Acylcarnitine measurement in blood spots: methodological aspects, problems and pitfalls with reference to the ERNDIM QA scheme

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Acylcarnitine measurement in blood spots ERNDIM QA scheme

- Acylcarnitine analysis
- Methodological aspects
- Overview of ERNDIM QA Scheme
- Problems and Pitfalls

Acylcarnitine analysis

- Electrospray MSMS
  - Precursor ion scan, m/z 85
- Blood spots/plasma/serum
- Rapid analysis
  - Flow injection analysis
  - Simultaneous acquisitions for aminoacids etc
- Range of disorders
  - Organic acidaemias, fatty acid oxidation disorders
  - Particularly useful for long chain fat ox
    - LCHADD, VLCADD

Chace, D. H., T. A. Kalas, et al. (2003). "Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns." Clinical Chemistry. 49(11): 1797-817

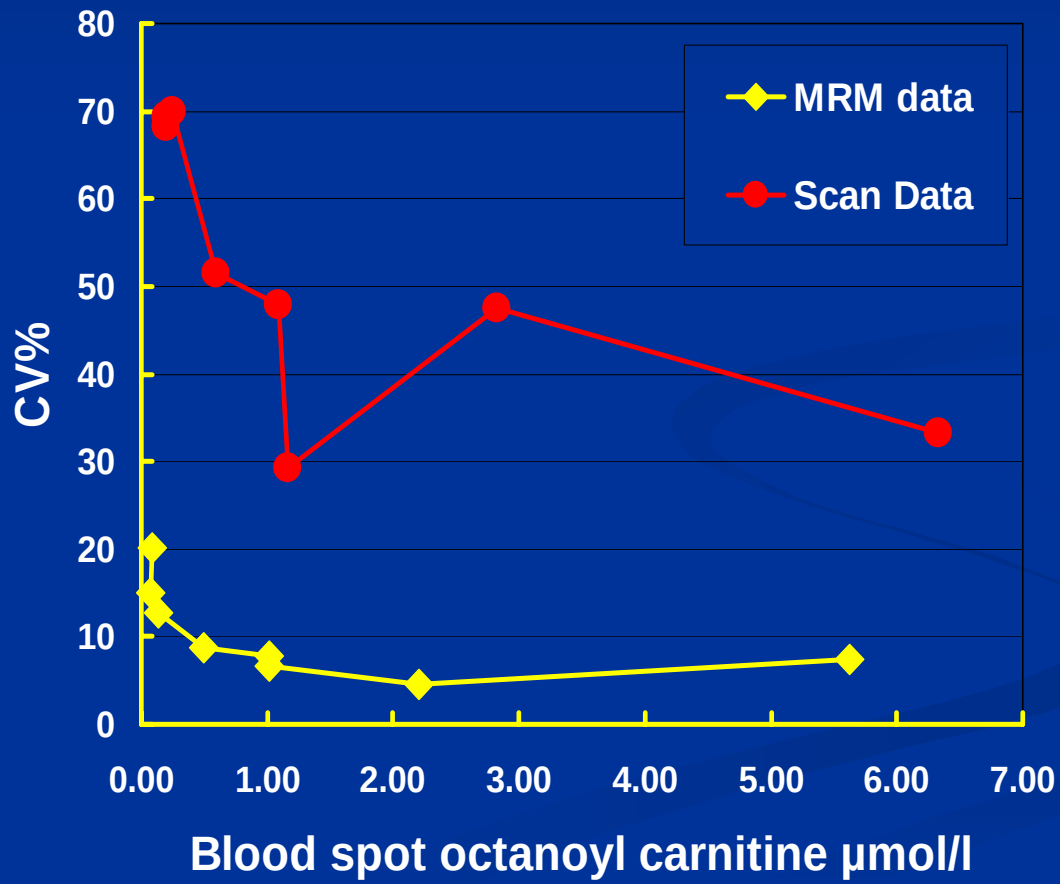
Acylcarnitine analysis
Quality control

"In –house"

stable isotope internal standards

quantitation - MRMs

Octanoyl carnitine within run precision profile: MRM v Scan



Acylcarnitine measurement in blood spots ERNDIM QA scheme

Methodological aspects

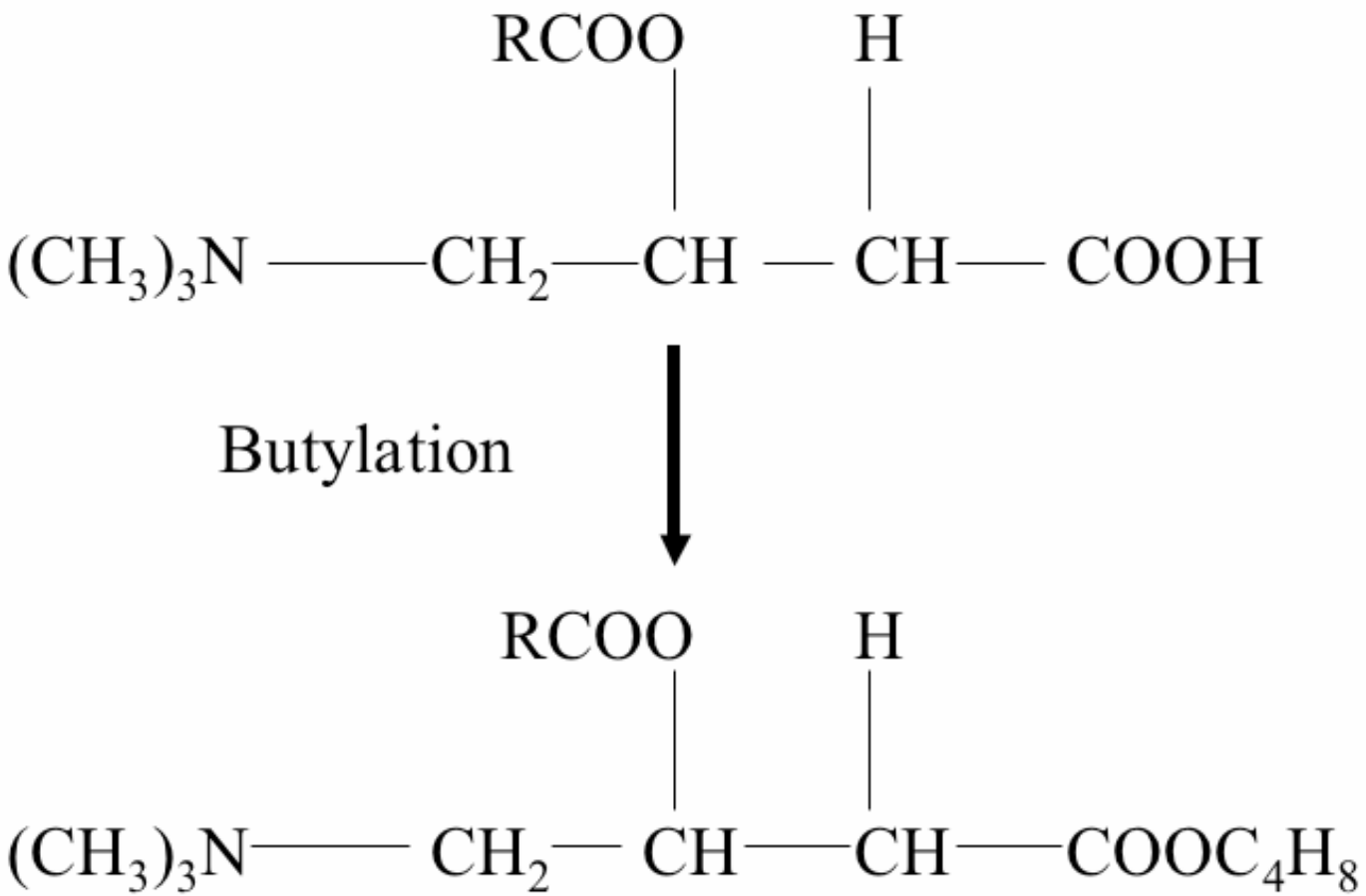
Butylation v direct assay

Questionnaire on 1st circulation

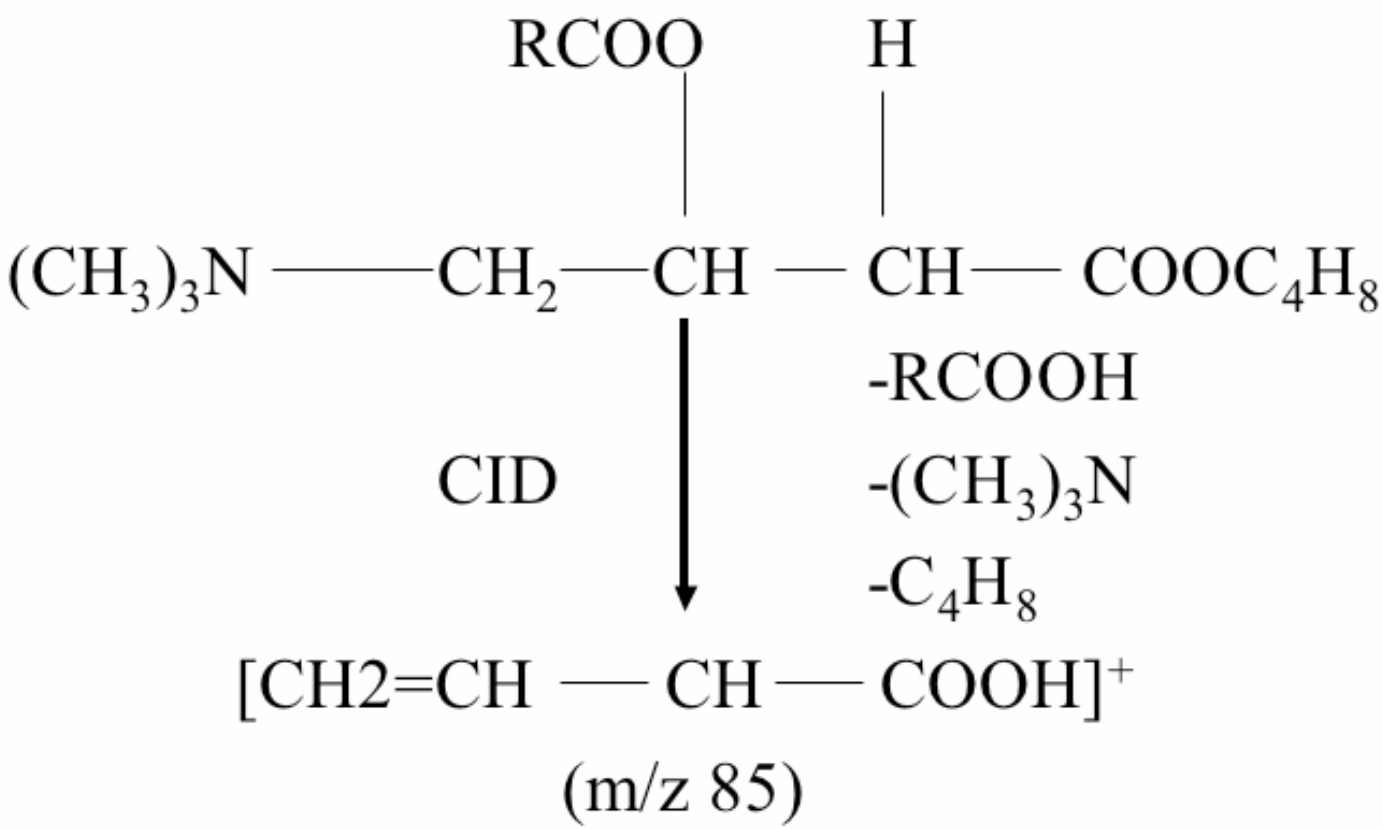
34/35 – butylation

1/35 - direct

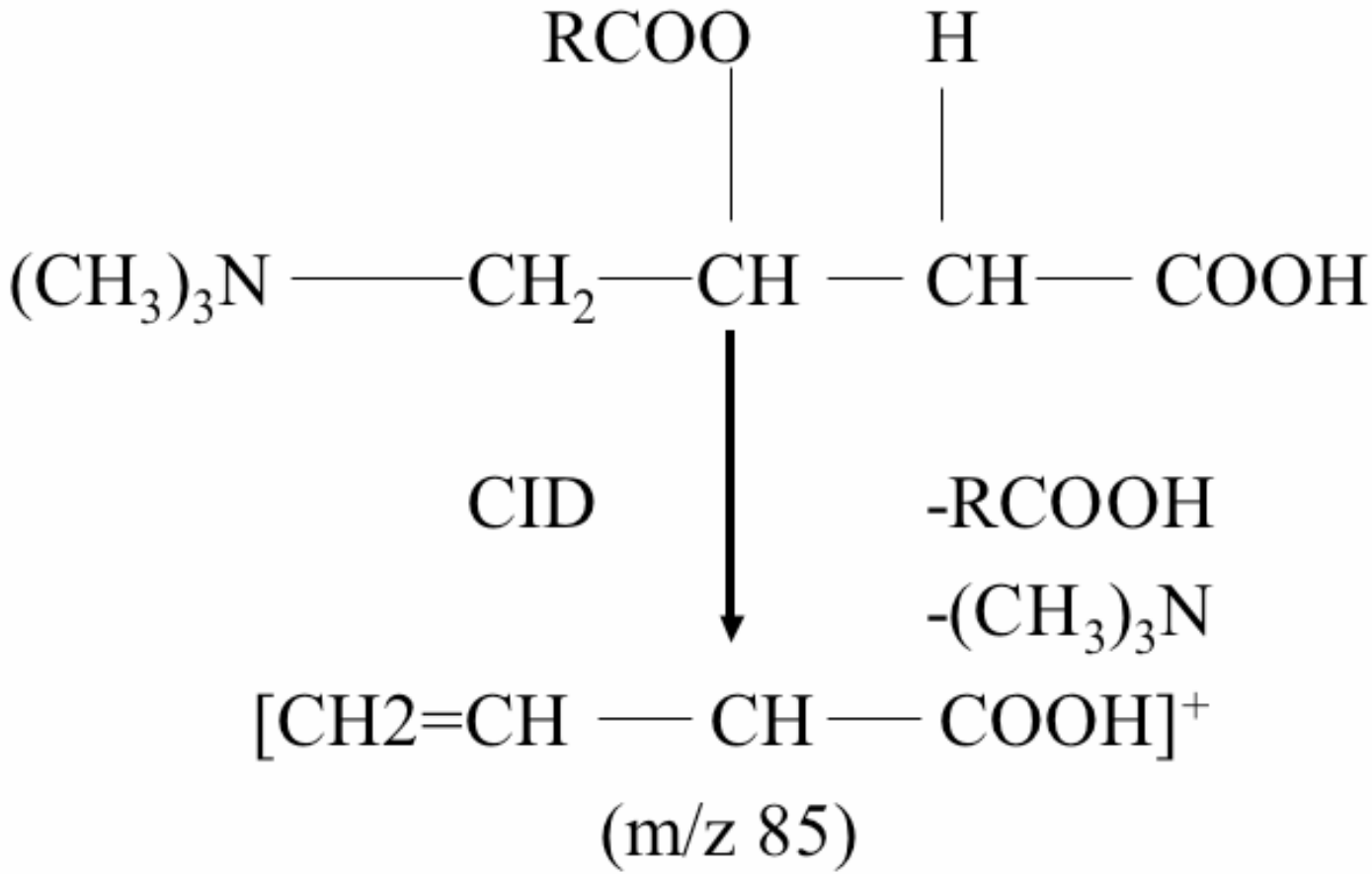
Acylcarnitines butylation



Acylcarnitines butylated fragmentation



Acylcarnitines underivatised fragmentation



Acylcarnitine measurement in blood spots ERNDIM QA scheme

Need for scheme?

CDC – excellent quantitative scheme

Spiked blood samples

Not specific for acylcarnitines

More suitable for control of neonatal screening programmes

No free carnitine

Acylcarnitine measurement in blood spots ERNDIM QA scheme

Need for scheme?

Personal

Methodological

Butylated v direct

Offered to set-up pilot for 1 year if sufficient interest

Questionnaire to see what users wanted

Acylcarnitine analysis
Quality control

ERNDIM

Clinical material (blood spot/serum/plasma)

Interpretative (qualitative/quantitative)

Learning exercise

Accreditation

Model: urine organic acid scheme

Acylcarnitine analysis
ERNDIM QA scheme

ERNDIM users canvassed

Over 30 laboratories expressed an interest

By the dispatch date, 4th April 2003 45 laboratories registered

Problems of blood volume and sample transport

scheme for plasma/serum excluded

Blood spots from real clinical cases

Quantitative special assays serum/urine includes free carnitine

Acylcarnitine measurement in blood spots ERNDIM QA scheme

Initially 30 laboratories expressed an interest

2003 45 laboratories registered
2004 59 laboratories registered
2005 67 laboratories registered

Acylcarnitine measurement in blood spots ERNDIM QA scheme

Patients

Presenting to the the metabolic service at Guy's Hospital (now Evelina Childrens Hospital, St Thomas's)

Diagnoses confirmed by enzymology or DNA sequencing

Routine blood sampling – written informed consent (patient/parent) obtained for use of excess blood for quality control purposes – authorised by the chairman of the Guy's Hospital Ethical Committee

Scheme participants encouraged to submit samples

1 donor so far + 2 recent offers

Acylcarnitine measurement in blood spots ERNDIM QA scheme

Samples

Lithium heparin anti-coagulated whole blood
40µl aliquots spotted onto Schleicher & Schuell 903 paper
Dried for 24h at RT before packing and despatch
Any delay stored at –80^{o}C

Current requirement for 1 circulation
approximately 4ml liquid whole blood
70+ dried blood spots

Acylcarnitine measurement in blood spots ERNDIM QA scheme

6 circulations to date

April 2003 – 45 sent, 35 returns (19 by due date)

January 2004 – 45 sent, 32 returns (25 by due date)

July 2004 – 59 sent, 41 returns (38 by due date)

Nov 2004-60 sent, 42 returns (38 by due date)

May 2005 - 67 sent, 53 returns (45 by due date)

Nov 2005- 67 sent, due date not yet passed

ERNDIM QA Scheme
Normal Samples
1a, 3b, 4c, 5b

- 5b was from diabetic patient hypoglycaemic due to insulin overdose
- Others from healthy subjects
- Most respondents reported acylcarnitine profile as normal
- Many suggested further tests

ERNDIM QA Scheme
MCADD
Samples 3a, 5a

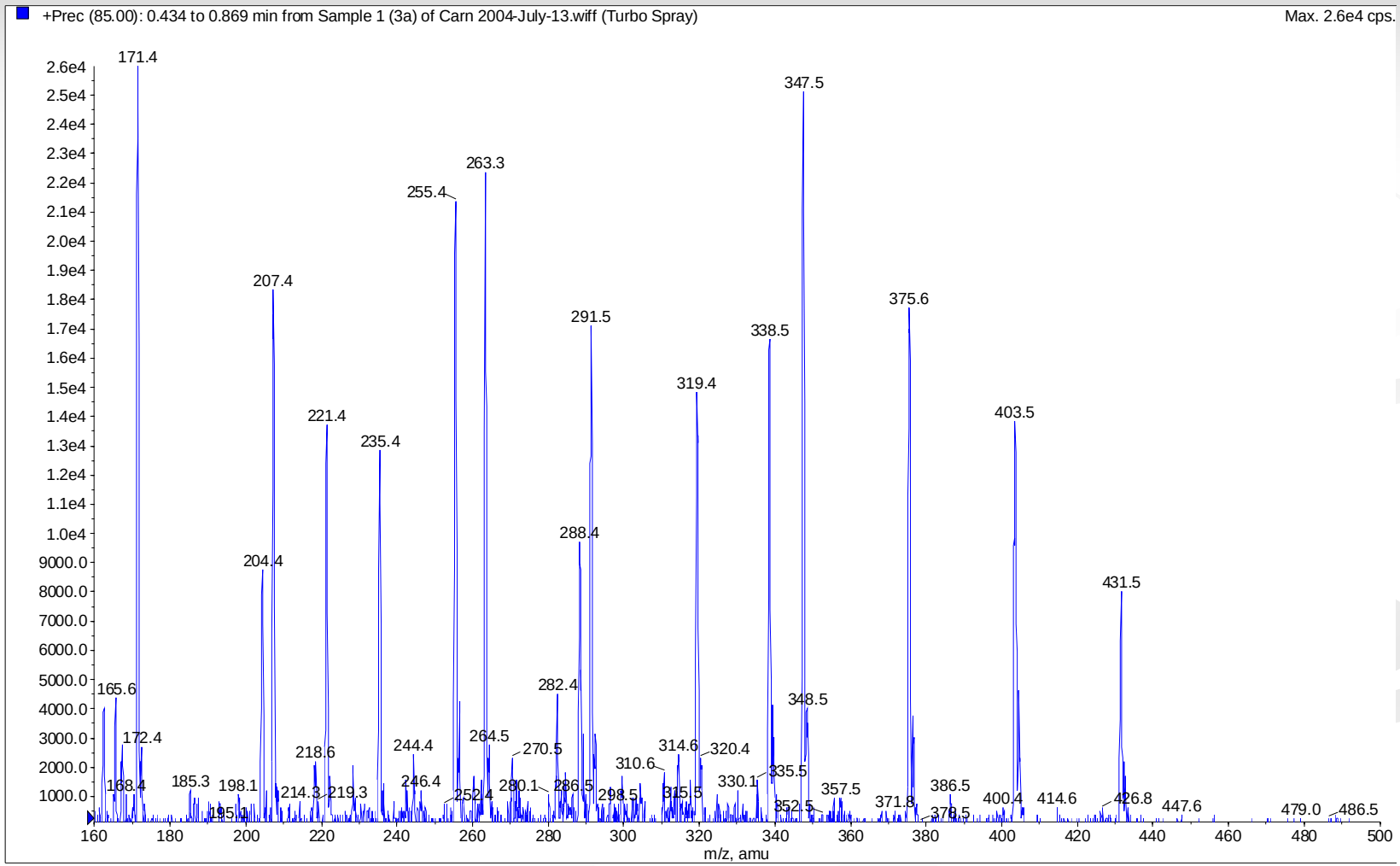
3a 3y old female, seizures and developmental delay

- 39/41 respondents noted a high excretion of octanoylcarnitine
- 27/39 respondents commented on raise C_6, C_{10}, and/or $C_{10:1}$. 24 noted a low or low normal free carnitine
- 32 labs quantitative C_8 – median 1.00µmol/l (0.51-2.26)
- 39 labs suggested medium chain acylCoA dehydrogenase deficiency as the diagnosis. 1 normal and 1 carnitine transporter defect.

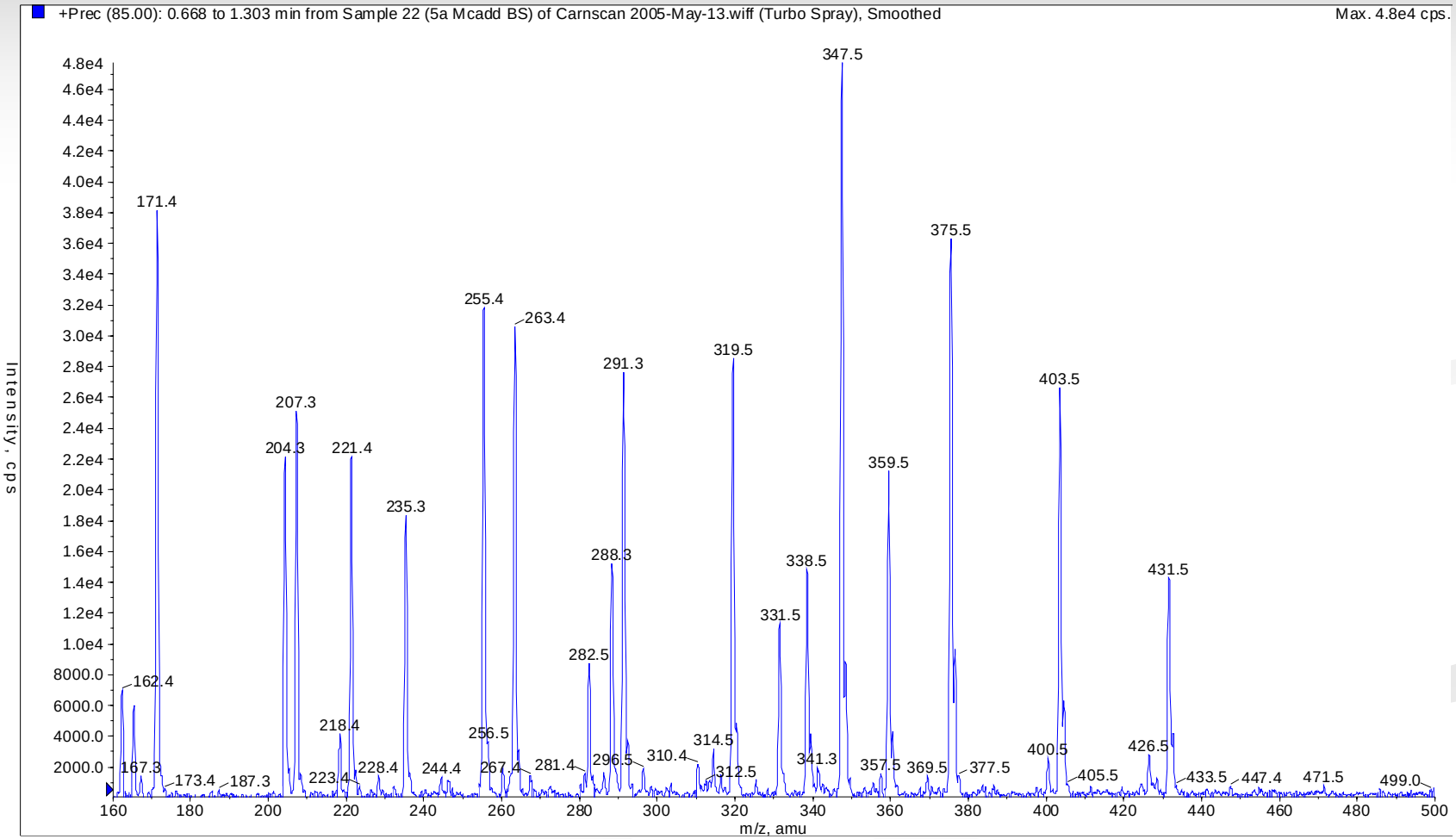
5a 3y old male, left side unresponsive to stimuli

- 53/53 respondents reported raised octanoyl carnitine and suggested MCADD as the probable diagnosis.
- 42 reported quantitative values (median 1.01, range 0.52-2.43micromol/l). 35 respondents commented upon the low free carnitine (31 gave quantitative results: median 8.4, range 4.4-32.4micromol/l),
- 24 reported a raised C10:1 carnitine and 16 a raised C6 carnitine.

Acylcarnitine analysis
ERNDIM QA scheme
Sample 3a



Acylcarnitine analysis
ERNDIM QA scheme
Sample 5a



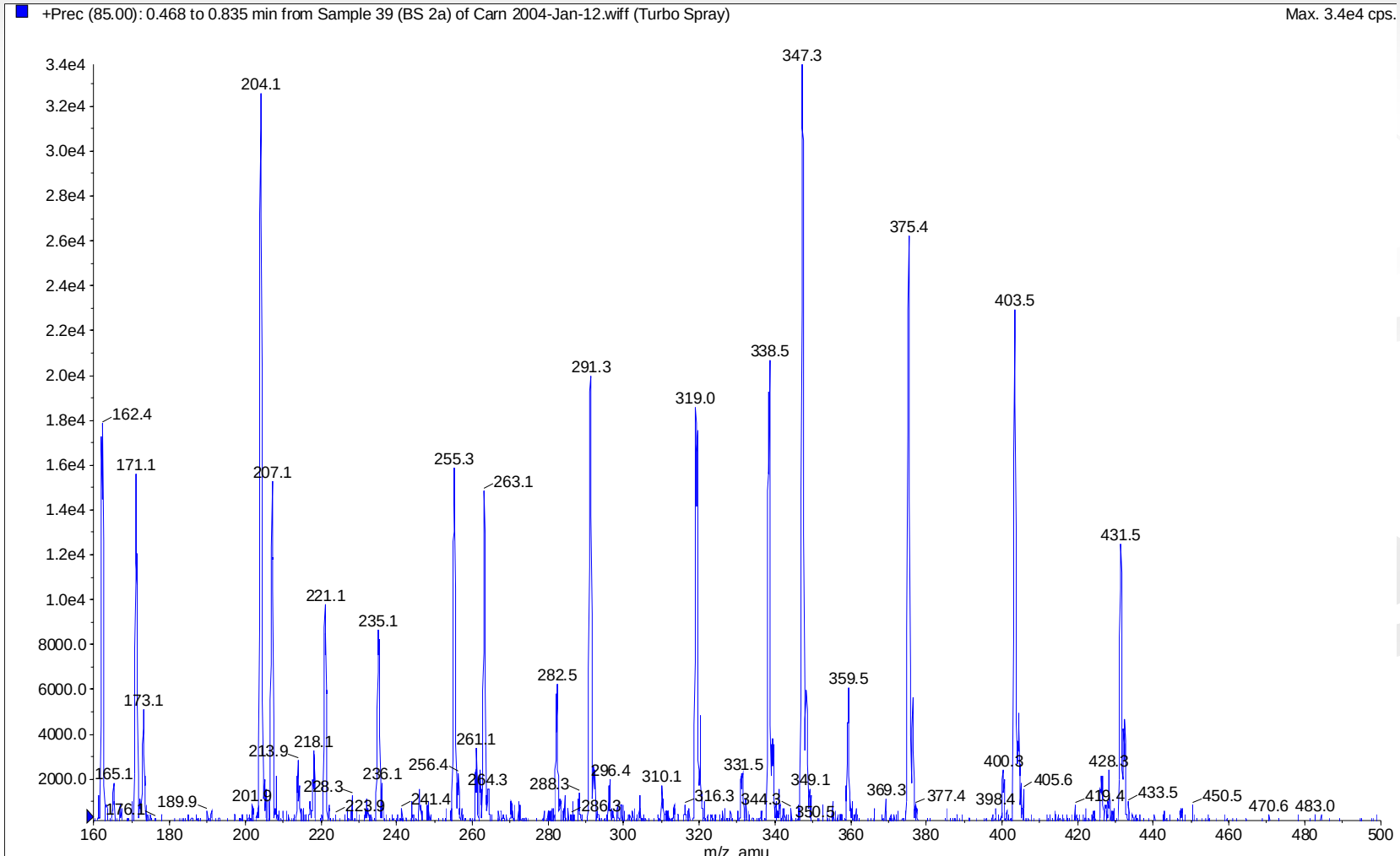
ERNDIM QA Scheme
Glutaric Aciduria Type 1
Samples 2a, 5c

2a 11month old male, collapse following intercurrent illness, large head with frontal bossing

- GlutarylCoA dehydrogenase deficiency (type 1 glutaric aciduria) – enzyme confirmed
- Bblood spot/plasma acylcarnitines consistently normal
- Urine organic acids - only once was 3OHglutarate suspicious
- 32/32 labs reported an essentially normal profile
- Only 8 respondents suggested further investigation to exclude type 1 glutaric aciduria

A normal acylcarnitine result does not exclude type 1 glutaric aciduria

Acylcarnitine analysis
ERNDIM QA scheme
Sample 2a



ERNDIM QA Scheme
Glutaric Aciduria Type 1
Samples 2a, 5c

5c 3y old male, intercurrent infection, altered consciousness, hypoglycaemia

52/53 ^ C5-dicarboxyl-carnitine and suggested a diagnosis of glutaryl-CoA-dehydrogenase deficiency.

40 quantitative results (median 1.2, range 0.39-3.1micromol/l)

34 quoted an upper limit of normal (median 0.15, range 0.03-0.5micromol/l).

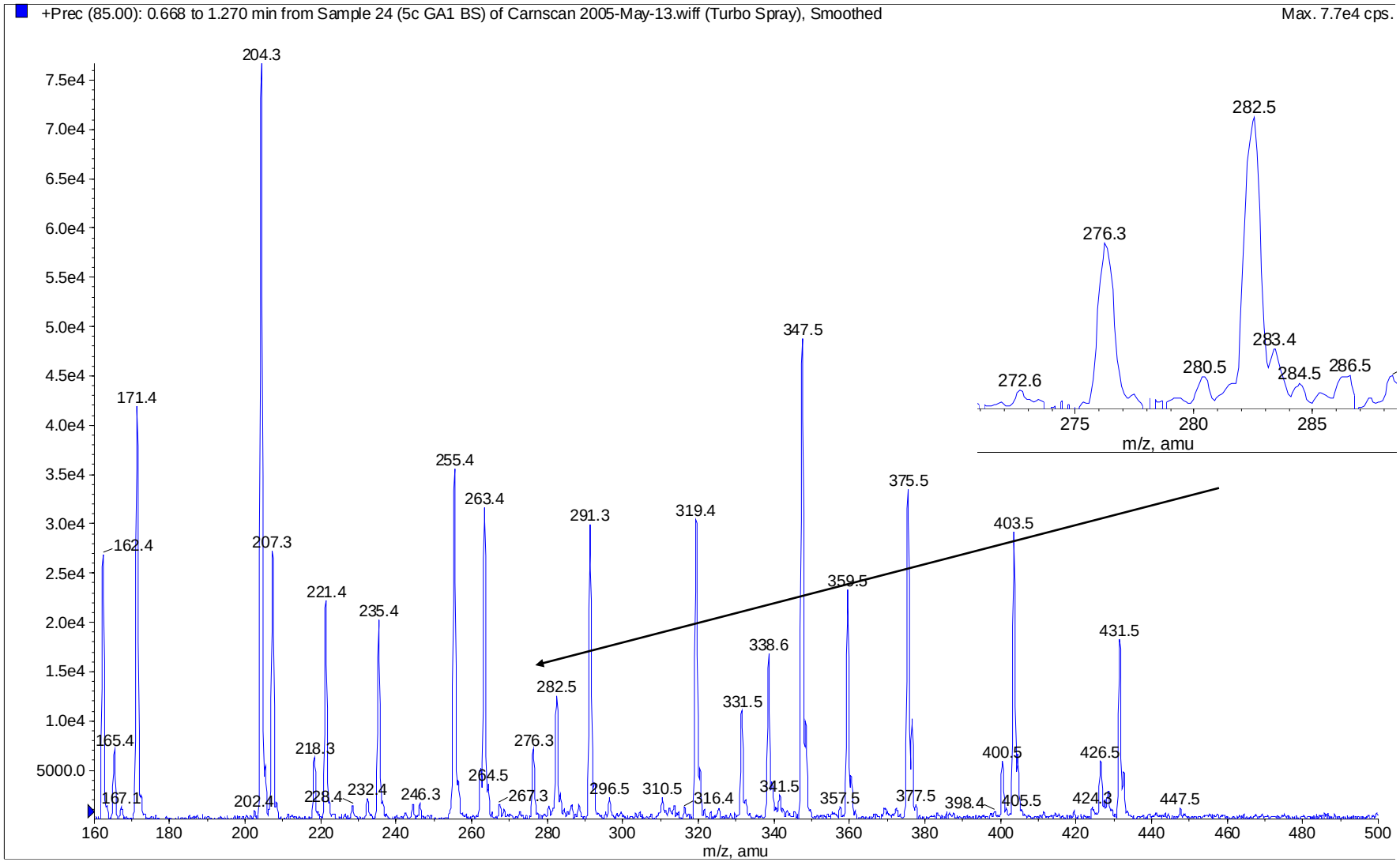
42 suggested followup tests, mainly urine organic acids & enzyme assay

17 respondents made suggestions for clinical management

1 respondent reported the sample as normal.

High awareness of GA1, unlikely to be missed if metabolite present

Acylcarnitine analysis
ERNDIM QA scheme
Sample 5c



Acylcarnitine analysis
ERNDIM QA scheme
Sample 2c

2month old female, Reye-like illness

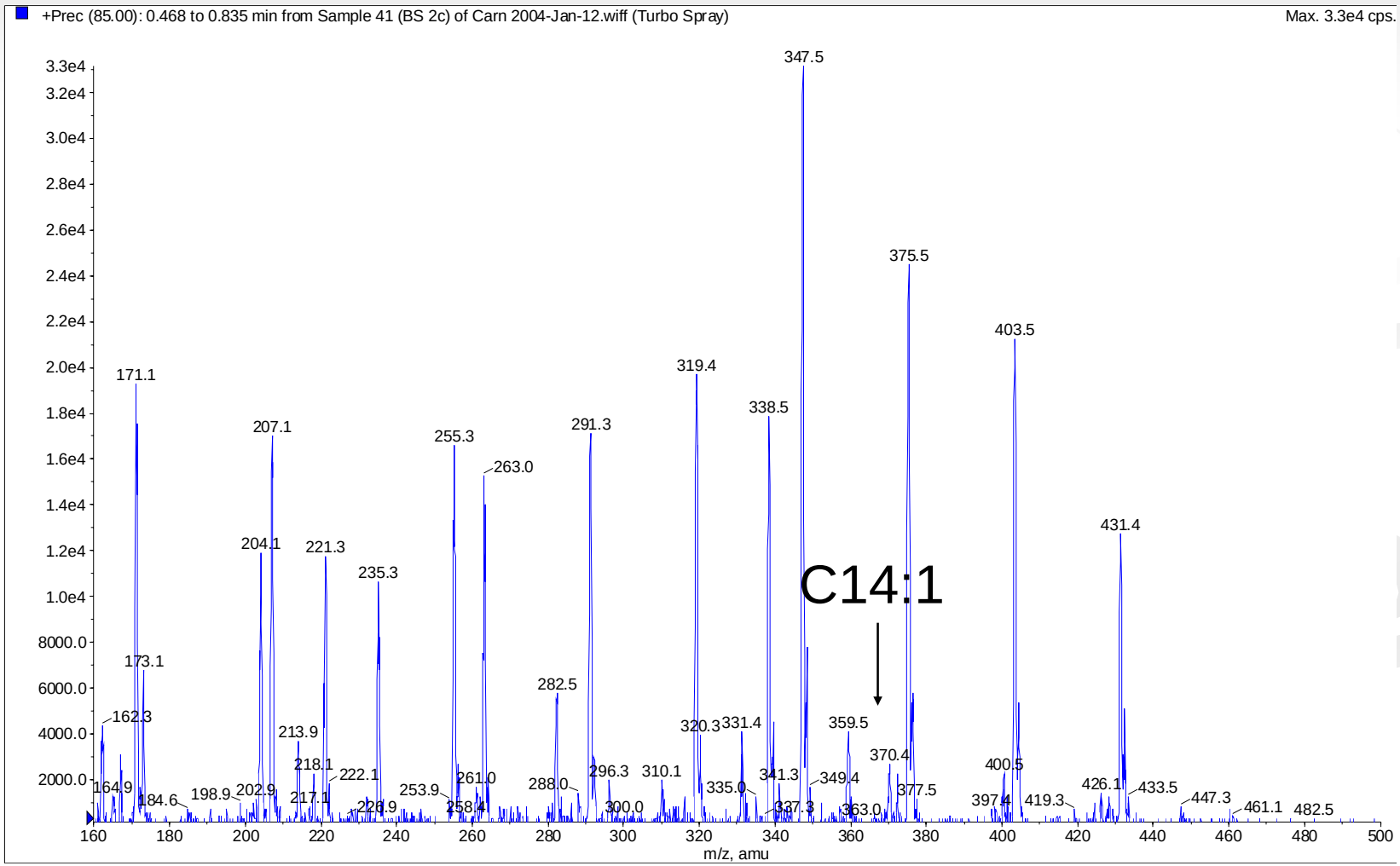
Very long chain acylCoA dehydrogenase deficiency (VLCADD) – enzyme and mutation analysis confirmed

- 16/32 respondents reported increased tetradecenoylcarnitine ($C_{14:1}$) and 12/16 provided quantitation (median 0.35µmol/l, 0.19-0.55), laboratory normal ranges, UL median 0.22, 0.07-0.55
- All 16 suggested VLCADD as the diagnosis
- 16 did not suggest VLCADD or comment on $C_{14:1}$ but 5/16 supplied scans in which peaks at m/z 370 (direct) and m/z 426 (butylated) were apparent
- 7/16 provided quantitation (median 0.27µmol/l, 0.09-0.38) – within the laboratory normal ranges, UL median 0.80, 0.54-1.07

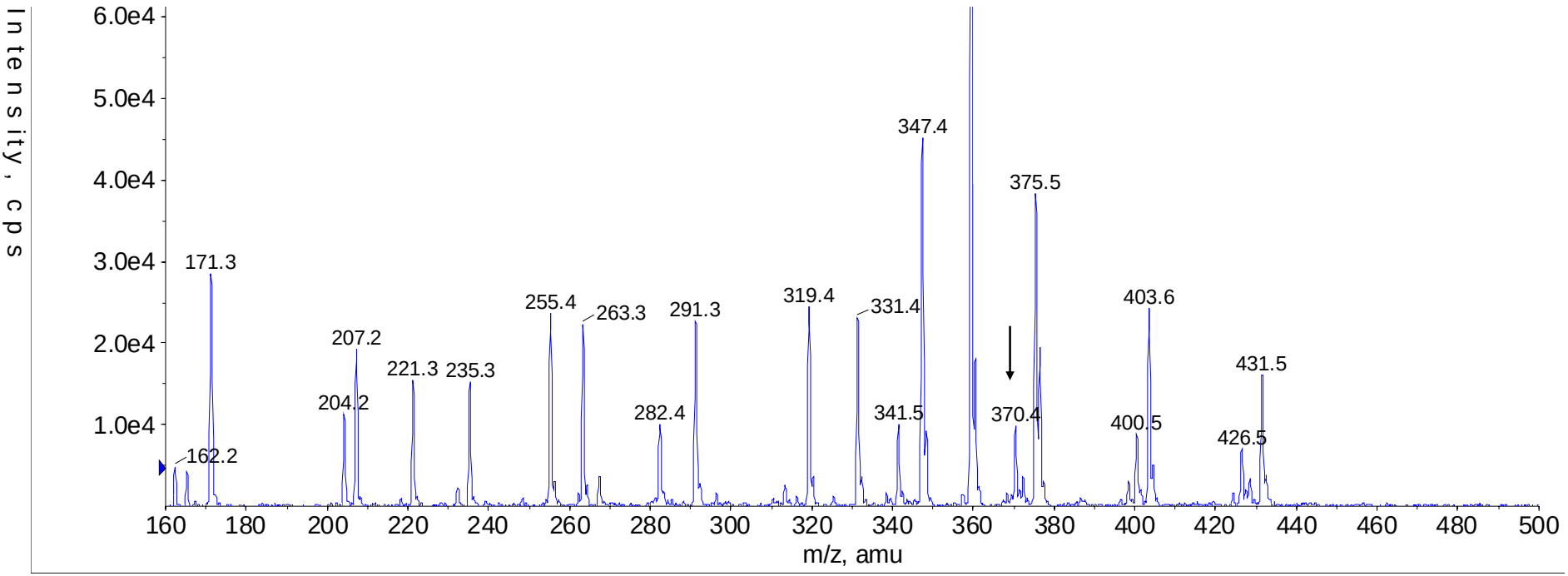
Patient in recovery phase may not show gross elevation of C14:1

Variation in normal ranges

Acylcarnitine analysis
ERNDIM QA scheme
Sample 2c



VLCADD when unwell



Acylcarnitine analysis
ERNDIM QA scheme
Sample 4b

6month old female, currently well. History of acute collapse at 4 days of age with apnoea, profound metabolic acidosis, hypoglycaemia, left ventricular hypertrophy

MalonylCoA decarboxylase deficiency

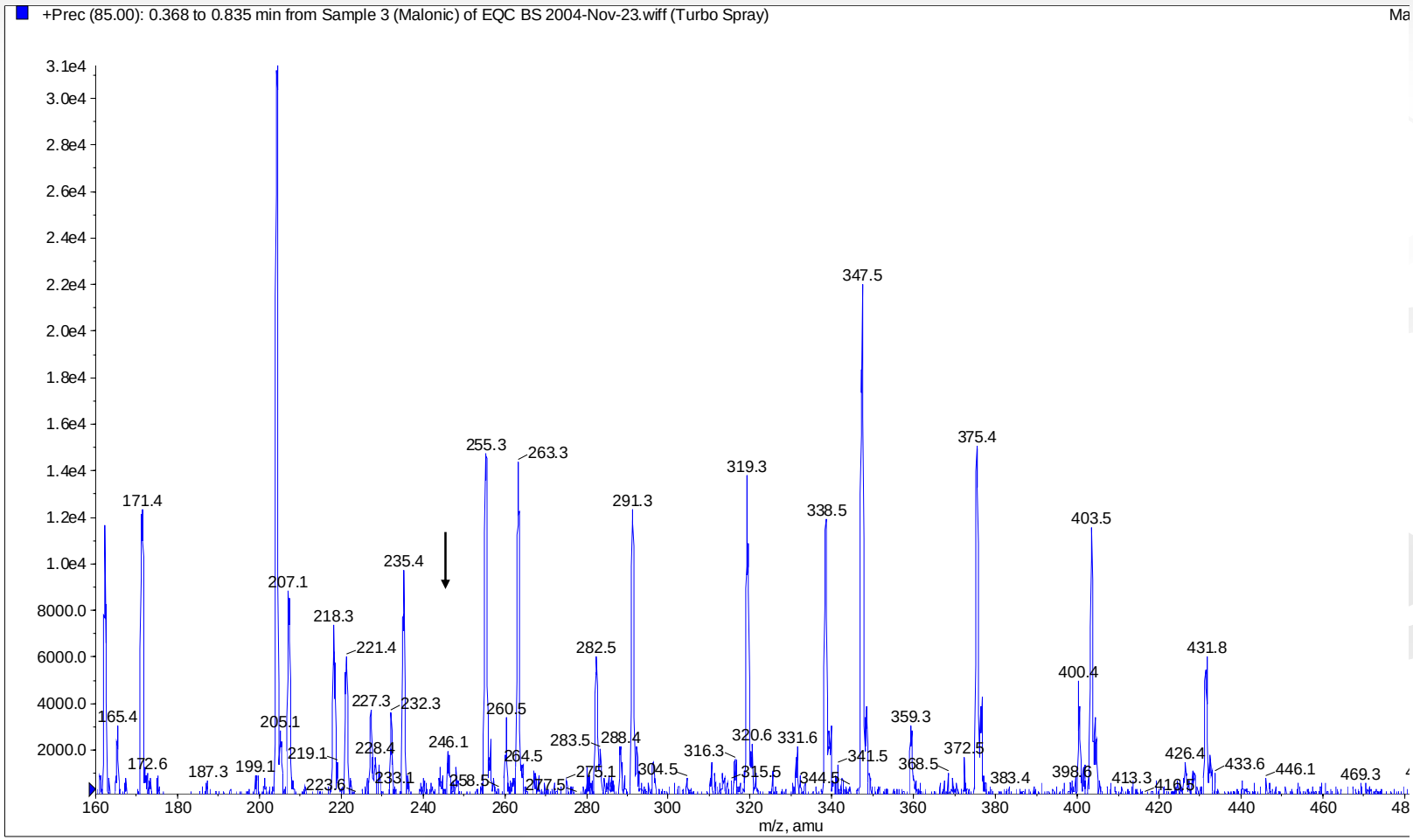
Increased malonylcarnitine

Acylcarnitine analysis
ERNDIM QA scheme
Sample 4b

- 20/42 malonic aciduria (malonyl CoA carboxylase deficiency)
  - ^C3DC, m/z 248 underivatised, m/z 360 butylated).
  - 15 quantitative values (median 1.1, range 0.35-2.2micromol/l),
  - 12 gave reference ranges (upper limit of normal: median 0.11, range 0.05-0.8micromol/l).

- 22 respondents did not suggest malonic aciduria
  - 11 supplied spectra of butylated sample in which the peak at m/z 360 was clearly visible.
  - 3 supplied MRM acquisition data which did not include malonyl carnitine.
  - 4 described the sample as normal

Acylcarnitine analysis
ERNDIM QA scheme
Sample 4b



Other Diagnoses

- Propionic acidaemia
- Isovalericacidaemia
- Carnitine transporter defect

Clear acylcarnitine profiles

Majority of laboratories made diagnoses

Summary of ERNDIM QA results

- Normal
  - Most labs report normal scans
  - Demonstrates difficulties of ruling out metabolic disease with single test
- MCADD, IVA, Propionic, Carnitine transporter
  - Clear diagnoses, no difficulty for most labs
- Methylmalonic
  - All labs pick up raised C3, C4DC less easy

Summary of ERNDIM QA results

- Malonic
  - Rare disorder not recognised by some
  - Some labs using MRM acquisition only did not include malonyl carnitine in panel
- VLCADD
  - Minor elevation of C14:1 in treated patients
  - Issues with normal ranges
- GA1
  - Some patients do not generate metabolites
  - When C5DC present well detected
    - No difference between butylators & non-butylators

Acylcarnitine analysis
Future perspectives

Technology

Greater sensitivity

Routine product ion scanning

Consolidation not revolution

Acylcarnitine analysis
Future perspectives

Use of electrospray MSMS more effectively and efficiently for diagnosis

Clinically targeted

Hyperammonaemia

Hypoglycaemia

Storage disorders

Liver failure

etc

Conclusions

- Acylcarnitine analysis
  - integral part of acute biochemical genetics laboratory investigation
- Butylation vs non-butylation
  - No clear diagnostic advantages either way
    - Underivatised simpler and faster for acute
    - More robust for population screening
- ERNDIM QA scheme
  - Real samples allow labs to build up experience of clinical variation
  - Useful forum to pool knowledge and share insight

Acknowledgements

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The patients and their families, other labs who have supplied samples

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Guy's & St Thomas' NHS Trust

Acylcarnitine analysis
ERNDIM QA scheme
Normal Samples

1a 49y old male, fatigue & muscle cramps: normal

- 34/36 labs reported normal, 27 labs suggested no further action

3b 50y old male, mild peripheral neuropathy, increased CK

Otherwise healthy subject with Charcot-Marie Tooth syndrome

- 31/41 respondents considered the profile normal
- 9/41 commented on elevated long chain acylcarnitines – 5 concluding probably normal, with 2 offering other tests.
- 2 LCHAD, 1 CPT-2 and 1 VLCADD, 1 ^ C3 & C4DC - MMA

4c 16y male, vacant episodes: no known metabolic disease

- 38/42 normal, 2 MADD, 1^ C3, 1 low carnitine

5b 31y old female, episodes of hypoglycaemia

- 44/53 normal, 7 mentioned LCFAO disorders not excluded if well
- 1 respondent suggested paired plasma insulin and glucose.
- 5 ^ C5OH carnitine 1^ C3 and 1 reduced C12 and C16 carnitines, both suggested repeating acylcarnitine analysis on plasma.