

Amino Acid Analysis of Spinach and Apple by UHPLC

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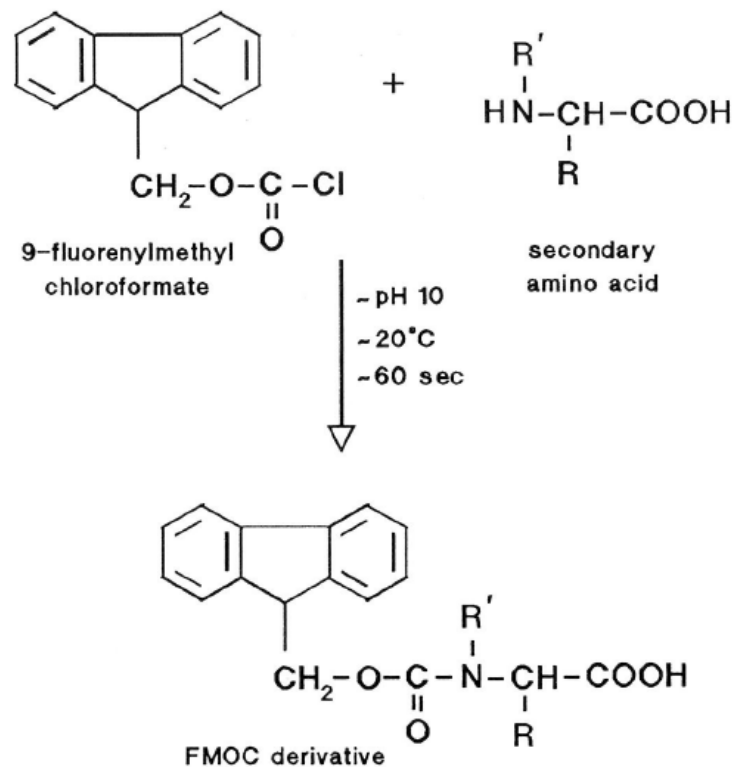
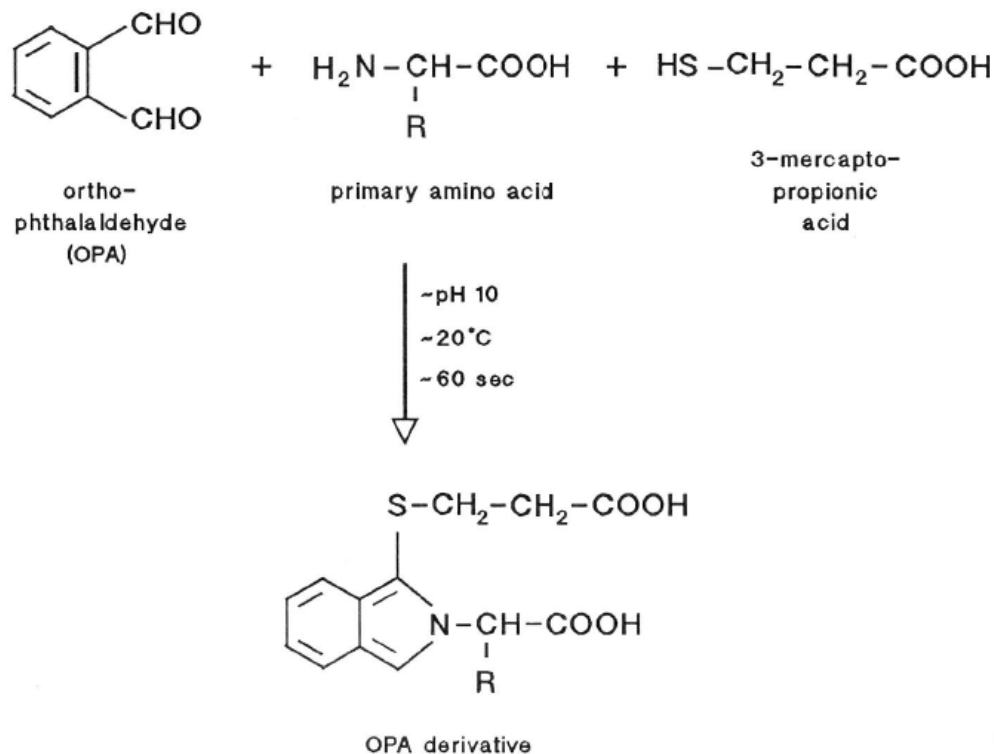


Outline

- Background of AAA on Eclipse Plus C18
- Transfer to UHPLC
- Chromatograms of Spinach and Apple
- Recent protein hydrolysate work

Automated Online Precolumn Derivatization

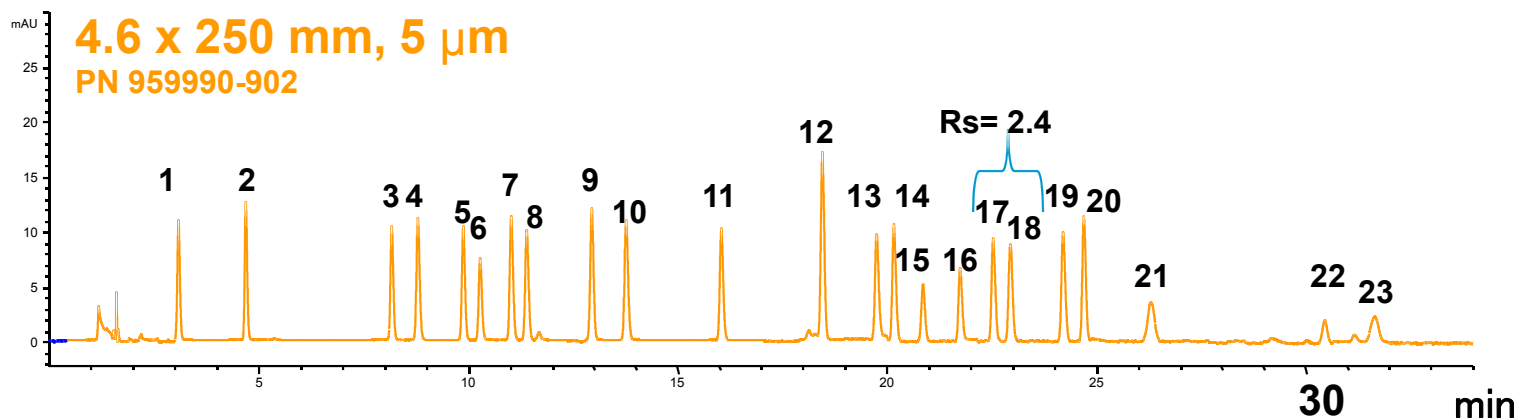
The primary amino groups react with ortho-phthalaldehyde (OPA) in the presence of 3-mercaptopropionic acid (3-MPA) at about pH 10 to form an isoindole derivative. Secondary amino groups do not react. The OPA derivatized amino acid is then detected by UV at 338 nm.



The secondary amino groups react with 9-fluorenylmethyl chloroformate (Fmoc) at pH 10 to form a secondary amide. The Fmoc derivatized amino acid is then detected by UV at 262 nm.

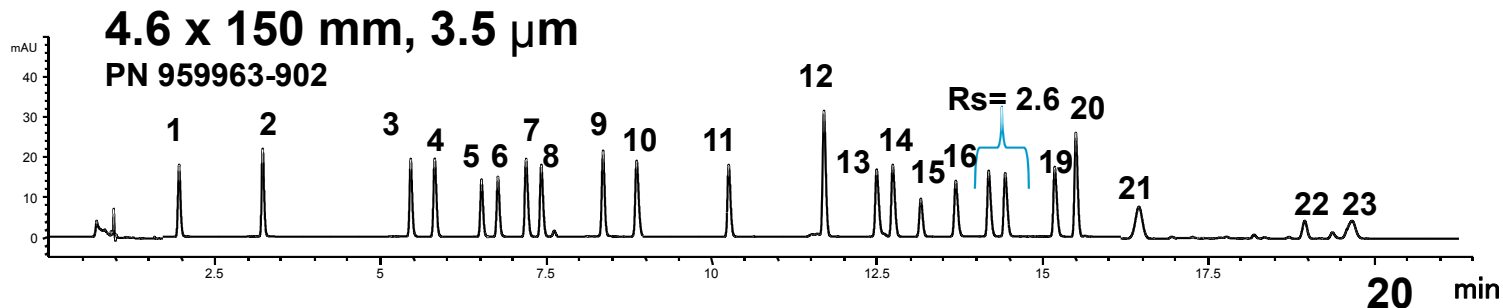
Amino Acid Analysis on Agilent 1200SL

An Eclipse Plus C18 5 μ m Option (40 min with re-equilibration)



1. Aspartic acid
2. Glutamic acid
3. Asparagine
4. Serine
5. Glutamine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
11. Tyrosine
12. Cystine
13. Valine
14. Methionine
15. Norvaline
16. Tryptophan
17. Phenylalanine
18. Isoleucine
19. Leucine
20. Lysine
21. Hydroxyproline
22. Sarcosine
23. Proline

A Rapid Resolution 3.5 μ m Option (24 min with re-equilibration)





What is Different About Columns for UHPLC?

Only Two Things:

1. Column Particle Size

- Smaller particle sizes are used (<2um)

2. Column Operating Pressure Limits

- Pressure limits for the column and LC instrument are > 400 bar, for UHPLC instruments, 600 – 1200 bar is typical (8700-17400 psi)

Everything else about the column remains the same



Amino Acid Analysis on 1290 Infinity UHPLC

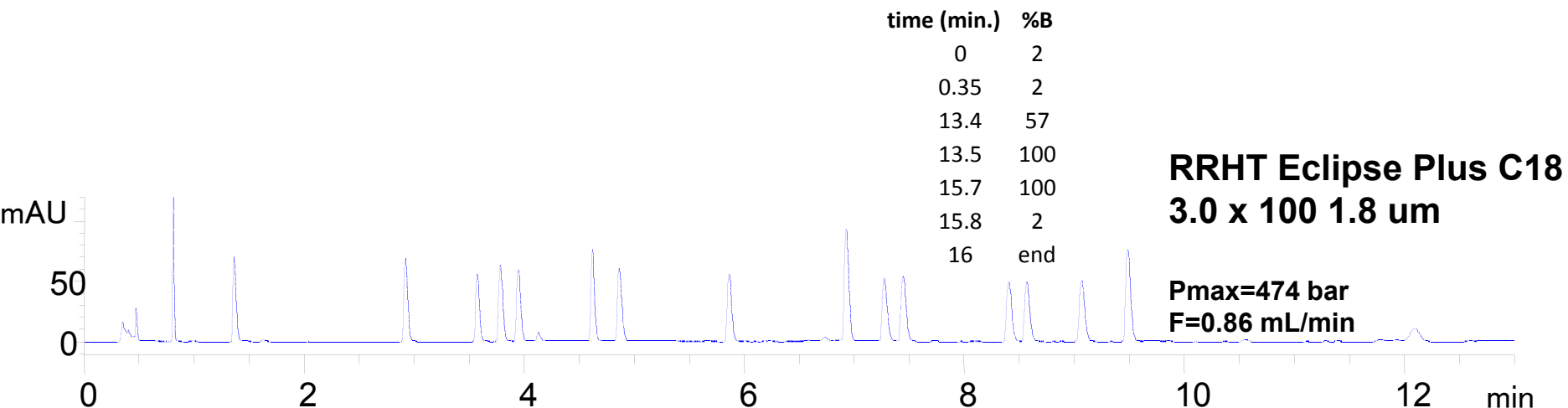
1200SL



1290 Infinity



17 Amino Acid Analysis on 1290 Infinity



original 1200 SL injection program 100 uL sample loop

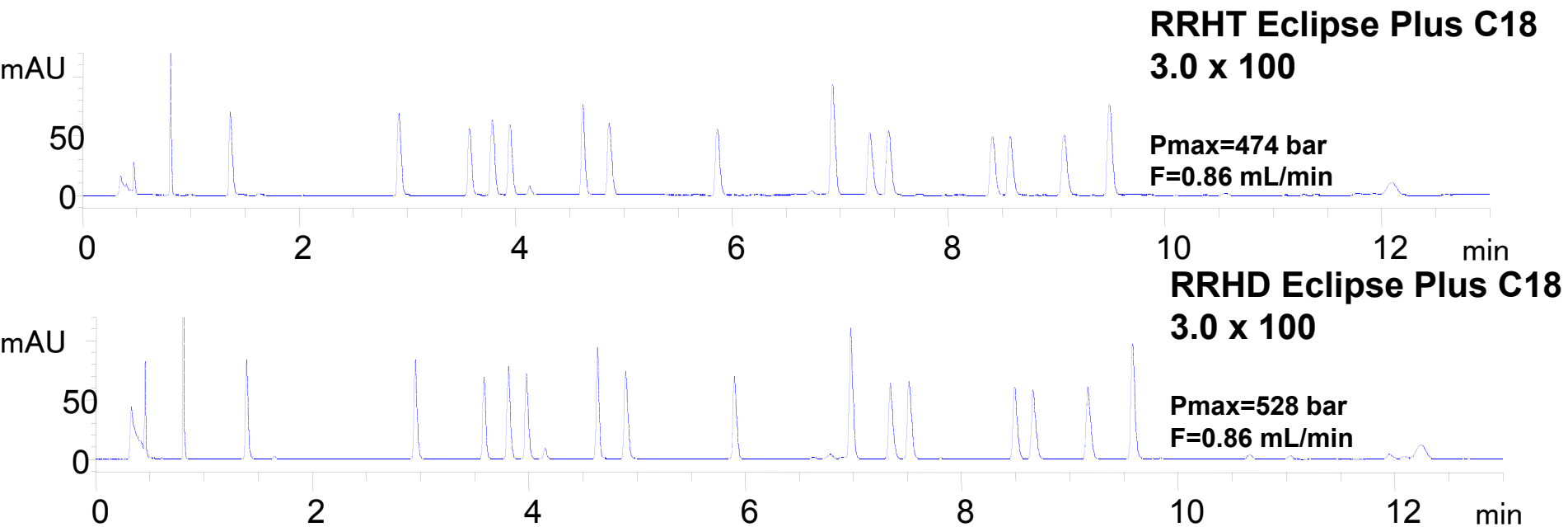
original 1290 Infinity injection program 20 uL sample loop (40 uL available)

- 1) Draw 2.5 μL from *Borate* vial (Agilent P/N 5061-3339)
- 2) Draw 1.0 μL from *Sample* vial
- 3) Mix 3.5 μL in washport 5X
- 4) Draw 0.5 μL from *OPA* vial (Agilent P/N 5061-3335)
- 5) Mix 4.0 μL in washport 10X max speed
- 6) Draw 0.4 μL from *FMOC* vial (Agilent P/N 5061-3337)
- 7) Mix 4.4 μL in washport 10X max speed
- 8) Draw 32 μL from *Injection Diluent* vial
- 9) Mix 20 μL in washport 8X
- 10) Inject

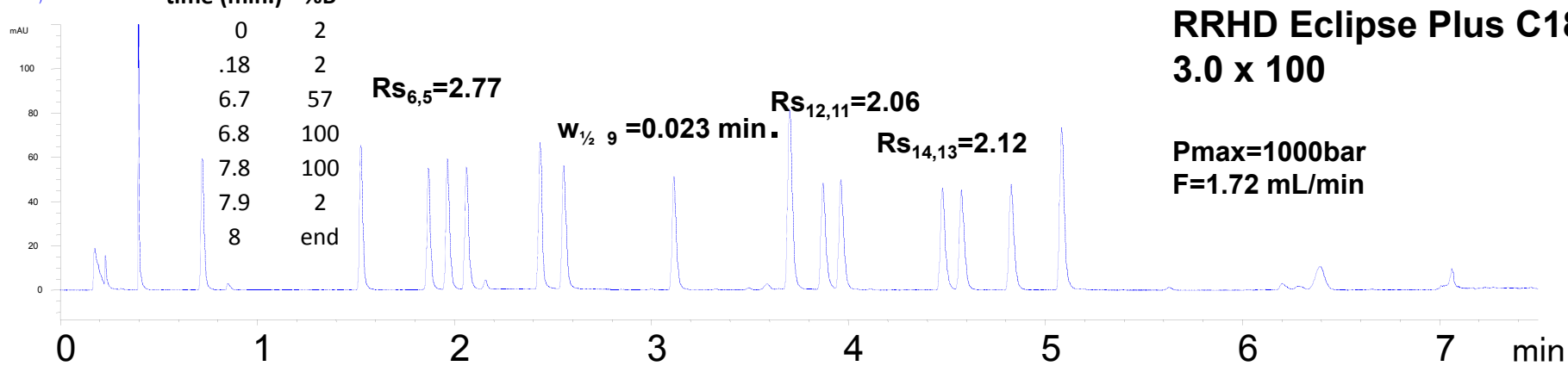
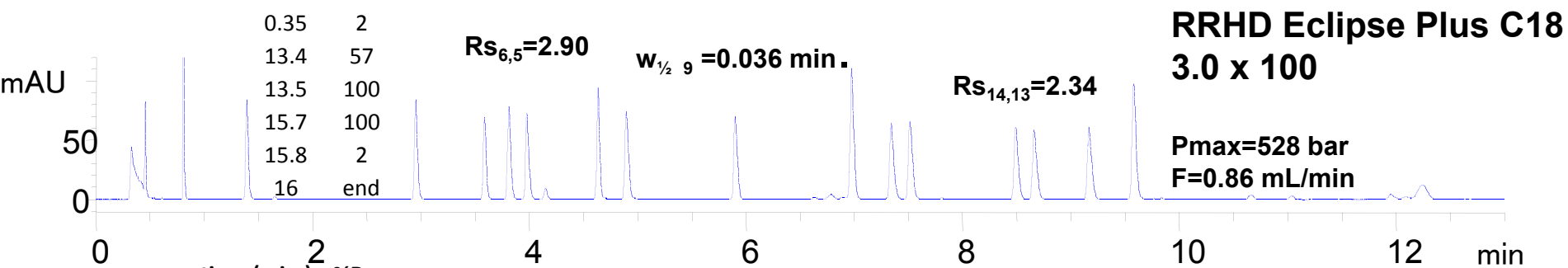
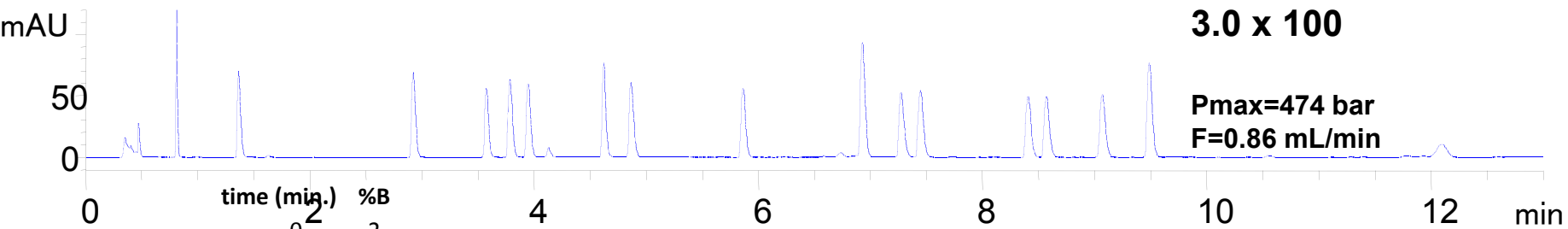
- 1) Draw 1.3 μL from *Borate* vial (Agilent P/N 5061-3339)
- 2) Draw 0.5 μL from *Sample* vial
- 3) Mix 1.8 μL in location P1C1 5X default speed, offset
- 4) Draw 0.5 μL from *OPA* vial (Agilent P/N 5061-3335)
- 5) Mix 2.3 μL in location P1C1 5X default speed, offset
- 6) Draw 0.2 μL from *FMOC* vial (Agilent P/N 5061-3337)
- 7) Mix 2.5 μL in in location P1C1 5X default speed, offset
- 8) Draw 7.4 μL from *Injection Diluent* vial
- 9) Mix 9.9 μL in location P1C1 5X default speed, offset
- 10) Inject



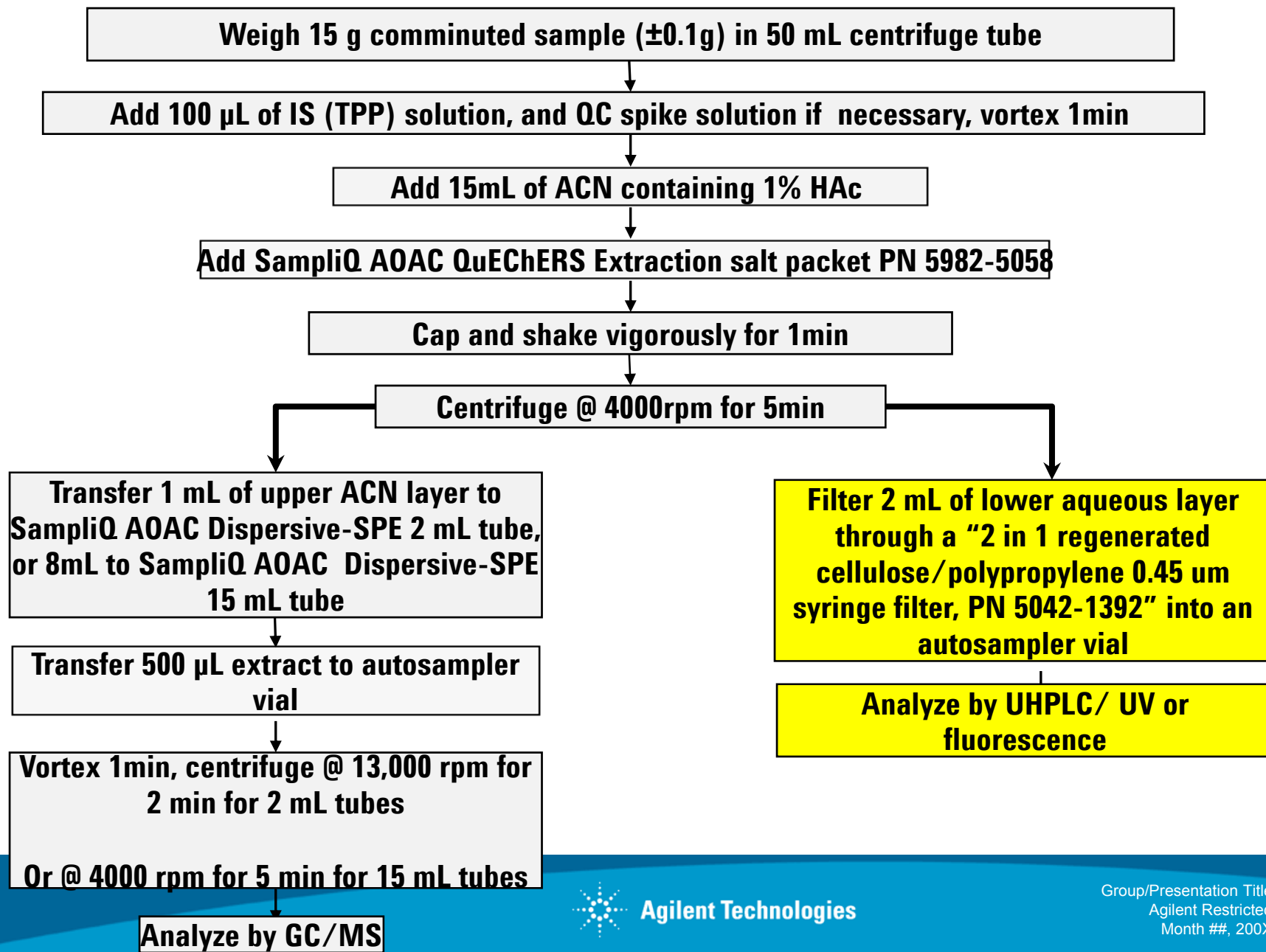
Transferability between RRHT and RRHD columns



Double Flow Rate and Halve Gradient Time to Double Throughput

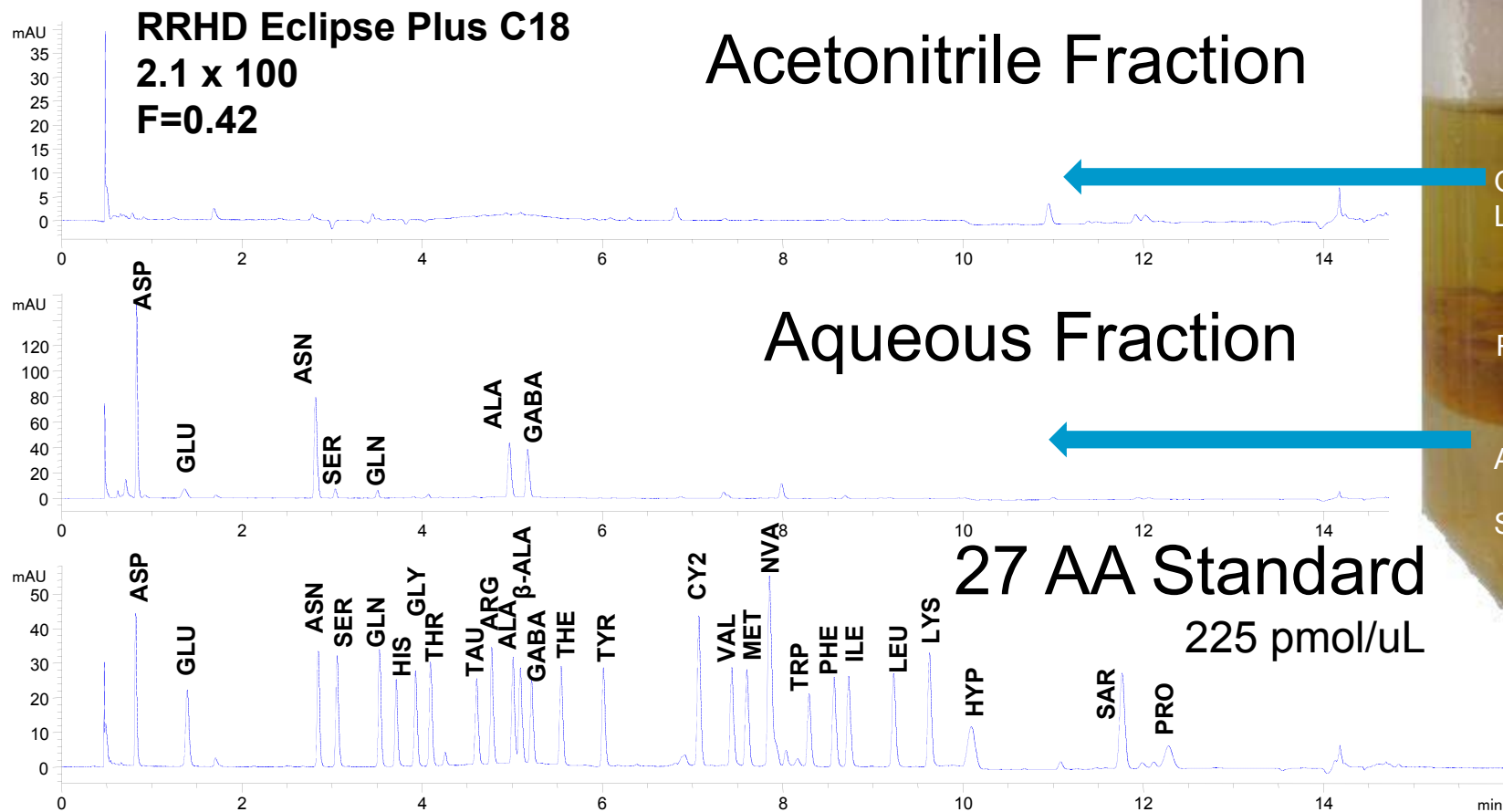


The QuEChERS Technique



Amino acids in Apple

from Quechers tube (AOAC Method 2007.01)



Includes: GABA, β-alanine, taurine, theanine, norvaline, hydroxyproline, sarcosine



GABA: It's a valuable component of free amino acid pool in most prokaryotic and eukaryotic organisms

In vertebrates:

GABA is the major neurotransmitter inhibitor in the central nervous system that prevents anxiety and stress related messages from reaching the motor centers of the brain

Inhibits the migration of colon carcinoma cells- paving the way to develop drugs to delay or inhibit cancer

In plants GABA might have a role in:

pH regulation

Nitrogen storage

Plant development

Plant defenses

Amino Acid and GABA content in different cultivars of *Momordica charantia* L.
S. Park, Y. Kim, H. Xu, H. Boo, S. Lee,
Journal of Medicinal Plants Research Vol. 3(11), pp.897-900 November 2009

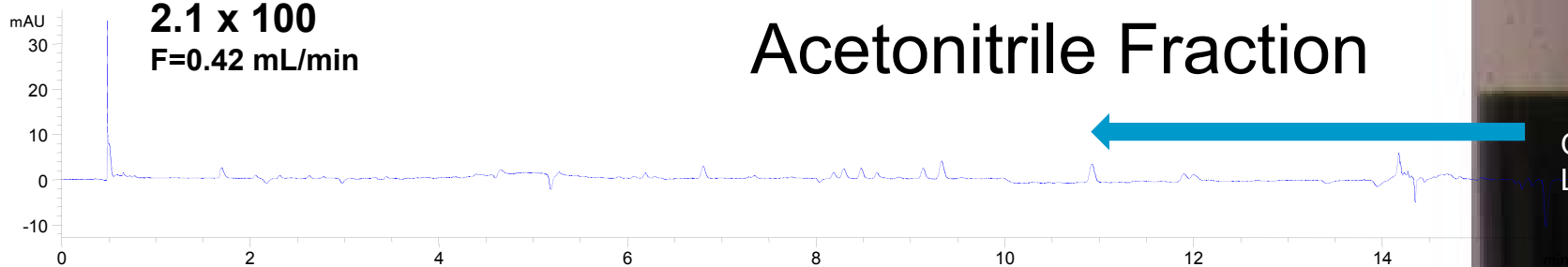
Amino acids in Spinach leaf from Quechers tube (AOAC Method 2007.01)

RRHD Eclipse Plus C18

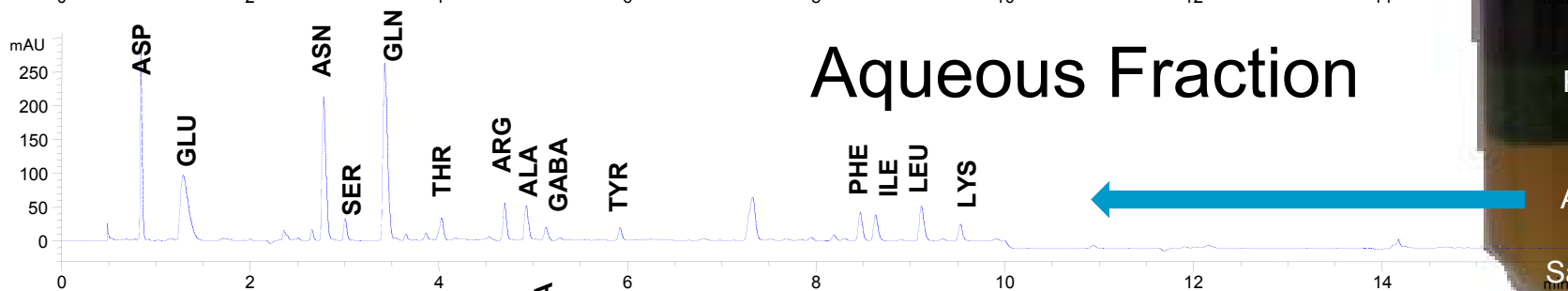
2.1 x 100

F=0.42 mL/min

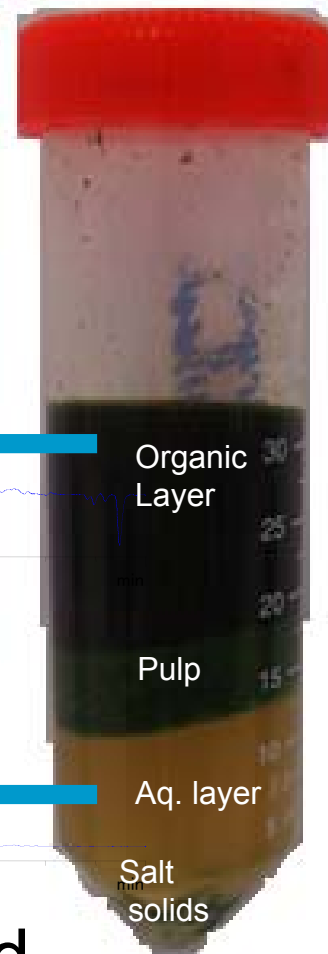
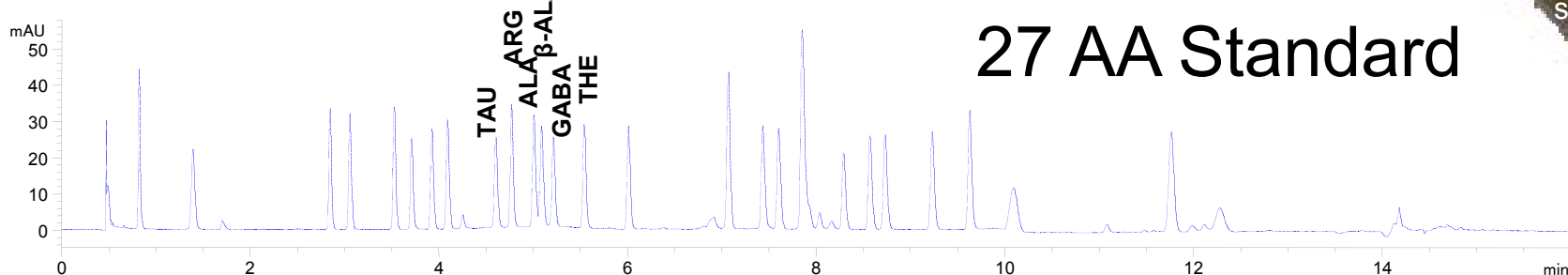
Acetonitrile Fraction



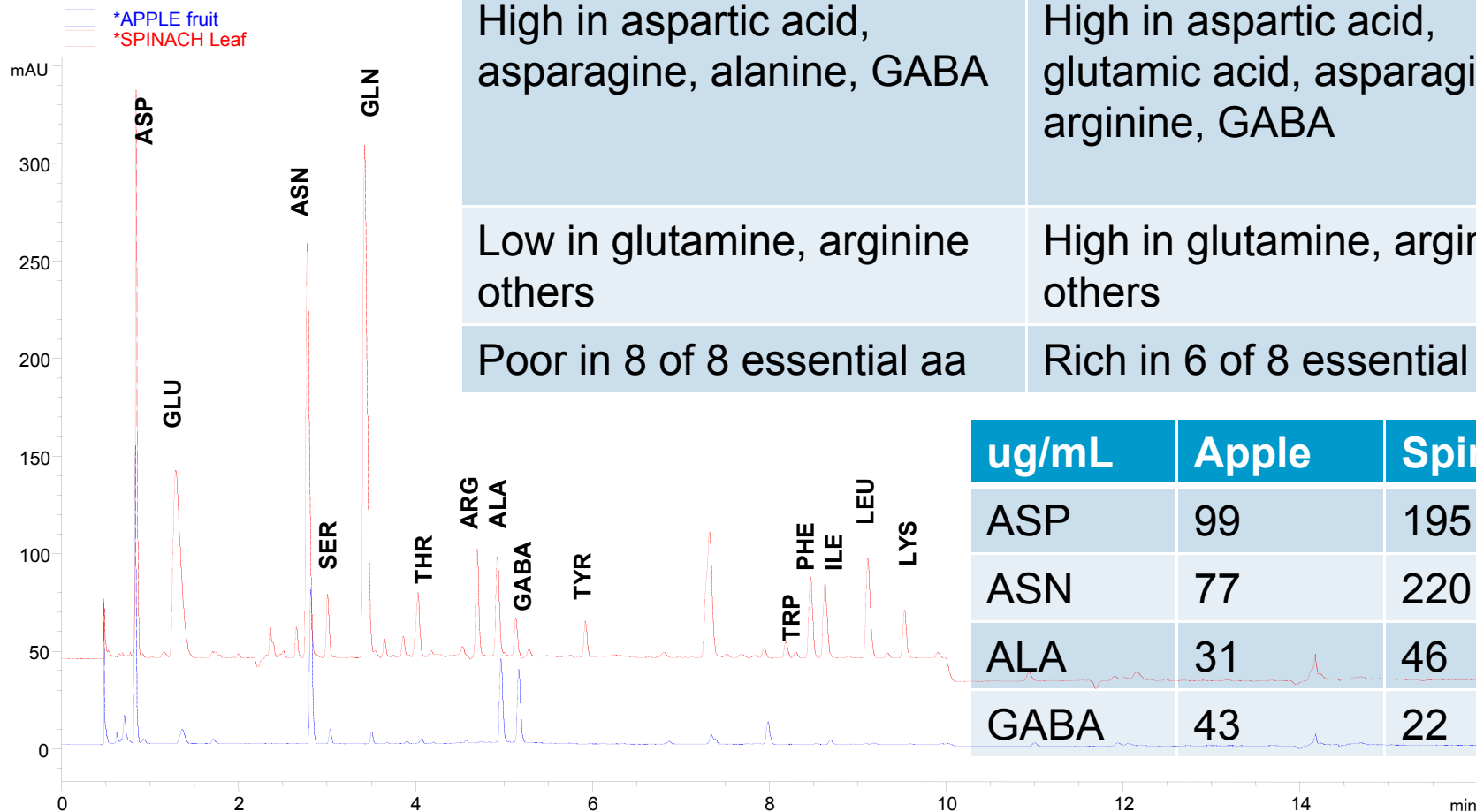
Aqueous Fraction



27 AA Standard



Comparing Amino Acid Profiles

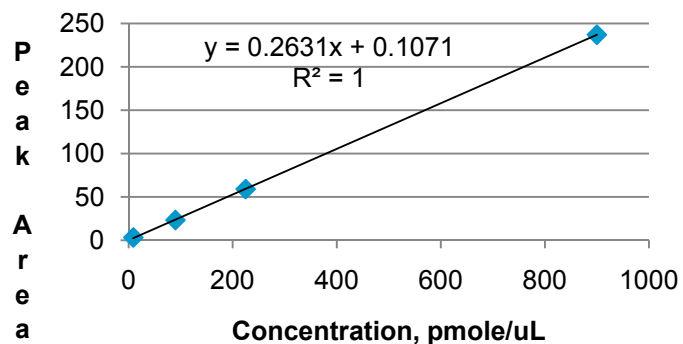


Apple	Spinach
High in aspartic acid, asparagine, alanine, GABA	High in aspartic acid, glutamic acid, asparagine, arginine, GABA
Low in glutamine, arginine others	High in glutamine, arginine others
Poor in 8 of 8 essential aa	Rich in 6 of 8 essential aa

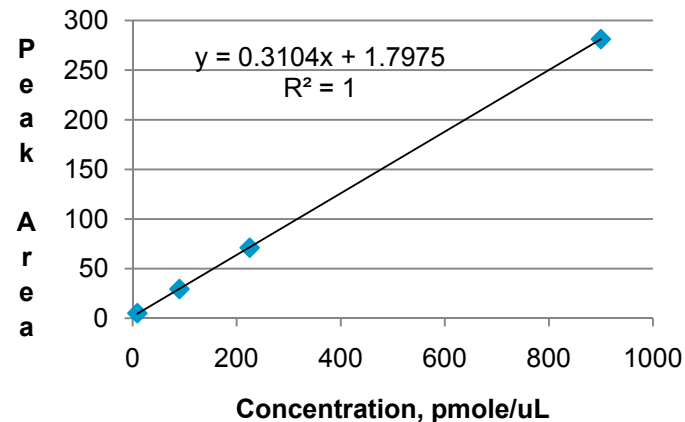
ug/mL	Apple	Spinach
ASP	99	195
ASN	77	220
ALA	31	46
GABA	43	22

Linearity (9-900 pmol/uL) of Early, Middle and Late Eluting Amino Acids

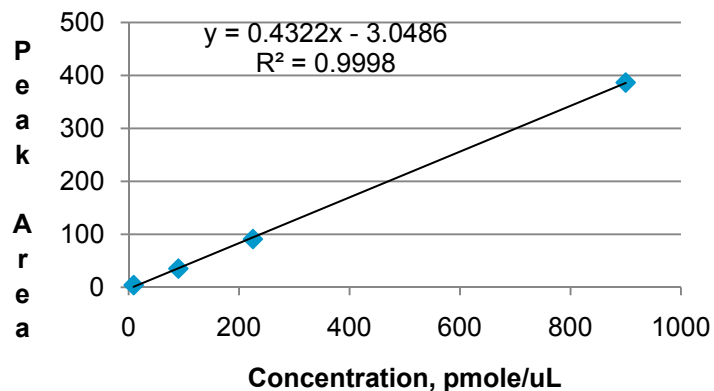
Glutamic Acid



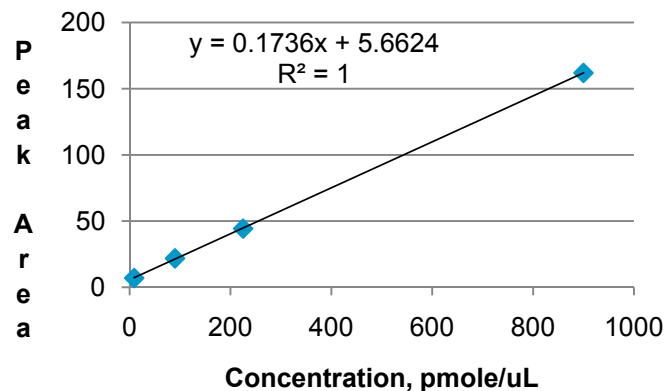
Tyrosine



Lysine



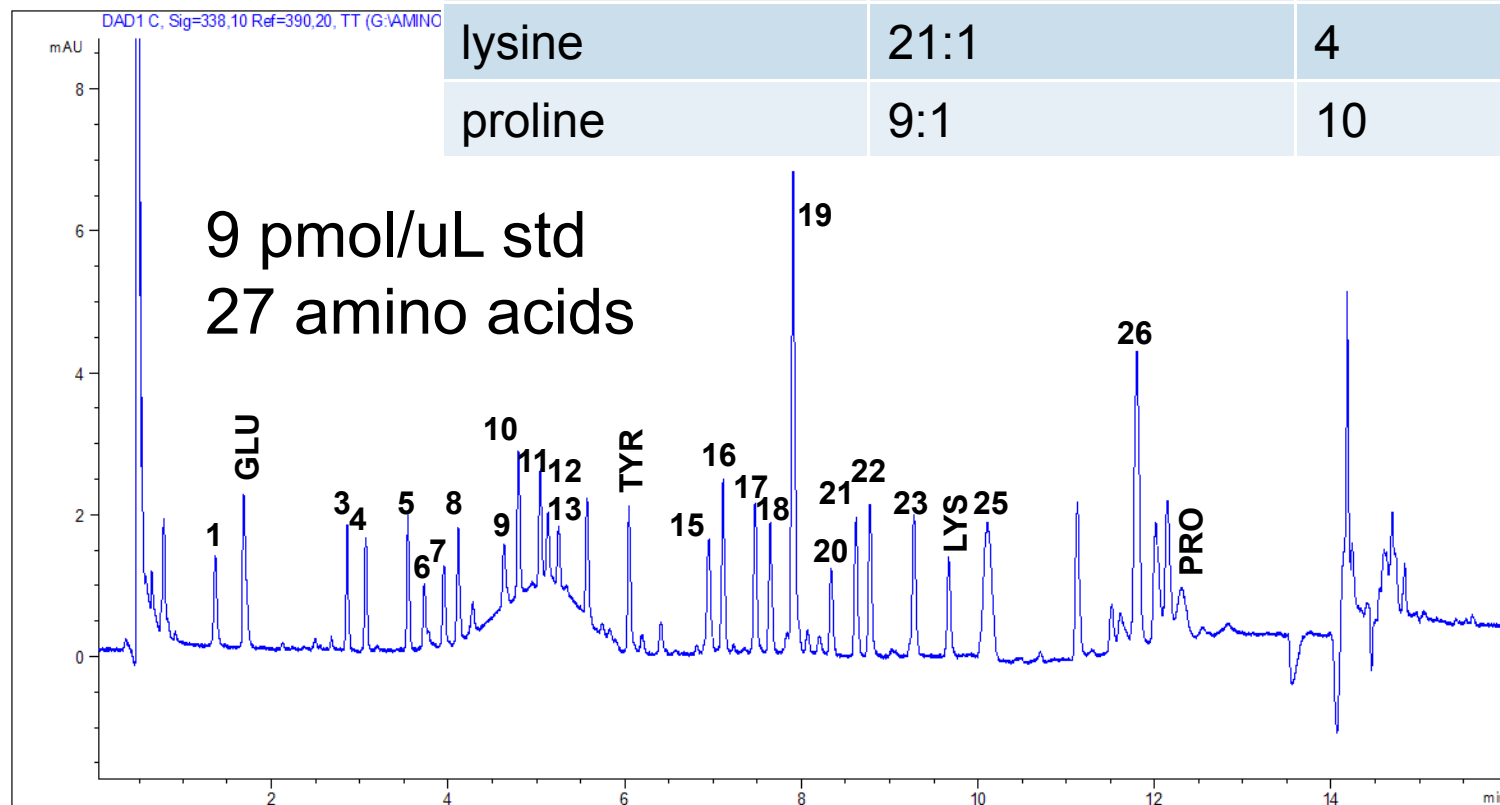
Proline



Quantitative Limit

UV detection with 10 mm Maxlight flow cell, 4nm slit

	S/N 9 pmol/uL	LOQ S/N 10:1
glutamic acid	21:1	4 pmol/ul
tyrosine	32:1	3
lysine	21:1	4
proline	9:1	10



Repeatability on 1290 Infinity

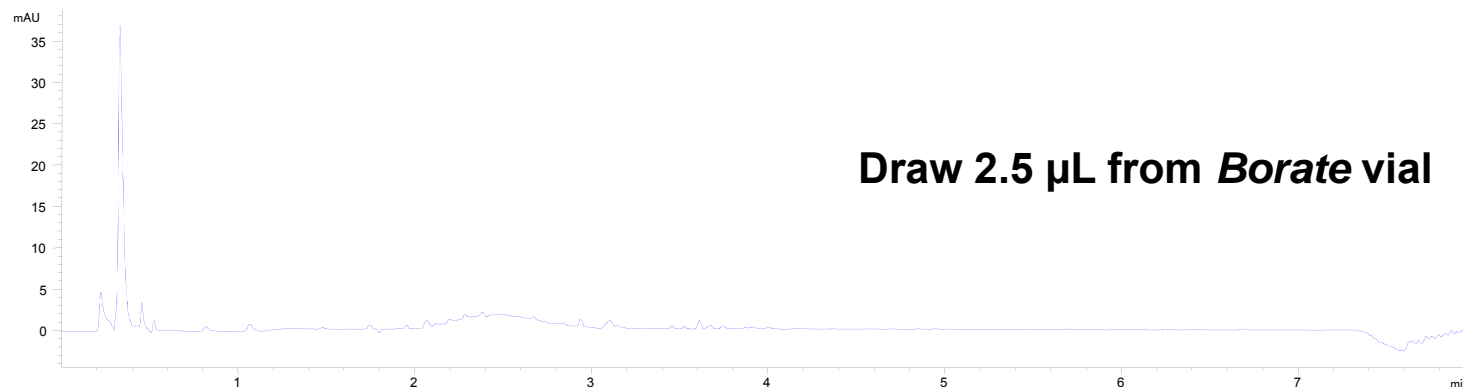
RRHD Eclipse Plus C18 2.1 x 100, 1.8 um

	asp	glu	ser	his	gly	thr	arg	ala	tyr	cy2	val	met	phe	ile	leu	lys	pro
dvd16002.d	136.8	139.3	162.5	122.7	163.2	153.4	172.2	171.7	157.1	258	165	173.5	159	160.1	170.9	243.8	77.5
dvd16003.d	136.4	138.1	161.9	121	162.2	152.7	171	171	154.5	256.4	164	172	158.2	159.1	170	243.2	78.8
dvd16004.d	136.7	138.7	161.3	120.8	163.5	153.1	172.7	182.9	156.4	257.9	164.4	173.4	158.7	160.8	170.6	241.3	82.5
dvd16005.d	137.9	140.1	162.3	121.3	162.1	153.5	173.1	184	157.4	258.7	166.4	174.9	159.5	161.4	172	239.3	82.3
dvd16006.d	135.2	137.4	160.6	118.6	161.3	151.4	170.9	182.3	153.9	255	163.7	171.9	157.1	159.5	169.7	238.2	77.5
dvd16007.d	136.6	139.3	161.9	119	161.7	152.7	172.6	184.8	155.5	258.3	164.5	173.8	159	160.2	171	236.2	76.3
stdev	0.865	0.954	0.688	1.327	0.855	0.764	0.924	6.337	1.414	1.405	0.958	1.140	0.842	0.828	0.815	2.962	2.640
avg	136.6	138.8	161.8	120.6	162.3	152.8	172.1	179.5	155.8	257.4	164.7	173.3	158.6	160.2	170.7	240.3	79.2
RSD	0.63	0.69	0.43	1.27	0.53	0.50	0.54	3.53	0.91	0.55	0.58	0.66	0.53	0.52	0.48	1.23	3.33

Eclipse Plus C18 2.1 x 150, 3.5 um

Peak Area											StDEV	Mean	%RSD
amino acid	Injection 1	Injection 2	Injection 3	Injection 4	Injection 5	Injection 6	Injection 7	Injection 8	Injection 9				
glu	90.7	93.7	92.4	93.1	93.8	94.7	92.5	95.7	93.2	1.43	93.3	1.5	
ala	109.9	113.2	111.8	112.1	113.3	114	111.6	116	113.3	1.72	112.8	1.5	
cy2	153.2	157	155	157	157.8	159	154.4	162.6	158	2.79	157.1	1.8	
lys	142.3	142.5	137.1	144.4	141.9	143.5	137.9	140.7	138.9	2.55	141.0	1.8	
pro	60.8	62.9	61.8	65.8	60.9	63.2	61.2	70.2	61	3.11	63.1	4.9	

Acidic Protein hydrolysates on 1200SL



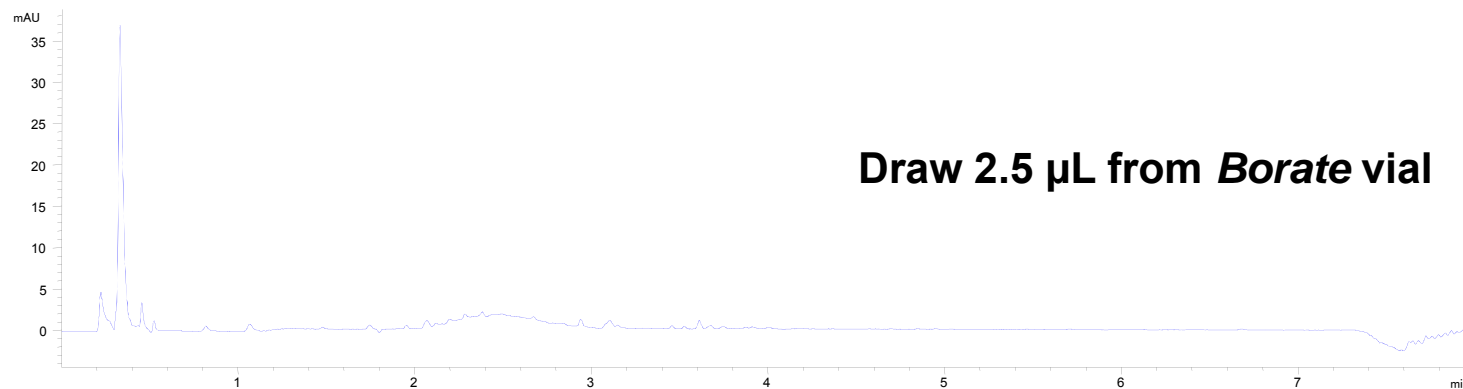
AOAC 994.12

- 0.5 g well ground animal feed +100 mL 6N HCl + phenol
- Boil 24 h
- Filter and dilute to 250 mL with water

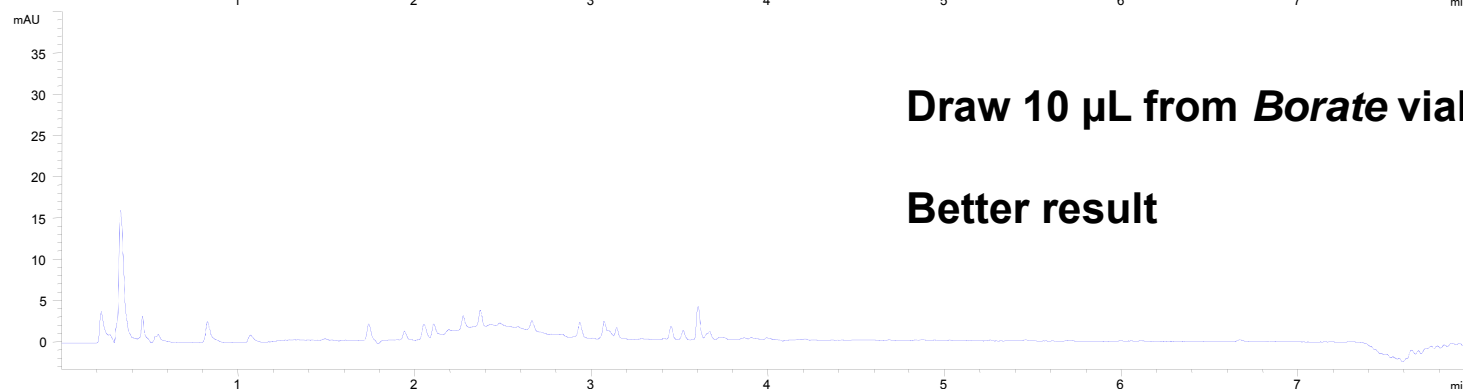
Transfer to autosampler vial and press start

- 1) Draw 2.5 μ L from *Borate* vial (Agilent P/N 5061-3339)
- 2) Draw 1.0 μ L from *Sample* vial
- 3) Mix 3.5 μ L in washport 5X
- 4) Draw 0.5 μ L from *OPA* vial (Agilent P/N 5061-3335)
- 5) Mix 4.0 μ L in washport 10X max speed
- 6) Draw 0.4 μ L from *FMOC* vial (Agilent P/N 5061-3337)
- 7) Mix 4.4 μ L in washport 10X max speed
- 8) Draw 32 μ L from *Injection Diluent* vial
- 9) Mix 20 μ L in washport 8X
- 10) Inject

Acidic Protein hydrolysate



Draw 2.5 μ L from *Borate* vial

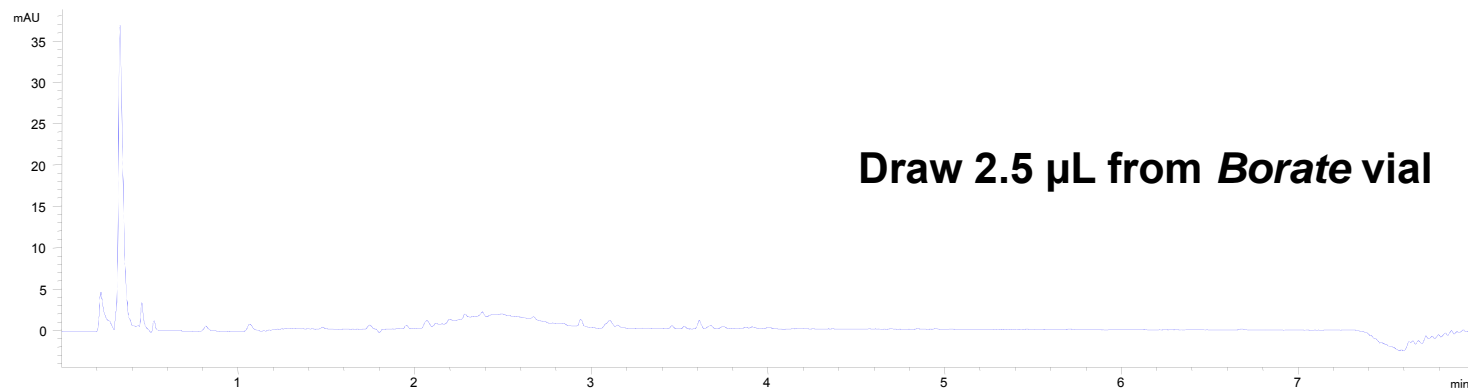


Draw 10 μ L from *Borate* vial

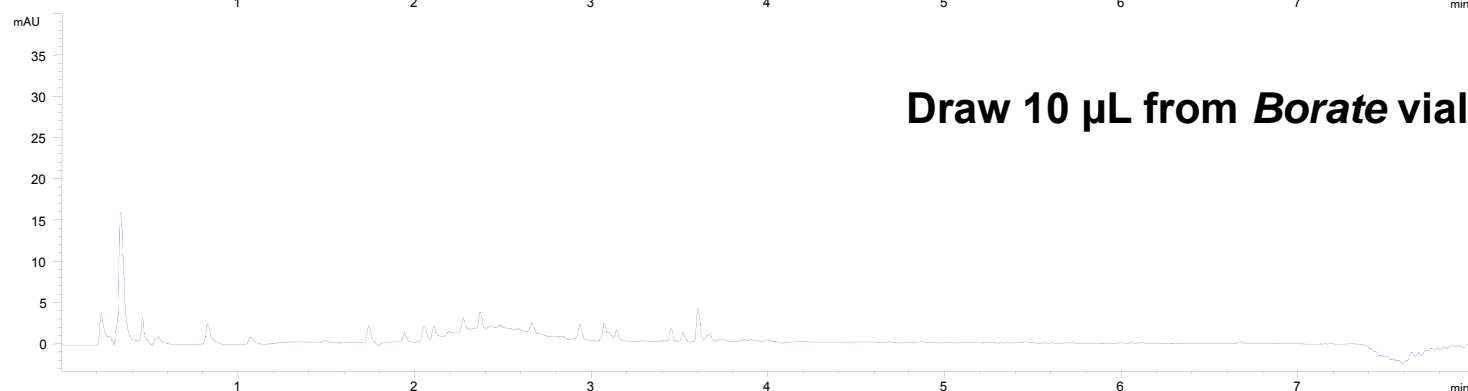
Better result

- 1) Draw 10 μ L from *Borate* vial (Agilent P/N 5061-3339)
- 2) Draw 1.0 μ L from *Sample* vial
- 3) Mix 11 μ L in washport 5X
- 4) Draw 0.5 μ L from *OPA* vial (Agilent P/N 5061-3335)
- 5) Mix 11.5 μ L in washport 10X max speed
- 6) Draw 0.4 μ L from *FMOC* vial (Agilent P/N 5061-3337)
- 7) Mix 11.9 μ L in washport 10X max speed
- 8) Draw 32 μ L from *Injection Diluent* vial
- 9) Mix 20 μ L in washport 8X
- 10) Inject

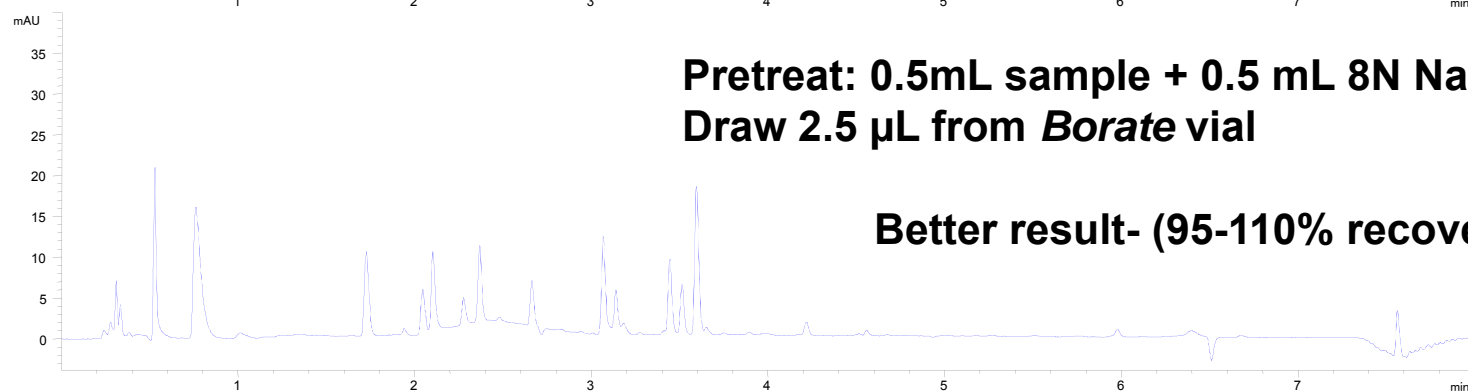
Acidic Protein hydrolysate



Draw 2.5 μ L from *Borate* vial



Draw 10 μ L from *Borate* vial



**Pretreat: 0.5mL sample + 0.5 mL 8N NaOH
Draw 2.5 μ L from *Borate* vial**

Better result- (95-110% recovery)

Conclusions

- An automated online derivatization method for amino acids using ZORBAX Eclipse Plus C18 was easily transferred from a 1200SL to a 1290 Infinity UHPLC
- Chromatographic performance improved over previous 1200 SL method (repeatability, linearity, and LOQ).
- QuEChERS extraction techniques may be a useful for analyzing fruit or vegetable for amino acids.
- If recovery is low for very acidic protein hydrolysates, neutralize to improve derivatization/recovery.
- Flexibility for optimization and customization: column choices, flow rates, injector programming