# Amino Acid Methods: Present and Future…

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# The Status Quo

- Questionnaire shows everybody using HPLC or ion-exchange amino acid analysers for routine quantitative services
- Some use tlc /HVE to pre-screen urine and /or plasma samples

# Why would we want to change?

- Long run times for AA analyser, significant maintenance to keep running, temperamental
- Significant sample preparation times for HPLC, co-eluting peaks
- Analysers /staff running at capacity (or beyond)
- Need for rapid results in urgent cases

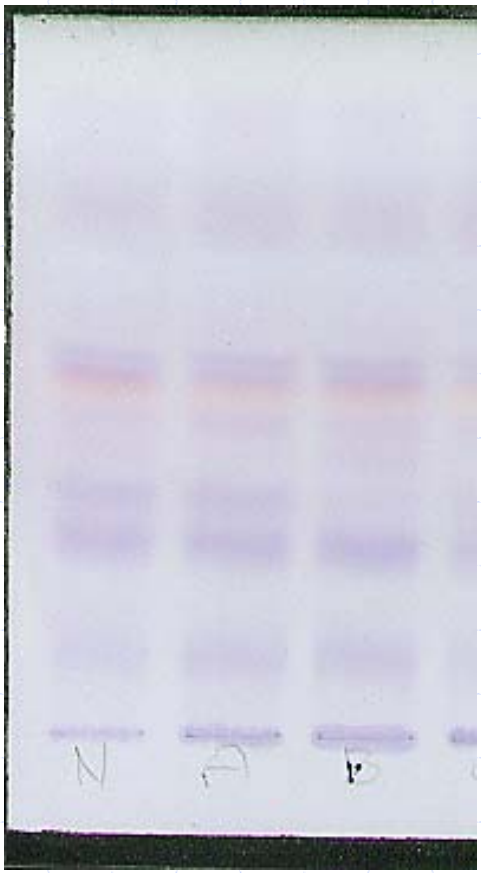
# Some current solutions to the capacity problem…

- Pre-screening samples using tlc and HVE to reduce number of samples that need to be quantitated
- Using HPLC as a semi-quantitative screen – (report as NSA or proceed to quant)
- Run two amino acid analysers
- Use tandem or underivatised HPLC method for PKU monitoring
- Use of short programs to reduce analyser time when only certain amino acids are required e.g. MSUD monitoring

# Why look for other alternatives?

- Screening by tlc has its own problems….
  - significant staff time
  - technically demanding
  - Interpretation and identification of amino acids requires significant experience
  - Have to have low threshold for quantitating samples to be confident of not missing something "significant" –and even then….

# Would you have spotted this?



Glycine = 110 µmol/L (200-600)
Serine = 30 µmol/L (50-350)

CSF glycine = <1 µmol/L (0-10)
CSF serine = 5 µmol/L (35-80)

# Why look for other alternatives?

- Cost of two instruments
- Run time is not just a capacity issue, also a need for quick turnaround times in certain circumstances

# The ideal analyser would be...

- Fast (run time <30 mins)
- Little or no sample preparation
- Separation and positive identification of all diagnostically relevant amino acids
- Robust equipment, easy to maintain
- Cheap

# What are the possible alternatives?

- GC-MS
- Tandem MS
- UPLC

# GC-MS

- ERNDIM returns indicate a small number of labs using GC-MS for amino acids, (? In house methods or commercial kits)
- Commercially available EZ:faast amino acid analysis kits for GC-MS

  -Sample derivatisation with propriety reagent and analysis within 15 mins

# Pros

- Fast
- Quick derivatisation
- Analyse 50 amino acids /dipeptides in an 8 min run time

# Cons

- Would need a dedicated GC-MS (can't use same column as organic acids)
- Citrulline and Arginine are NOT two of the 50

# Tandem-MS

- Use well established in Neonatal Screening
- Increasingly being used as a solution to amino acid analysis in specific circumstances or specific problem analytes

  -e.g. Rapid screen in acutely ill child, monitoring of treatment in decompensated MSUD, sulphocysteine
- Small number of labs are using it for routine analysis

# Rapid Screen of the acutely ill child

- A number of diagnostically useful amino acids can be easily measured by underivatised MS/MS method
- Can be measured on the same injection as acylcarnitines
- 10 minutes sample preparation and 3 minute run can give a host of information and either provide a diagnosis or narrow it down considerably
- What can be measured is limited by the problem of isobaric / isomeric compounds

# Monitoring in Decompensated MSUD

- A probable diagnosis can be made from the simple MS/MS method
- Need a different method to measure leucine, isoleucine and allo-isoleucine separately to allow planning and monitoring of treatment
- A chromatographic step is required for separation of the isomers

# Possible chromatographic options…

- Macrocyclic glycopeptides eg Chirobiotic TAG (teicoplanin aglycone)
- Porous graphite column (Hypercarb)

-columns easily attached between sample manager and tandem with an extra piece of tubing

-adds a few minutes to the run time

-allows rapid measurement of leucine and its isomers, ideal for monitoring treatment during crisis when frequent sampling is required

# Routine Amino Acid Analysis

- Small number of labs are using tandem methods for routine amino acid analysis

e.g. Piraud et al, Lyon (SSIEM abstract 140-P 2005)

-using ion-pairing reverse phase liquid chromatographic method with MS/MS as the detector

-run time is ~25mins

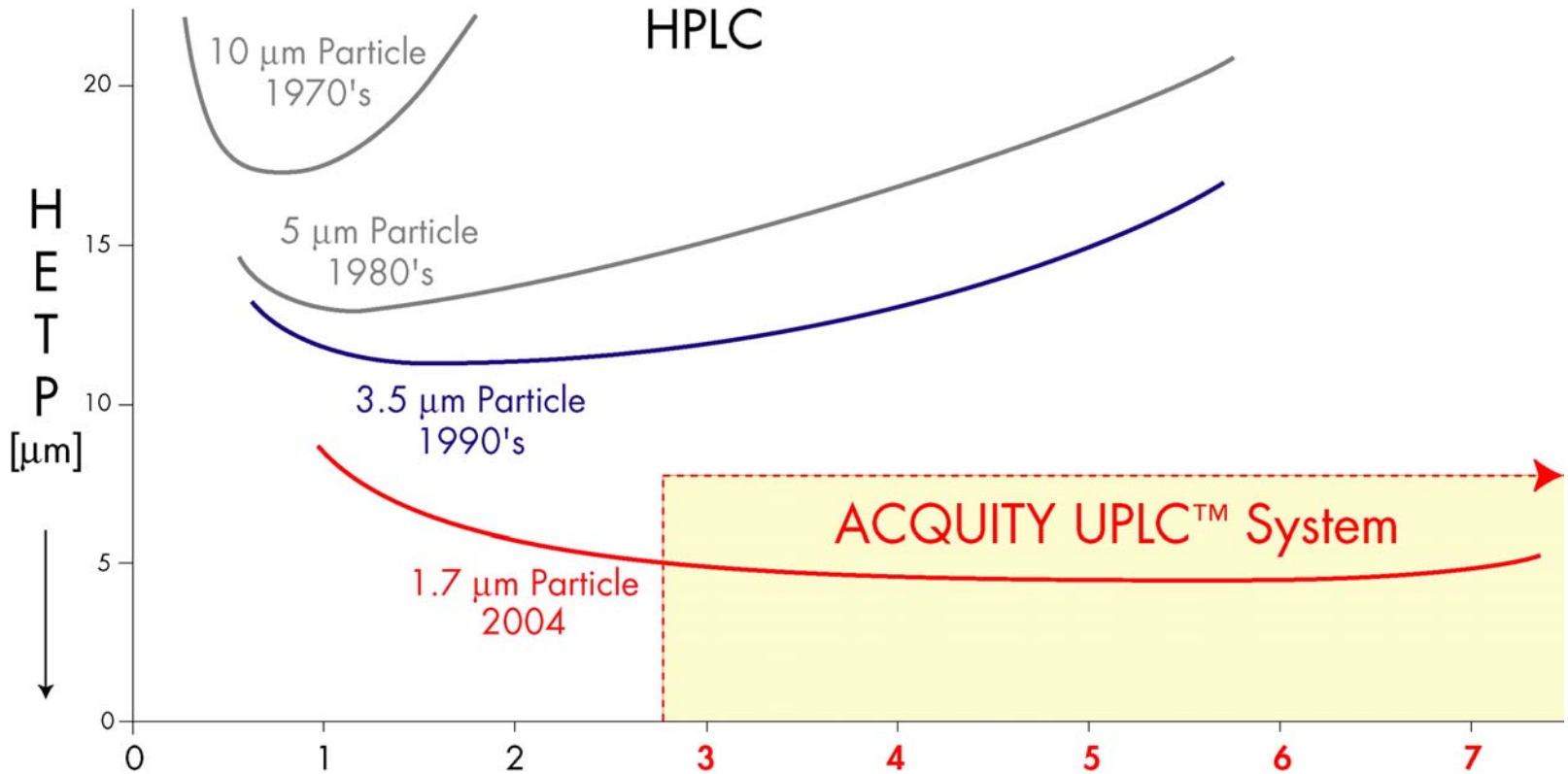
-in routine use for 18 months, several thousand samples

-have diagnosed and monitored wide range of disorders without problems

# UPLC (ultra performance liquid chromatography)

- Souped up version of HPLC
- Relies on even smaller particle size (made possible by new material) and a system designed to tolerate very high pressures to provide better resolution and increased speed

## *The promise of the van Deemter plot*

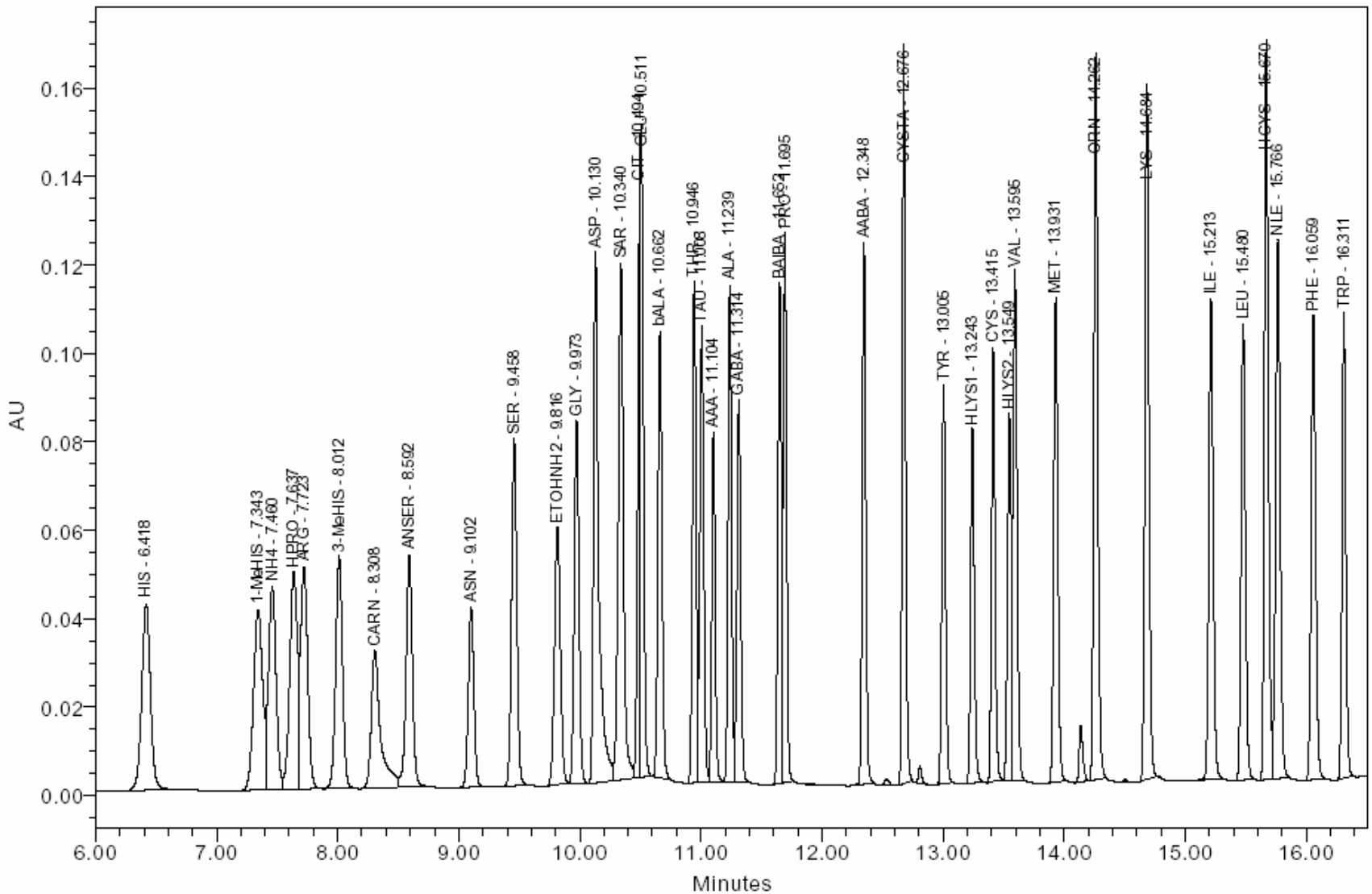



| *Flow Rate [mL/min]:* | | | | | | | |
|---|---|---|---|---|---|---|---|
| ID = 1.0 mm | 0.04 | 0.07 | **0.10** | **0.13** | **0.17** | **0.20** | **0.24** |
| ID = 2.1 mm | 0.15 | 0.3 | **0.45** | **0.6** | **0.75** | **0.9** | **1.05** |
| *ID = 4.6 mm* | *0.7* | *1.4* | *2.1* | *2.8* | *3.5* | *4.2* | *4.9* |

# UPLC cont…

- Run time <20 mins
- Better resolution than HPLC
- Requires derivatisation step but this is much shorter than the PITC method (<30 mins)
- Cost of equipment similar to current amino acid analysers
- Appears to be capable of analysing all necessary amino acids
- Unfortunately amino acid analysis by UPLC is still very much in development

# UPLC Physiological Amino Acids UV Detection @ 250 nm



# One stage further: UPLC-MS/MS

- Using MS/MS as the detector would allow positive identification of peaks

  -Best of both worlds, UPLC separates isobaric compounds, MSMS "separates" co-eluting peaks
- No derivatisation of samples required
- Rapid, high through-put system suitable for diagnosis, monitoring and urgent analysis with minimal sample preparation
- Same system could be used for other assays –would open up possibilities for the larger, specialist labs

# The Future….

- New technologies could alter the way we operate allowing more flexibility
- Higher through-put systems could put an end to pre-screening samples by tlc etc
- In the next few years newer technologies will become more and more of a realistic alternative –which way labs decide to go will ultimately depend on staff expertise, costs, existing facilities and equipment, workloads and clinical needs