The world's first specialty column for intact amino acid analysis via LC-MS

Intrada Amino Acid

Imtakt

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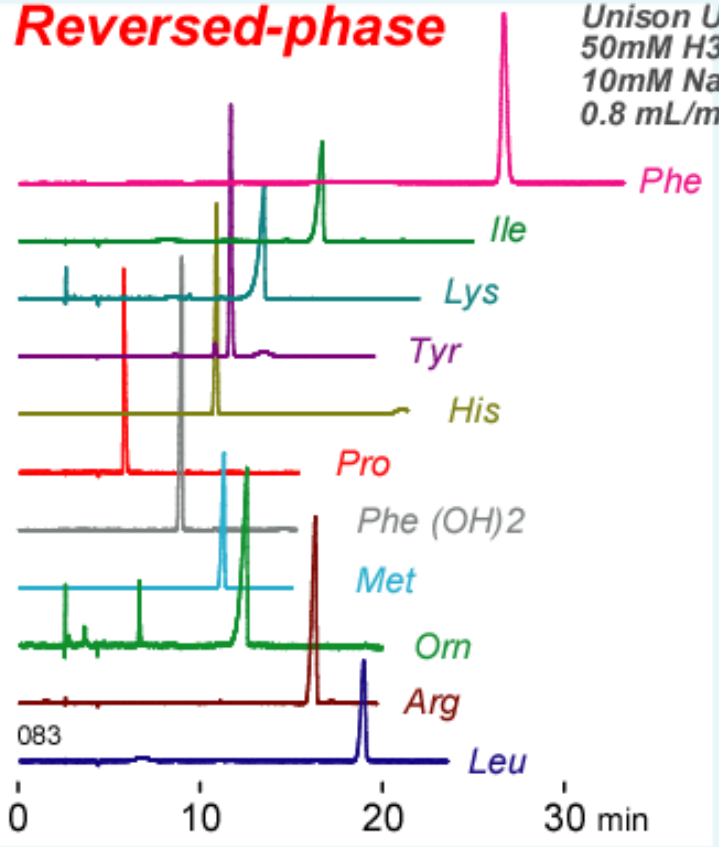
Imtakt Corporation

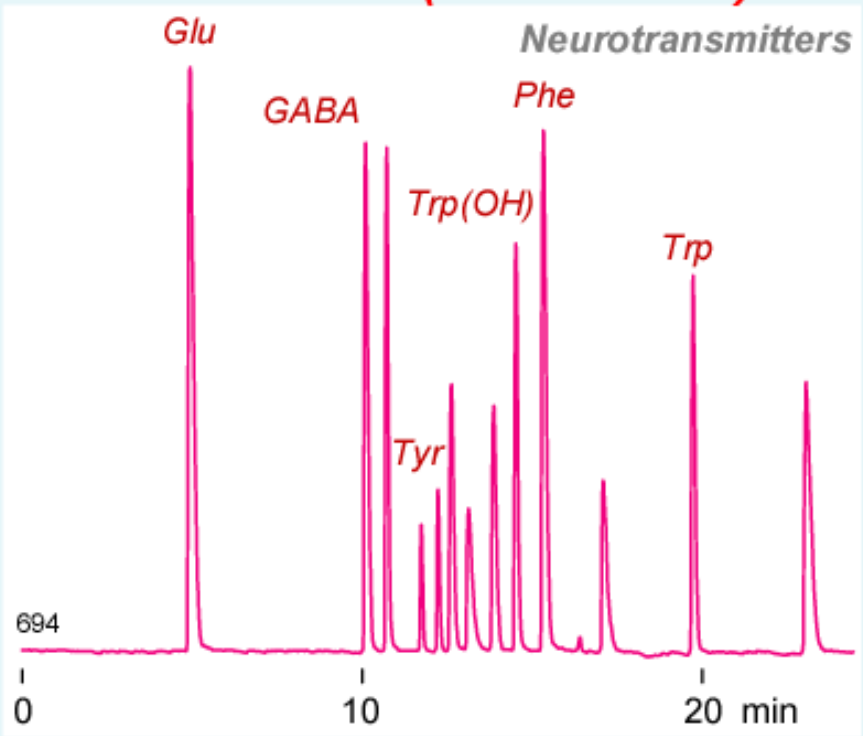
Various separation modes for amino acids direct analysis

Reversed-phase

Unison UK-C18, 250 x 4.6 mm
50mM H3PO4 +
10mM Na-heptanesulfonate /ACN = 85 /15
0.8 mL/min, 37 °C, 14.2 MPa, 210 nm



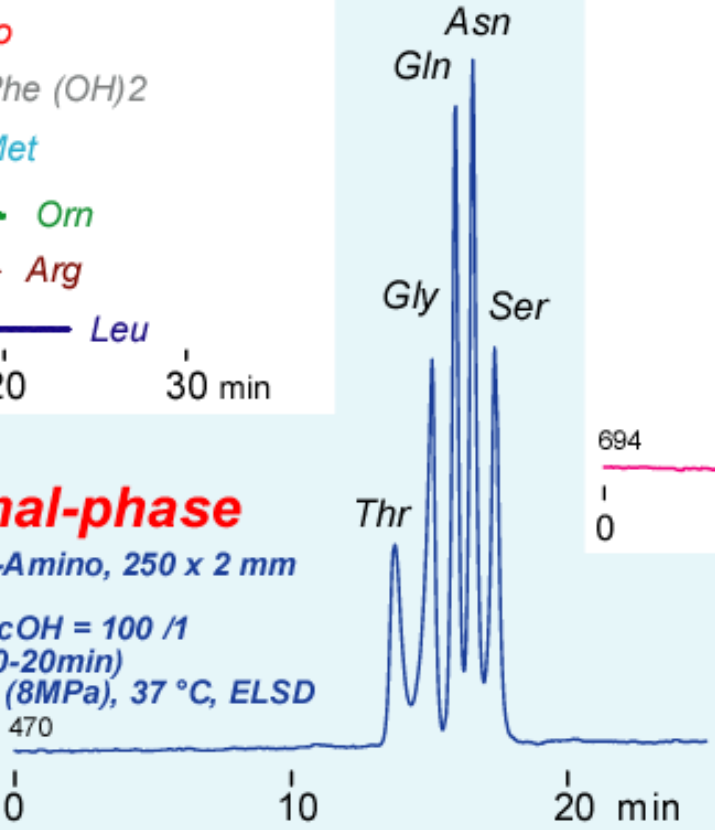
Multi-mode (RP+AX+CX)



Scherzo SS-C18, 150 x 3 mm
A: water /HCOOH = 100 / 0.5
B: 45mM HCOONH4 /ACN = 65 / 35
10-100 %B (0-30 min)
0.4 mL/min, 37 deg.C, ELSD

Normal-phase

Unison UK-Amino, 250 x 2 mm
A: ACN
B: water /AcOH = 100 /1
20-25 %B (0-20min)
0.2 mL/min (8MPa), 37 °C, ELSD



Packing materials and separation mode

3 µm pure spherical silica particles
Specially designed stationary phase for amino acids separation with LC-MS
Mixed-mode with Normal Phase + IEX

ANALYTICAL CONDITIONS PROTOCOL

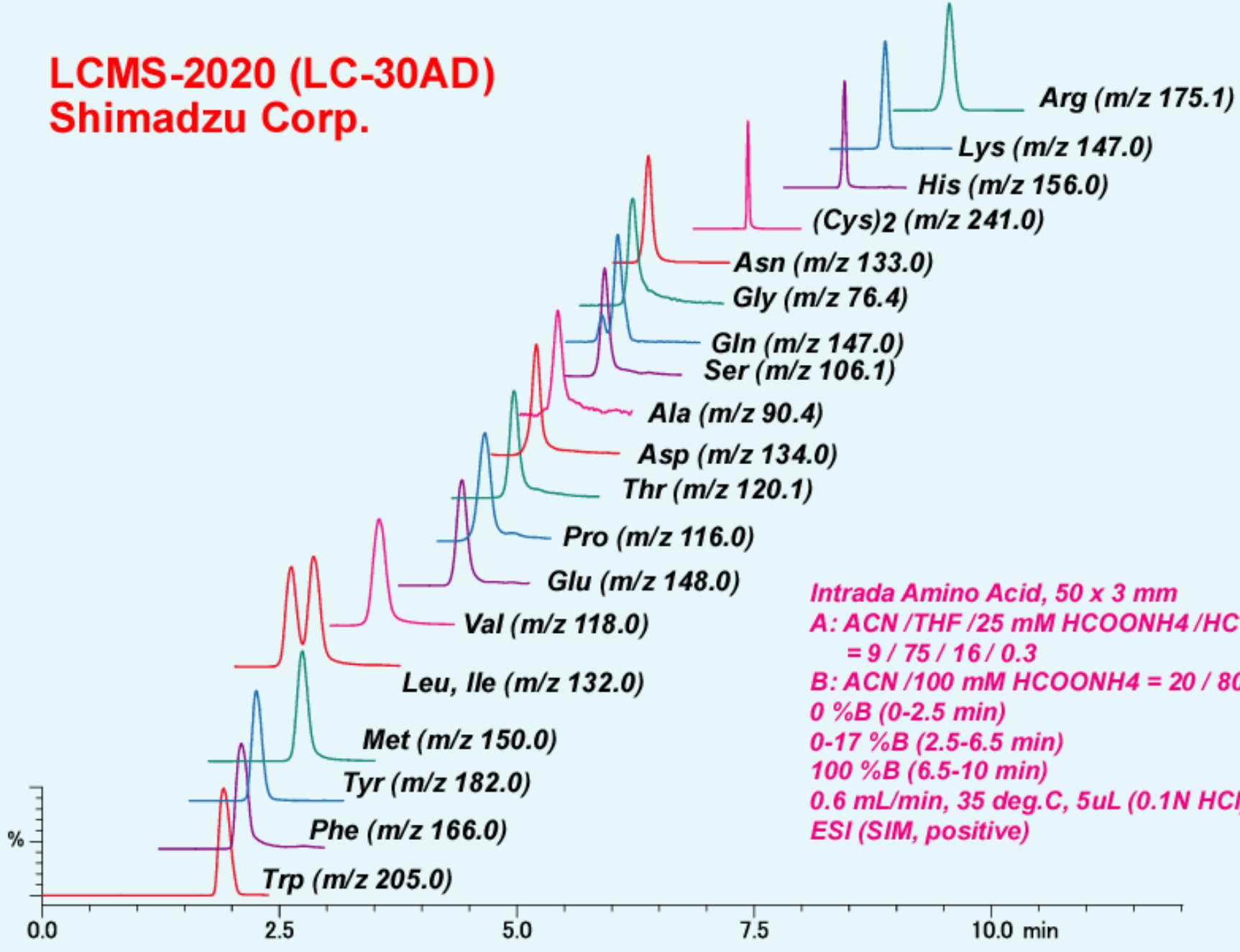
A: ACN (THF, MeOH) /HCOOH = 100 / (0.1 - 0.5), v/v
B: (50-200mM) HCOONH4

Initial - Final %B (Gradient Time)
Flow Rate: depends on column I.D.
Temperature: up to 65°C
Injection Solution: 0.1N HCl or 0.1 - 2% HCOOH
MS detection: ESI, positive

Imtakt Intrada Amino Acid

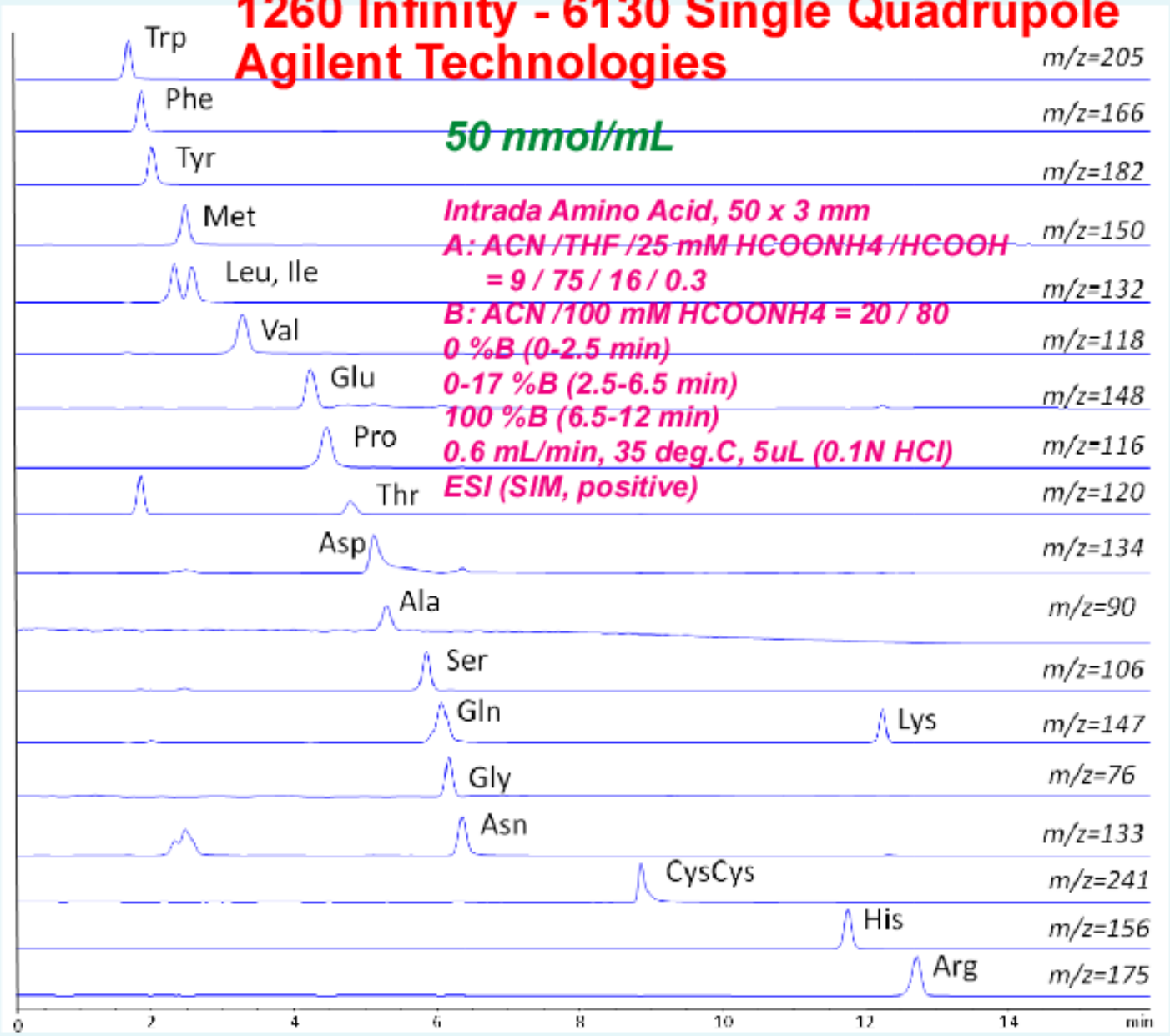
LC-MS analysis for intact amino acids

LCMS-2020 (LC-30AD)
Shimadzu Corp.



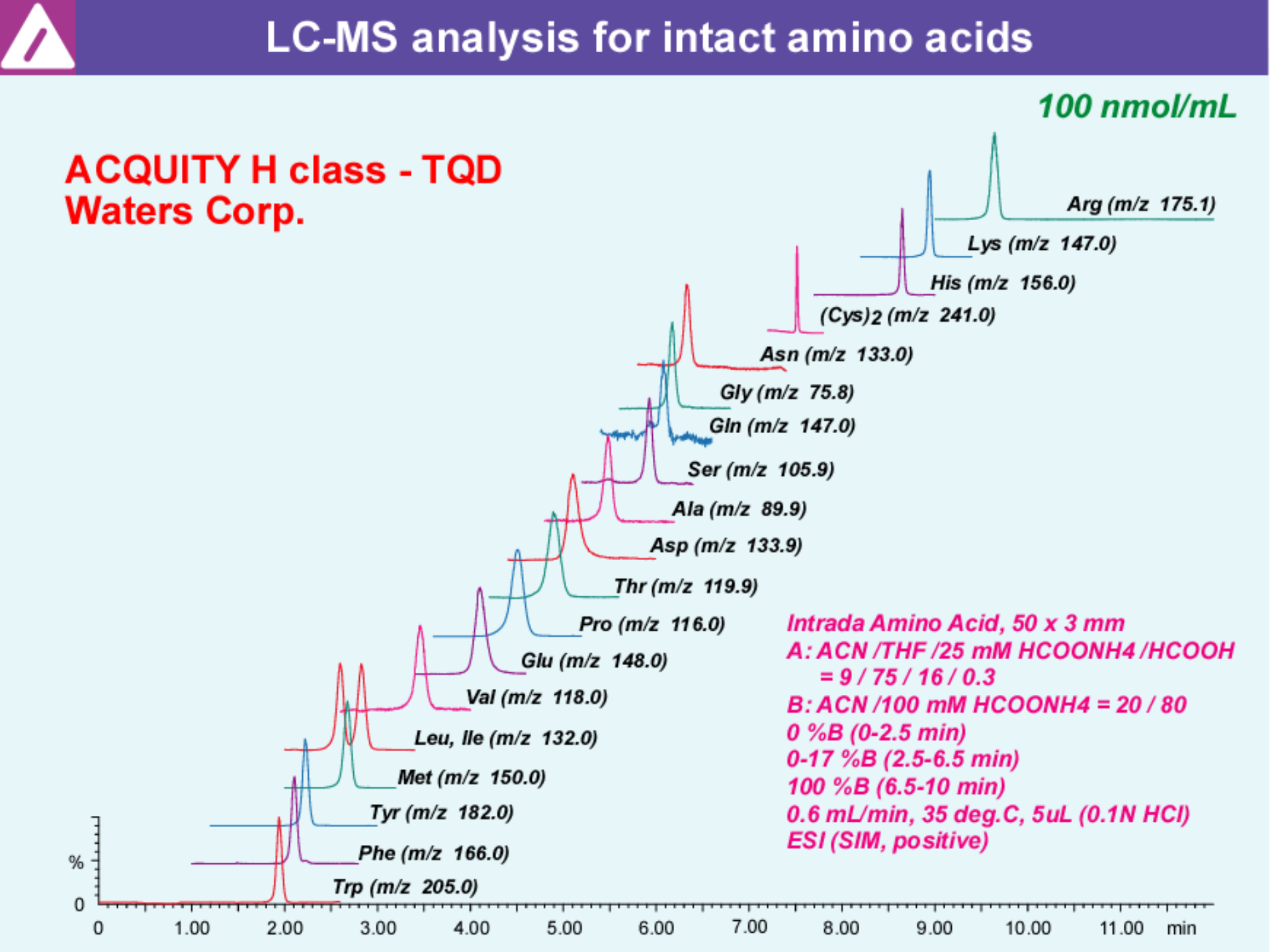
Intrada Amino Acid, 50 x 3 mm
A: ACN /THF /25 mM HCOONH4 /HCOOH
= 9 / 75 / 16 / 0.3
B: ACN /100 mM HCOONH4 = 20 / 80
0 %B (0-2.5 min)
0-17 %B (2.5-6.5 min)
100 %B (6.5-10 min)
0.6 mL/min, 35 deg.C, 5uL (0.1N HCl)
ESI (SIM, positive)

LC-MS analysis for intact amino acids

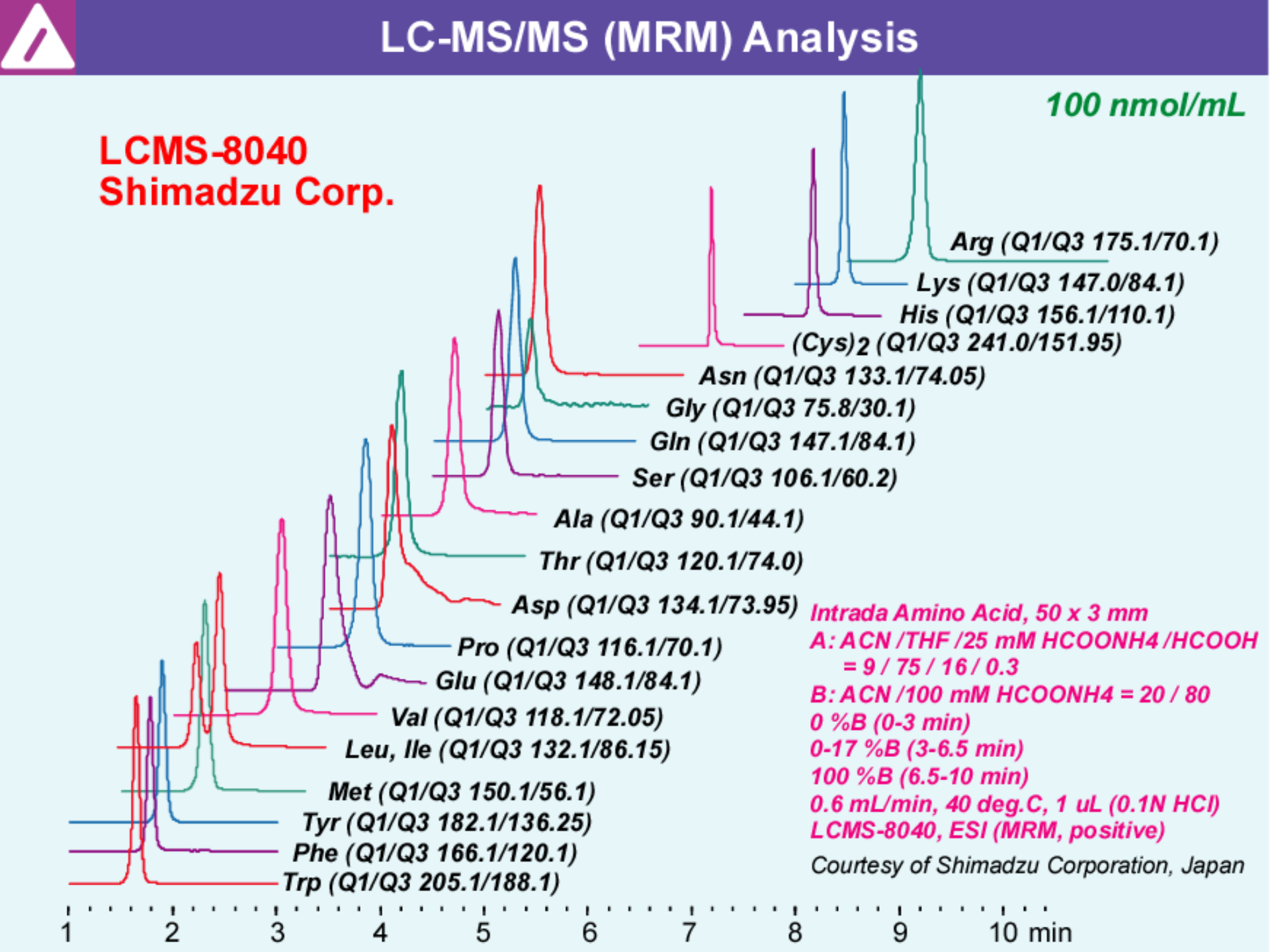


Courtesy of Agilent Technologies

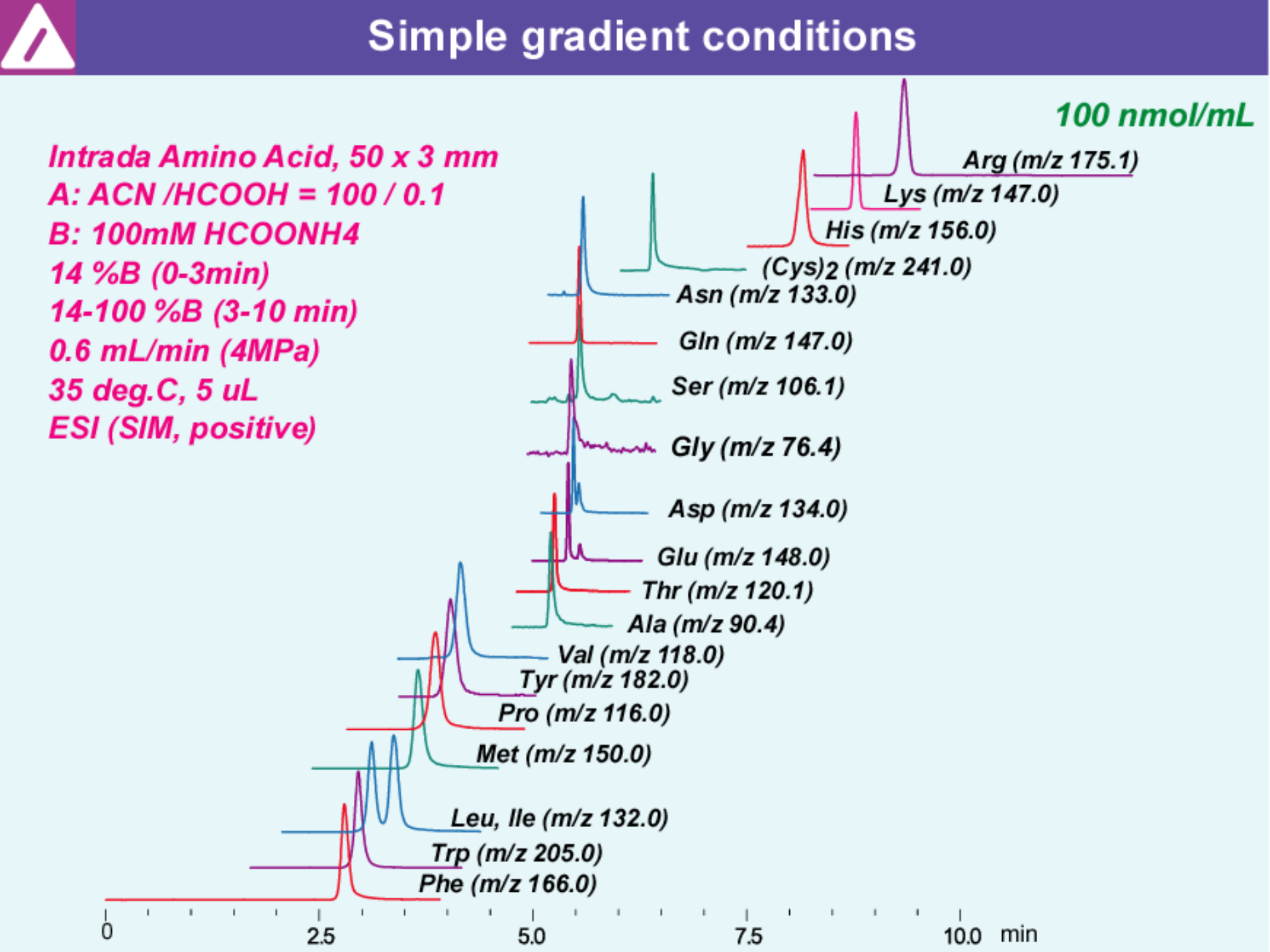
LC-MS analysis for intact amino acids



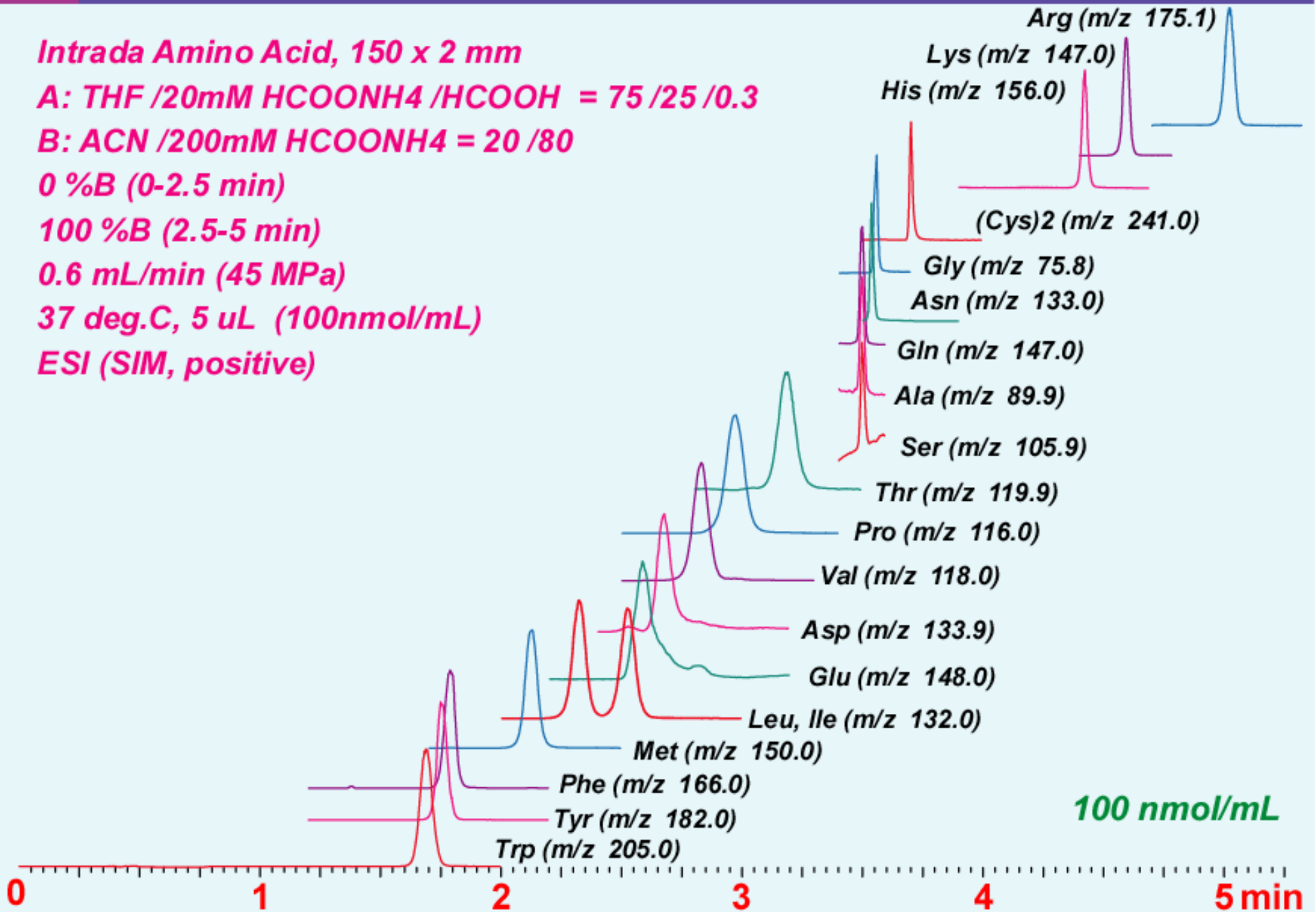
LC-MS/MS (MRM) Analysis



Simple gradient conditions



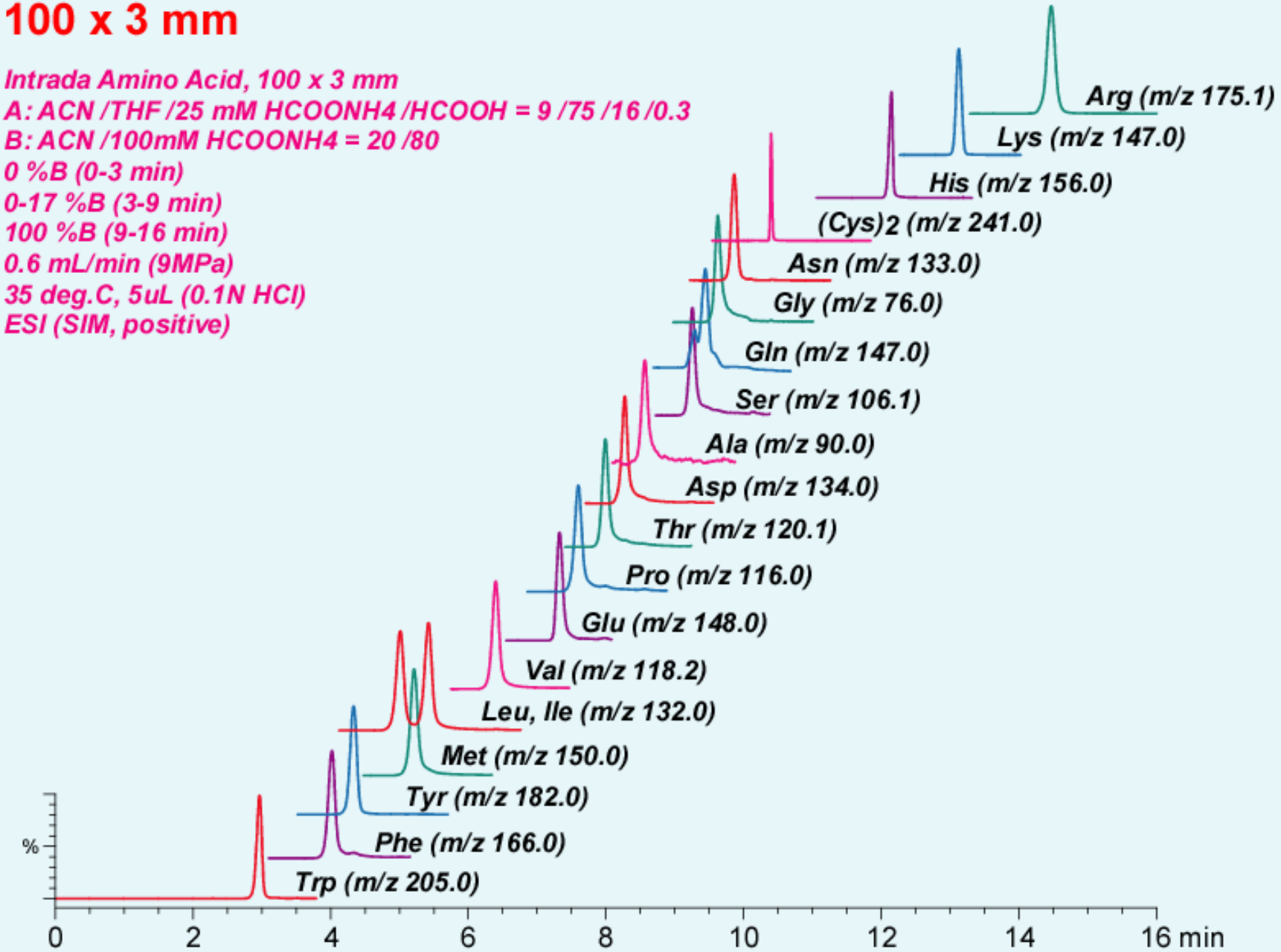
5min High Throughput Analysis



Column dimension (100 x 3 mm)

100 x 3 mm

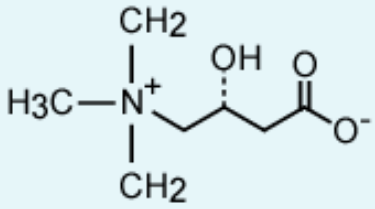
Intrada Amino Acid, 100 x 3 mm
A: ACN /THF /25 mM HCOONH4 /HCOOH = 9 /75 /16 /0.3
B: ACN /100mM HCOONH4 = 20 /80
0 %B (0-3 min)
0-17 %B (3-9 min)
100 %B (9-16 min)
0.6 mL/min (9MPa)
35 deg.C, 5uL (0.1N HCl)
ESI (SIM, positive)

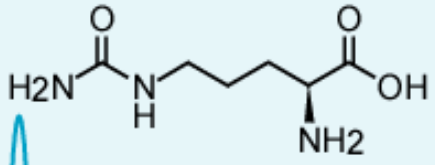


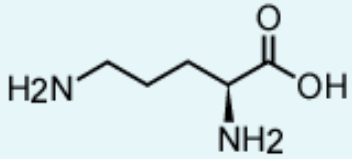
1min High Throughput Analysis

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





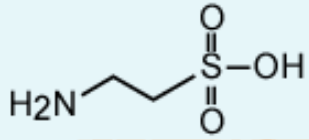


m/z 133.1 (240 nmol/mL) — **ornithine**

m/z 176.1 (170 nmol/mL) — **citrulline**

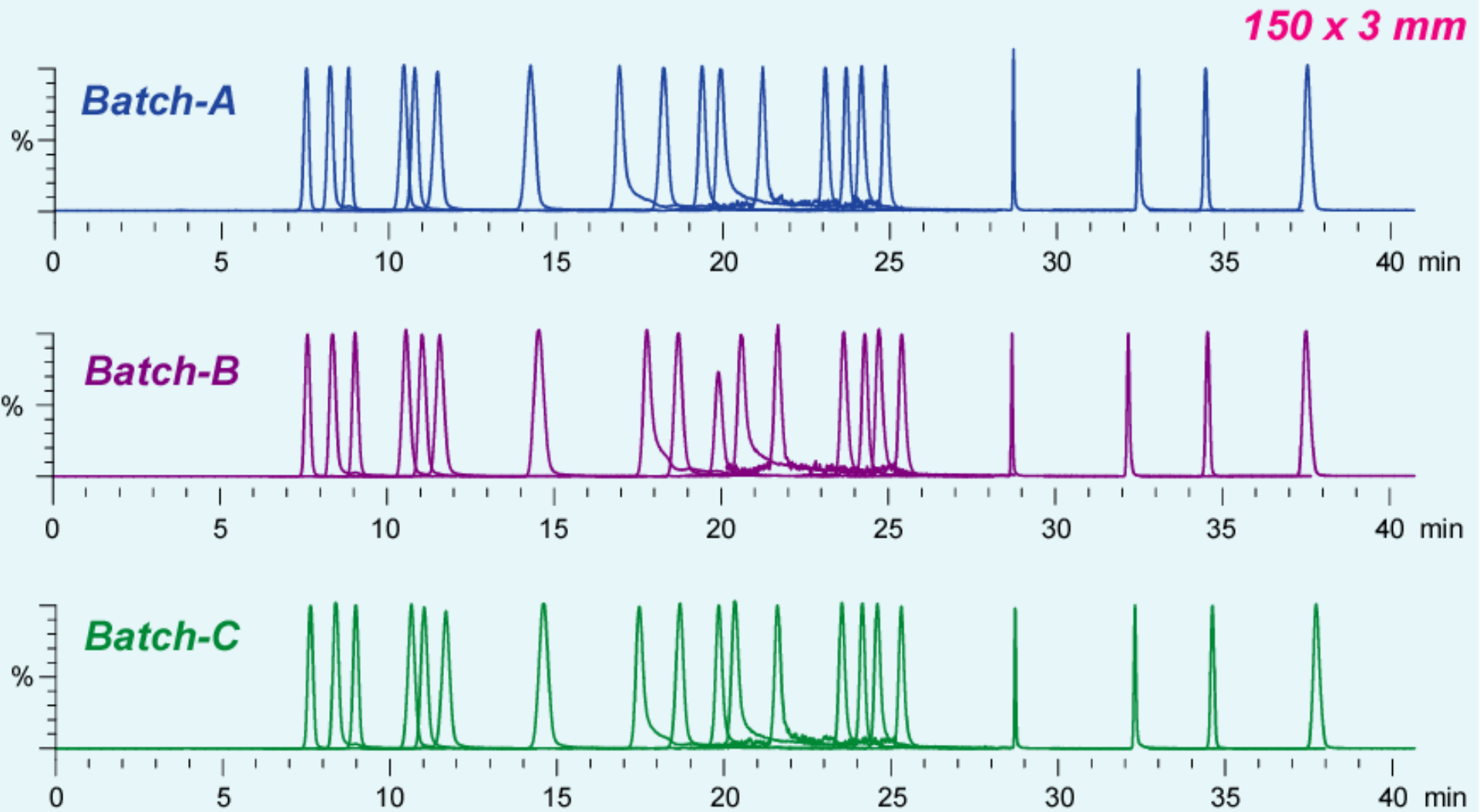
m/z 162.1 (300 pmol/mL) — **carnitine**

m/z 126.1 (1.6 umol/mL) — **taurine**



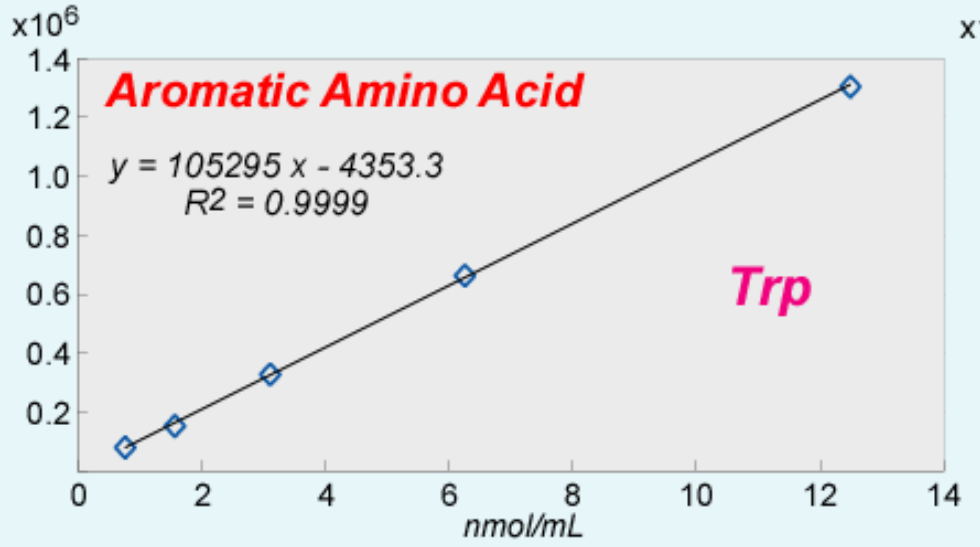
0 *12* *24* *36* *48* *60 sec*

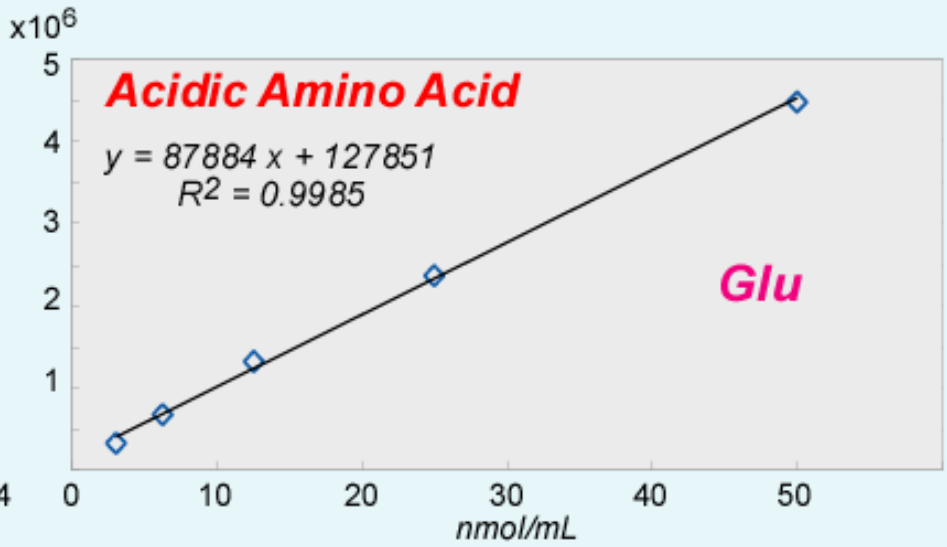
Batch-to-Batch Reproducibility (20 AA)

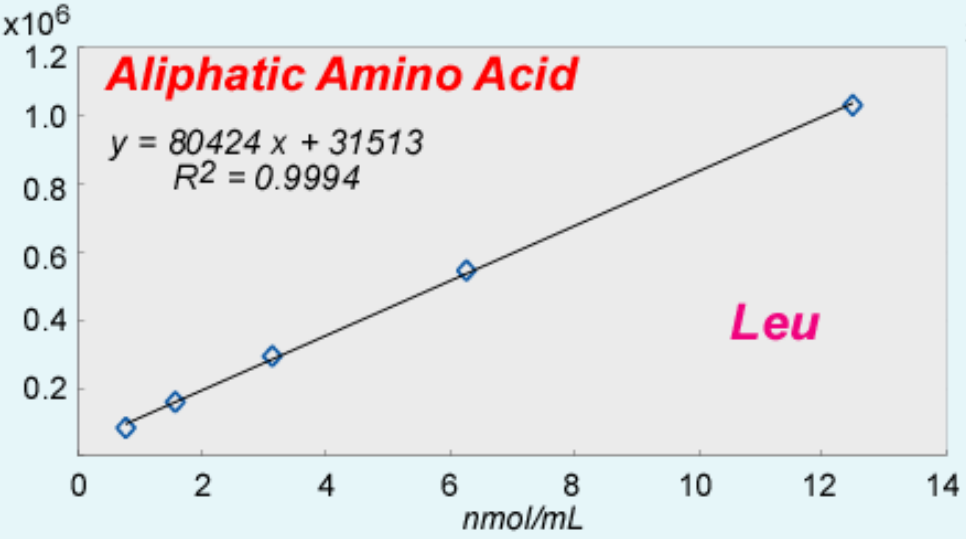


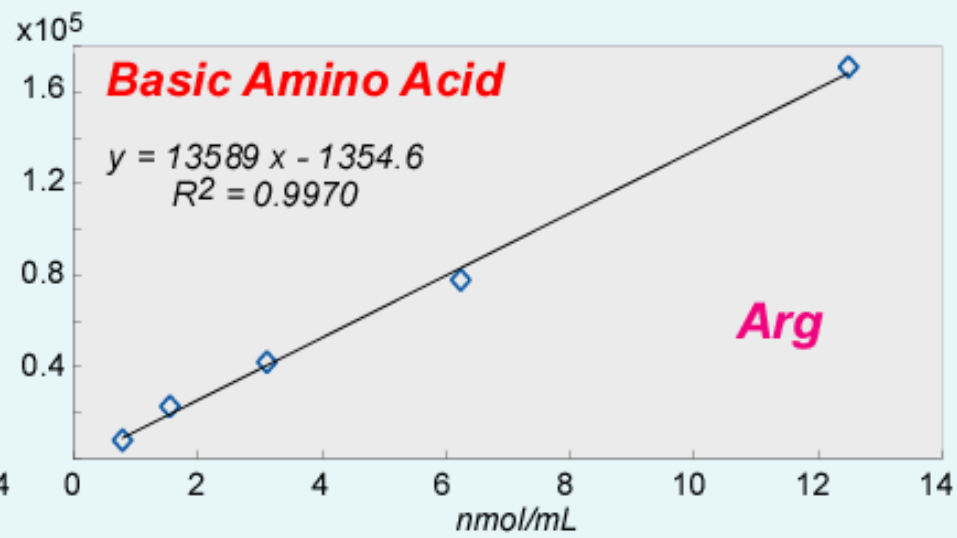
Intrada Amino Acid, 150 x 3 mm
A: ACN /THF /25mM HCOONH4 /HCOOH = 9 / 75 / 16 / 0.3 (v/v/v/v)
B: ACN /100mM HCOONH4 = 20 / 80 (v/v)
0 %B (0-12 min), 0-17 %B (12-26 min), 100 %B (26-45 min)
0.4 mL/min (7MPa), 35 deg.C, 1.2 uL (1 umol/mL in 0.1N HCl), ESI, positive

linearity


x10^6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Aromatic Amino Acid
y = 105295 x - 4353.3
R2 = 0.9999
Trp
0
2
4
6
8
10
12
14
nmol/mL


x10^6
5
4
3
2
1
Acidic Amino Acid
y = 87884 x + 127851
R2 = 0.9985
Glu
0
10
20
30
40
50
nmol/mL


x10^6
1.2
1.0
0.8
0.6
0.4
0.2
Aliphatic Amino Acid
y = 80424 x + 31513
R2 = 0.9994
Leu
0
2
4
6
8
10
12
14
nmol/mL


x10^5
1.6
1.2
0.8
0.4
Basic Amino Acid
y = 13589 x - 1354.6
R2 = 0.9970
Arg
0
2
4
6
8
10
12
14
nmol/mL

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

55 Amino Acids for Intrada Amino Acid/LC-MS

alanine (Ala, m/z 90.0)

β-alanine (β-Ala, m/z 90.0)

α-aminoadipic acid (m/z 162.1)

α-aminobutyric acid (m/z 104.0)

β-aminobutyric acid (m/z 104.0)

β-aminoisobutyric acid (m/z 104.0)

γ-aminobutyric acid (GABA, m/z 104.0)

2-aminoethanol (EtOHNH2, m/z 62.1)

5-aminolevulinic acid (m/z 132.0)

5-aminovaleric acid (m/z 118.1)

α-aminopimelic acid (Apm, m/z 162.1)

anserine (Ans, m/z 241.1)

arginine (Arg, m/z 175.1)

asparagine (Asn, m/z 133.0)

aspartic acid (Asp, m/z 134.0)

carnosine (Car, m/z 227.1)

citrulline (Cit, m/z 176.1)

cysteic acid (Cys(O3H), m/z 170.0)

cysteine (Cys, m/z 122.0)

cystine ((Cys)2, m/z 241.0)

cystathionine (Cysthi, m/z 223.1)

glutamic acid (Glu, m/z 148.0)

glutamine (Gln, m/z 147.0)

glycine (Gly, m/z 76.4)

glycylproline (Gly-Pro, m/z 173.1)

histidine (His, m/z 156.0)

homoserine (m/z 120.1)

hydroxylysine (Hylys, m/z 163.0)

hydroxyproline (Hyp, m/z 116.0)

isoleucine (Ile, m/z 132.0

allo-isoleucine (m/z 132.0)

leucine (Leu, m/z 132.0)

lysine (Lys, m/z 147.0)

methionine (Met, m/z 150.0)

methionine sulfone (m/z 182.0)

1-methylhistidine (1-Mehis, m/z 170.1)

3-methylhistidine (3-Mehis, m/z 170.1)

o-methylserine (m/z 120.1)

norleucine (m/z 132.0)

norvaline (m/z 118.0)

ornithine (Orn, m/z 133.1)

phenylalanine (Phe, m/z 166.0)

o-phosphoethanolamine (m/z 142.0)

o-phosphoserine (Ser (P), m/z 186.0)

proline (Pro, m/z 116.0)

sarcosine (Sar, m/z 90.0)

serine (Ser, m/z 106.1)

taurine (Tau, m/z 126.0)

theanine (m/z 175.1)

threonine (Thr, m/z 120.1)

allo-threonine (allo-Thr, m/z 120.1)

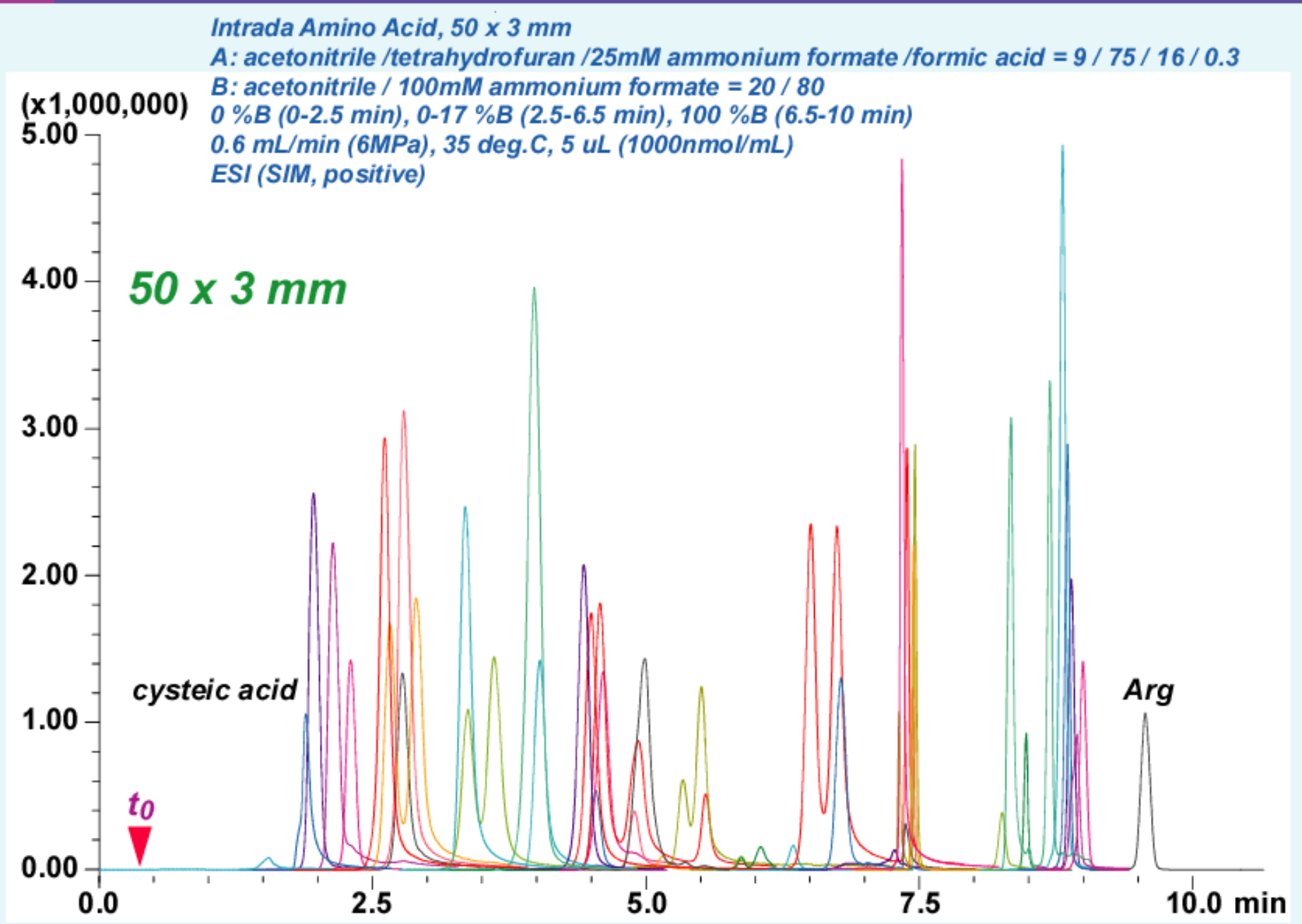
thioproline (m/z 134.0)

tryptophan (Trp, m/z 205.0)

tyrosine (Tyr, m/z 182.0)

valine (Val, m/z 118.0)

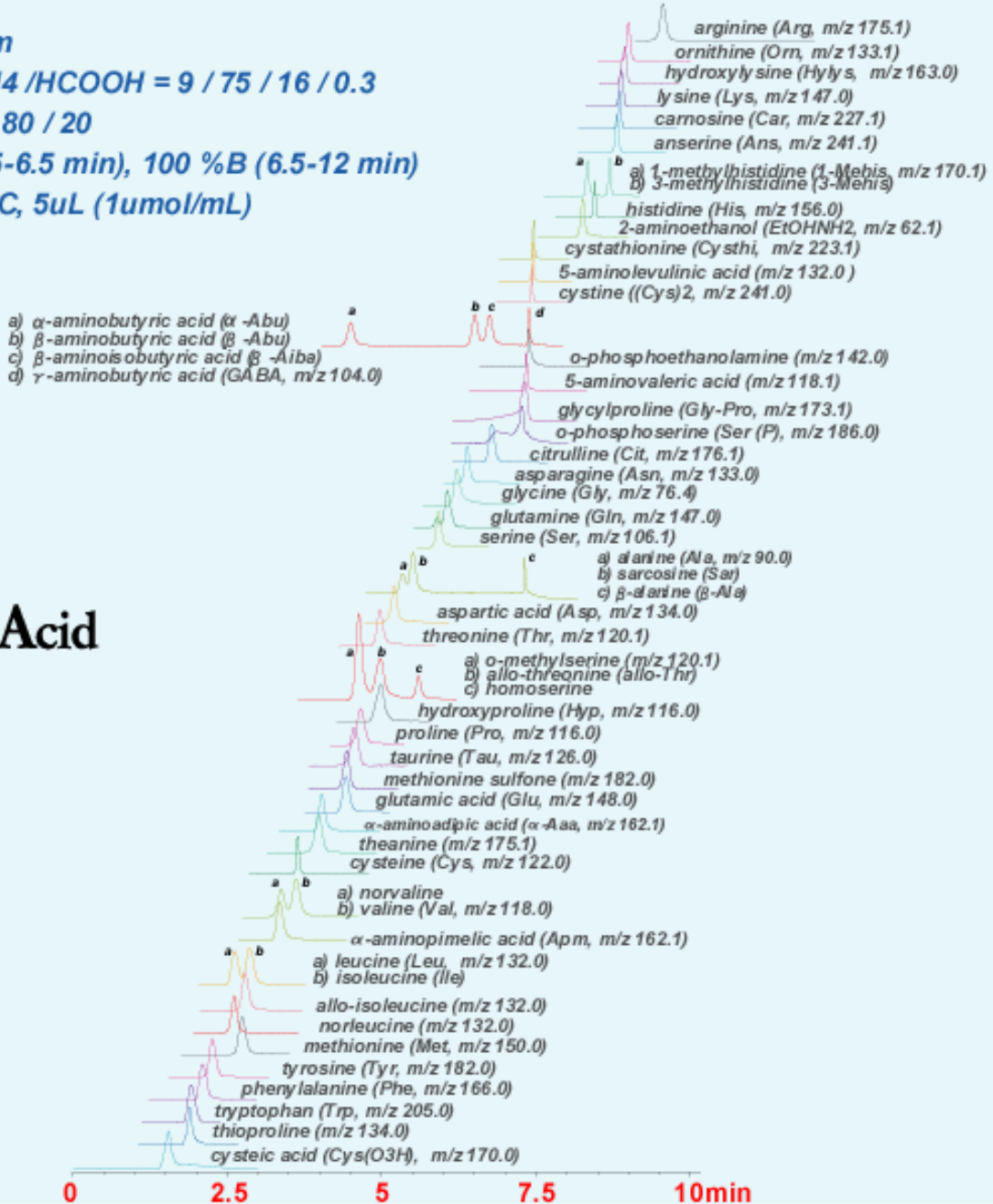
LC-MS analysis for 55 Amino Acids



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)

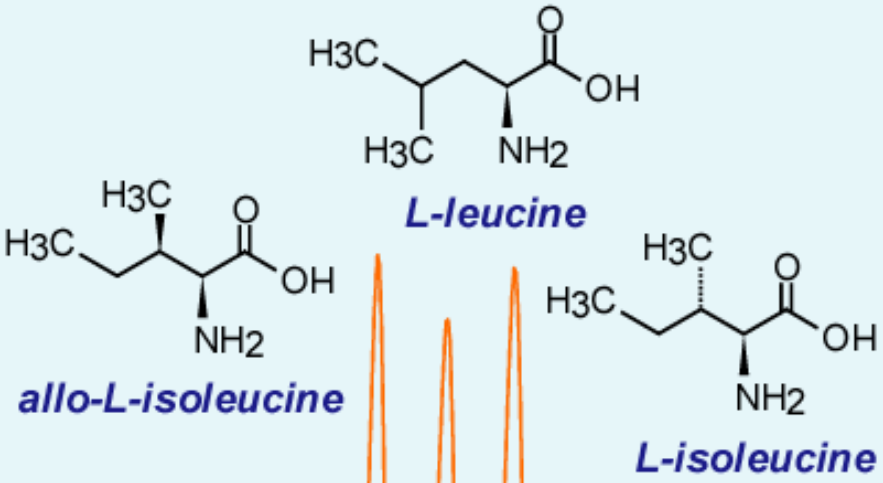
Intrada Amino Acid

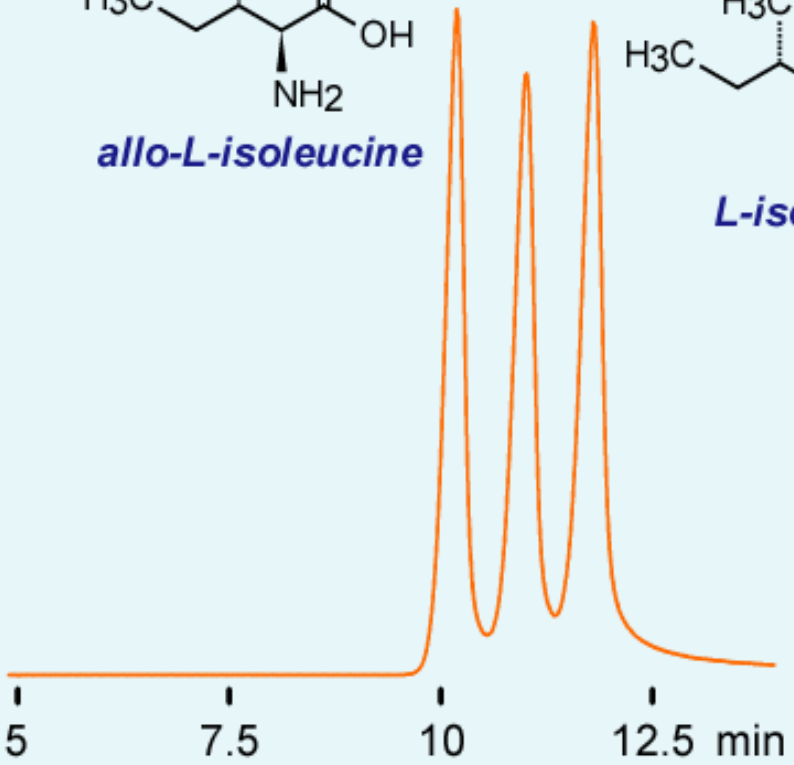


Leucine Isomers (131Da)

m/z 132.0

150 x 3 mm



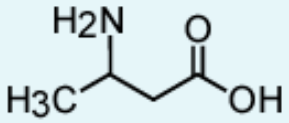


Intrada Amino Acid, 150 x 3 mm
A: MeOH /water /HCOOH = 85 / 15 / 0.3
B: ACN /100mM HCOONH4 = 20 / 80
12-13 %B (0-12 min)
100 %B (12-15 min)
0.4 mL/min (11 MPa)
35 deg.C, 5 uL (0.1N HCl)
ESI (positive, m/z 132.0)

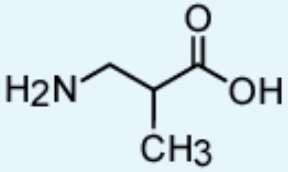
Alpha, Beta, Gamma Amino Acid Isomers (103Da)

m/z 104.1

100 x 3 mm

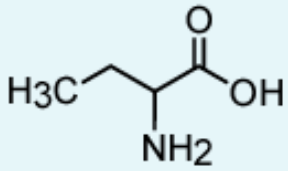


3-aminobutyric acid
(beta-aminobutyric acid)

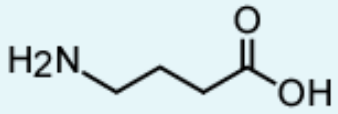


3-aminoisobutyric acid
(beta-aminoisobutyric acid)

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

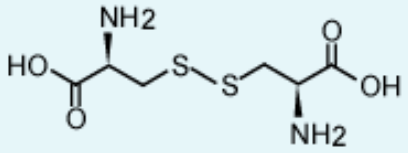


2-aminobutyric acid
(alpha-aminobutyric acid)



Intrada Amino Acid, 100 x 3 mm
A: ACN /HCOOH = 100 / 0.3
B: 100mM HCOONH4
25-30 %B (0-12 min)
100 %B (12-15min)
0.4 mL/min (4MPa)
37 deg.C, 5 uL (0.1N-HCl aq.)
ESI (positive, m/z 104.1)

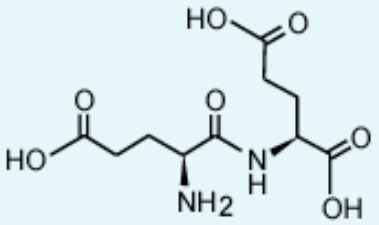
Dipeptides



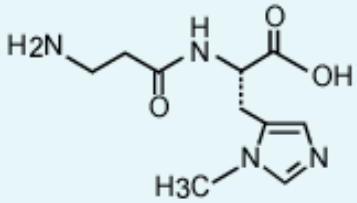
L-cystine ((Cys)2)

75 x 2 mm

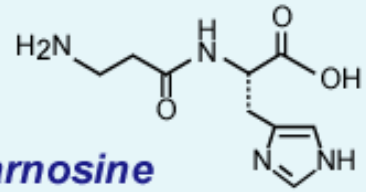
Intrada Amino Acid, 75 x 2 mm
A: acetonitrile / formic acid = 100 / 0.3
B: 100 mM ammonium formate
20-100 %B (0-10 min), 100 %B(10-12 min)
0.3 mL/min (5 MPa), 35 deg.C
2 uL (0.02-0.32 ug, 0.1N HCl)
ESI (SIM, positive)



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

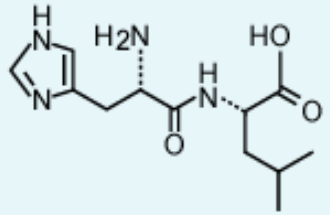


L-anserine

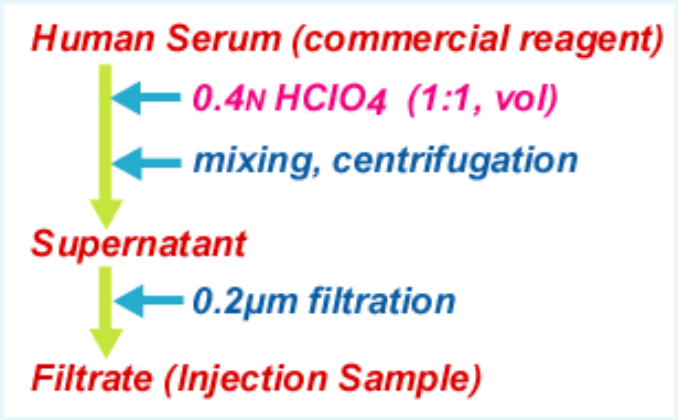


L-carnosine

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

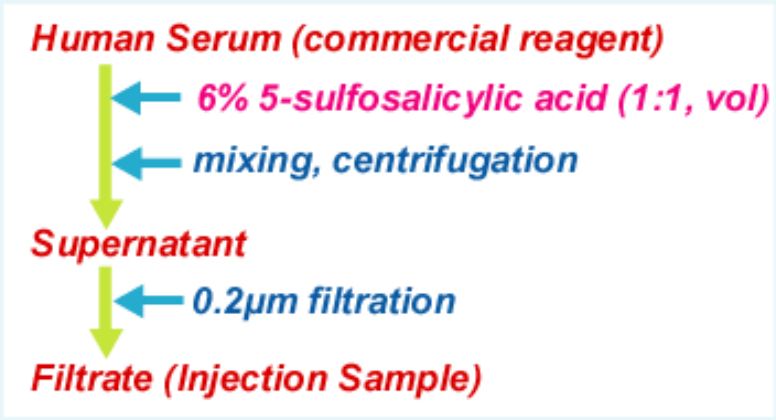


Amino acids in human serum (HClO4)



Intrada Amino Acid, 50 x 3 mm
A: ACN /THF /25mM HCOONH4 /HCOOH = 9 /75 /16 /0.3
B: ACN /100mM HCOONH4 = 20 /80
0 %B (0-2.5 min), 0-17 %B (2.5-6.5 min), 100 %B (6.5-10 min)
0.6 mL/min (6MPa), 35 deg.C, 5 uL
ESI (SIM, positive)

Amino acids in human serum (SULFOSALICYLIC ACID)



Intrada Amino Acid, 50 x 3 mm
A: ACN /THF /25mM HCOONH4 /HCOOH = 9 /75 /16 /0.3
B: ACN /100mM HCOONH4 = 20 /80
0 %B (0-2.5 min), 0-17 %B (2.5-6.5 min), 100 %B (6.5-10 min)
0.6 mL/min (6MPa), 35 deg.C, 5 uL
ESI (SIM, positive)

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

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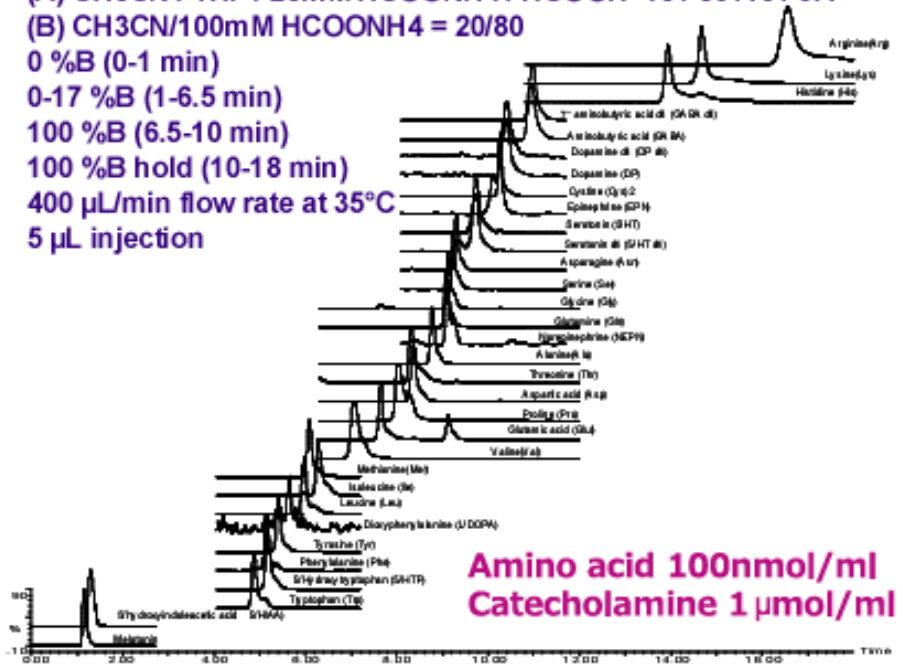
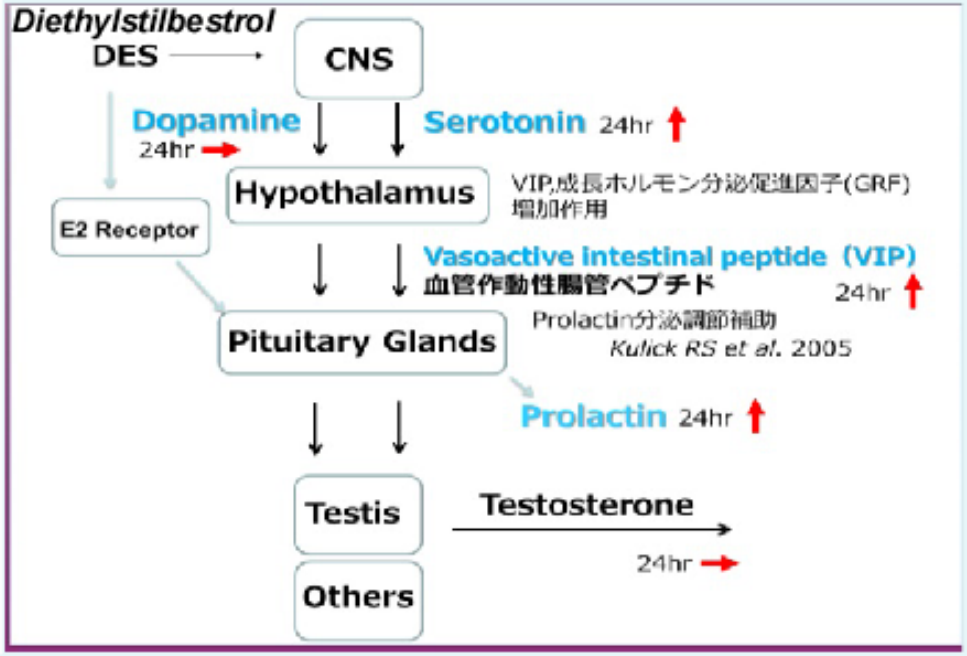
ACMS2013 (61st), Sept 10, 2013, Tsukuba, Japan

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

Conclusions

- *Intact amino acid analysis column for LC-MS*
- *Analysis time was less than 10min*
- *Available for both Single MS and Triple MS system*
- *Good results for linearity and repeatability*
- *Various column dimensions for isomers and dipeptides*
- *Available for practical samples like serum*