

Disclosure

- R Neil Dalton
- is a director and minority shareholder in
 - **SpotOn Clinical Diagnostics**

Targeted metabolic profiling

ERNDIM Workshop meeting

Manchester, November 21st – 22nd 2017



**Evelina
London**

R Neil Dalton
WellChild Laboratory
Evelina London Children's Hospital

WellChild

Guy's and St Thomas'



NHS Foundation Trust

Targeted metabolic profiling

Diagnosis of inherited metabolic diseases

Confirmatory testing

Therapeutic monitoring

Potential biochemical test panels for inherited metabolic disease screening, diagnosis and confirmation are increasing rapidly

NGS is mooted as the most important technique for current and future investment

Critical that we continuously review our analytical practices and seek alternatives

better (accuracy, specificity, and sensitivity), faster, cheaper

Targeted metabolic profiling

Aim of any future “test”

maximise disease coverage, simplify laboratory logistics, and
minimise the requesting dilemmas of clinicians

Robust mass spectrometric and nuclear magnetic resonance analytical
platforms are available

Important to review these technologies and,
where cost benefit without analytical compromise can be established,
validate and introduce them into routine clinical practice

Early diagnosis, effective treatment, improved clinical outcomes

Targeted metabolic profiling

- Targeted metabolic profiling using liquid chromatography electrospray stable isotope dilution tandem mass spectrometry
 - Know the IEMs that have been investigated
 - Know the IEMs that have not been investigated
 - Quantify analytes with accuracy and precision
 - Define linearity, LOQs, and expanded uncertainties
- Lisbon 2008, described an MSMS based amino acid analyser
 - Barcelona 2013, described an IEM analyser

Targeted metabolic profiling

- Combines a range of quantitative specific analyte assays,
 - that we had developed previously, into a single assay

amino acids, acylcarnitines, organic acids, purines and pyrimidines,
and special assays

- At present 107 analytes are quantified
- more than 50 routine samples can be analysed overnight
 - urgent stat samples can be added at any time.



QC: "in house" and retained ERNDIM AA and special assays samples

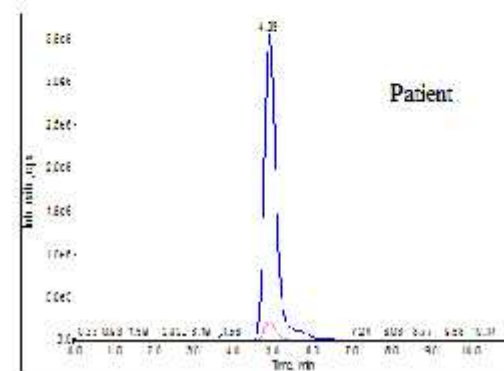
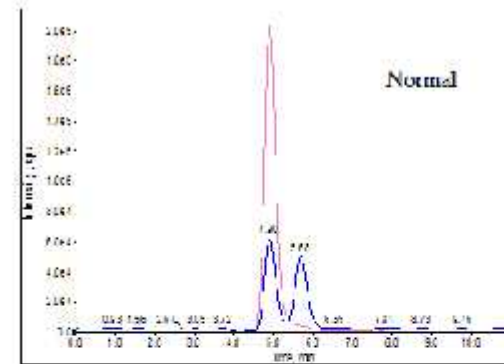
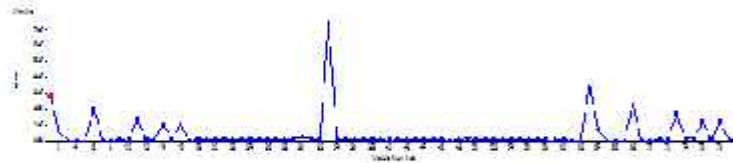
Targeted metabolic profiling

amino acid analyser

Citrullinaemia

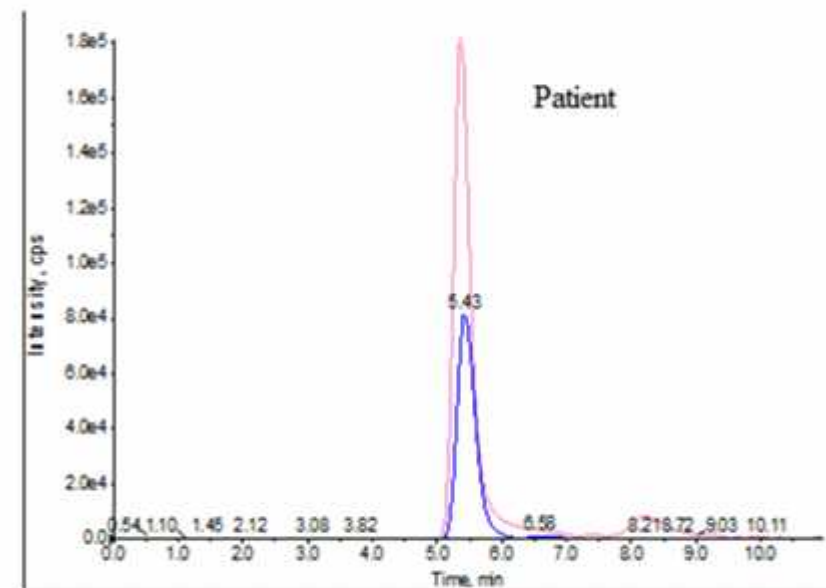
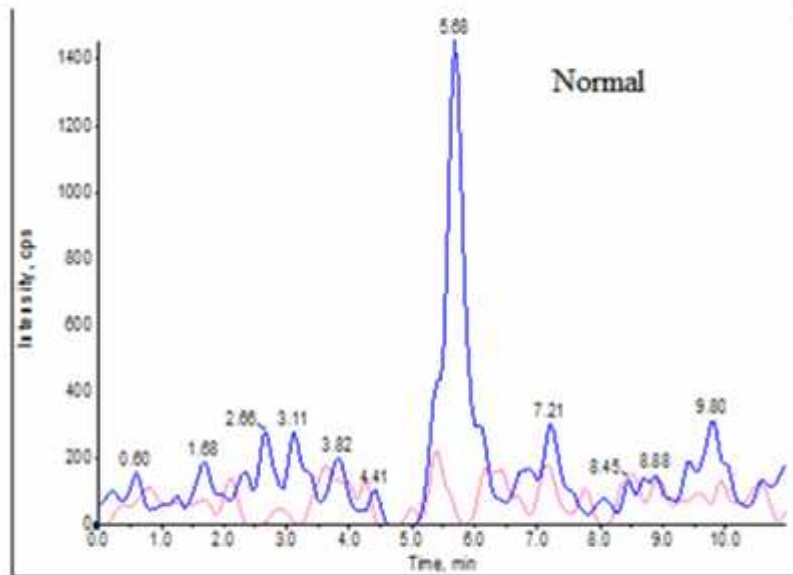
citrulline (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma citrulline
concentrations



Time, min

Targeted metabolic profiling
amino acid analyser
Argininosuccinic acidemia
argininosuccinic acid (red) & anhydride (blue)



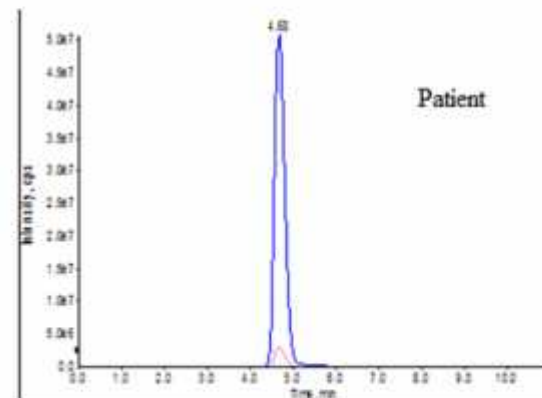
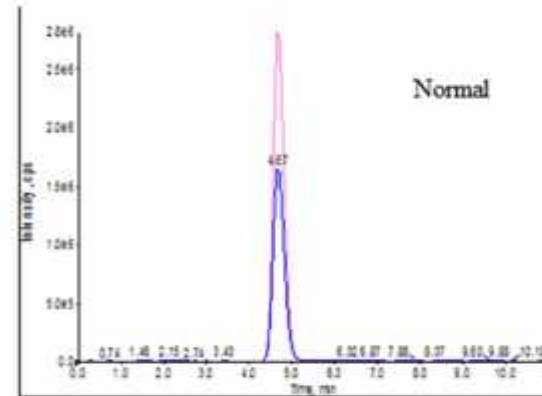
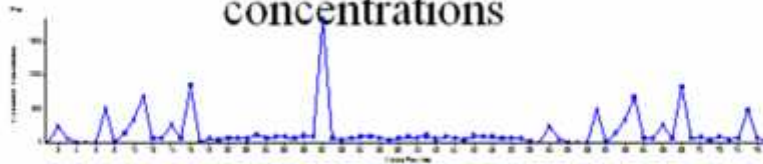
Targeted metabolic profiling

amino acid analyser

Phenylketonuria

phenylalanine (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma
phenylalanine
concentrations



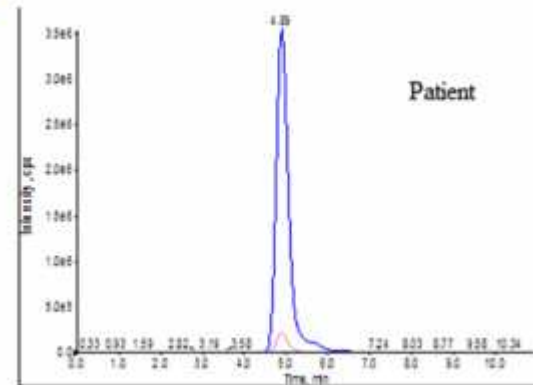
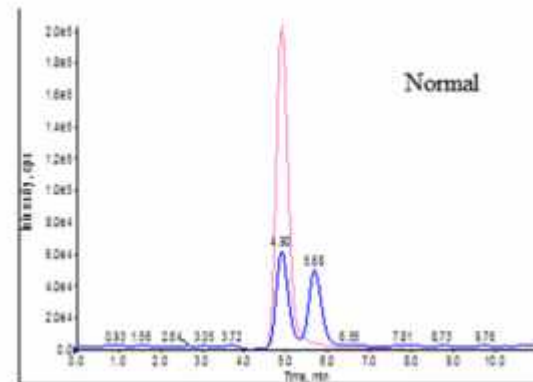
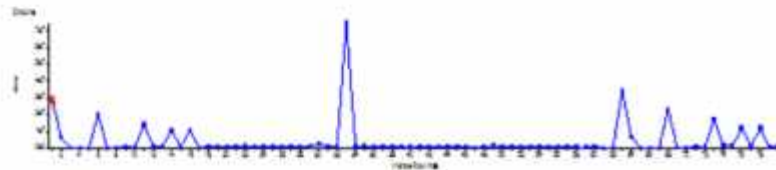
Targeted metabolic profiling

amino acid analyser

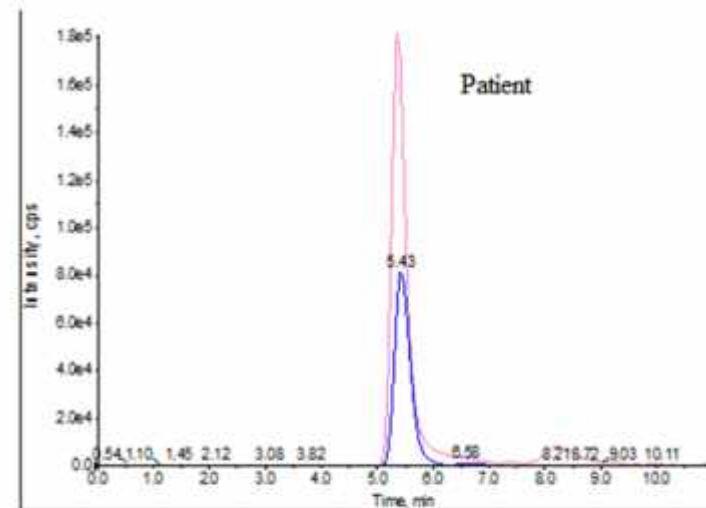
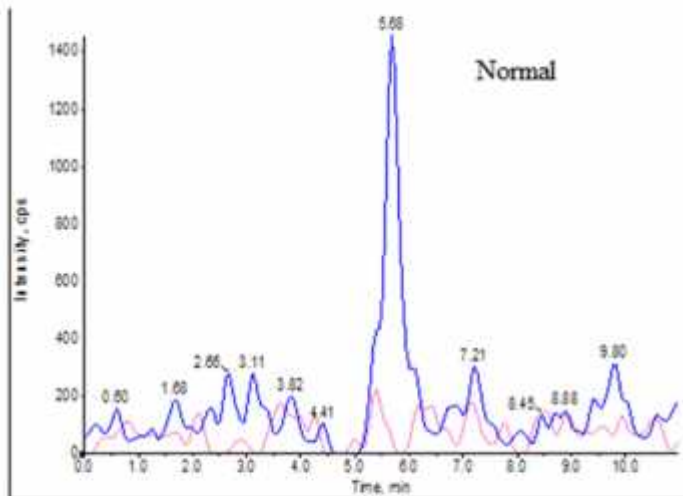
Citrullinaemia

citrulline (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma citrulline
concentrations



Targeted metabolic profiling
amino acid analyser
Argininosuccinic acidemia
argininosuccinic acid (red) & anhydride (blue)

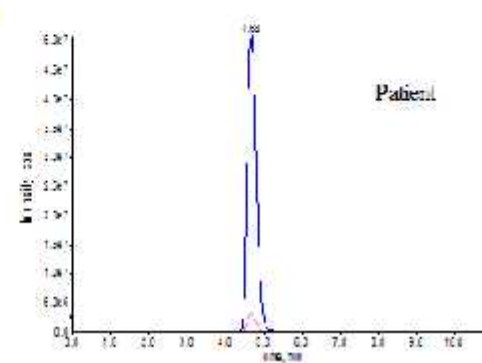
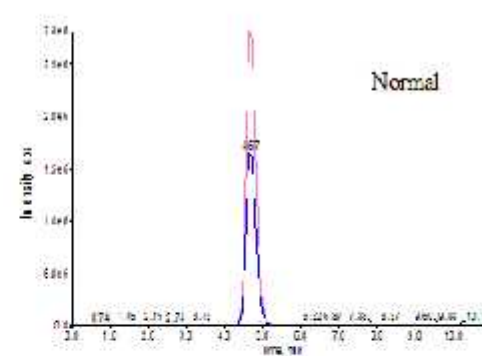
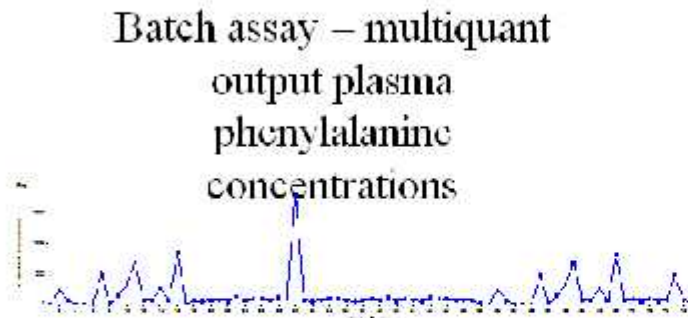


Targeted metabolic profiling

amino acid analyser

Phenylketonuria

phenylalanine (blue), stable isotope internal standard (red)



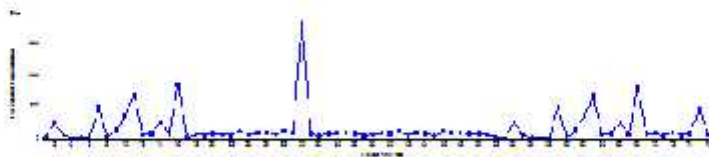
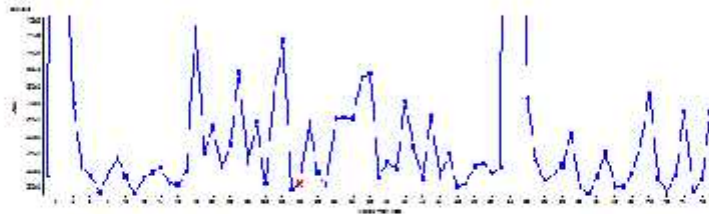
Targeted metabolic profiling

amino acid analyser plus

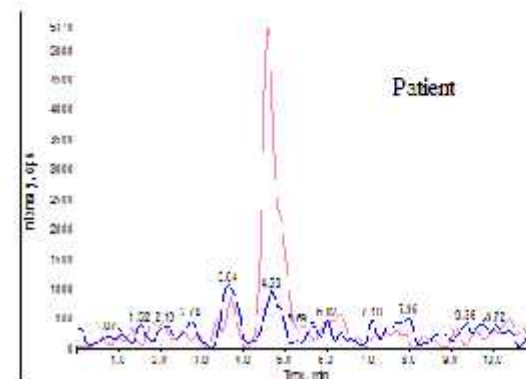
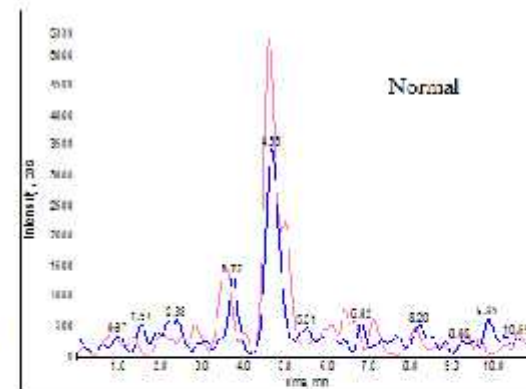
Phenylketonuria

3-O-methyl-L-DOPA (blue), stable isotope internal standard (red)

Batch assay multiquant output
plasma 3-O-methyl-L-DOPA
concentrations



Batch assay – multiquant
output plasma
phenylalanine
concentrations



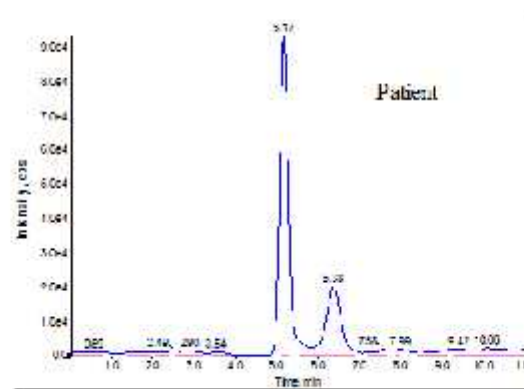
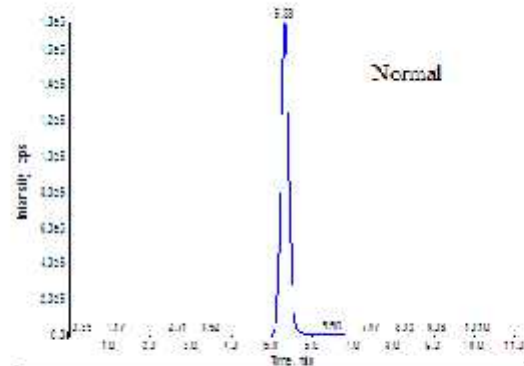
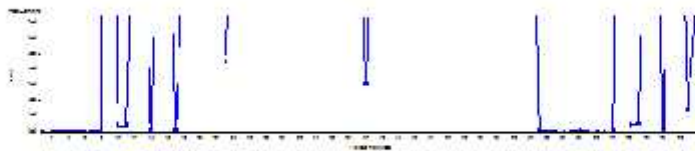
Targeted metabolic profiling

amino acid analyser plus

VLCAD

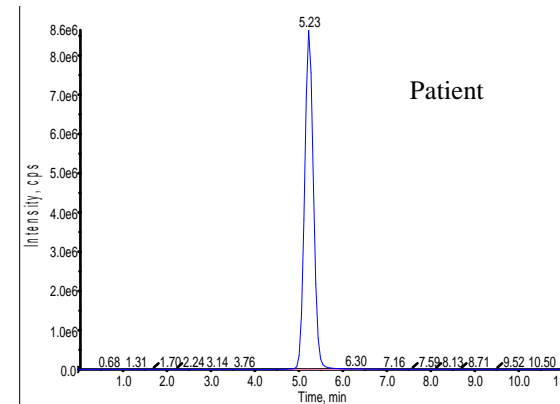
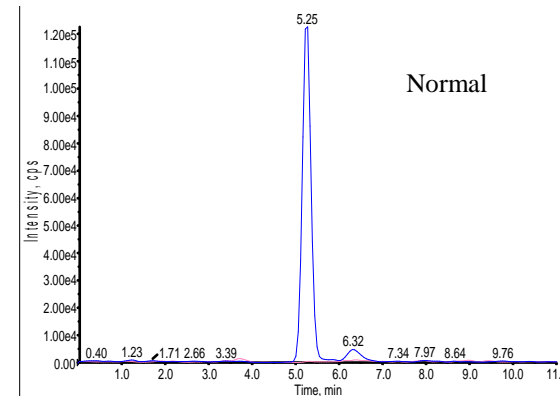
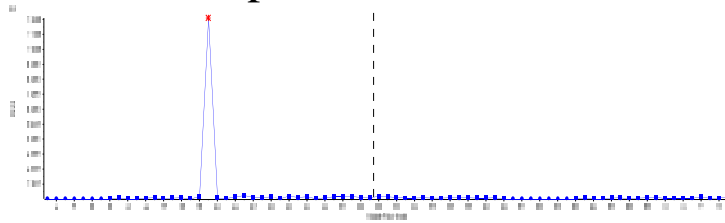
free carnitine (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma free carnitine
peak areas



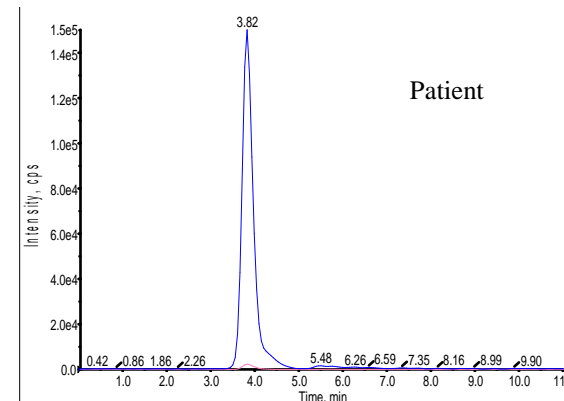
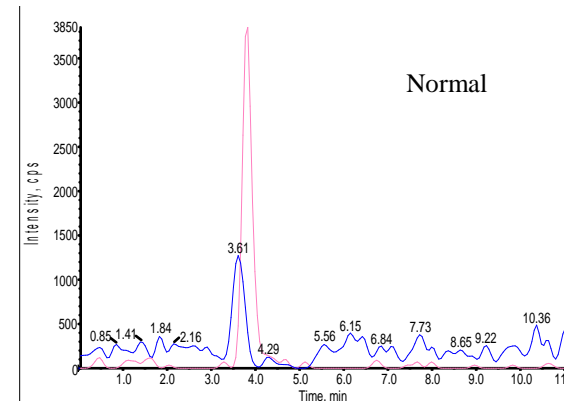
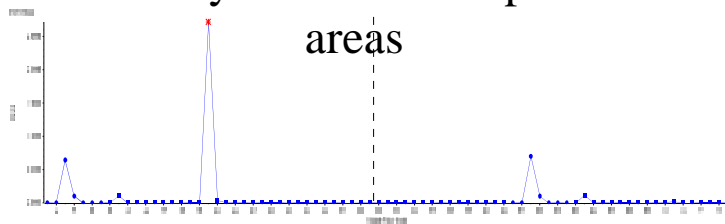
Targeted metabolic profiling amino acid analyser plus Methylmalonic acidaemia C3-carnitine (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma C3 carnitine
peak areas



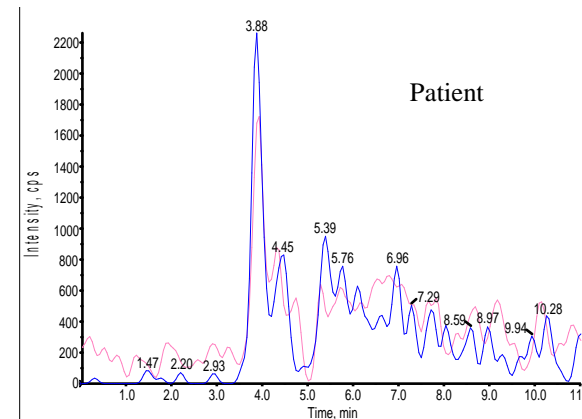
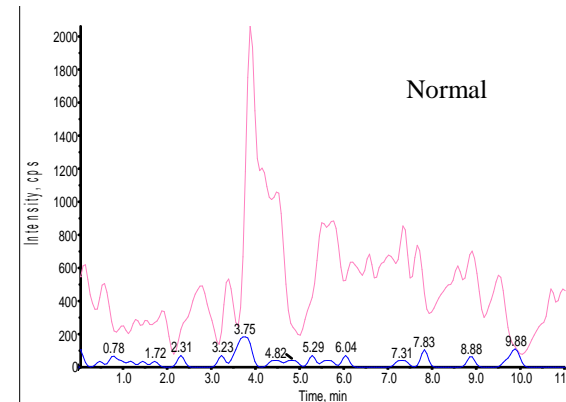
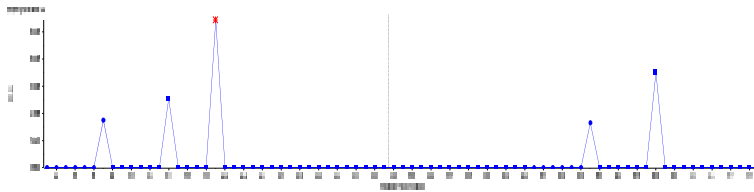
Targeted metabolic profiling amino acid analyser plus Methylmalonic acidaemia methylmalonic acid (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma
methylmalonic acid peak
areas



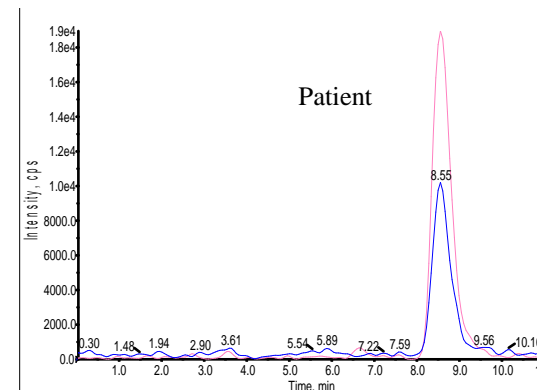
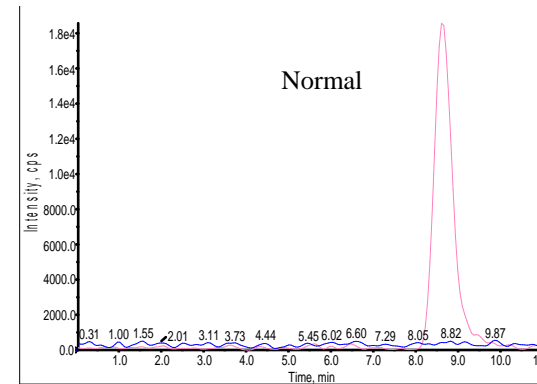
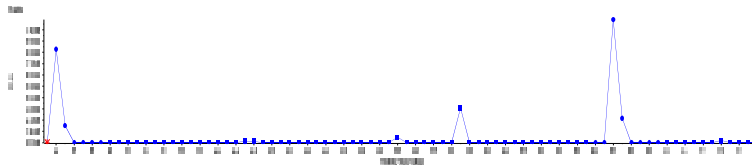
Targeted metabolic profiling amino acid analyser plus Methylmalonic acidaemia methylcitrate (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma methylcitrate
peak areas



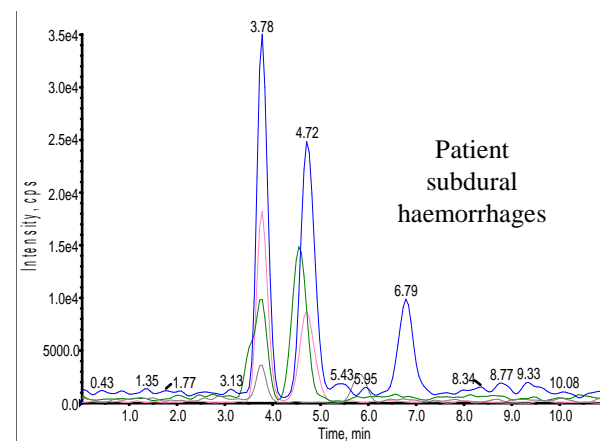
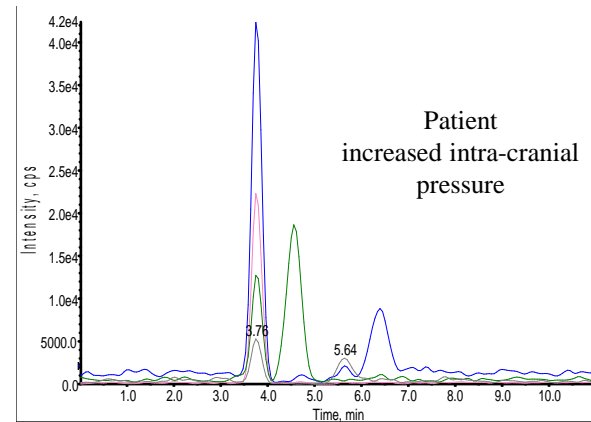
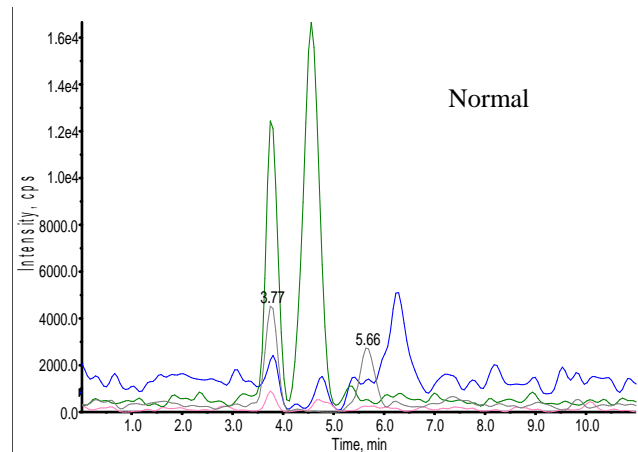
Targeted metabolic profiling
amino acid analyser plus
Ornithine transcarbamylase deficiency
orotic acid (blue), stable isotope internal standard (red)

Batch assay – multiquant
output plasma orotic acid
peak areas



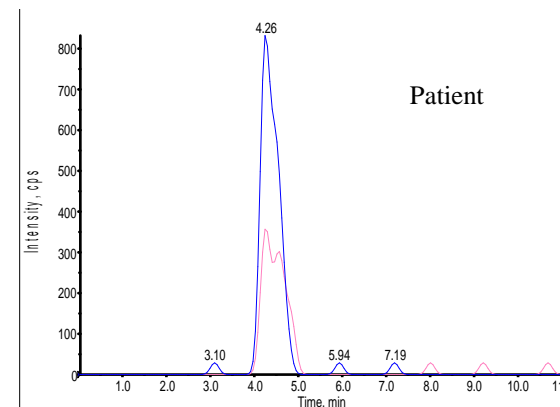
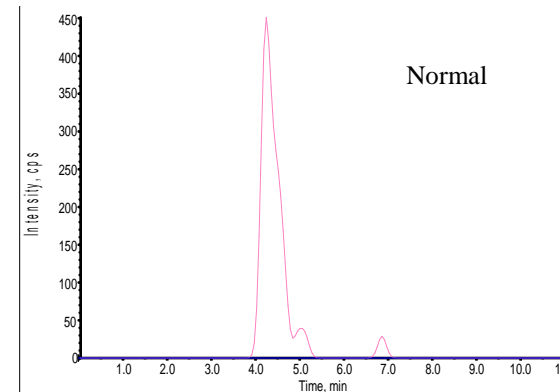
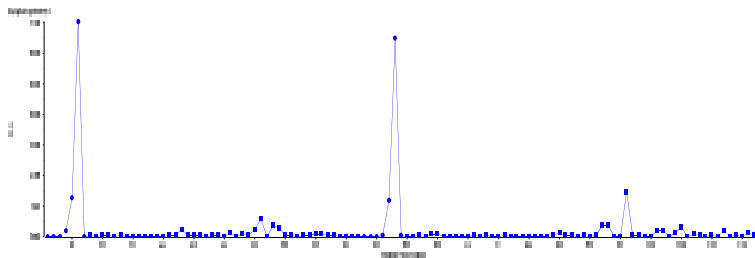
Targeted metabolic profiling amino acid analyser plus Brain injury or Canavan's disease – aspartoacylase deficiency?

N-acetylaspartic acid (blue, red), stable isotope internal
standard (green, grey)



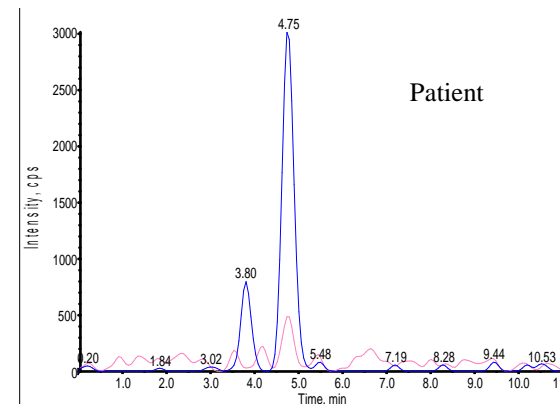
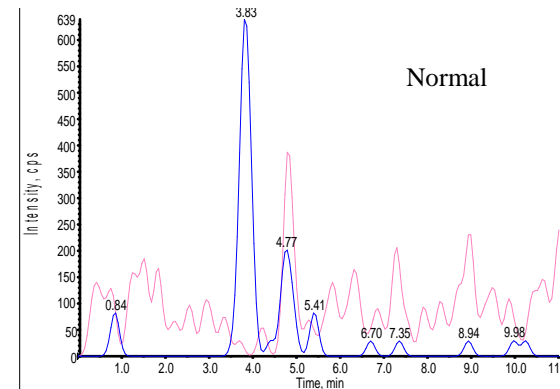
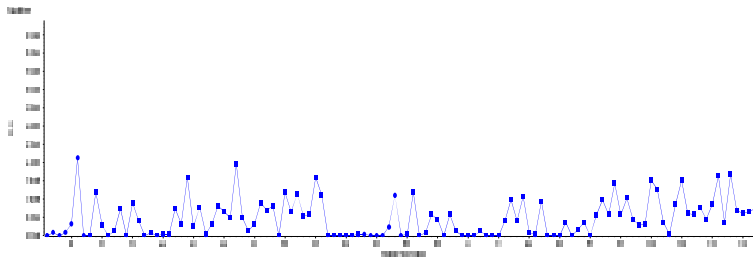
Targeted metabolic profiling amino acid analyser plus Molybdenum cofactor deficiency sulphocysteine (blue), stable isotope internal standard (red)

Batch assay – multiquant
output
plasma sulphocysteine peak
areas



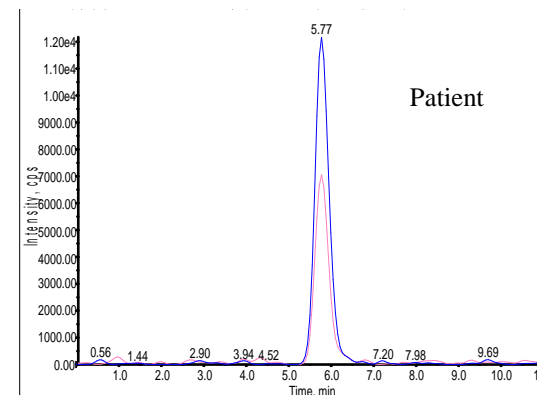
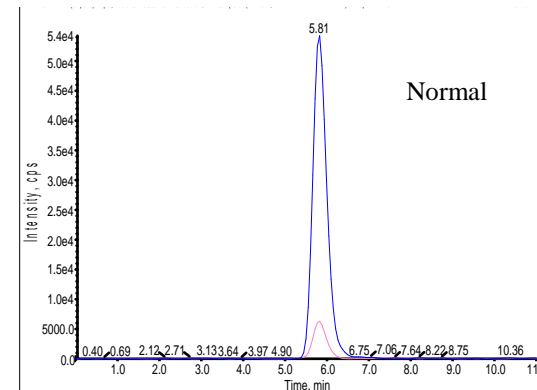
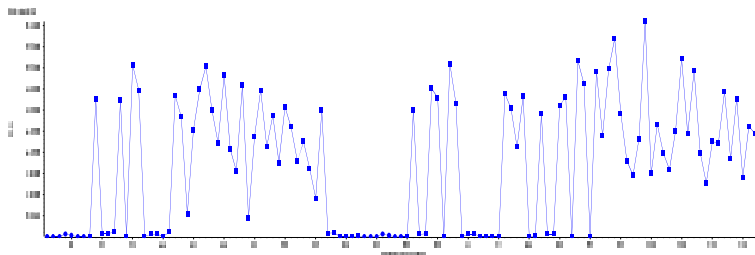
Targeted metabolic profiling amino acid analyser plus Molybdenum cofactor deficiency xanthine (blue), stable isotope internal standard (red)

Batch assay – multiquant
output
plasma xanthine peak areas



Targeted metabolic profiling amino acid analyser plus Molybdenum cofactor deficiency uric acid (blue), stable isotope internal standard (red)

Batch assay – multiquant
output
plasma uric acid peak areas

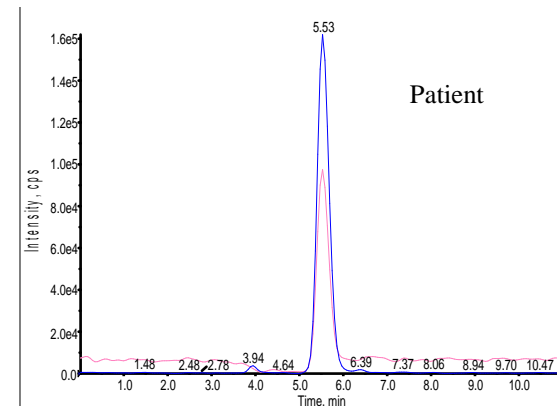
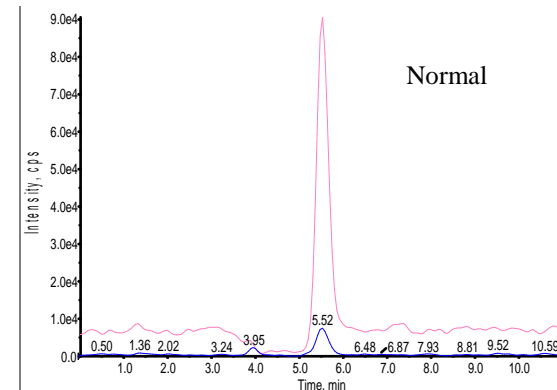
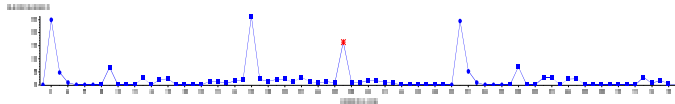


Targeted metabolic profiling

amino acid analyser plus

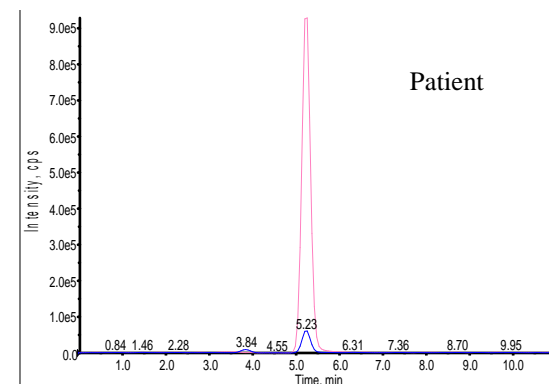
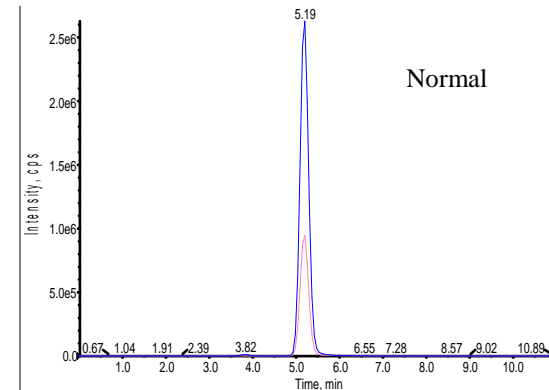
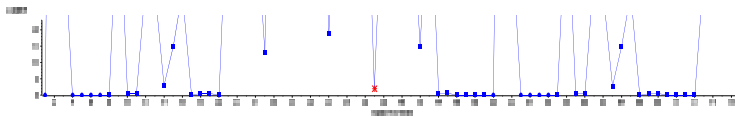
Guanidinoacetate methyl transferase (GAMT) deficiency
guanidinoacetate (blue), stable isotope internal standard (red)

Batch assay – multiquant
output
plasma guanidinoacetate
concentrations



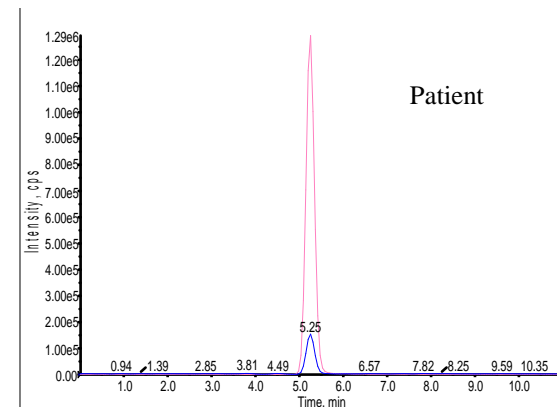
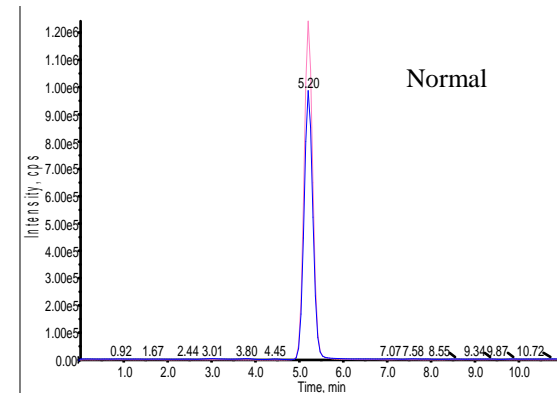
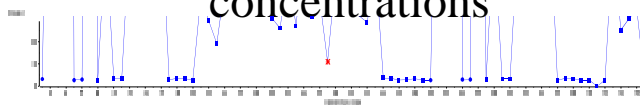
Targeted metabolic profiling amino acid analyser plus Guanidinoacetate methyl transferase (GAMT) deficiency creatinine (blue), stable isotope internal standard (red)

Batch assay – multiquant
output
plasma creatinine concentrations



Targeted metabolic profiling amino acid analyser plus Guanidinoacetate methyl transferase (GAMT) deficiency creatinine (blue), stable isotope internal standard (red)

Batch assay – multiquant
output
plasma creatinine
concentrations

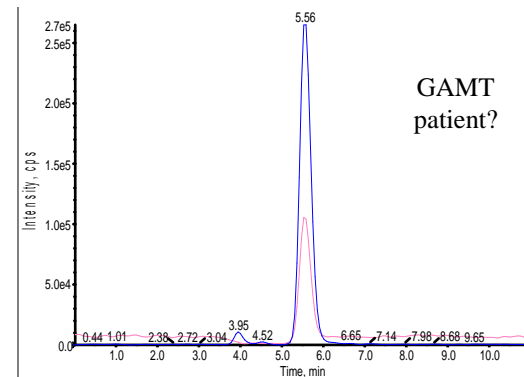
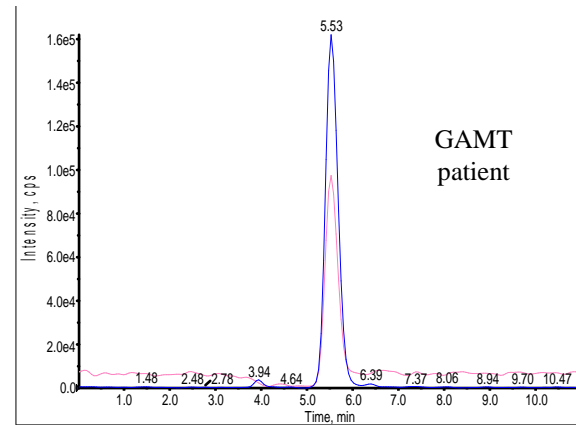
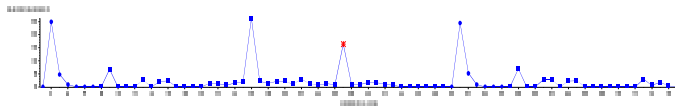


Targeted metabolic profiling

amino acid analyser plus

Guanidinoacetate methyl transferase (GAMT) deficiency?
guanidinoacetate (blue), stable isotope internal standard (red)

Batch assay – multiquant
output
plasma guanidinoacetate
concentrations



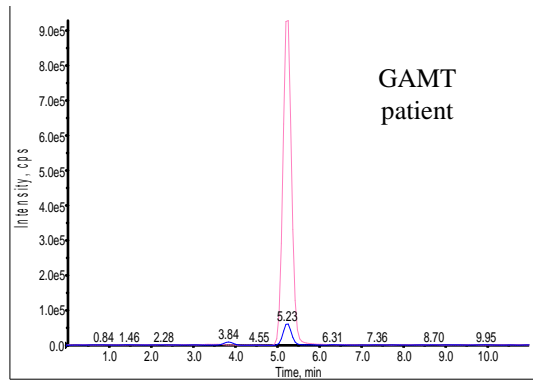
Targeted metabolic profiling

amino acid analyser plus

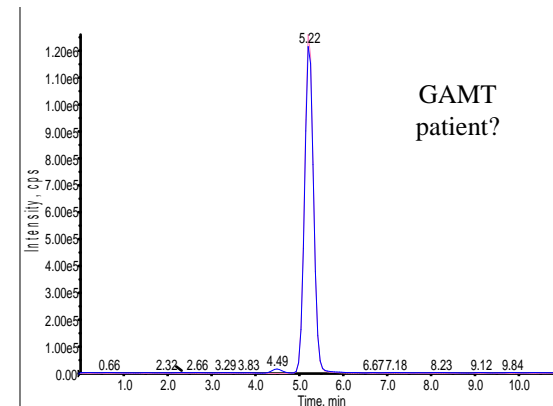
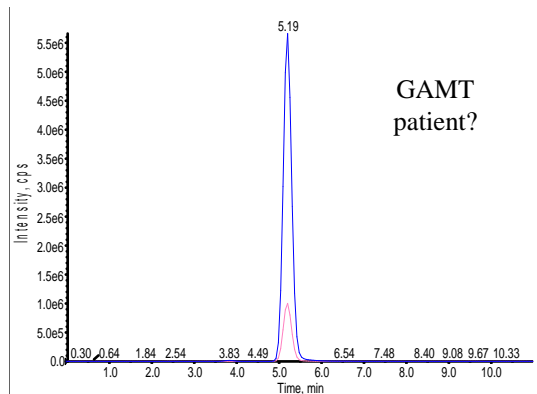
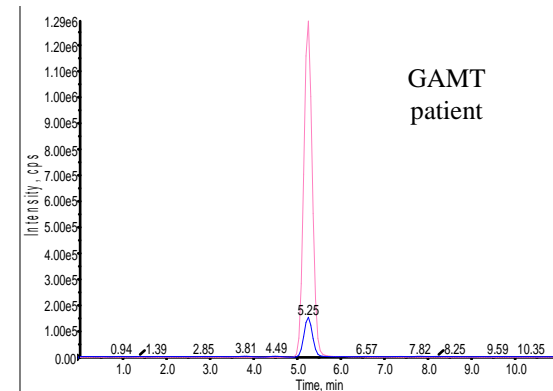
Guanidinoacetate methyl transferase (GAMT) deficiency?

creatinine, creatinine (blue), stable isotope internal standards (red)

Creatine



Creatinine



Targeted metabolic profiling

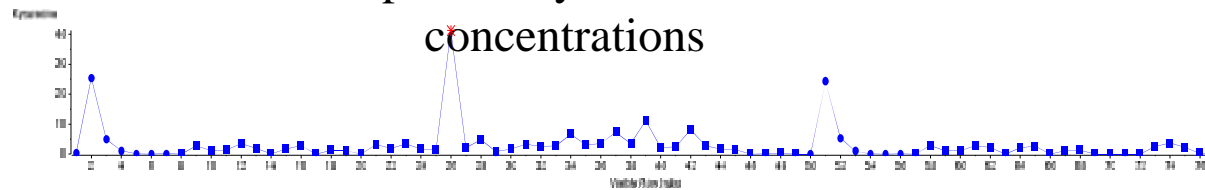
amino acid analyser plus

Reason for increased guanidinoacetate – infection?

kynurenine/tryptophan ratio increased in infection

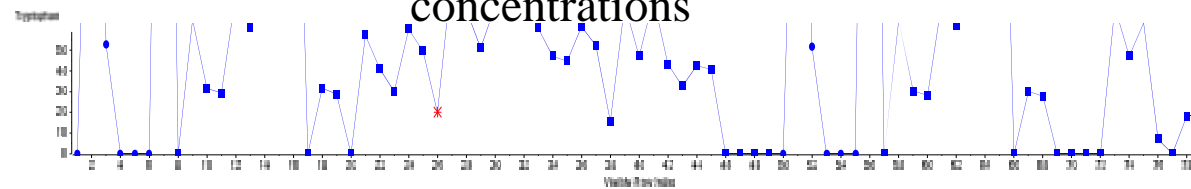
Batch assay – multiquant
output

plasma kynurenine
concentrations



Batch assay – multiquant
output

Plasma tryptophan
concentrations



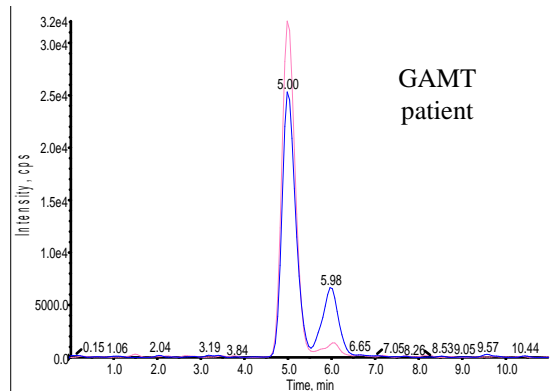
Targeted metabolic profiling

amino acid analyser plus

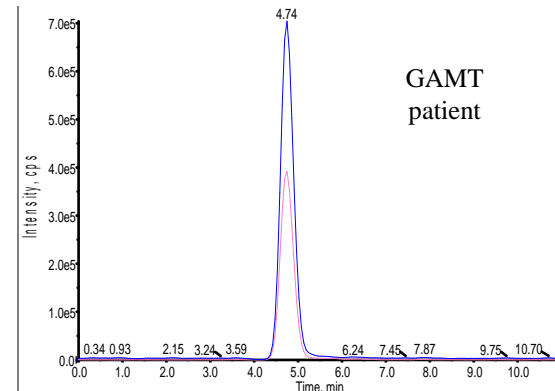
Reason for increased guanidinoacetate – infection

kynurenine, tryptophan (blue), stable isotope internal standards (red)

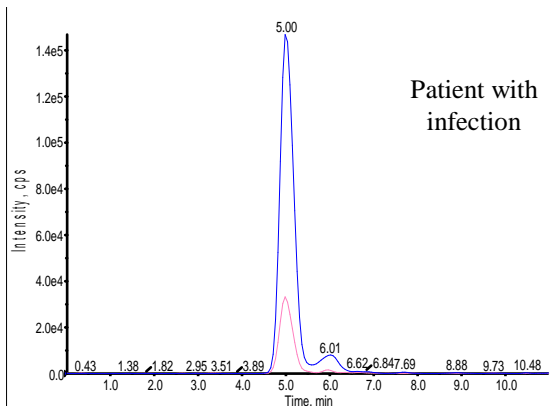
Kynurenine



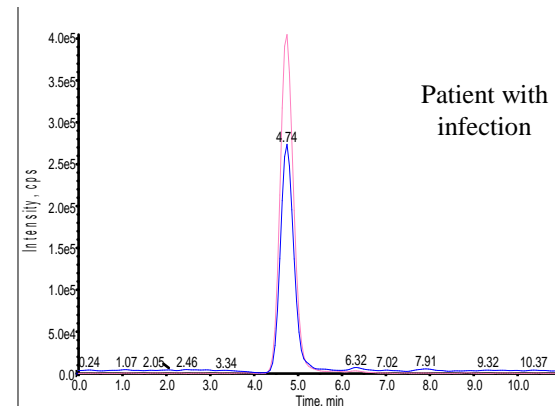
Tryptophan



Patient with infection



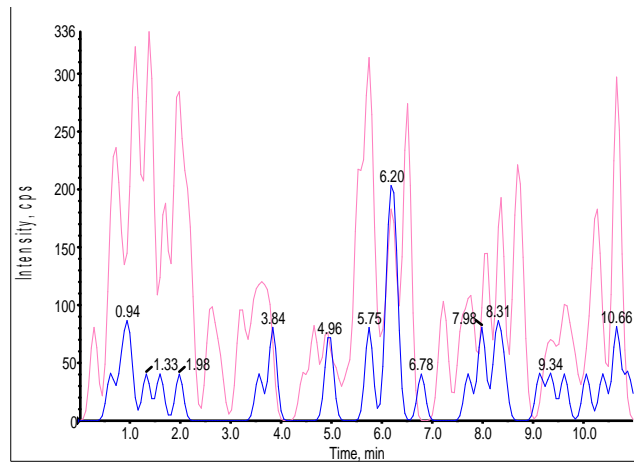
Patient with infection



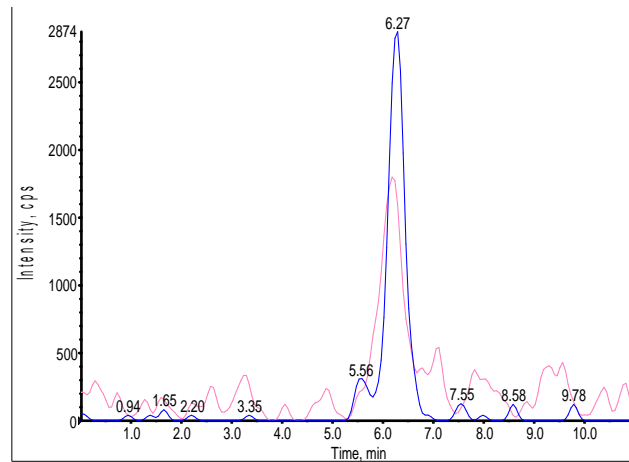
Targeted metabolic profiling amino acid analyser plus

Reason for increased guanidinoacetate – infection
neopterin, 2 transitions

GAMT
patient



Patient with subsequently confirmed
HSV infection



Targeted metabolic profiling

amino acid analyser plus

Neonatal fits, responsive to biotin/pyridoxal phosphate

3-O-methyl-L-DOPA (blue, red), stable isotope internal standard (green, grey)

Plasma 3-O-methyl-L-DOPA
monitored in real time during
treatment

26/10/2017

3310 nmol/L

27/10/2017

3020 nmol/L

29/10/2017

1880 nmol/L

02/11/2017

646 nmol/L

03/11/2017 14:30

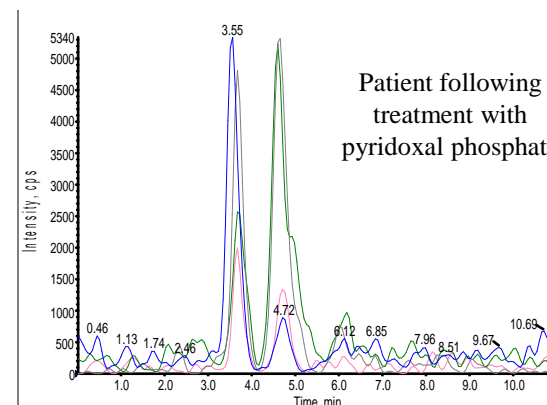
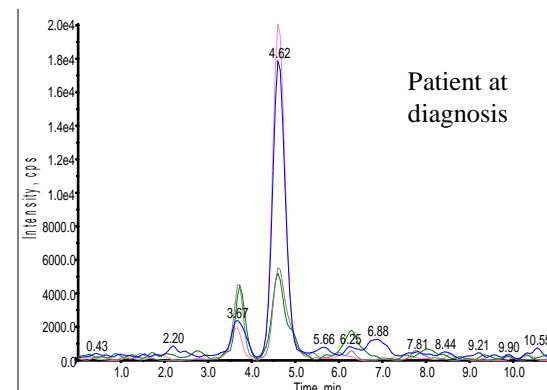
705 nmol/L

04/11/2017 14:15

729 nmol/L

06/11/2017 10:30

868 nmol/L



Conclusions

Targeted metabolic profiling

Simple, rapid sample (10 μ l) preparation, 10min

Rapid LC-MSMS analysis, 11min

Significantly faster than current amino acid analysis systems
and provides, based on ERNDIM returns, at least equivalent
results

Includes free and acylcarnitines, a range of organic acids, some
purine and pyrimidines, and a range of special assay
analytes

Additional information on infection, liver function, renal
function, brain injury

Conclusions

Targeted metabolic profiling

Analytical cost

comparable to the current cost of an amino acid profile
alone

Better

Faster

Cheaper



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Guy's & St Thomas' Charity
Evelina London Children's Hospital Appeal

Guy's and St Thomas' NHS Foundation Trust