Amino Acid Analysis-
Back to basics….

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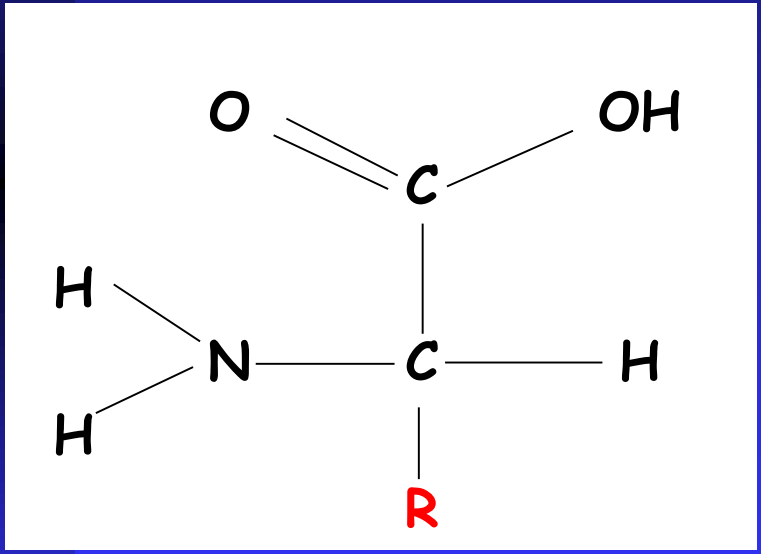
Amino acid analysis

- Why are amino acids important
- When to consider amino acid analysis
- Available methodology
  - Limitations and pitfalls

Amino Acid Structure



- Amino group (-NH_2)
- Carboxyl group (-COOH)
- Distinctive R group

Amino Acids

- Essential
  - Phenylalanine
  - Threonine
  - Methionine
  - Lysine
  - Tryptophan
  - Leucine
  - Isoleucine
  - Valine
  - Histidine
- Non-essential
  - Tyrosine
  - Aspartate
  - Asparagine
  - Alanine
  - Serine
  - Glycine
  - Cysteine
  - Glutamine
  - Glutamate
  - Proline
  - Arginine

Amino acid disorders

- Clinically and biochemically heterogeneous
- Can present at any age
- Characterised by
  - Pathological accumulation of normal metabolites
  - Presence of non-physiological metabolites
- Combined incidence 1:6000

Primary amino acid disorders

- Phenylketonuria
- Tyrosinaemia (I/II/III)
- Maple Syrup Urine Disease
- Homocystinuria
- Non-Ketotic Hyperglycinaemia
- Hyperprolinaemia (I/II)
- Sulphite oxidase def
- OAT deficiency

- Urea Cycle Disorders
  - OTC deficiency
  - CPS deficiency
  - Citrullinaemia
  - Argininosuccinic aciduria
  - Argininaemia
  - NAGS deficiency
  - HHH

Primary renal amino acid disorders

- Cystinuria
  - Cystine, Ornithine, Arginine, Lysine
- Hartnup disease
  - Neutral amino aciduria
- Lysinuric protein intolerance
  - Lysine, Ornithine, Arginine
- Iminoglycinuria
  - Proline, Hydroxyproline, Glycine

Secondary causes of increased amino acids

Generalised aminoaciduria

- Fanconi Syndrome
- Galactosaemia
- Tyrosinaemia type I
- Cystinosis

Increases in urine

- Glycine- renal immaturity, anticonvulsant Rx

Increases in plasma

- Alanine- lactic acidaemia
- Glutamine- hyperammmonaemia
- Methionine/tyrosine- liver disease
- Isoleu/leu/val- ketosis

Some pitfalls to avoid

- Not always increased amino acids
  - Serine deficiency
- Free amino acids
  - Homocystinuria
  - Urine homocystine not sensitive
  - Analysis of choice is total homocysteine

When to consider amino acid analysis

- Neonate- Lethargy/coma/seizures/vomiting
- Hyperammonaemia
- Hypoglycaemia
- Ketosis
- Metabolic acidosis or lactic acidaemia
- Metabolic decompensation/encephalopathy
- Unexplained Liver disease
- Unexplained developmental delay
- Renal disorders- Calculi, Tubulopathy

Specific considerations

- Gyrate atrophy of retina
  - Ornithine Amino Transferase deficiency
- Marfan-like appearance/Vascular abnormalities
  - Homocystinuria (Cystathione B Synthase def)
- Hyperkeratosis
  - Tyrosinaemia Type II

Choice of sample

- Plasma
  - Most informative
  - Often not the sample of choice by families
- Urine
  - AA concentrations much more variable
  - Prone to interference from medication
  - Necessary for diagnosis of renal transport disorders
- CSF
  - Useful in specific disorders
  - Paired with plasma

Amino acid analysis

- Spot test
- Qualitative screening
  - TLC
  - HVE
- Quantitative analysis
  - HPLC
  - AAA
  - TMS

Spot tests

- Ferric Chloride
  - Reacts with a number of compounds to form a colour
  - PKU, Tyrosinaemia, MSUD
- Cyanide/Nitroprusside
  - Reacts with sulphur containing amino acids
  - Homocystinuria, Cystinuria
- 2,4 Dinitrophenylhydrazine
  - Reacts with branch-chain keto acids and phenylketones
  - MSUD, PKU

Spot tests

ADVANTAGES

- Cheap
- Easy
- No expensive equipment required

LIMITATIONS

- Prone to interference
- Neither sensitive or specific
- May mislead investigations
- Health and safety issues

Qualitative analysis

- Thin Layer Chromatography
  - 1D/2D
  - Ninhydrin to visualise
  - Selective staining increases number of compounds identified
- High Voltage Electrophoresis

Qualitative screening

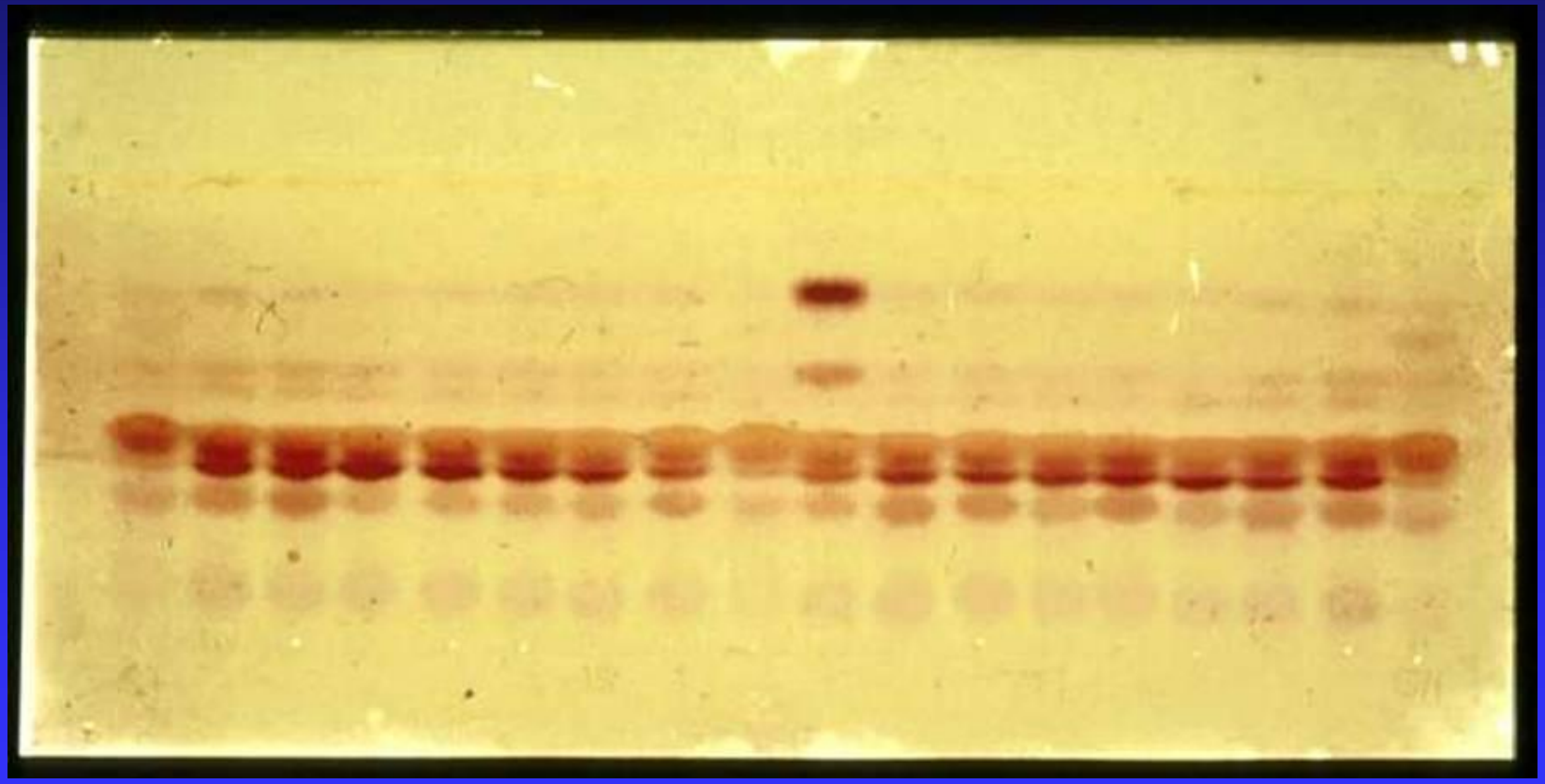
ADVANTAGES

- Cheap
- Can be used to pre-screen samples before referring

LIMITATIONS

- Significant staff time
- Technically demanding
- Interpretation requires experience
- Does not identify all compounds of interest
- May only detect gross abnormalities

TLC- Maple Syrup Urine Disease



Quantitative analysis

- Separation of free amino acids
- Identification of compounds
  - UV detection- retention time
  - MS detection
- Quantitation of compounds
  - Comparison to standards

Amino acid analyser (AAA)

Quantitative analysis- AAA

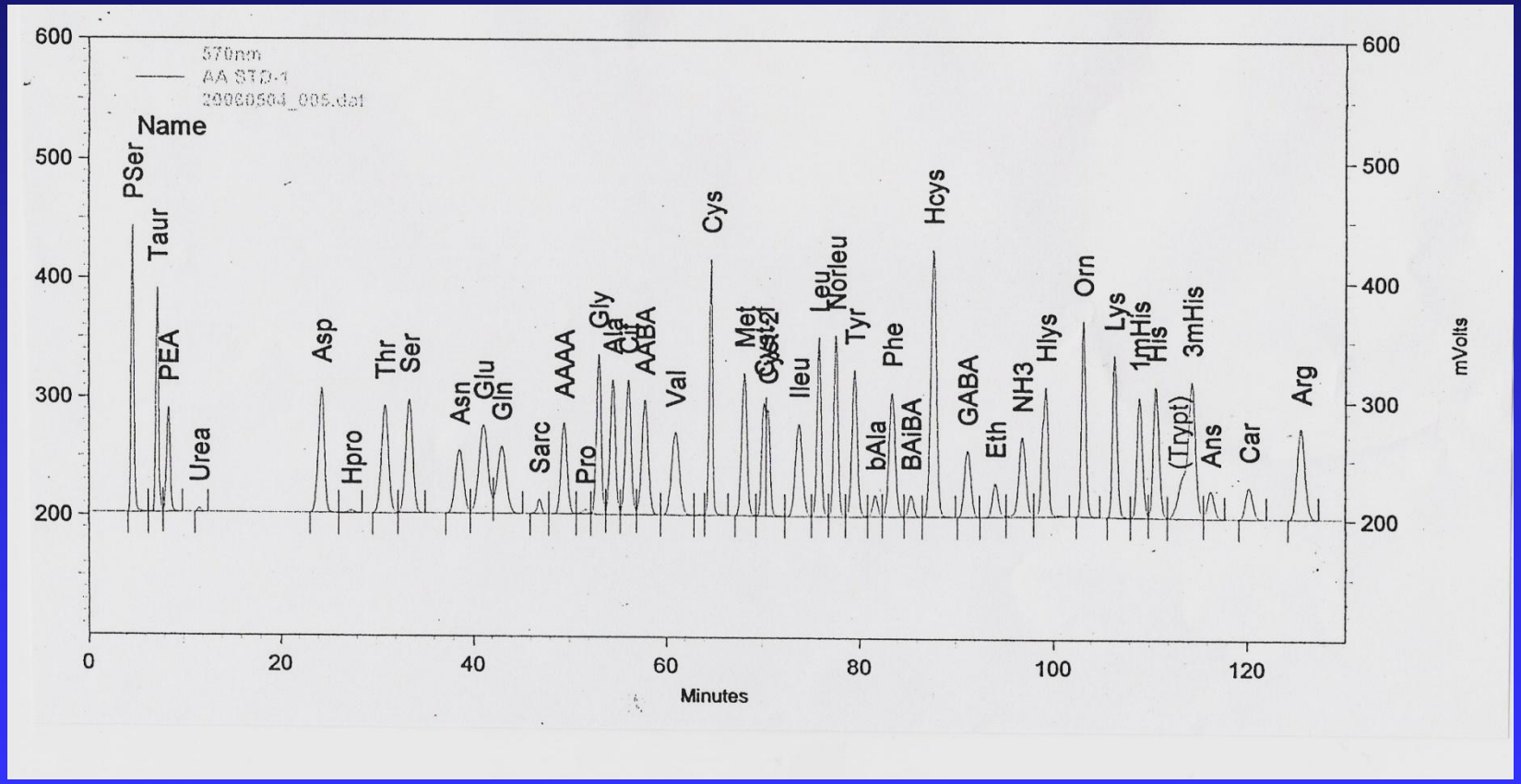
ADVANTAGES

- Dedicated instrument
- Specific for amino acids
- Will identify all compounds of interest

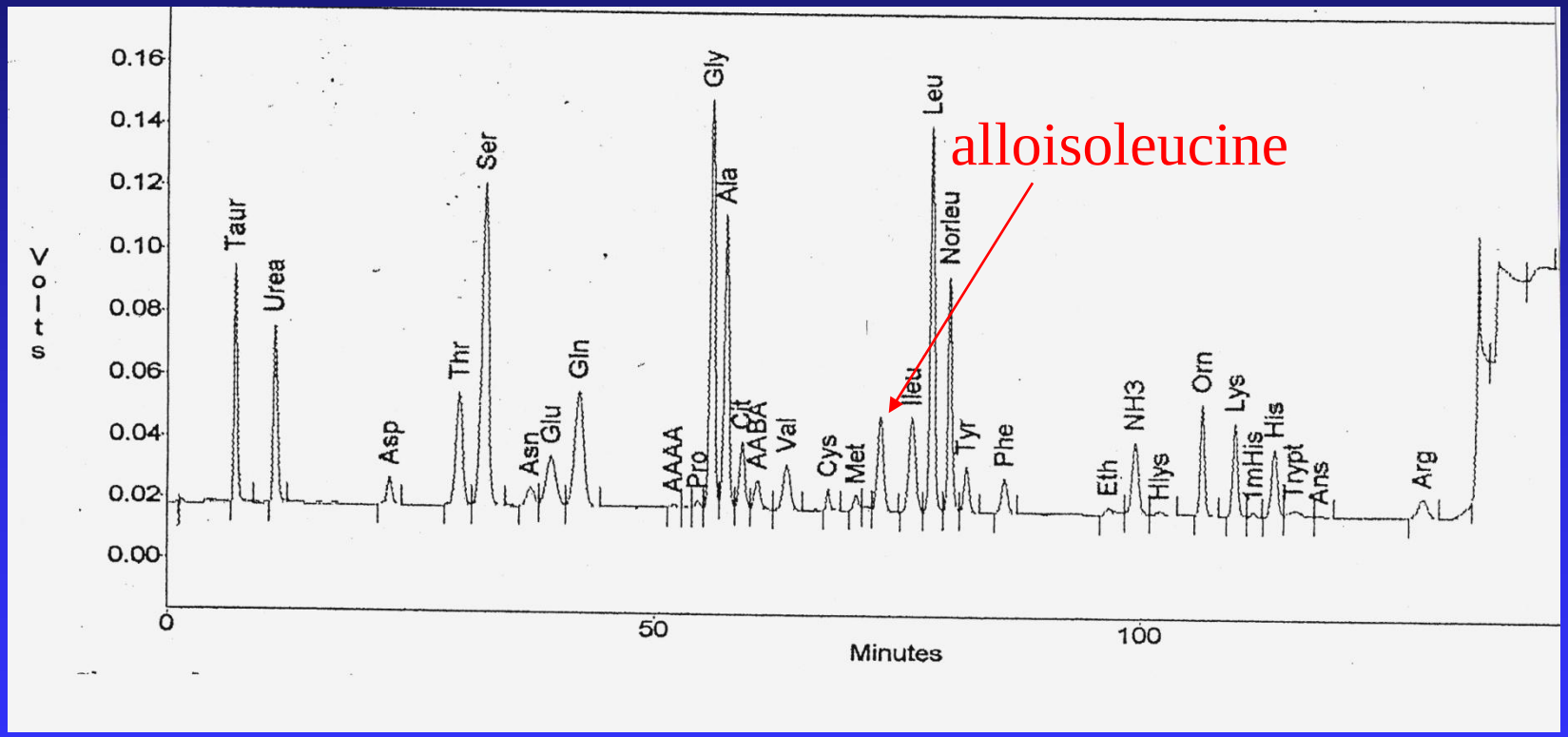
LIMITATIONS

- Long run times
- Significant maintenance
- Often running at capacity
- Urgent cases need rapid results

AAA- separation



AAA- Maple Syrup Urine Disease



Quantitative analysis- TMS

Quantitative analysis- TMS

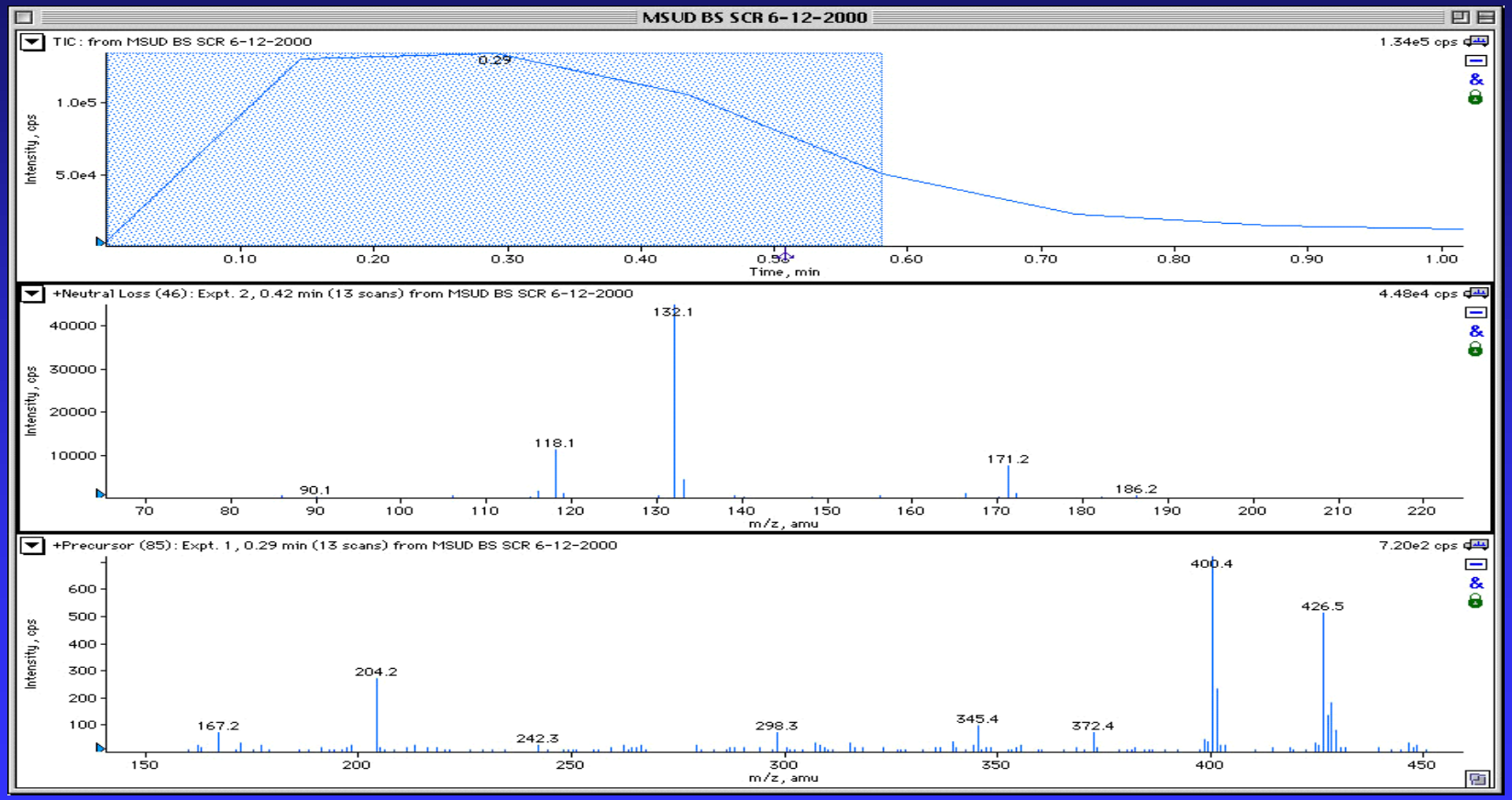
ADVANTAGES

- Established in IEM field
- Can measure other compounds of interest on same injection
- Simple sample prep
- Rapid results
- Ideal for targeted screen

LIMITATIONS

- Expensive capital cost
- Expertise in technology required
- Isobaric/isomeric compounds require separation

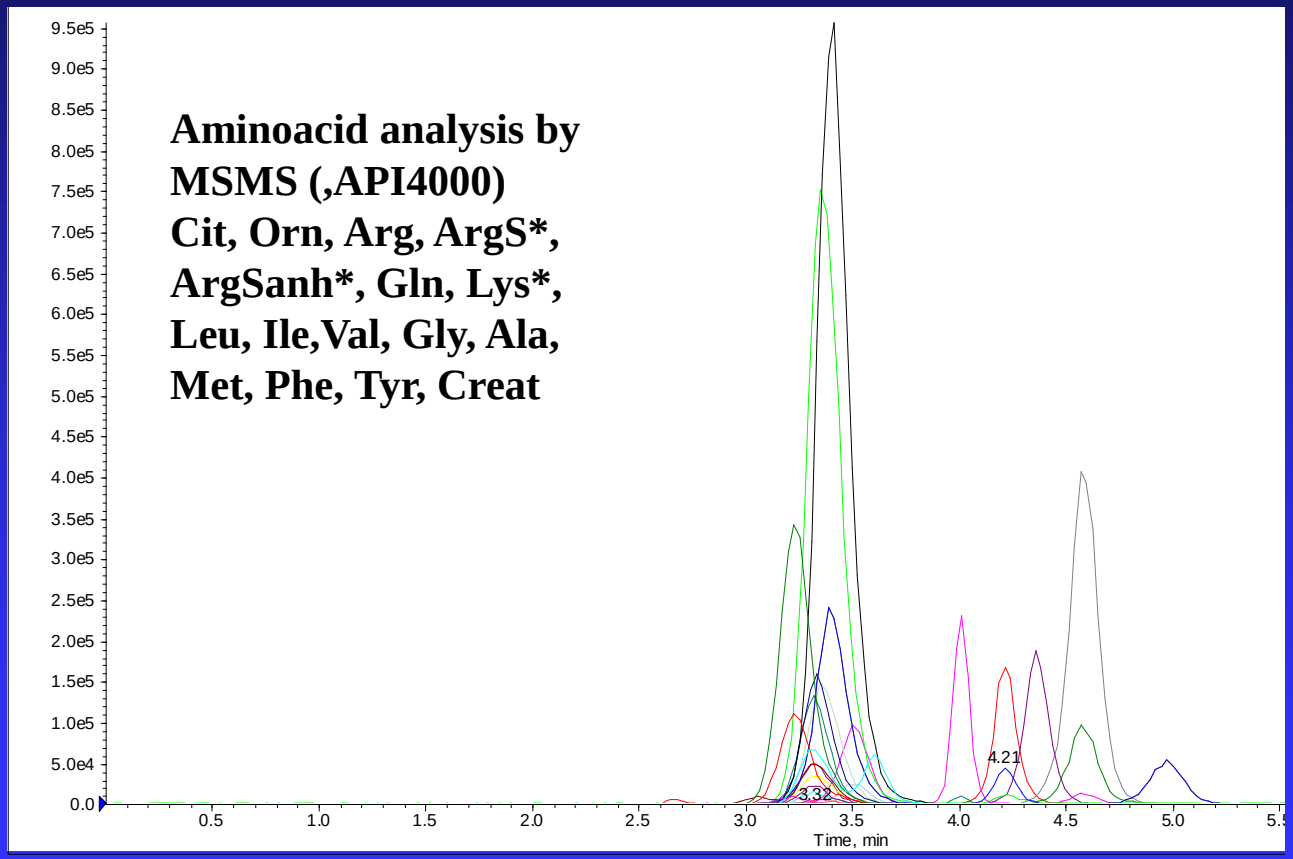
TMS-Maple Syrup Urine Disease



TMS- Future of routine AA analysis

- Rapid Commun in Mass Spec
  Piraud et al 19(22):3287-97
- 76 Amino acids detected
- Ion pairing reversed phase LC linked to positive electrospray MS
- Throughput of 2 samples per hour

TMS-Amino acid analysis



Conclusion

- Understand the limitations of strategy
  - State which disorders are confidently excluded
- In clinical emergency
  - Rapid targeted TMS testing
  - Good communication to specialist centre