Clinical Validation of the Waters Kairos™ Amino Acid Kit using Liquid Chromatography Single Quadrupole Mass Spectrometry (LC-MS).

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The All Wales Inherited Metabolic Disease Service

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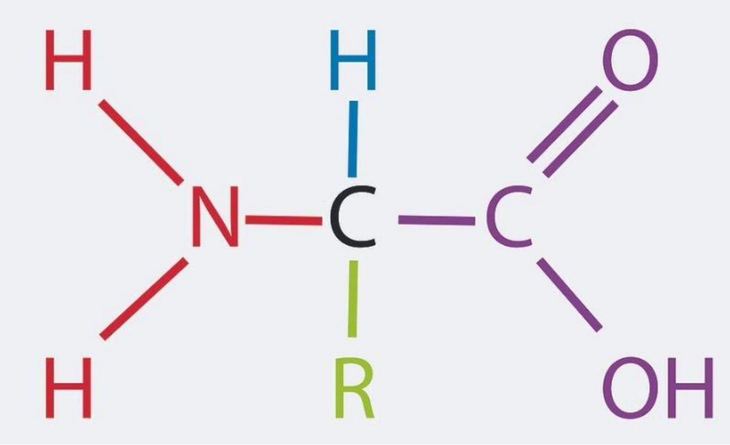
PRIFYSGOL CAERDYDD

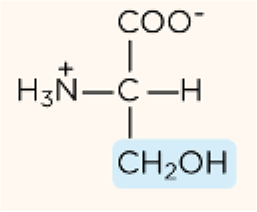
Amino Acids

- Metabolic and cellular functions.
  - Protein building blocks,
  - Metabolic intermediates,
  - Energy source
- Consist of:
  - Amino group
  - Carboxyl group
  - Central carbon
  - Hydrogen atom
  - R group (e.g. sulfhydryl, OH, 2°amino- or COOH group).

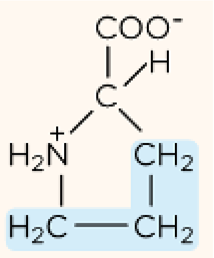


Amino Acids

Polar – R groups

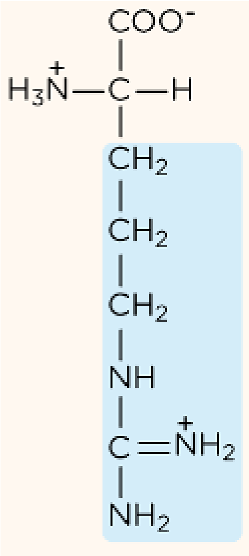


Serine

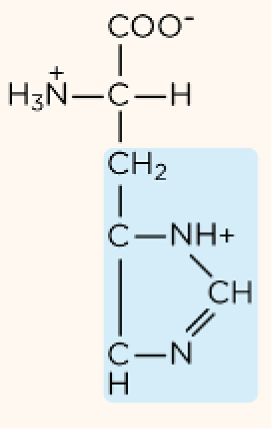


Proline

+ve charged R groups

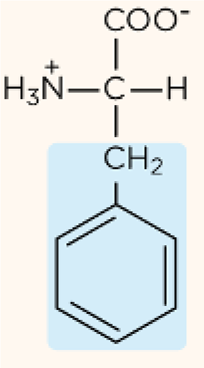


Arginine

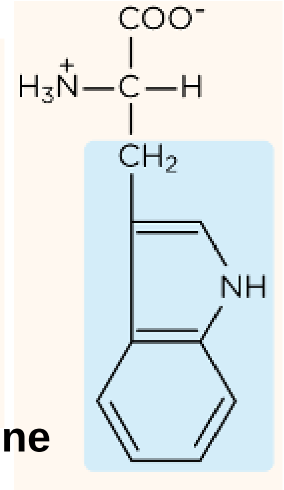


Histidine

Non-polar – aromatic R groups

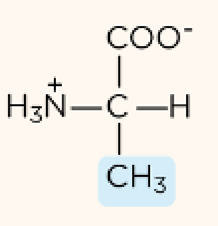


Phenylalanine

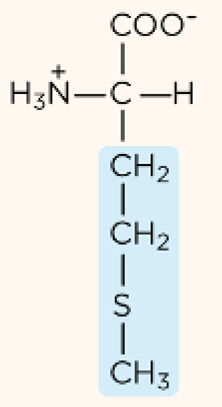


Tryptophan

Non-polar – R groups

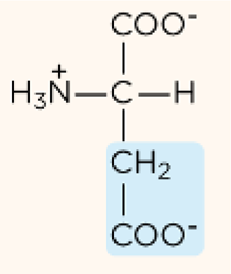


Alanine

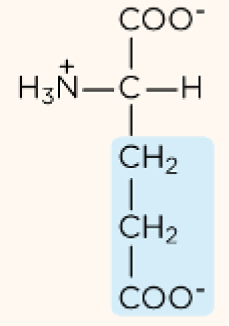


Methionine

-ve charged R groups



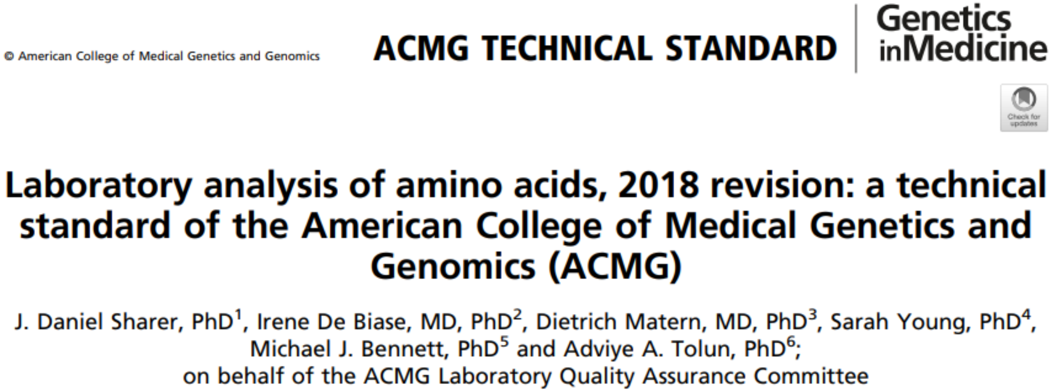
Aspartate



Glutamate

Analytical requirements for amino acid analysis (AAA)

- Robust method
- Accurate & precise
- Wide linear range
- >20 key AAs
- Minimal sample prep
- Rapid TAT
- Plasma, CSF, urine & DBS
- UKAS accredited
- UKCA (IVDR)



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ACMG TECHNICAL STANDARD

Genetics inMedicine

Laboratory analysis of amino acids, 2018 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG)

J. Daniel Sharer, PhD[1], Irene De Biase, MD, PhD[2], Dietrich Matern, MD, PhD[3], Sarah Young, PhD[4], Michael J. Bennett, PhD[5] and Adviye A. Tolun, PhD[6];
on behalf of the ACMG Laboratory Quality Assurance Committee

METBIONET GUIDELINES FOR AMINO ACID ANALYSIS

Methods for plasma AAA

- Ion exchange chromatography (UV detection)
- GC-MS
- HPLC (UV or FLD)
- FIA-MS/MS
- LC-MS/MS
- LC-MS

The Status Quo

Method	2001	2007	2015	2020	2024
IEC (%)	85	82.6	70.8	53.1	41.1
LC-MS/MS (%)	0	2.8	9.9	25.6	41.5
LC-MS (%)	0	0	0	4.4	5.9
HPLC (%)	12.7	13.5	14	12.5	10
Other (%)	2.3	1.1	5.3	4.5	1.5
Labs (n=)	134	178	243	273	270

Data courtesy of ERNDIM Quantitative AA scheme

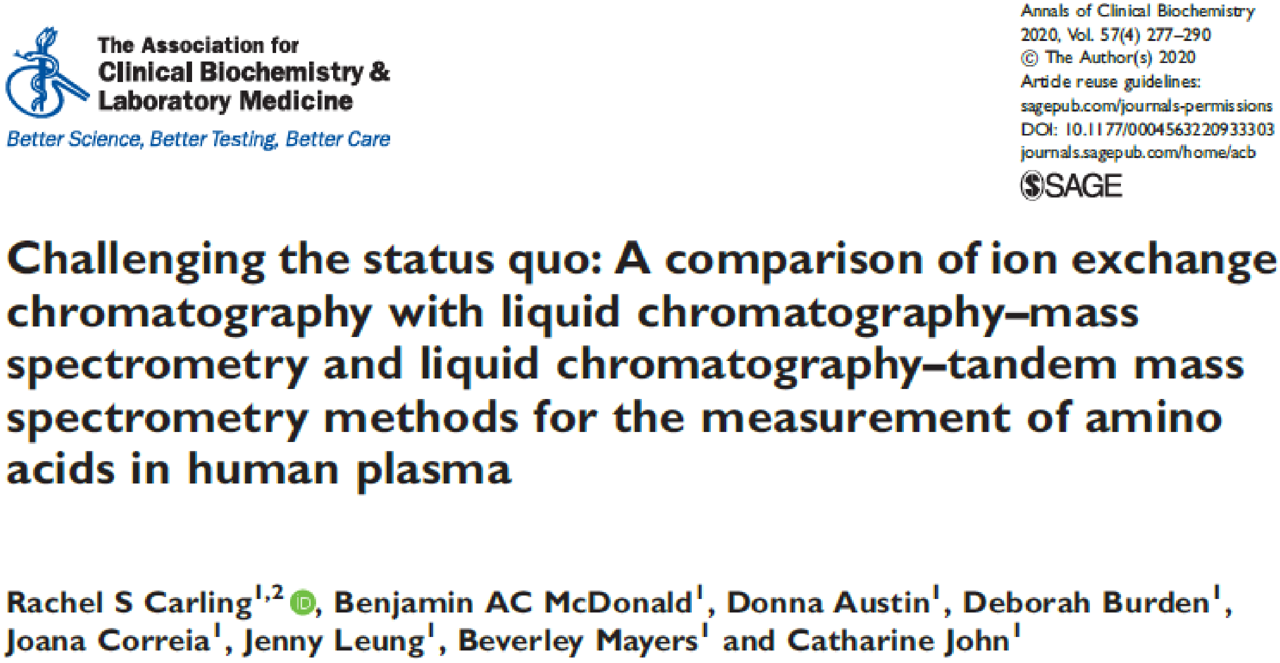
Ion exchange chromatography - disadvantages

- Analysis time ~2.5 hours / sample
- Dedicated analyser
- Expertise required
- Single calibrator
- Structural analogue internal standard
- Lacks selectivity
  - Identification based on RT
  - Co-elution of drugs / ninhydrin reactive compounds
  - Poor resolution of analytes e.g. sulphocysteine, ASA etc

The need for change in Cardiff?!

- Maintenance issues – inadequate support!
- Analyser & staff running at capacity
- Require STAT for urgent cases

IEC vs LC-MS/MS vs LC-MS



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Challenging the status quo: A comparison of ion exchange chromatography with liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry methods for the measurement of amino acids in human plasma

Rachel S Carling[1,2], Benjamin AC McDonald[1], Donna Austin[1], Deborah Burden[1], Joana Correia[1], Jenny Leung[1], Beverley Mayers[1] and Catharine John[1]

Evaluated - Mass Chrom and SpOtOn Kits using LC-MS/MS (underivatized methods) and Waters AccQ Tag ULTRA kit using LC-MS

Results from Carling *et al* 2020 study

Method	CCC ≥0.95	CCC 0.90- 0.95	CCC <0.90
Mass Chrom LC-MS/MS	10/21	3/21	7/21
SpOtOn LC-MS/MS	10/21	2/21	8/21
AccQ Tag LC-MS	6/21	5/21	9/21

Method	Inter-assay CV <10%
Mass Chrom LC-MS/MS	High IQC only
SpOtOn LC-MS/MS	Low & High IQC
AccQ Tag LC-MS	>10% in low IQC & 25% AAs >10% in High IQC

Method	ERNDIM Bias >10%
Mass Chrom LC-MS/MS	3/15
SpOtOn LC-MS/MS	4/15
AccQ Tag LC-MS	8/15

Results from Carling *et al* 2020 study

- Recommendations from Carling *et al* study
  - LC-MS/MS Kits fit for use only!

- Limitations of Carling *et al* study
  - Retrospective study – sample stability issues
  - ACQUITY UPLC H-Class used for LC-MS method
  - Lacking stable isotope for a number of key AAs

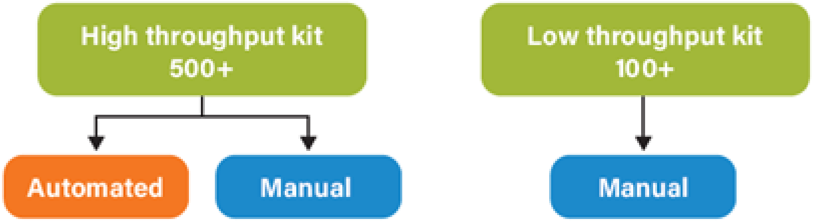
Advantages of Kairos LC-MS vs LC-MS/MS (underivatized) kits

- Derivatisation improves chromatographic separation and MS properties of small m/z analytes e.g. glycine, serine, proline & alanine.
- High sensitivity
- Stable retention times of analytes
- Resolution of all analytes i.e. isobaric BCAAs
- Lower cost of instrumentation vs LC-MS/MS

Dis-advantages of Kairos LC-MS vs LC-MS/MS (underivatized) kits

- Derivatisation time (10 mins) ? Precluding real time analysis.
- Poor yields / variable derivatisation & unstable derivatives?!
- Lack of MS/MS specificity

Waters Kairos AA Kit

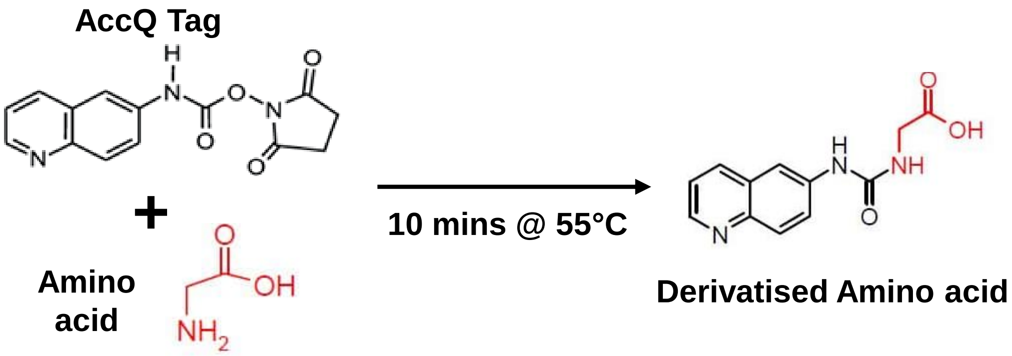

High throughput kit 500+

Low throughput kit 100+

Automated

Manual

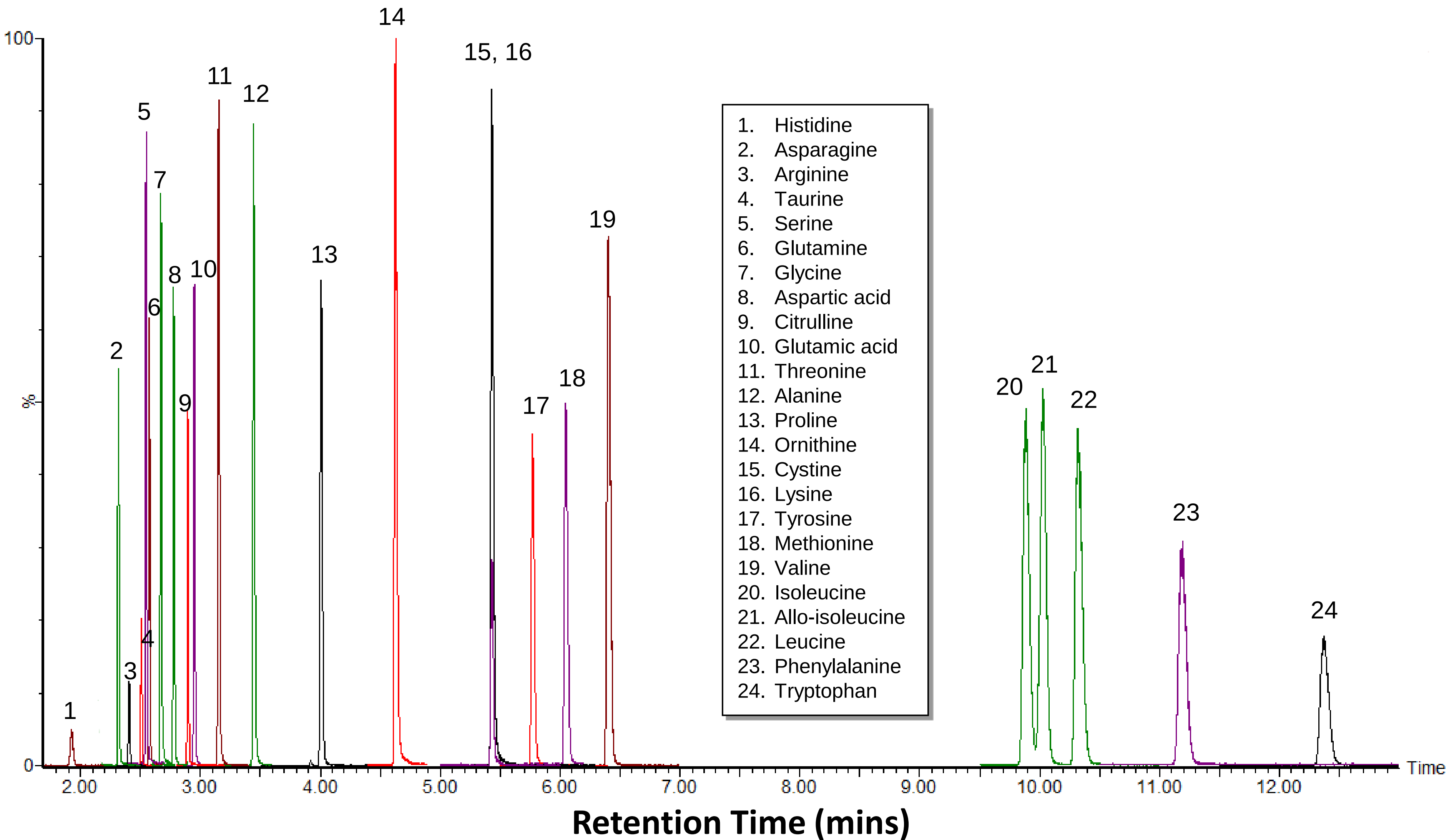
Manual



Cardiff Evaluation Study

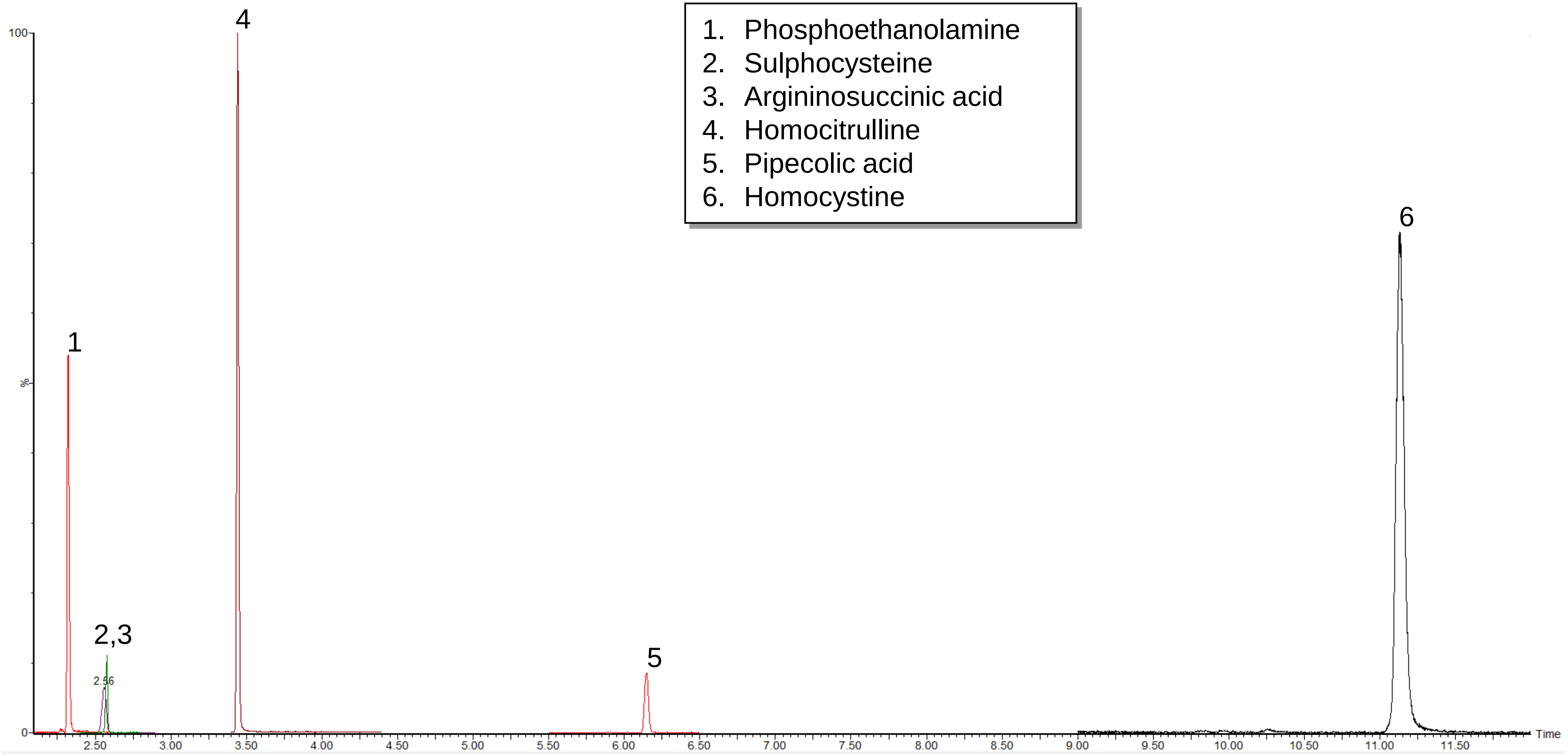
- Assessed performance of the Waters Kairos AA kit using Waters UPLC I-Class with QDA (single Quad MS).
- Prepared samples as per Waters protocol
- Increased analytical run time from 9 to 14 mins
- Parallel study vs IEC (n=115 patients samples)
- Commercial IQC – Recipe ClinChek
- TraceCert SRM & Biochrom Calibrators
- ERNDIM EQA material

Cardiff study – Kairos AA Chromatogram



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

AA chromatogram



1. Phosphoethanolamine
2. Sulphocysteine
3. Argininosuccinic acid
4. Homocitrulline
5. Pipecolic acid
6. Homocystine

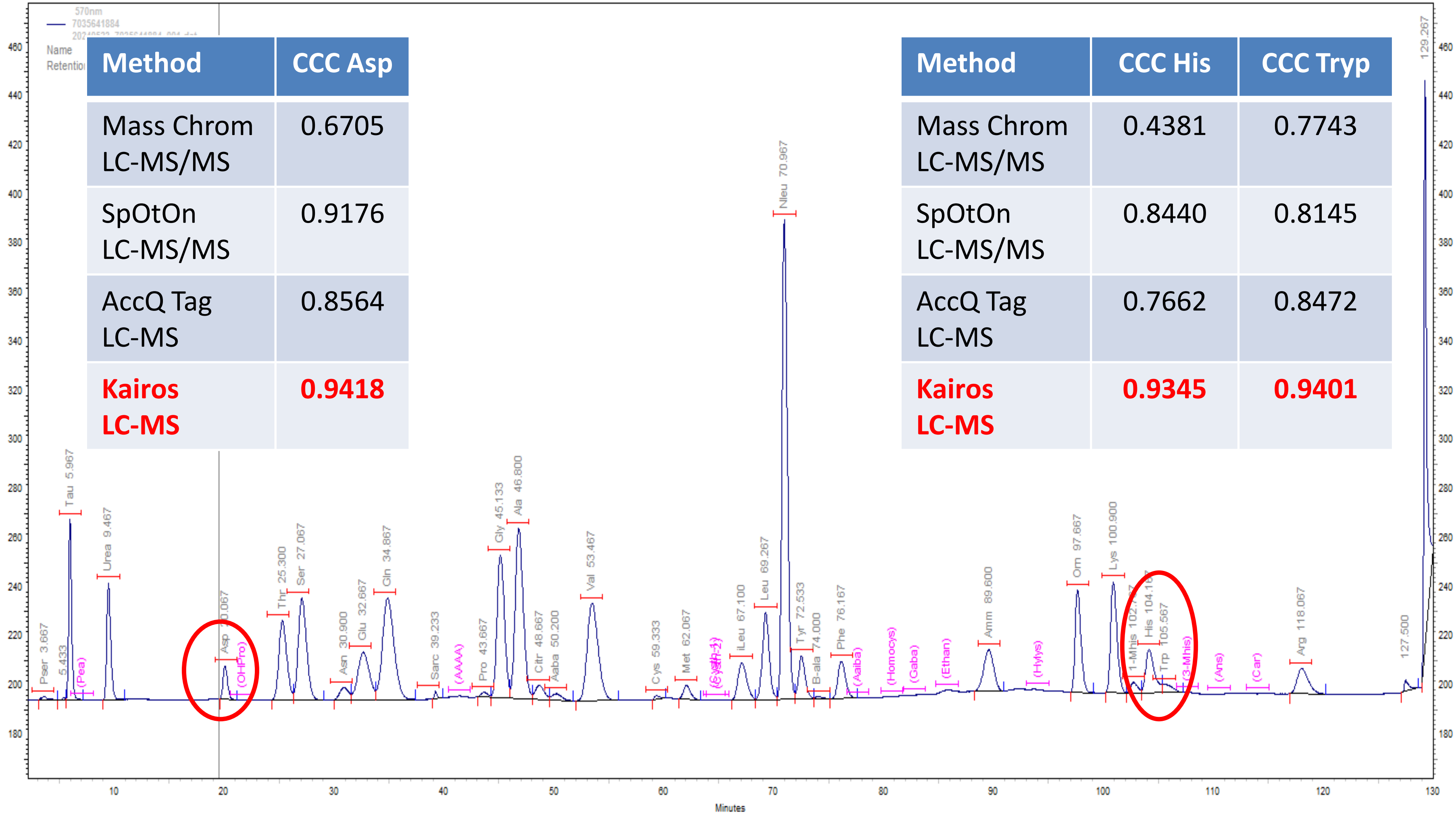
Results from Cardiff evaluation – Concordance

Analyte	Viapath (n=68)	Cardiff Validation 2024 (n=115)
	CCC	CCC
Alanine	0.7696	0.9890
Arginine	0.9001	0.9877
Asparagine	N/A	0.9599
Aspartic acid	0.8564	0.9418
Citrulline*	0.8507	0.9978
Cystine	N/A	0.9947
Glutamine	N/A	0.9721
Glutamic acid	N/A	0.9862
∑Gln + Glu	0.377	
Glycine	0.7696	0.9957
Histidine	0.7662	0.9345
Isoleucine	0.994	0.9974
Leucine	0.9973	0.9963
Lysine	0.6424	0.9877
Methionine	0.9915	0.9979
Ornithine*	0.9066	0.9948
Phenylalanine	0.9994	0.9997
Proline	0.8179	0.9741
Serine	0.9049	0.9853
Taurine*	0.9421	0.9832
Threonine	0.9447	0.9859
Tryptophan	0.8474	0.9401
Tyrosine	0.9947	0.9991
Valine	0.9913	0.9914

Method	**CCC ≥0.95**	**CCC 0.90-0.95**	**CCC <0.90**
Mass Chrom LC-MS/MS	10/20	3/20	7/20
SpOtOn LC-MS/MS	10/20	2/20	8/20
AccQ Tag LC-MS	6/20	5/20	9/20
Kairos LC-MS	**20/23**	**3/23**	**0/23**

Mean bias between IEC vs Kairos = -1.2%

Chromatogram for AA profile on Biochrom 30+ amino acid analyser



Method	CCC Asp
Mass Chrom LC-MS/MS	0.6705
SpOtOn LC-MS/MS	0.9176
AccQ Tag LC-MS	0.8564
Kairos LC-MS	**0.9418**

Method	CCC His	CCC Tryp
Mass Chrom LC-MS/MS	0.4381	0.7743
SpOtOn LC-MS/MS	0.8440	0.8145
AccQ Tag LC-MS	0.7662	0.8472
Kairos LC-MS	**0.9345**	**0.9401**

Results from Cardiff evaluation – precision, LLOQ & linearity

Amino Acid	[a]RT	[b]LLOQ	[c]QC1 mean	[c]QC1 %CV	[c]QC2 mean	[c]QC2 %CV	[d]Linearity
Alanine	3.41	0.63	200.7	3.14	893.2	3.25	4008
Alloisoleucine	9.92	1.25	30.9	4.31	171.7	3.39	2808
Arginine	2.37	1.25	62.5	4.61	263.2	3.91	2613
Asparagine	2.30	0.63	39.7	4.10	195.5	2.29	2357
Aspartic acid	2.70	2.50	40.0	4.46	193.5	3.83	2546
Citrulline	2.90	0.63	34.1	4.62	169.3	3.10	2694
Cystine	5.37	0.31	4.5	9.22	36.7	5.26	1311
Glutamine	2.50	2.50	193.6	4.76	881.0	3.68	3693
Glutamic acid	2.95	2.50	76.5	4.12	338.8	3.32	2363
Glycine	2.60	2.50	196.2	4.46	895.7	4.00	3783
Histidine	1.90	2.50	70.4	4.03	340.4	3.08	2720
Isoleucine	9.80	2.50	54.6	4.72	254.0	3.34	3804
Leucine	10.20	2.50	103.0	5.16	485.5	2.94	3846
Lysine	5.40	2.50	81.7	3.61	339.3	2.94	2204
Methionine	6.00	0.63	52.3	4.80	271.8	3.39	2480
Ornithine	4.60	2.50	71.4	5.73	345.8	2.79	2702
Phenylalanine	11.10	2.50	115.5	4.72	607.5	2.81	3926
Proline	4.00	2.50	140.4	4.76	652.9	3.37	2635
Serine	2.50	2.50	67.8	5.03	301.8	4.34	4032
Taurine	2.50	2.50	67.7	4.89	345.9	4.97	2374
Threonine	3.10	2.50	72.3	4.51	309.0	2.65	3516
Tryptophan	12.30	2.50	29.3	5.15	135.0	3.51	2501
Tyrosine	5.70	2.50	37.1	4.73	179.8	3.27	3724
Valine	6.40	2.50	136.7	4.47	614.9	3.10	3898

Imprecision

Method	**Inter-assay CV <10%**
Mass Chrom LC-MS/MS	High IQC only
SpOtOn LC-MS/MS	Low & High IQC
AccQ Tag LC-MS	>10% in low IQC
Kairos LC-MS	**Low IQC – 25/25** **High IQC – 25/25**

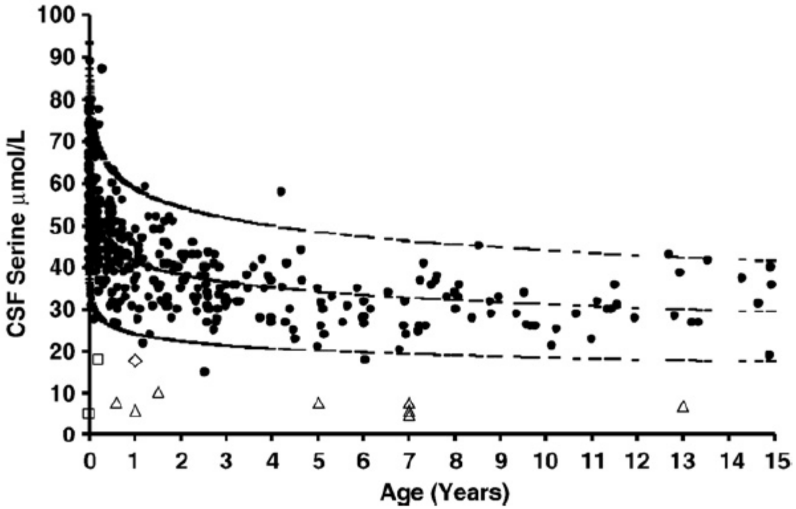
LLOQ

Amino Acid	Kairos	Mass Chrom	SpOtOn
Alanine	**0.63**	127	25
Glycine	**2.50**	130	25
Proline	**2.50**	59	10
Serine	**2.50**	50	25
Threonine	**2.50**	53	2.5

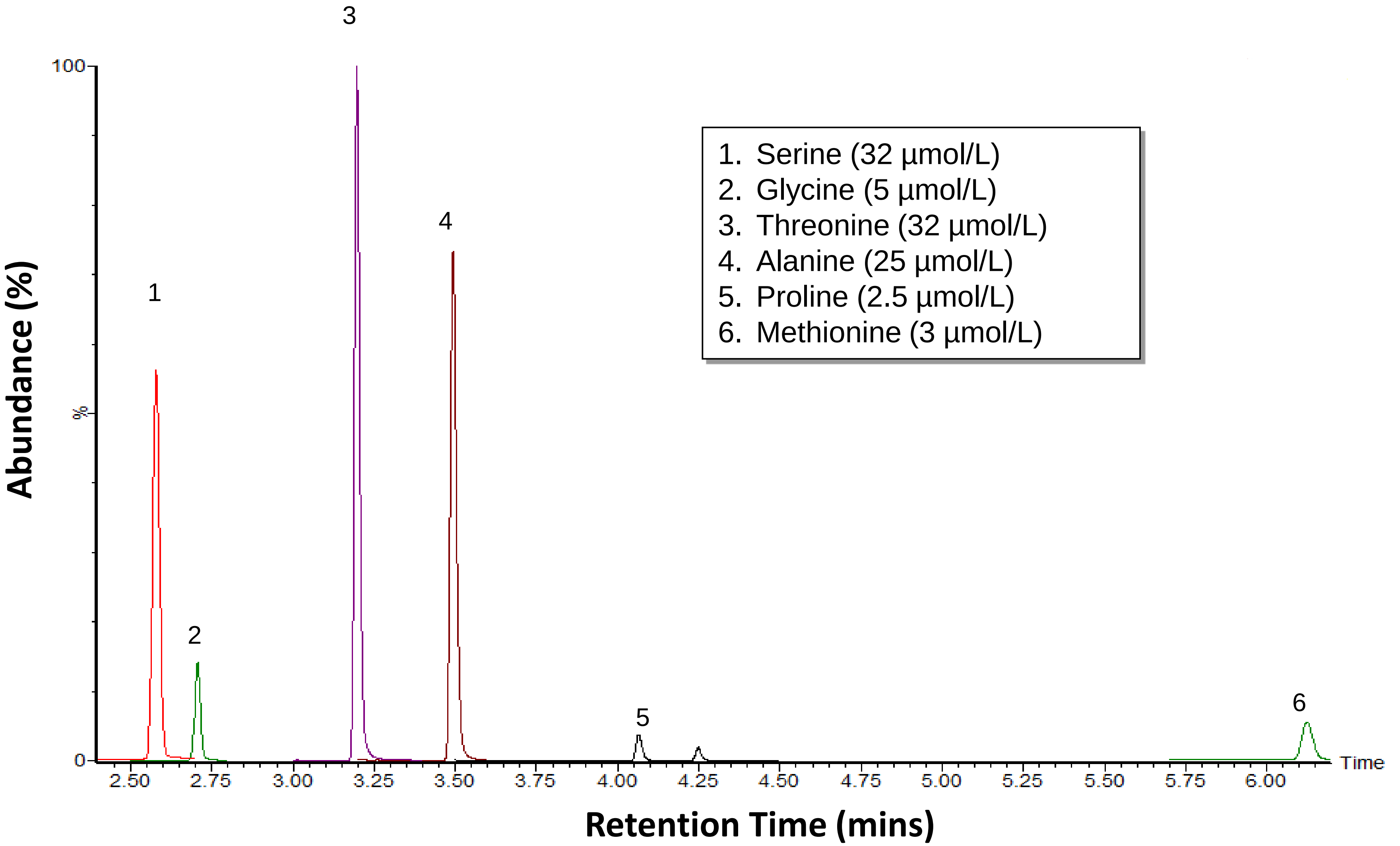
Implications for CSF amino acids

CSF RIs (µmol/L)

Alanine – 13-41
Glycine – 3 -10
Proline – <5
Serine – 18-44
Threonine – 12-55



CSF AA chromatogram



Results from Cardiff evaluation-Accuracy

Method	**ERNDIM Bias >10%**
Mass Chrom LC-MS/MS	3/15
SpOtOn LC-MS/MS	4/15
AccQ Tag LC-MS	8/15
Kairos LC-MS	**1/23**
Biochrom IEC	**5/23**

- No matrix matched certified reference material (CRM) for AAs in plasma
- Aqueous CRM (SigmaTrace Cert)
- ERNDIM scheme reports against derivation of ALTM not enriched value!

Results from Cardiff evaluation- Stability of derivatised products

Mean difference of derivatised products stored at 2-8°C, analysed on day 0, 7, 10 and 17.

Amino acid	Day 7 (%)	Day 10 (%)	Day 17 (%)	Overall (%)
Alanine	-0.7	-1.2	-1.4	-1.1
Allo-isoleucine	1.0	-0.3	0.5	0.4
Cystine	0.2	-0.6	0.3	-0.1
Arginine	-1.2	-2.8	-2.9	-2.3
Asparagine	1.5	0.2	6.2	2.6
Aspartic acid	1.8	-1.1	0.9	0.5
Citrulline	-2.4	3.1	5.1	1.9
Glutamine	0.7	-4.1	-1.7	-1.7
Glutamic acid	-4.5	-4.8	-4.2	-4.5
Glycine	-6.0	-4.0	-5.2	-5.1
Histidine	-0.4	-1.7	-2.0	-1.4
Hydroxyproline	2.0	6.7	5.2	4.6
Isoleucine	2.1	6.5	-2.0	2.2
Leucine	1.2	2.2	0.0	1.1
Lysine	3.4	5.6	2.7	3.9
Methionine	-0.2	0.7	-0.3	0.1
Ornithine	-1.4	0.9	-2.4	-1.0
Phenylalanine	-2.5	-1.7	-2.0	-2.1
Proline	-0.5	-0.2	-0.3	-0.4
Serine	-6.5	-4.9	-8.6	-6.7
Threonine	-1.8	-1.2	0.1	-0.9
Tryptophan	-0.2	0.5	1.0	0.4
Tyrosine	-0.6	-1.2	-0.4	-0.7
Valine	0.4	1.2	0.1	0.5

Overall change -0.4%

Comparison of Biochrom vs Kairos Kit using QDA LC-MS

- LC-MS 1.2 x more expensive vs Biochrom.
- LC-MS analyse 5x as many specimens.
- LC-MS – open access - Vit A&E analysis.
- Reagent costs for Kairos 50% more expensive vs biochrom – staff time is less using Kairos
- Cardiff requires TWO Biochroms for AA service and an HPLC for Vit A&E service
- 2 x QDA LC-MS systems more cost effective!!

Conclusions

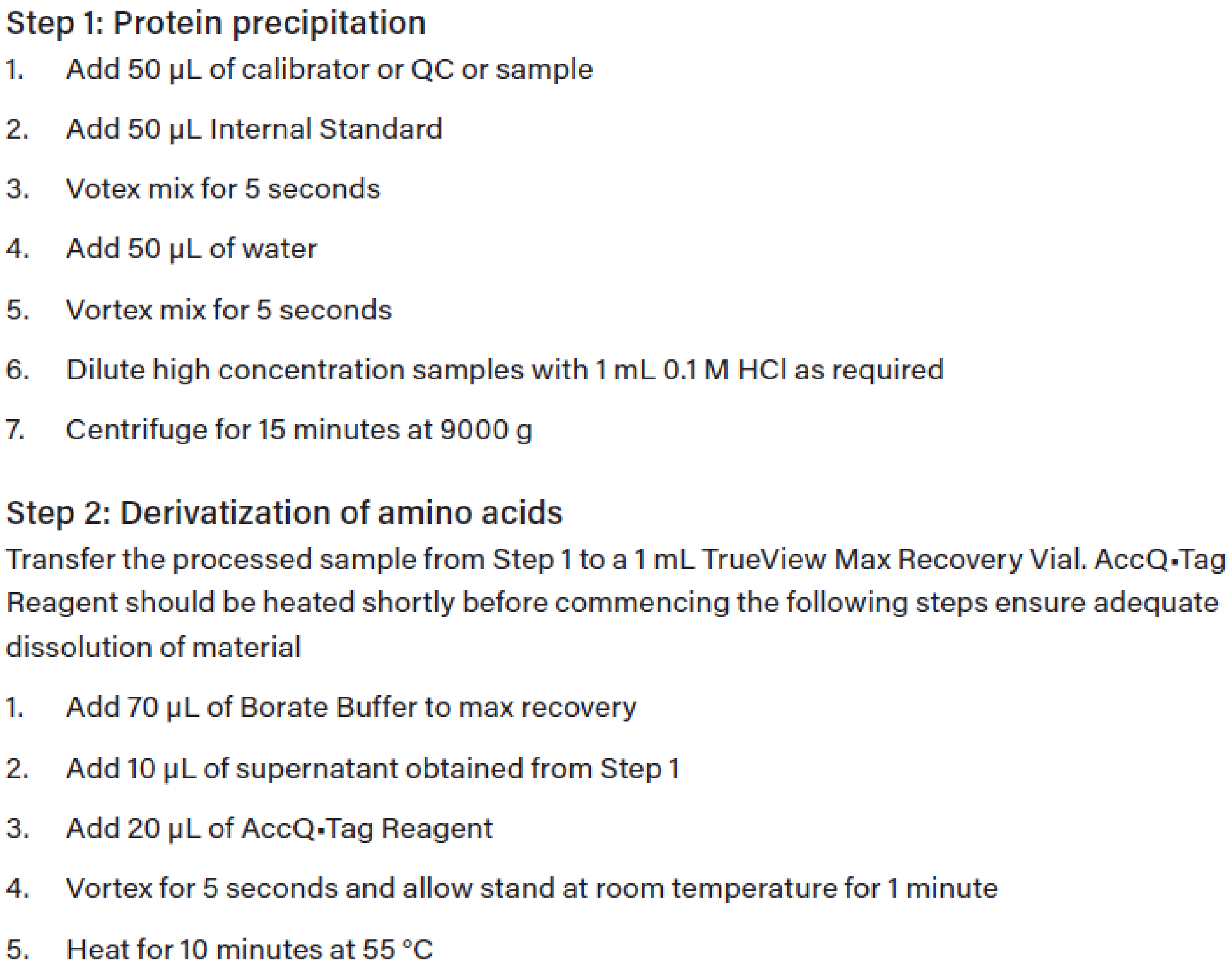
- Plasma AAA – challenging!
- Kairos AA kit using LC-MS methodology superior to IEC performance.
- Decreased run time means improved TAT and greater capacity.
- Stable calibration <5% over 3 month period
- RT stable <0.4% over 3 month period
- Implemented Kairos kit into Cardiff Lab (July 2024).
- Currently validating Kairos kit for CSF and urine panels.

Acknowledgements

- Wales NBS & Metabolic Team
    - Rowan Hellier, Chandra Sundas
- Waters Team
    - Luke Griffiths, Adam Cryar, Pete Christensen, Padhraic Rossiter, Lisa Calton, Heather Brown & Alex Hunt.
- Rachel Carling – Synnovis

Thank you!

Sample Prep



Step 1: Protein precipitation

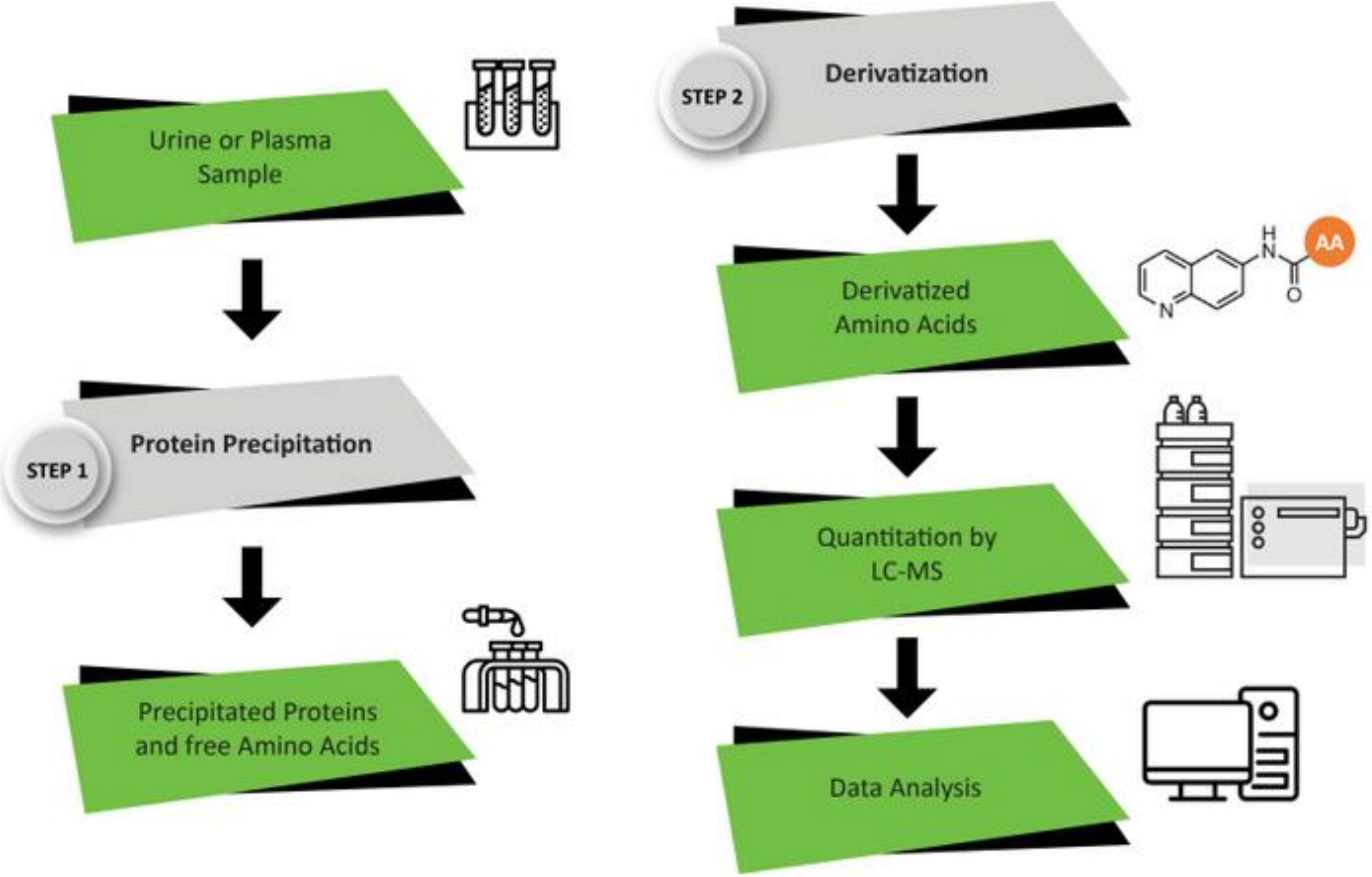
1. Add 50 µL of calibrator or QC or sample
2. Add 50 µL Internal Standard
3. Votex mix for 5 seconds
4. Add 50 µL of water
5. Vortex mix for 5 seconds
6. Dilute high concentration samples with 1 mL 0.1 M HCl as required
7. Centrifuge for 15 minutes at 9000 g

Step 2: Derivatization of amino acids

Transfer the processed sample from Step 1 to a 1 mL TrueView Max Recovery Vial. AccQ•Tag Reagent should be heated shortly before commencing the following steps ensure adequate dissolution of material

1. Add 70 µL of Borate Buffer to max recovery
2. Add 10 µL of supernatant obtained from Step 1
3. Add 20 µL of AccQ•Tag Reagent
4. Vortex for 5 seconds and allow stand at room temperature for 1 minute
5. Heat for 10 minutes at 55 °C

Sample preparation



Urine or Plasma Sample

STEP 1 Protein Precipitation

Precipitated Proteins and free Amino Acids

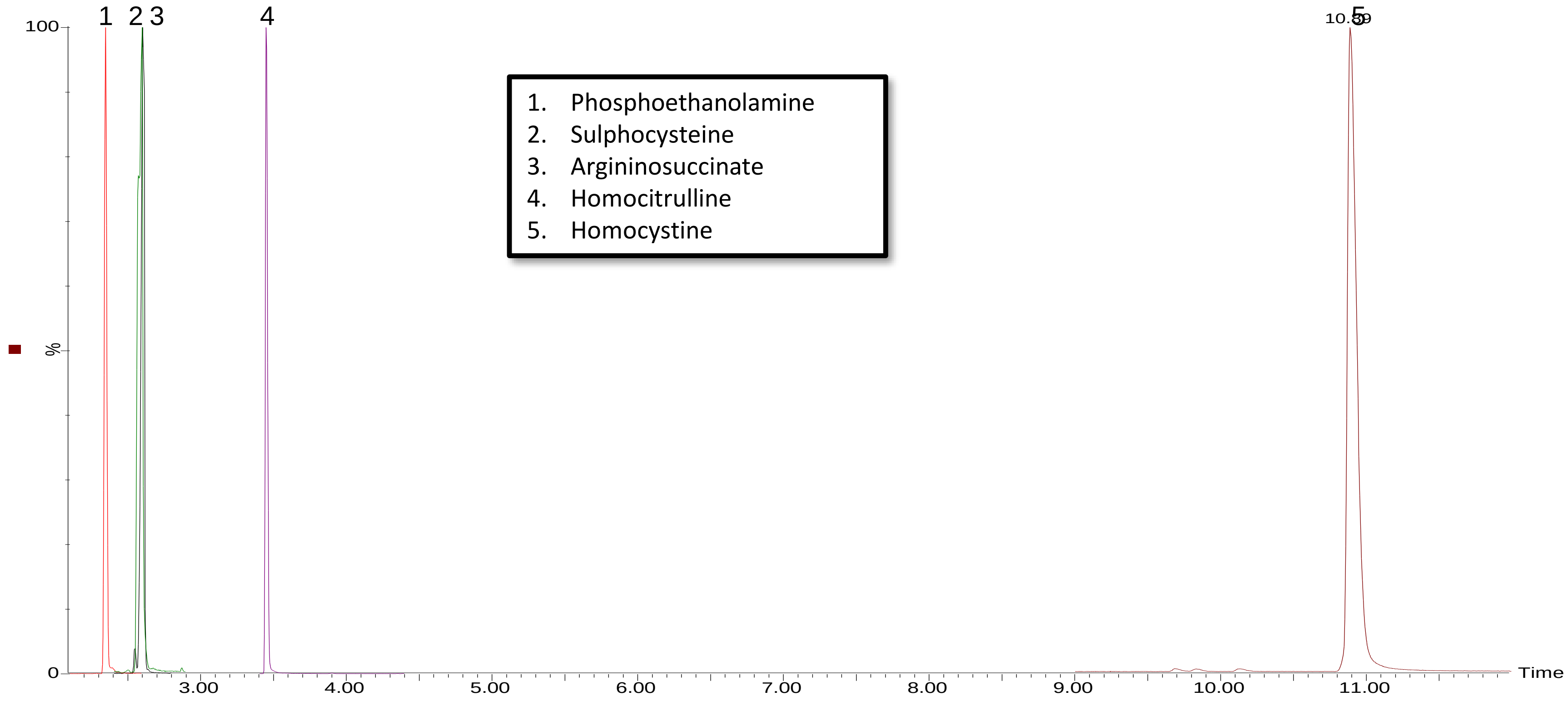
STEP 2 Derivatization

Derivatized Amino Acids

Quantitation by LC-MS

Data Analysis

Other important diagnostic AAs



1. Phosphoethanolamine
2. Sulphocysteine
3. Argininosuccinate
4. Homocitrulline
5. Homocystine