min)
0.6 mL/min (6MPa), 35 deg.C, 5 uL (1000nmol/mL)
LCMS-2020 (Single quad.), ESI (SIM, positive)



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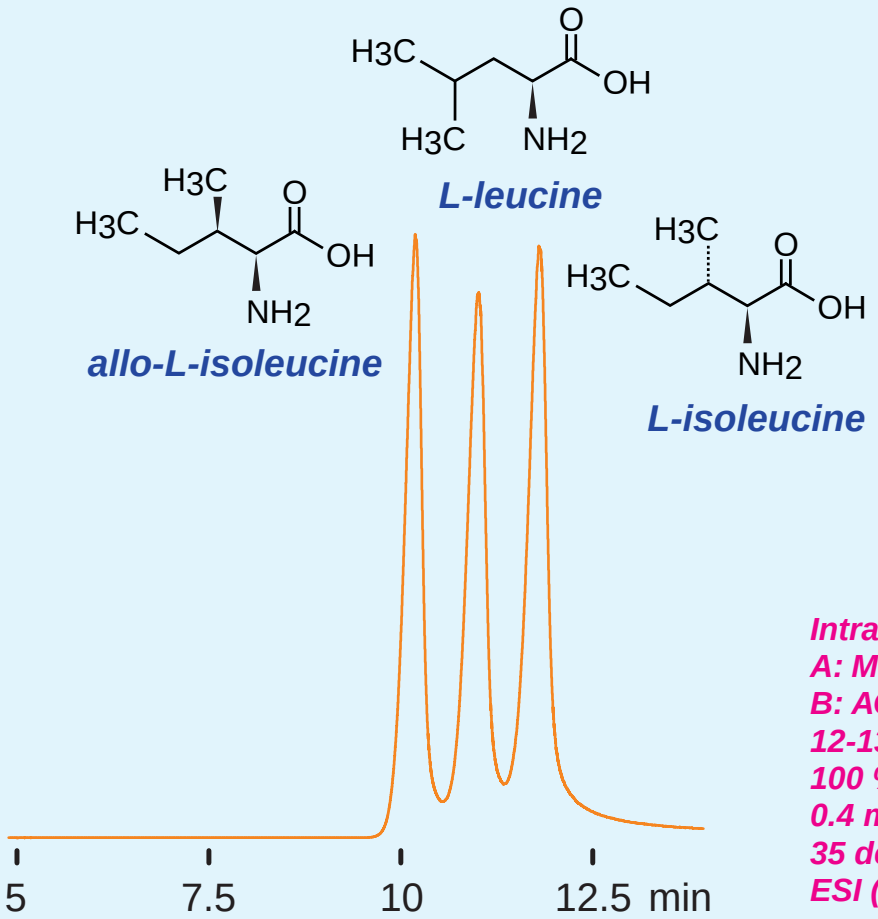
55 standard amino acids were separated and detected on LC-MS. Almost amino acids were analyzed within 10 minutes.

Leucine Isomers (131Da)

m/z 132.0

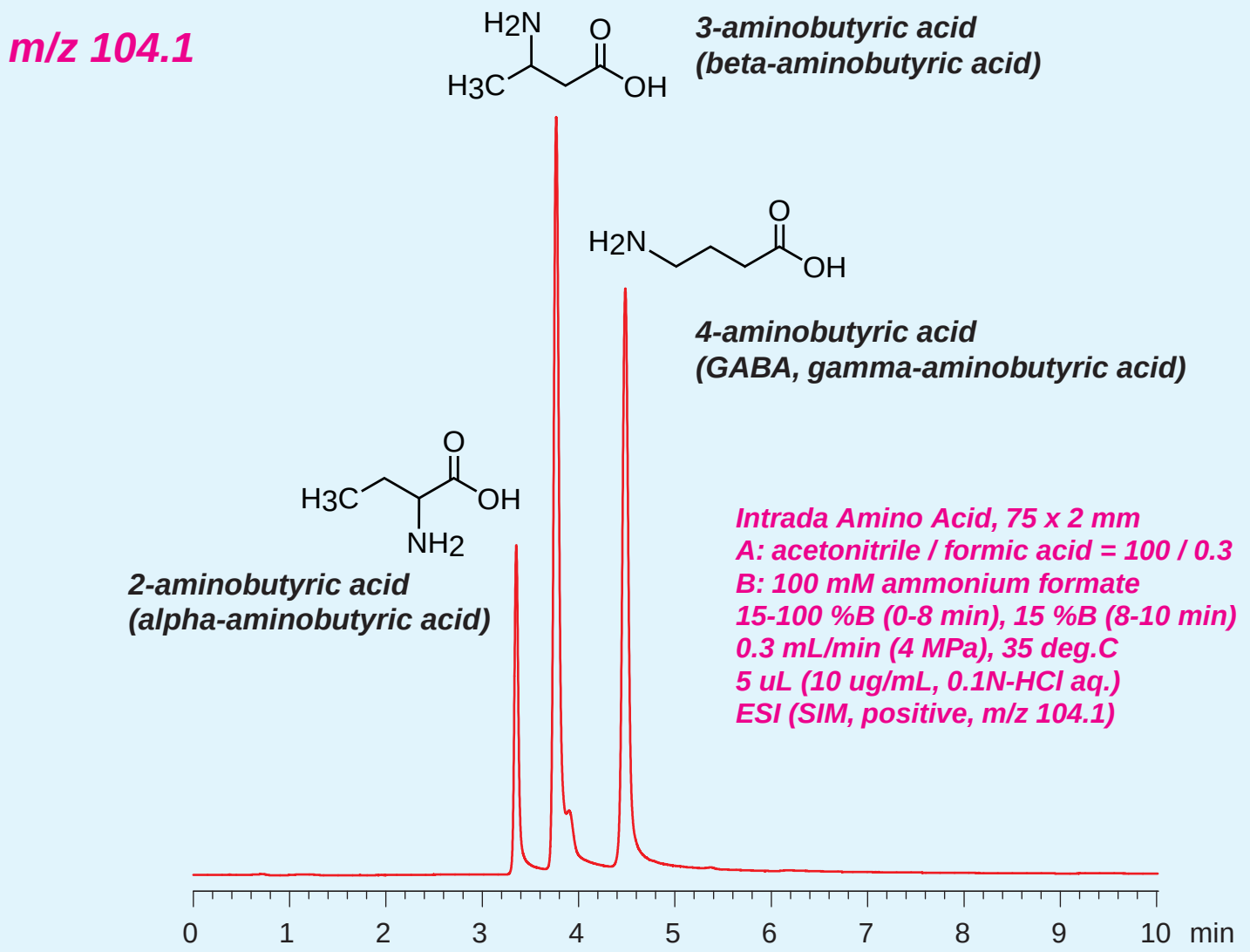
150 x 3 mm



Intrada Amino Acid, 150 x 3 mm
A: MeOH /water /HCOOH = 85 / 15 / 0.3
B: ACN /100mM HCOONH4 = 20 / 80
12-13 %B (0-12 min)
100 %B (12-15 min)
0.4 mL/min (11 MPa)
35 deg.C, 5 uL (0.1N HCl)
ESI (positive, m/z 132.0)

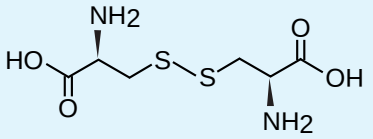
Leucine isomers, same m/z value, were well separated in short time.

Alpha, Beta, Gamma Amino Acid Isomers



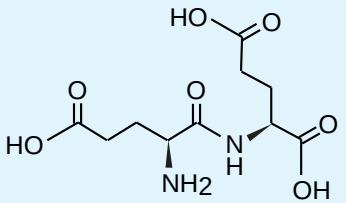
Not only alpha-amino acids but also beta- and gamma-amino acids isomers were well separated.

Dipeptide Analysis

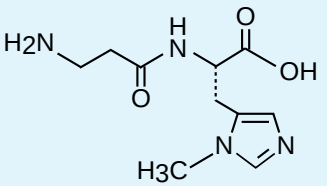


L-cystine ((Cys)2)

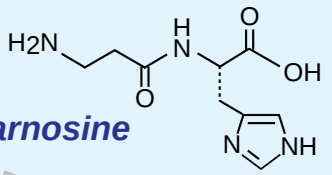
Intrada Amino Acid, 75 x 2 mm
A: acetonitrile / formic acid = 100 / 0.3
B: 100 mM ammonium formate
20-100 %B (0-10 min), 100 %B(10-12 min)
0.3 mL/min (5 MPa), 35 deg.C
2 uL (0.02-0.32 ug, 0.1N-HCl aq.)
ESI (SIM, positive)



N-(L-alpha-glutamyl)
-L-glutamic acid
(Glu-Glu)

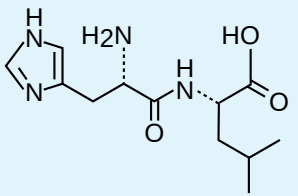


L-anserine



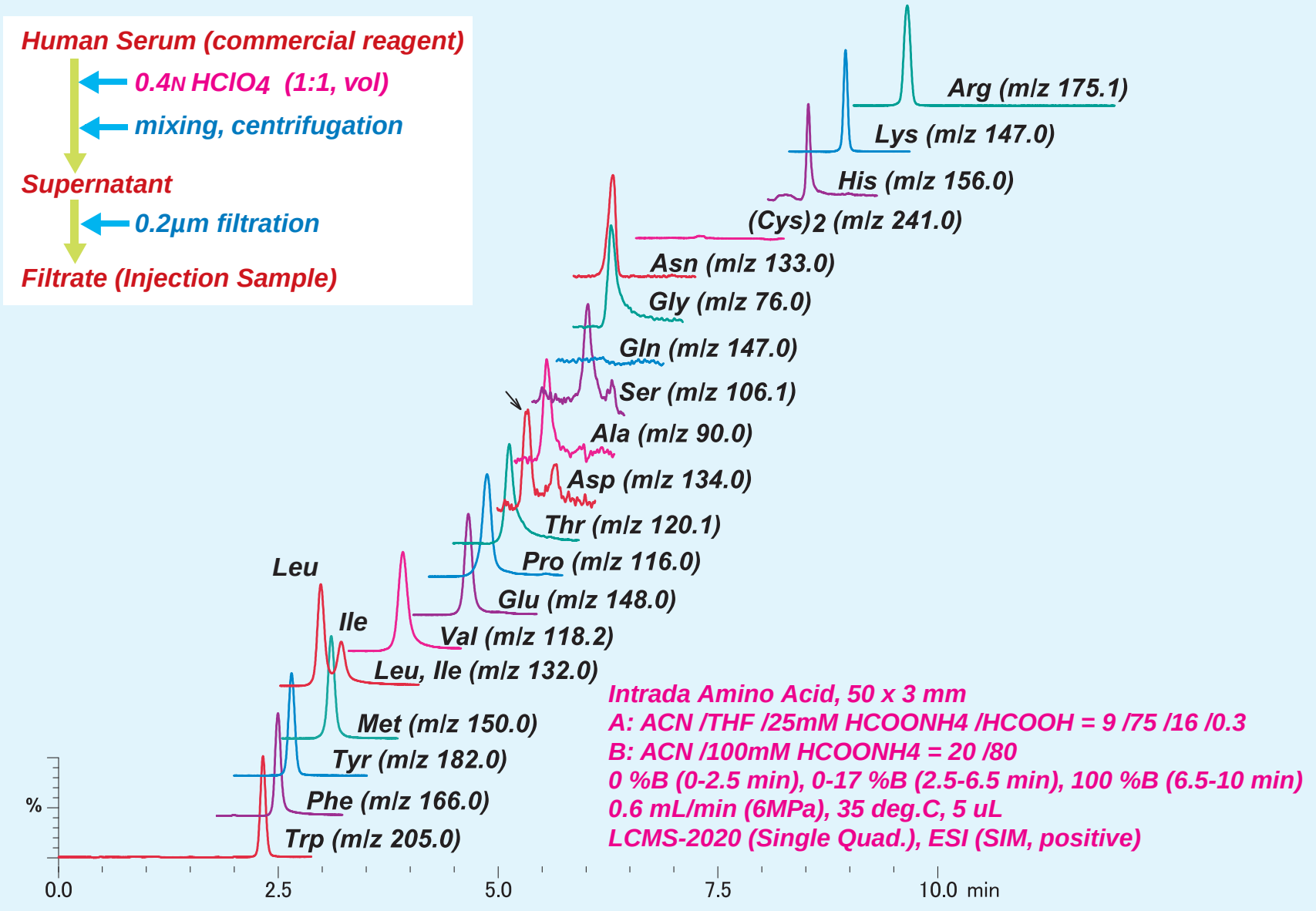
L-carnosine

N-L-histidyl-L-leucine
(His-Leu)



The novel amino acid analysis column could be applied for dipeptides.

Amino acids in human serum (HClO4)



The novel amino acid analysis column was applied for amino acids in serum and almost amino acids were detected.

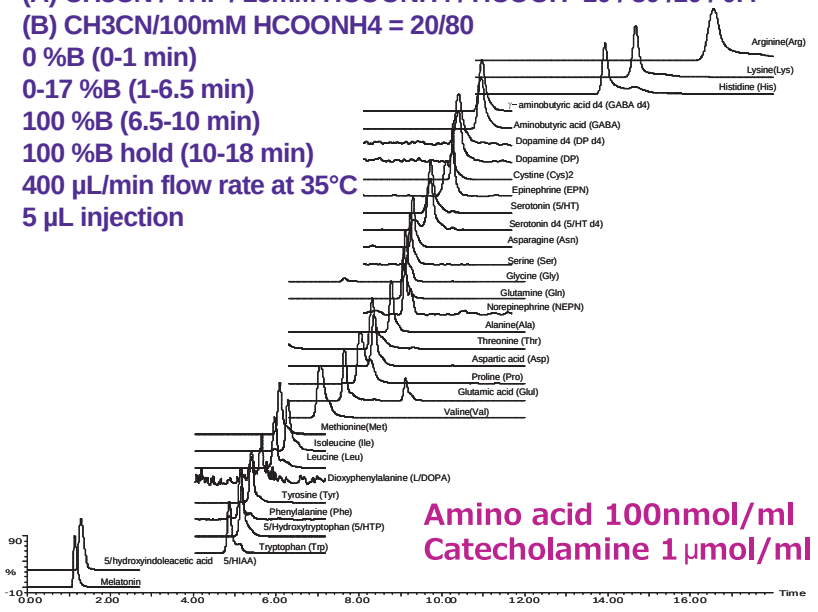
15 LC-MS Analysis for amino acids and catecholamines in rat pituitary

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

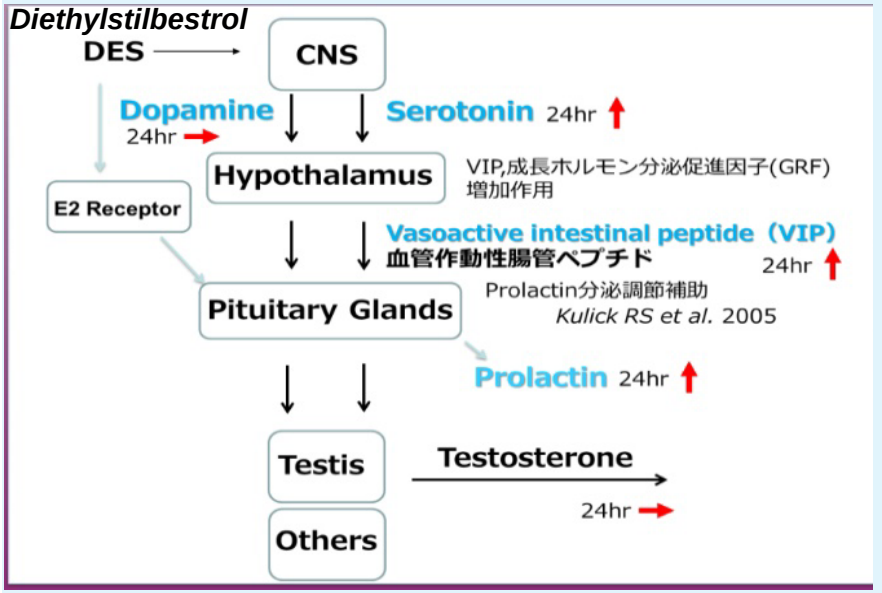
Naoyuki Maeda1,2, Emi Tanaka1, Kanae Masu1, Kanako Okumura2 Yuki Ikeda2, Taku Miyasho2, Satoko Haeno2 and Hiroshi Yokota2.
1) Japan Meat Science & Technology Institute
2) Rakuno Gakuen University

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

The separation was achieved using an Intrada Amino Acid (100 x 3 mm 3µm 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 µL/min flow rate at 35°C
5 µL injection



Amino acid 100nmol/ml
Catecholamine 1 µmol/ml



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.

Courtesy of Dr. Maeda, Japan Meat Science & Technology Institute, Japan