A rough guide to Acylcarnitines

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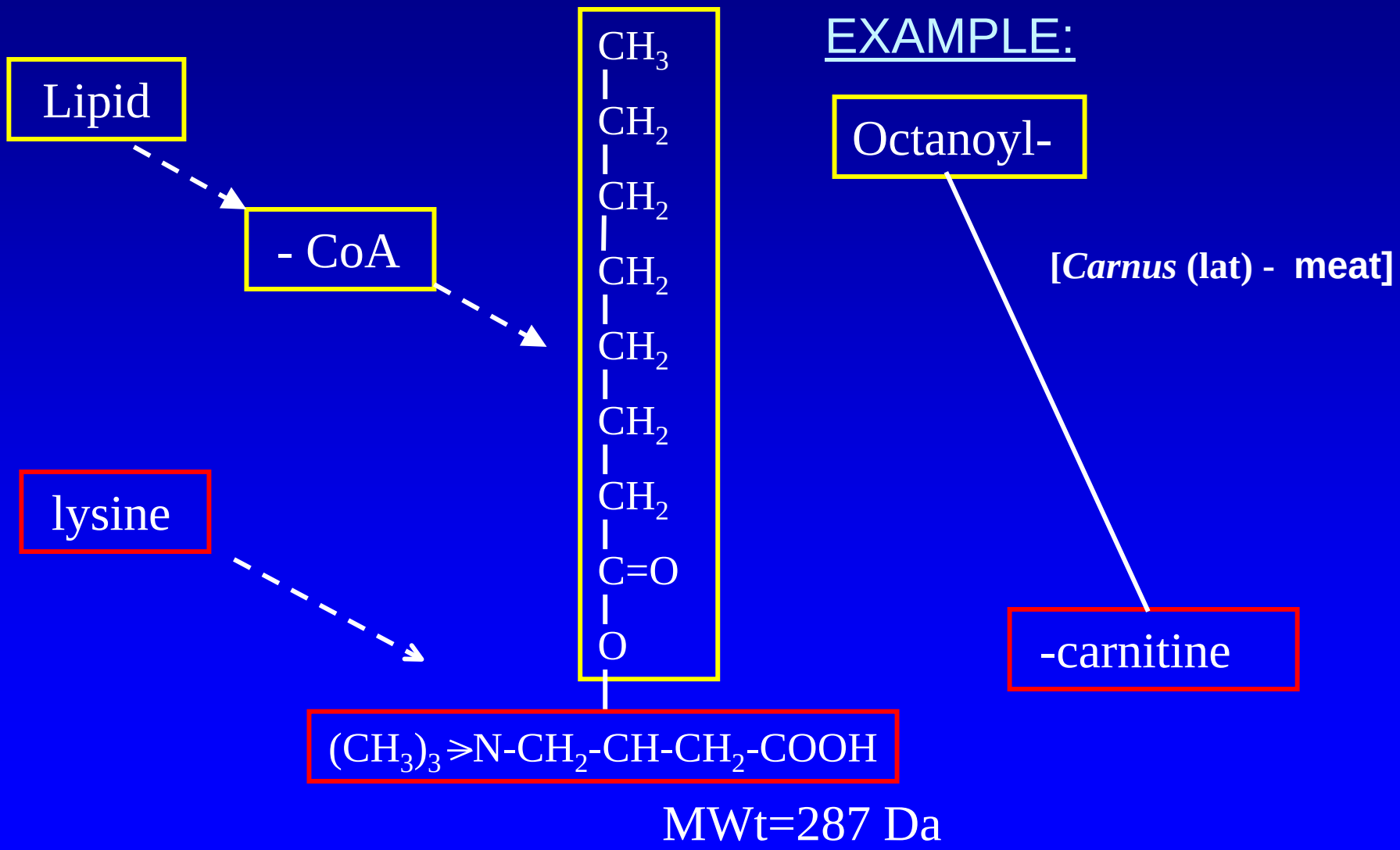
Menu

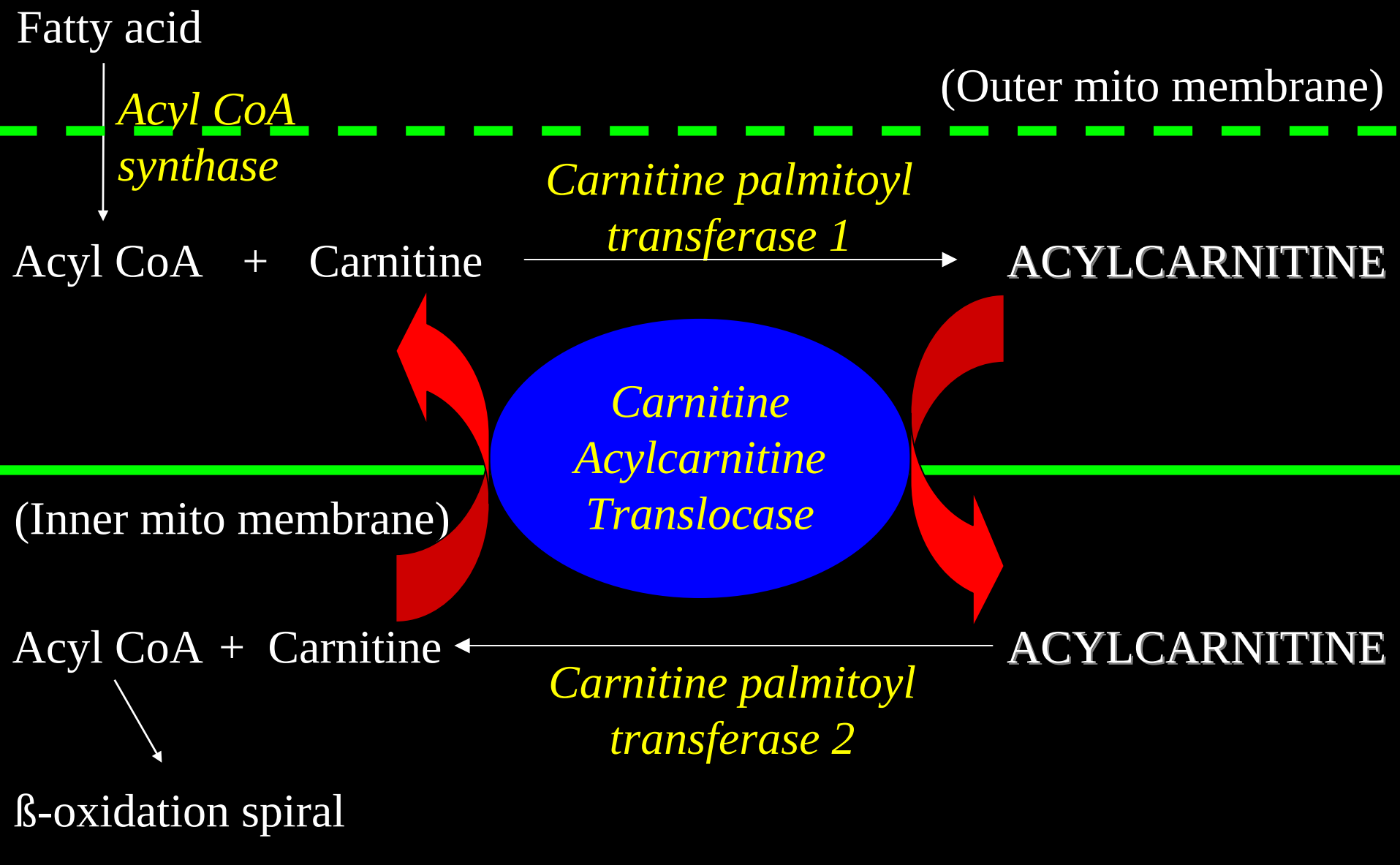
What are acylcarnitines?

- Fatty acyl ester of L-carnitine
- Facilitate entry of long-chain fatty acids (LC-FA) into the mitochondrion via the Carnitine Shuttle
  - LC-FA's act as important fuels for many tissues (e.g. skeletal & cardiac muscle) via ß-oxidation
- In fatty-acid oxidation defects, acylcarnitine species accumulate and are released into the circulation
  - pattern of acylcarnitine species can be diagnostic for a number of ß-oxidation defects

What are Acylcarnitines?



Carnitine shuttle

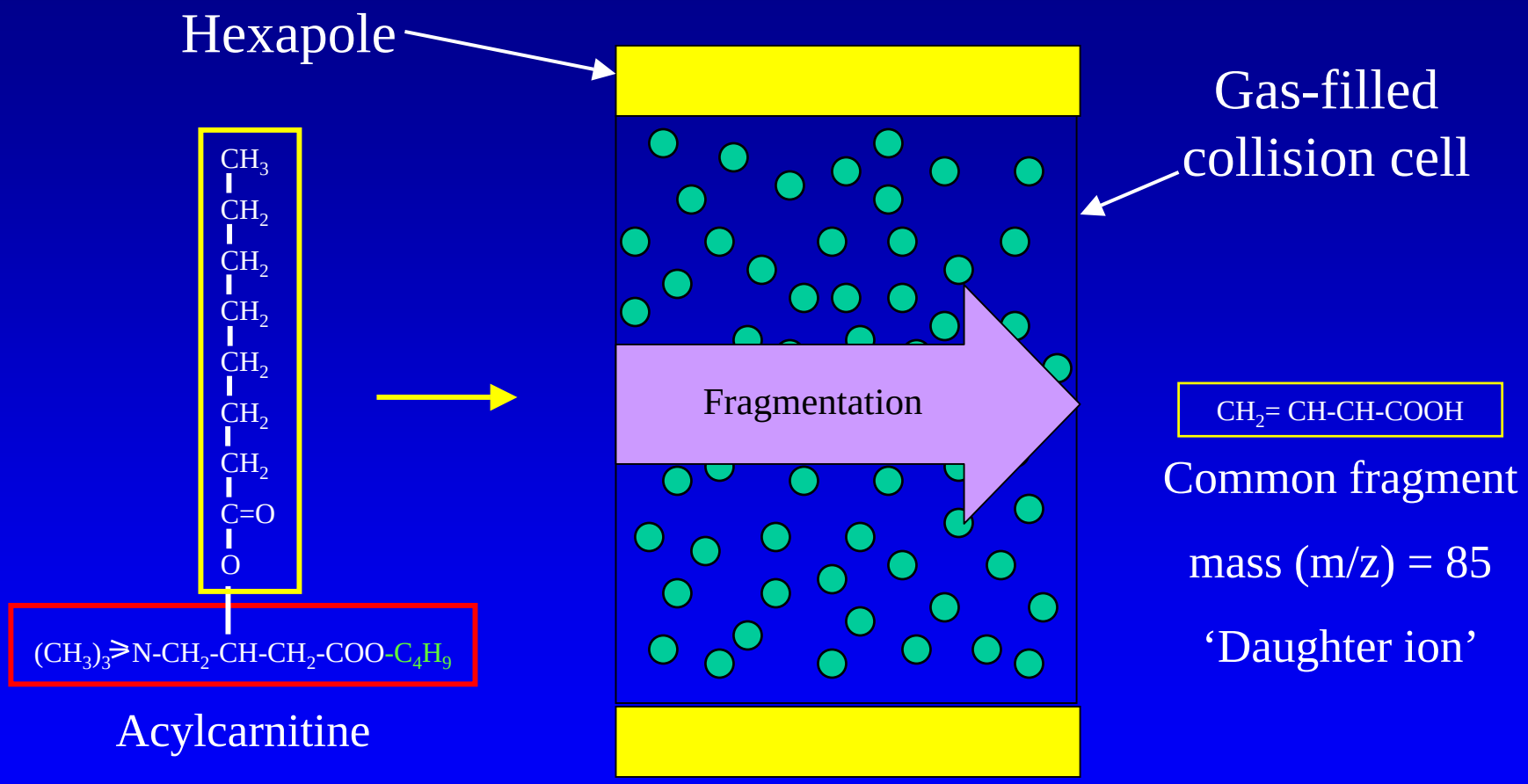


Acylcarnitine analysis

- Early acylcarnitine detection methods were GC-MS based
  - time consuming
  - required laborious sample preparation
- Introduction of Tandem-MS eliminated need for chromatographic separation
  - lowered analysis time
  - increased throughput
  - possible screening tool

- Method replies on fragmentation of acylcarnitine within the Tandem MS forming a common fragment with a mass of *m/z*=85 (daughter ion)
- Scanning parent ions with a daughter ion m/z 85 can predict the acylcarnitine species present → identification

Formation of m/z 85 'daughter ion'



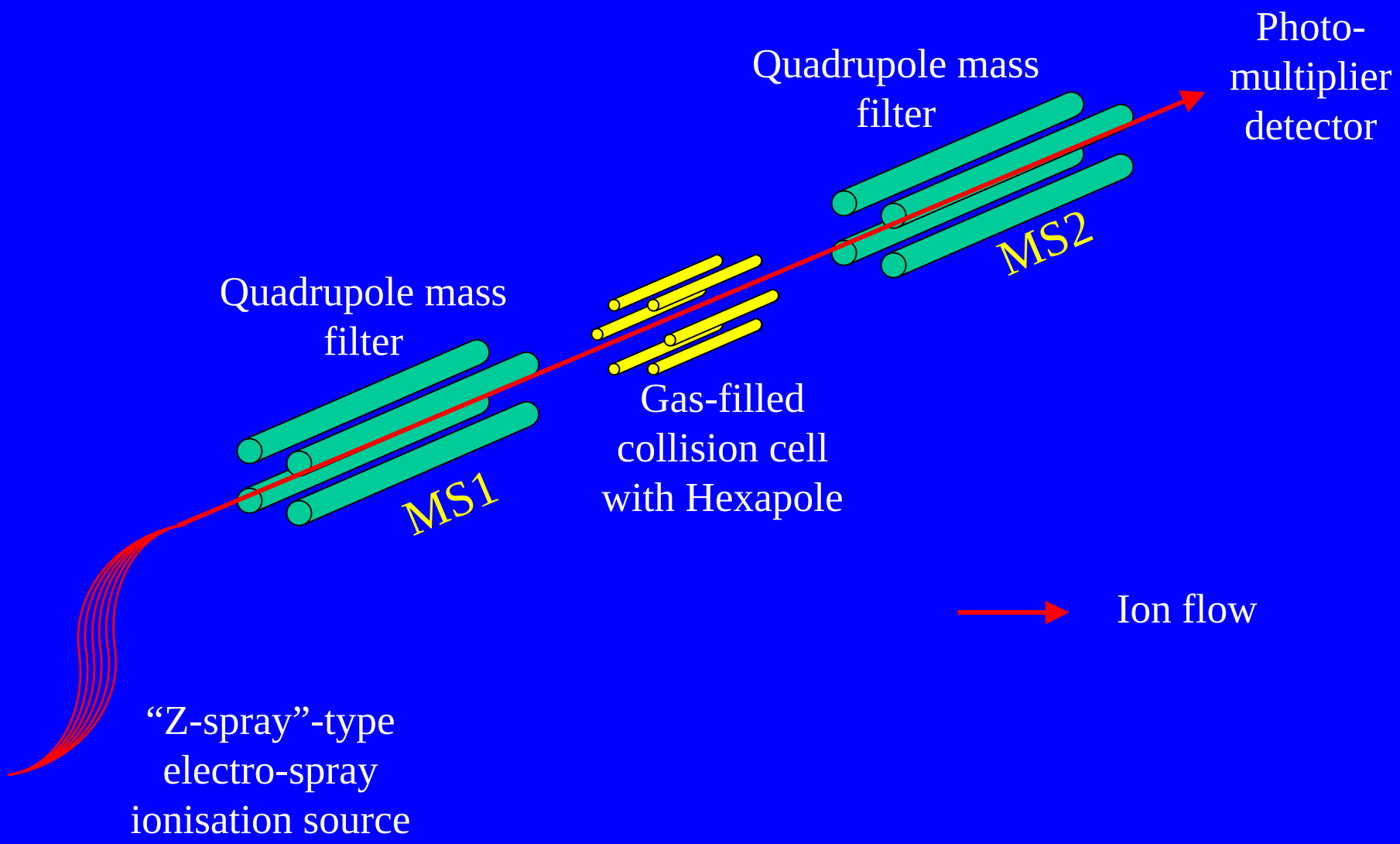
Profiling by Tandem-MS

- Electrospray Tandem-MS (also termed ESI- MS/MS or LC-MS/MS) = Electrospray ionisation (with or without Liquid chromatographic separation) with Tandem Mass Spectrometric detection
- Stages in Tandem-MS/MS:
  1. ESI = Electrospray ionisation → molecular ions (positive or negatively charged ions)
  2. Separation by quadrupole mass-spectrometer → mass filter allows only ions of only 1 mass/charge ratio (m/z) [termed '**Parent ions**'] to pass through at any one time

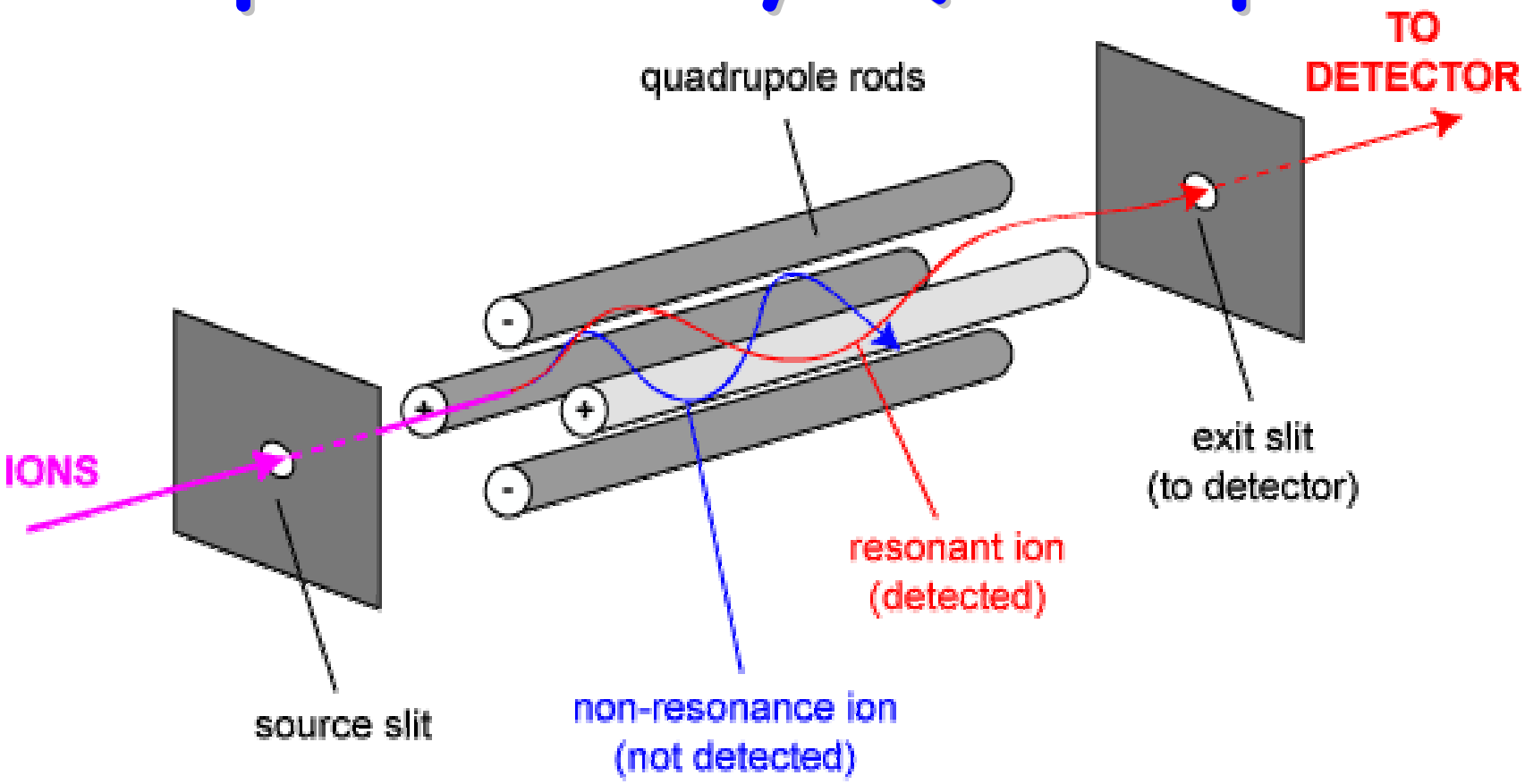
Profiling by Tandem-MS

3. Fragmentation of Parent ion within an inert gas (e.g. argon) containing collision cell situated between the 2 quadrupole mass filters
4. Separation by second quadrupole mass filter (allows only ions of only 1 m/z [termed '**Daughter ions**'])
5. Electron- or photo-multiplier detection → identification and/or quantitation by stable isotope dilution

Schematic of Tandem MS



Separation by Quadrupole



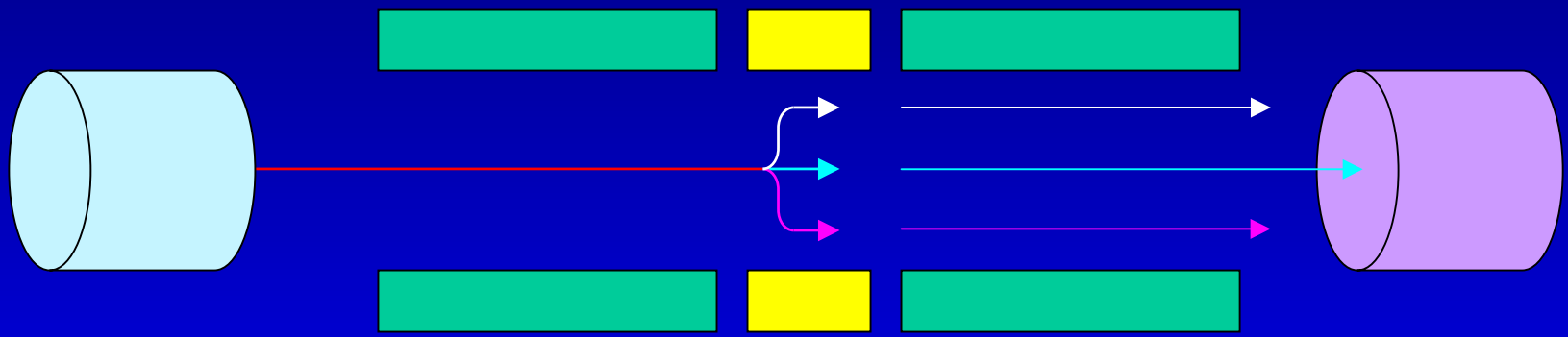
- Fluctuating charges on quadrupole rods, under control of radio frequency generator and direct current supply
- Ions effectively spiral in 3 dimensions along entire quadrupole length

Tandem-MS modes

(Shown diagrammatically on subsequent slides)

- Daughter ion spectrum
  - mainly assay development
- Parent ion spectrum
  - used for Acylcarnitine analysis
- Neutral loss spectrum
  - used for amino acid analysis
- Multiple reaction monitoring (MRM)
  - used for quantitation eg Phe & Tyr, octanoylcarnitine for MCADD (newborn screening)

Daughter ion spectrum



Ion source

MS1

(Static at set m/z)

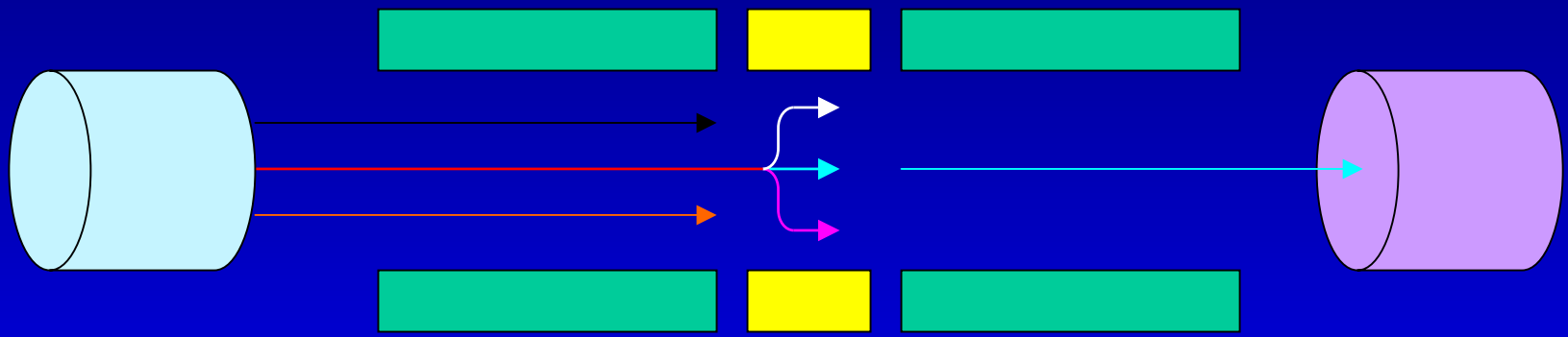
Collision cell

MS2

(Scan across mass range)

Detector

Parent ion spectrum



Ion source

MS1

(Scan across mass range)

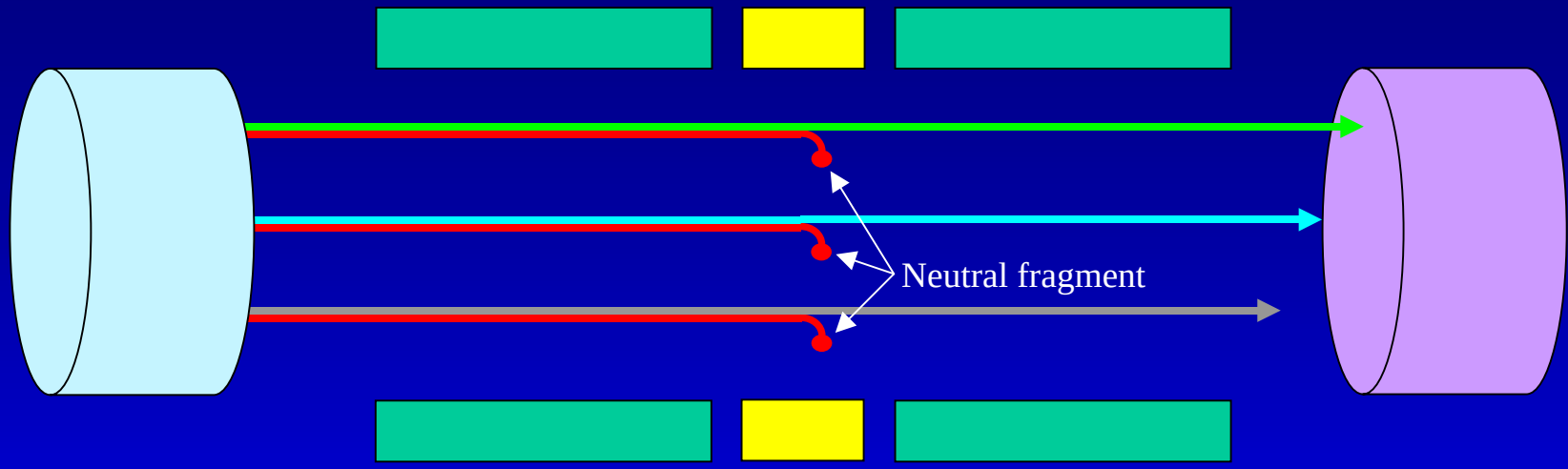
Collision cell

MS2

(Static at set m/z)

Detector

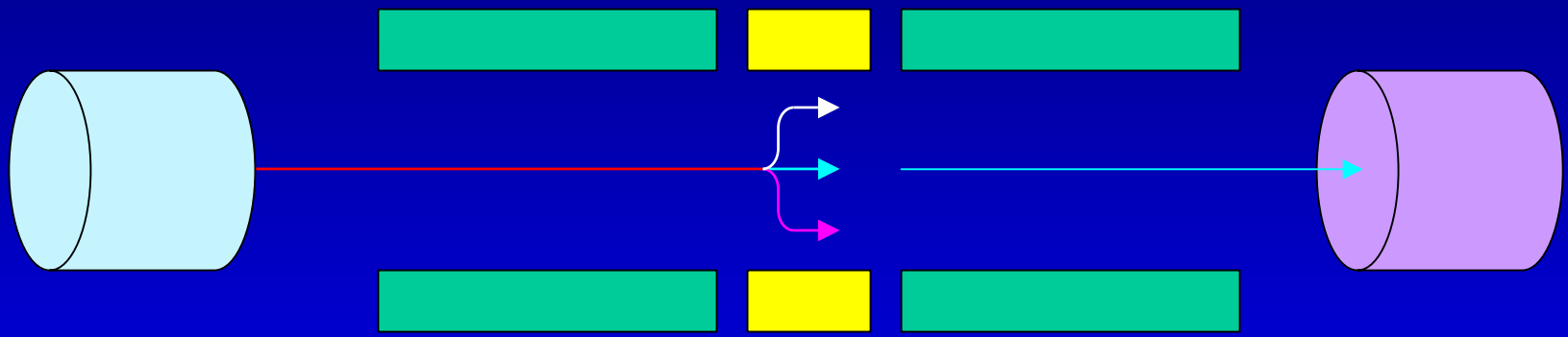
Neutral loss spectrum



Ion source | MS1 (Scan across mass range) | Collision cell | MS2 (Scan across mass range synchronised with MS1 at offset equal to mass of neutral fragment) | Detector

- As the neutral fragment (●) carries no charge, it does not travel through MS2

Multiple reaction monitoring (MRM)



Ion source

MS1

(Static at set m/z)

Collision cell

MS2

(Static at m/z different set-point to MS1)

Detector

(Similar in concept to Single Ion Monitoring (SIM) in GC-MS)

Plasma / DBS sample preparation for acylcarnitine analysis

3mm blood spot

10µl plasma

Add Stable Isotope Internal Standards in methanol

1 hour elution

‘protein crash’

(Derivatised)

Dry

Butylate

Dry

Add CH_3CN

(Underivatised)

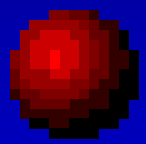
(Underivatised)

(Fast track!)

Direct flow injection ESI -Tandem MS (MS/MS)

Plasma/DBS sample preparation timings
(from receipt of sample to injection)

3mm blood spot



(Derivatised method) → ~90 mins

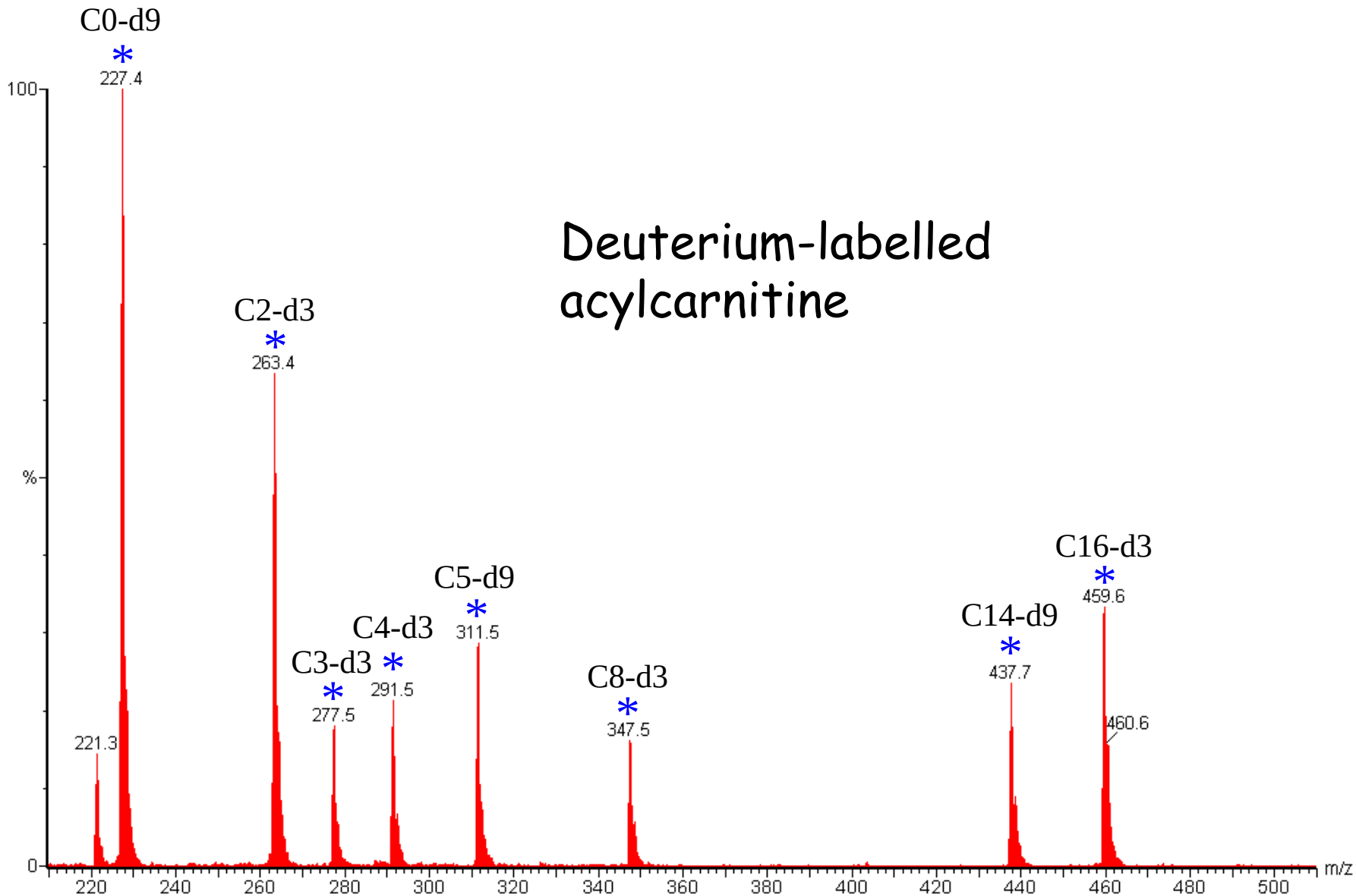
(Underivatised method) → ~60 mins

10µl plasma

(Derivatised method) → ~30 mins

(Underivatised method) → ~5 mins

Internal Standards



Deuterium-labelled acylcarnitine

Short-chain acyl-CoA dehydrogenase deficiency (SCADD)

- Rare & poorly understood
- Autosomal recessive inheritance
- Defect is reduced level of mitochondrial flavo-enzyme (catalyses initial reaction in short-chain ß-oxidation)
- Unlike 'classical' disorders of fatty acid oxidation, does not present with hypoketotic hypoglycaemia

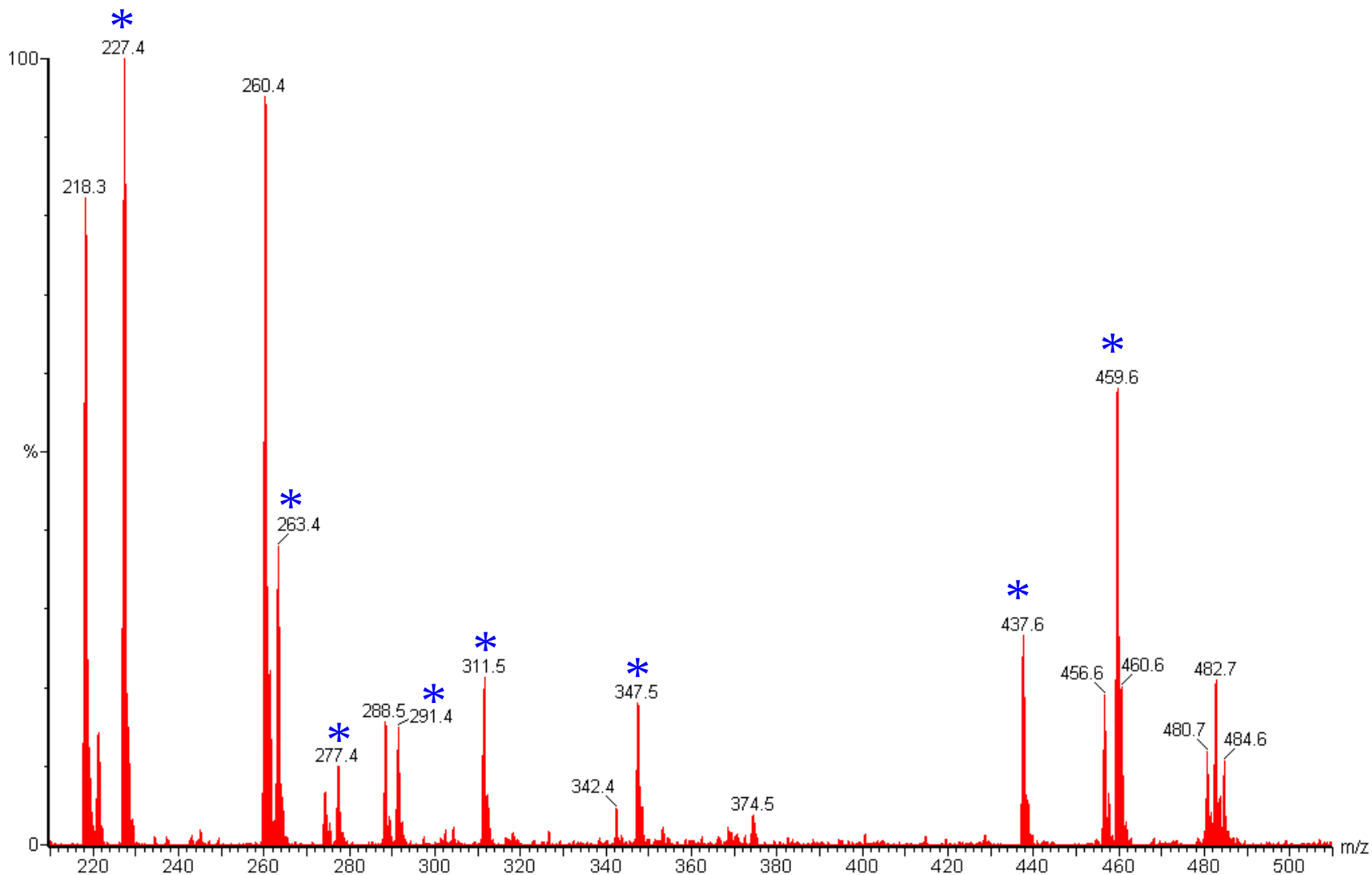
SCADD

- Varied presentation in neonatal period:
  - metabolic acidosis
  - hypotonia
  - developmental delay
  - seizures
  - myopathy
- Severe cases:
  - encephalopathy
  - hypoglycaemia
  - hepatic disease

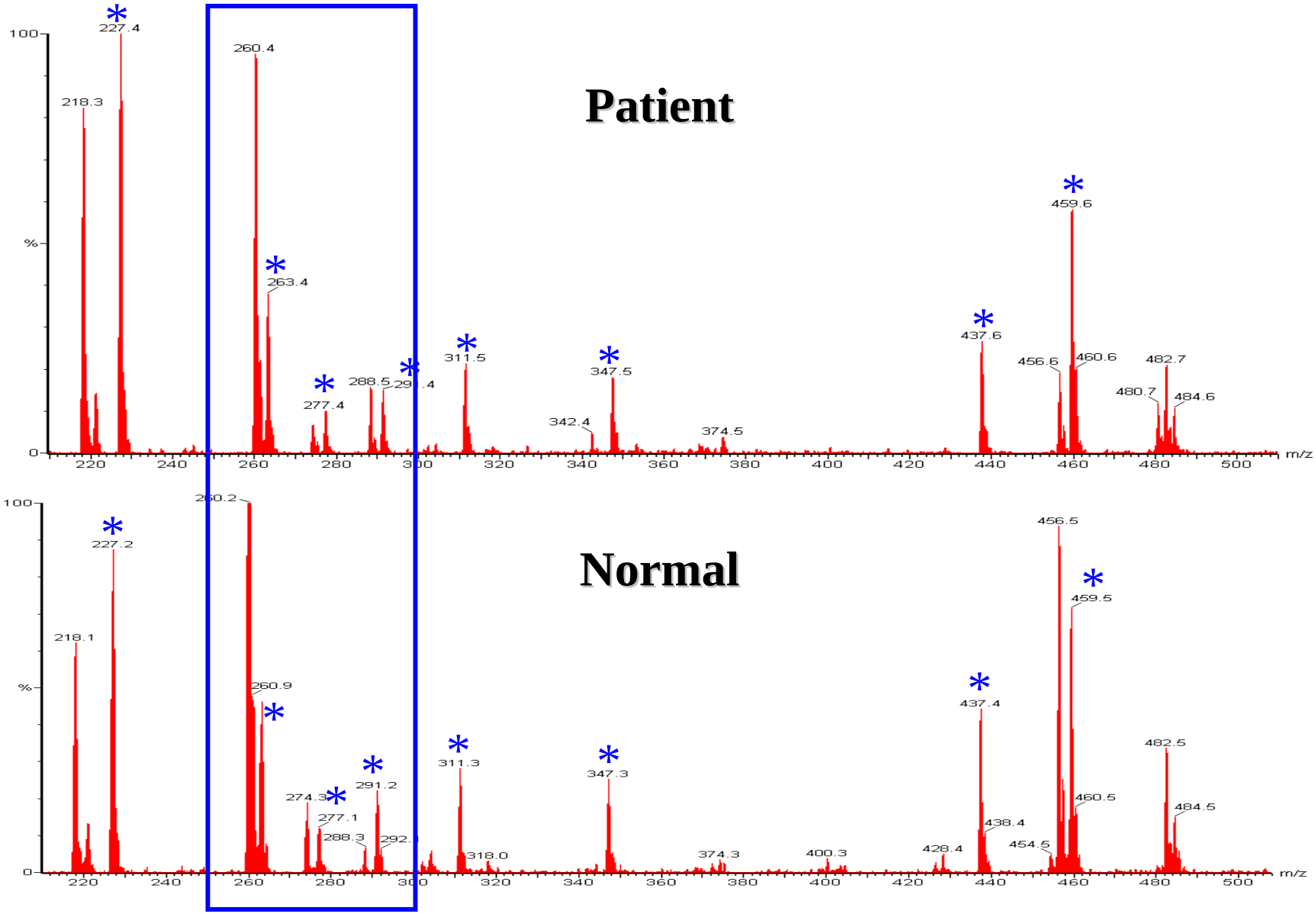
SCADD

- Urine organic acids:
  - ethymalonate (nb also seen I patients with ethylmalonic aciduria & GA-2)
  - methylsuccinate
  - butyrylglycine
- Acyl-carnititne profile:
  - elevated C4 (butyrylcarnitine)
- Treatment:
  - dietary fat restriction
  - carnitine supplementation
  - riboflavin supplements (in some patients)

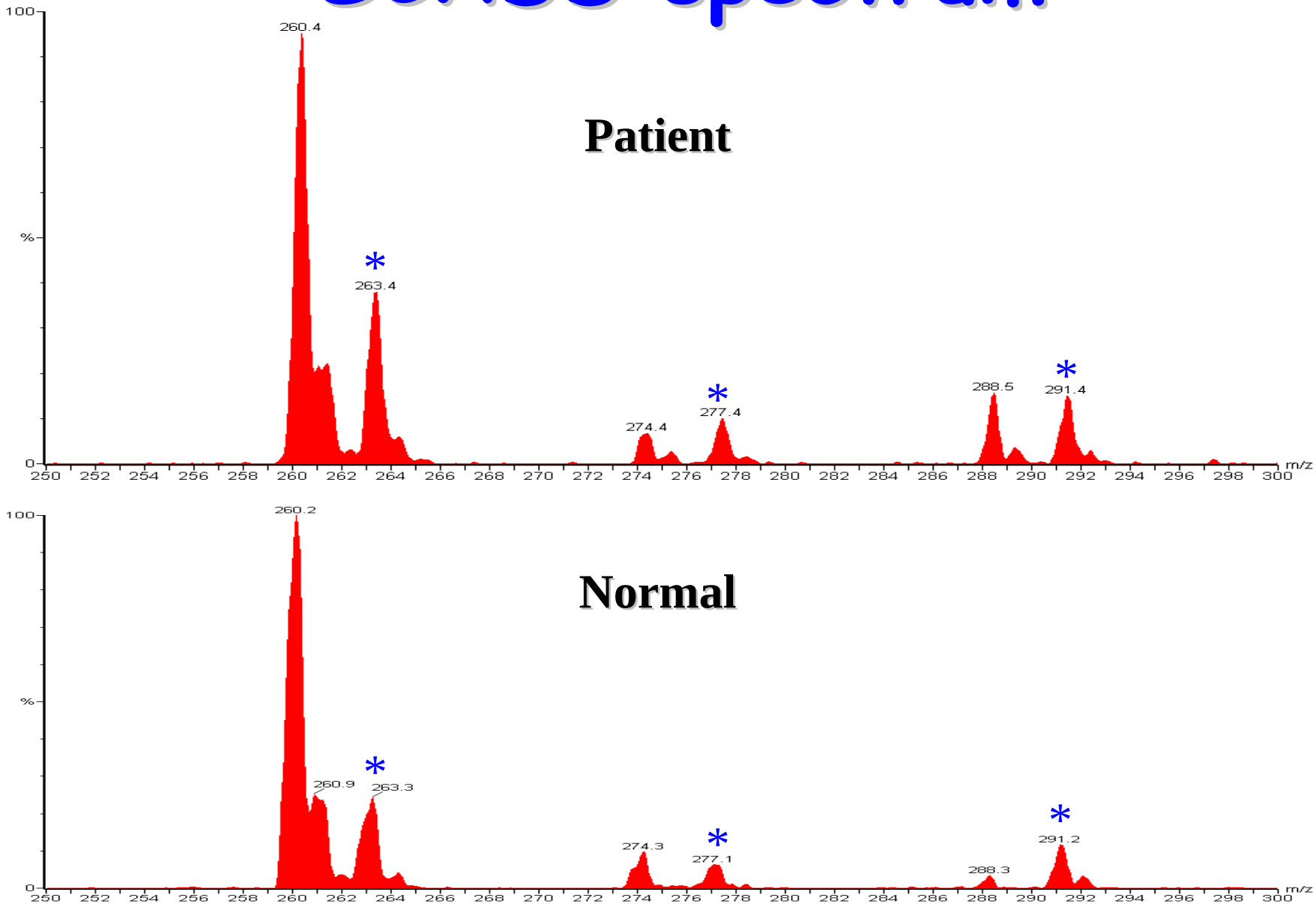
SCADD spectrum



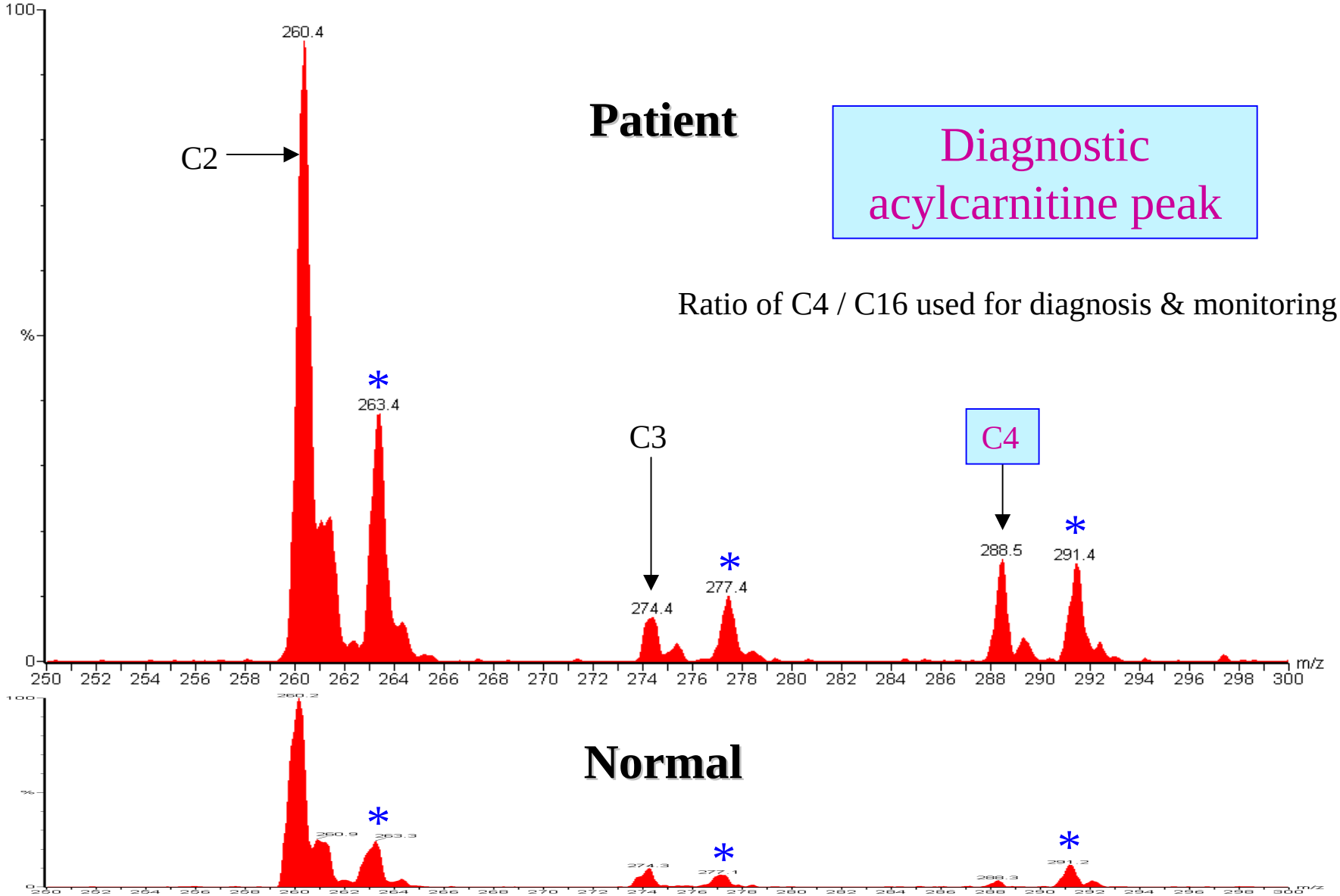
SCADD spectrum



SCADD spectrum



SCADD



Diagnostic acylcarnitine peak

Ratio of C4 / C16 used for diagnosis & monitoring

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)

- Commonest fatty-acid oxidation defect
- Autosomal recessive inheritance
- Incidence 1 in 10,000-20,000 births (depending on population)
- First crisis is fatal in 20-25% of cases
- Mean age of presentation is 12 months
- ~85% of cases are due to the mutation K304E
- Presentation often follows periods of intercurrent illness or vomiting

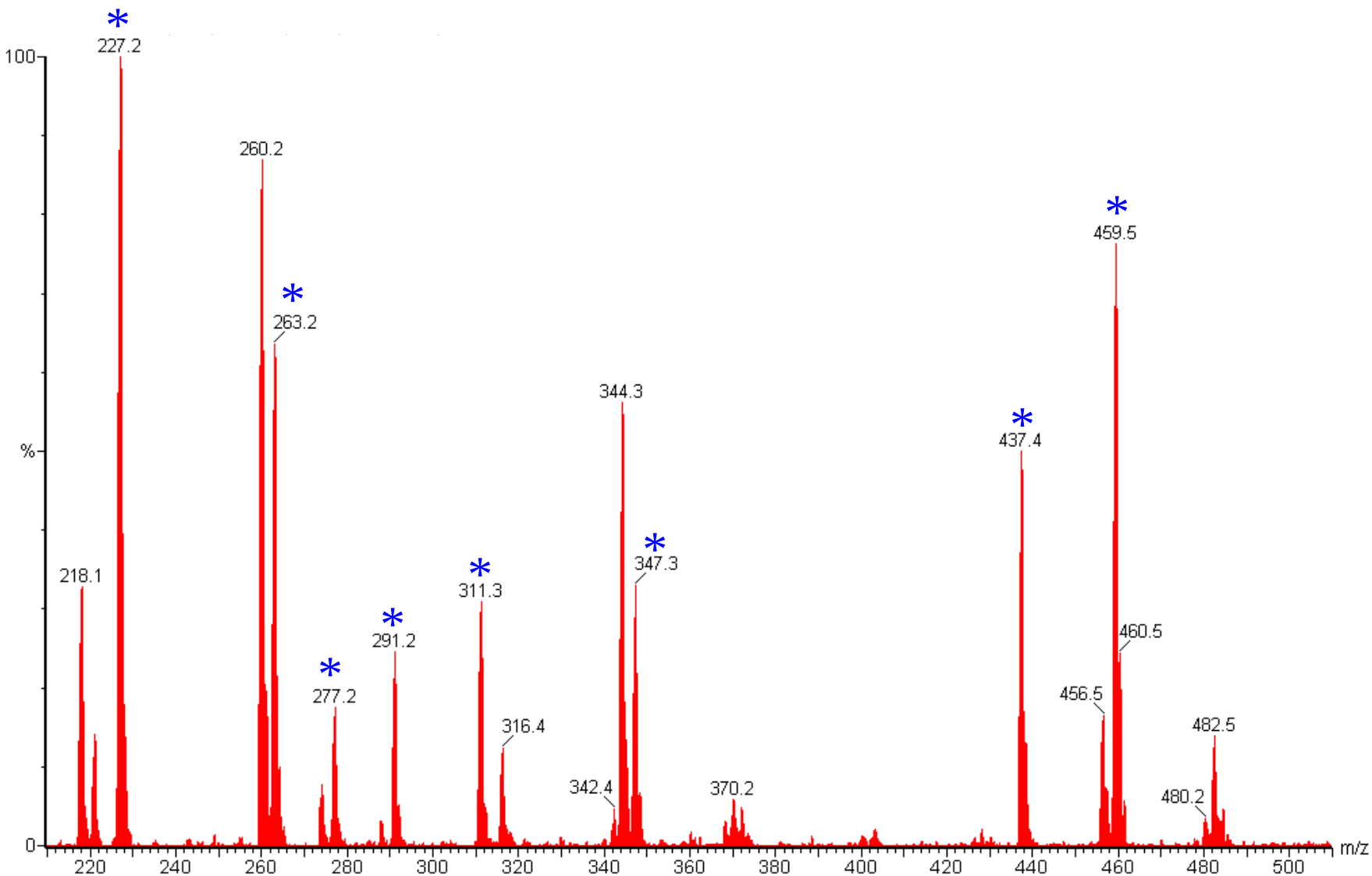
MCADD

- Presentation (episodic):
  - hypoketotic hypoglycaemia
  - myopathy or cardiomyopathy
  - hyperammonaemia
  - hypotonia
  - lethargy
  - encephalopathy
  - hepatomegaly

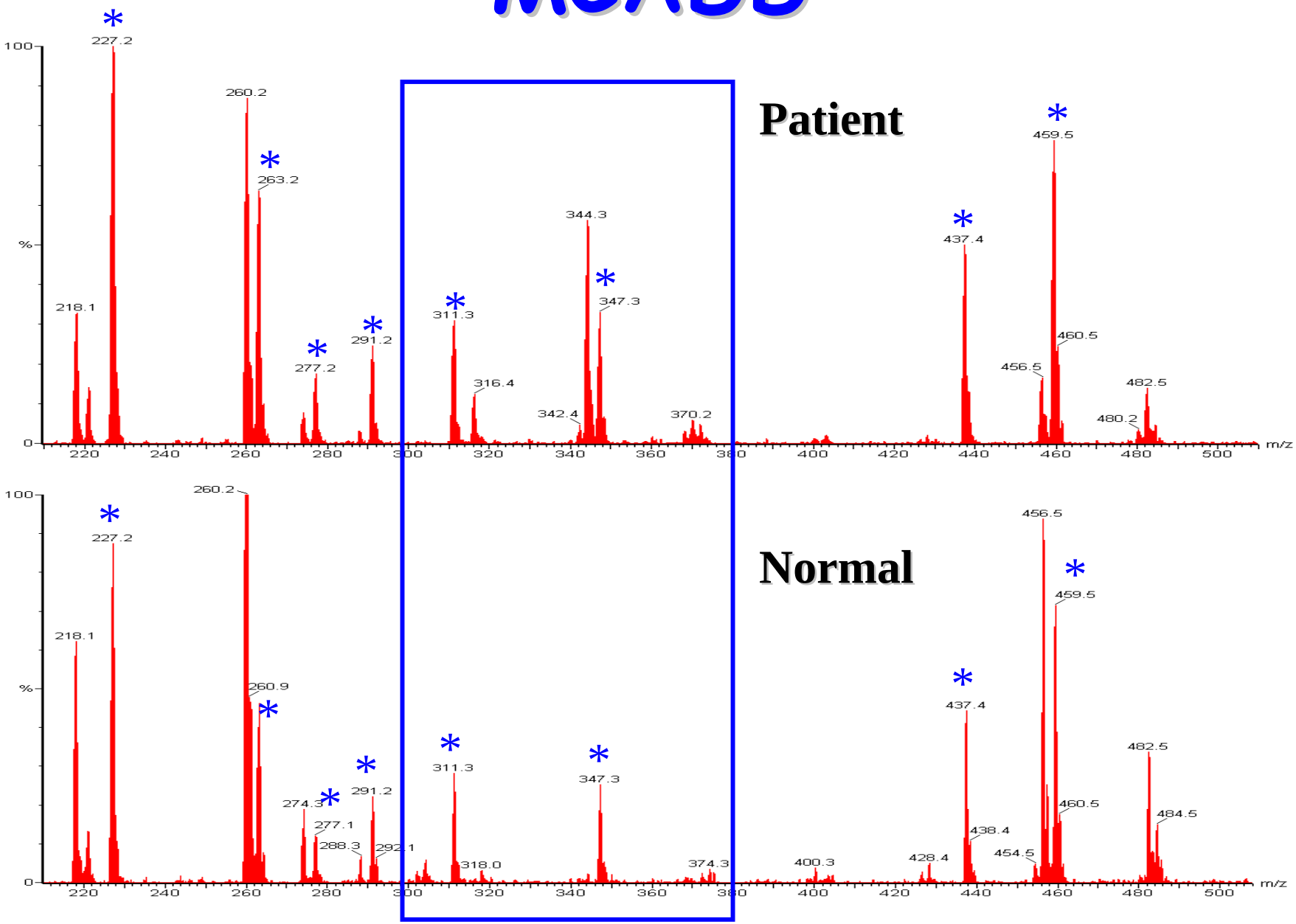
MCADD

- Urine organic acids:
  - increased medium-chain dicarboxylic acids
  - hexanoyl-, suberyl- and phenylpropionyl-glycines
- Acylcarnitine profile:
  - elevated C6, C10:1 & C8 (octanoylglycine)
- Treatment:
  - avoid prolonged fasting,
  - carnitine supplementation (during crisis)
  - cornstarch [slow release carbohydrate] supplementation (during crisis)

MCADD spectrum



MCADD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227.2
260.2
263.2
218.1
277.2
291.2
311.3
316.4
344.3
347.3
342.4
370.2
437.4
459.5
460.5
456.5
482.5
480.2
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m/z
Normal
100
%
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227.2
260.2
218.1
260.9
274.3
277.1
288.3
292.1
291.2
311.3
318.0
347.3
374.3
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428.4
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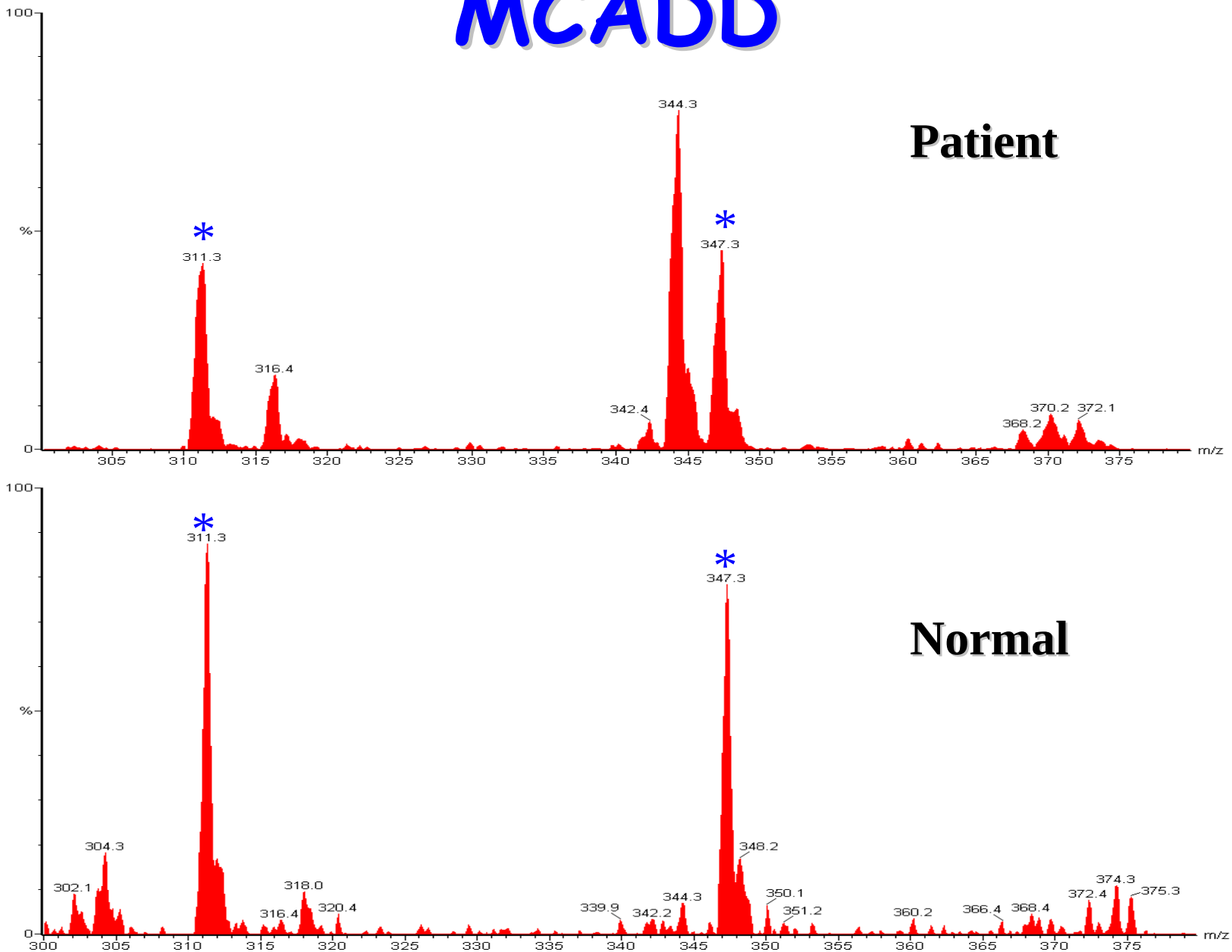
m/z

MCADD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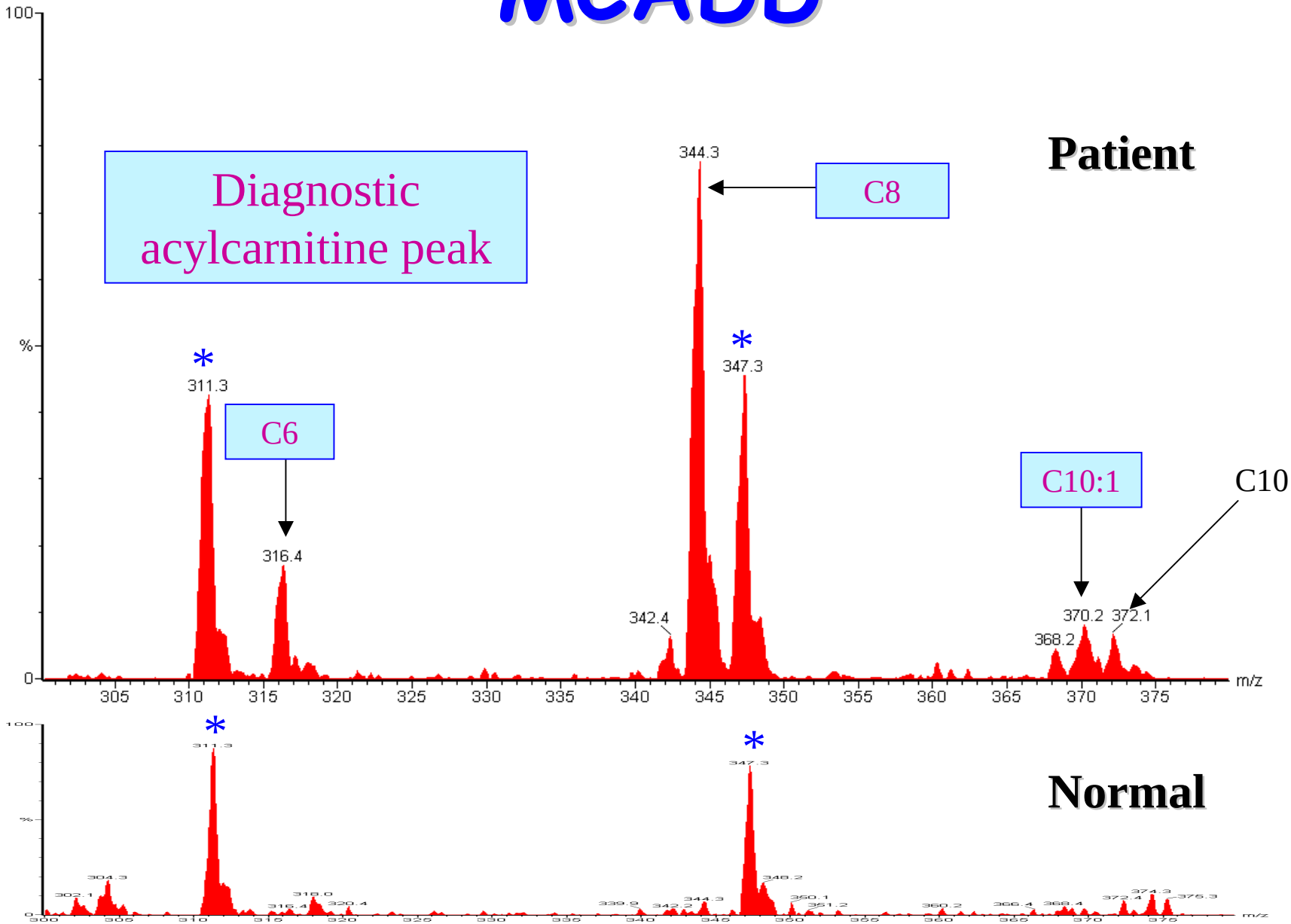
m/z

MCADD


Diagnostic acylcarnitine peak
Patient
C8
C6
C10:1
C10
344.3
347.3
311.3
316.4
342.4
370.2
372.1
368.2
100
%
0
m/z
305
310
315
320
325
330
335
340
345
350
355
360
365
370
375
Normal

Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)

- Multi-enzyme protein complex containing enzyme activities:
  - L-3-hydroxyacyl-CoA DHG
  - 2-enoyl-CoA hydratase
  - 3-oxoacylCoA thiolase
- 2 disorders described:
  - Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)
  - deficiency in all 3 enzymes of the tri-functional protein complex (MTP)

LCHAD/MTP Deficiency

- LCHADD is more common than MTP deficiency
- Association of LCHADD with maternal HELLP syndrome (haemolysis, elevated liver enzymes, low platelets)
- Defect is metabolism of long chain fatty acids (C-12 to C-16 in length)

LCHAD/MTP Deficiency

- Marked clinical heterogeneity associated with LCHADD, but presentation may include:
  - acute hypoketotic hypoglycaemic encephalopathy
  - hypotonia
  - cardiomyopathy
  - hepatomegaly leading to:
    - cirrhosis
    - fulminant liver failure

LCHAD/MTP Deficiency

- Late onset presentation:
  - exercise-induced myopathy & rhabdomyolysis
  - cardiomyopathy

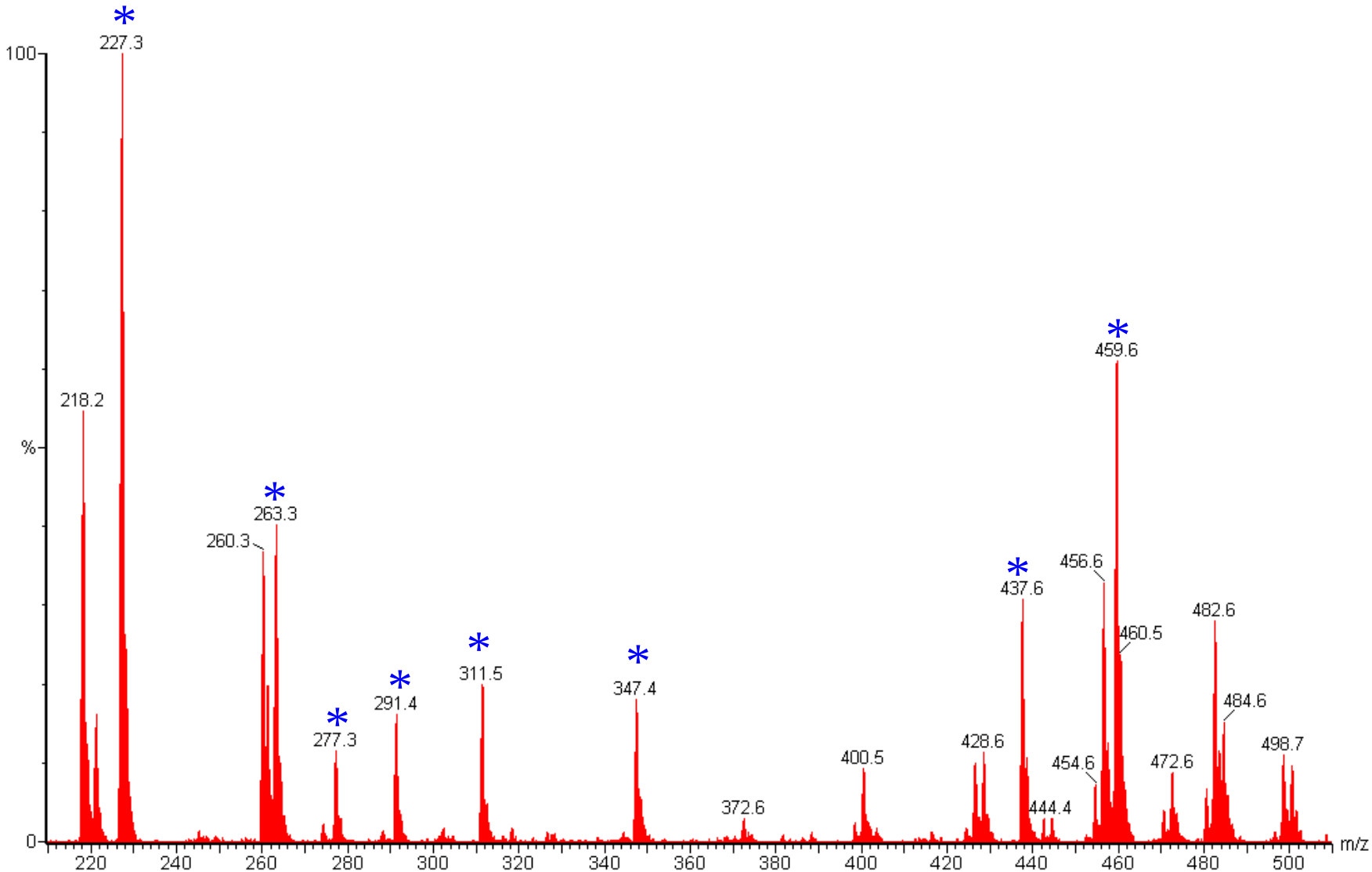
LCHAD/MTP Deficiency

- Urine organic acids:
  - 3-hydroxydicarboxylicaciduria
- Elevated CK during acute illness
- Acylcarnitine profile:
  - elevated C14:1, C16(OH), C16:1(OH), C18:1(OH), C18:2(OH)

LCHAD/MTP Deficiency

- Treatment:
  - restricted long-chain fat intake
  - avoid prolonged fasting
  - uncooked starch supplementation
  - Medium Chain Triglyceride (MCT) diet
  - carnitine supplementation

LCHADD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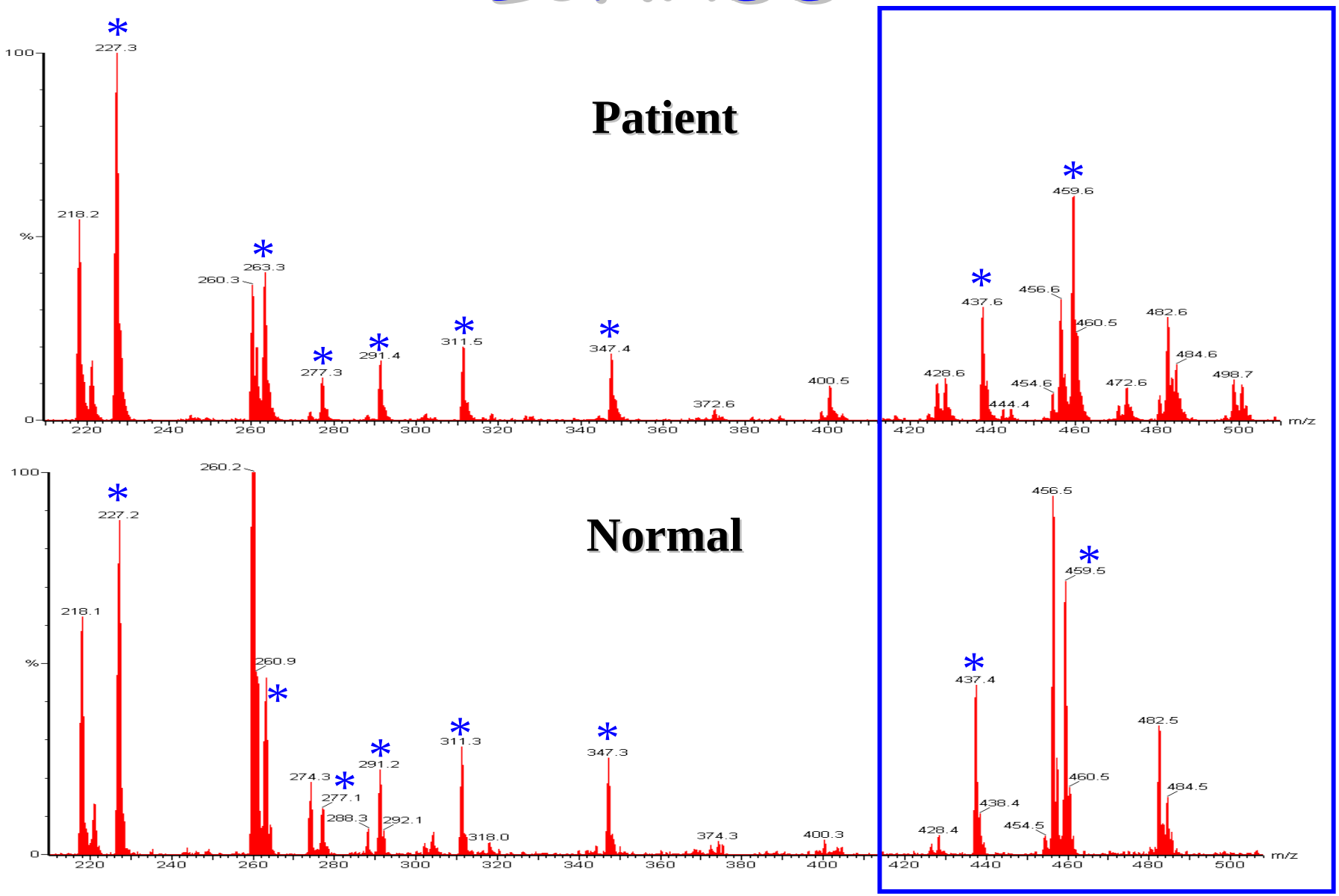
LCHADD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Normal
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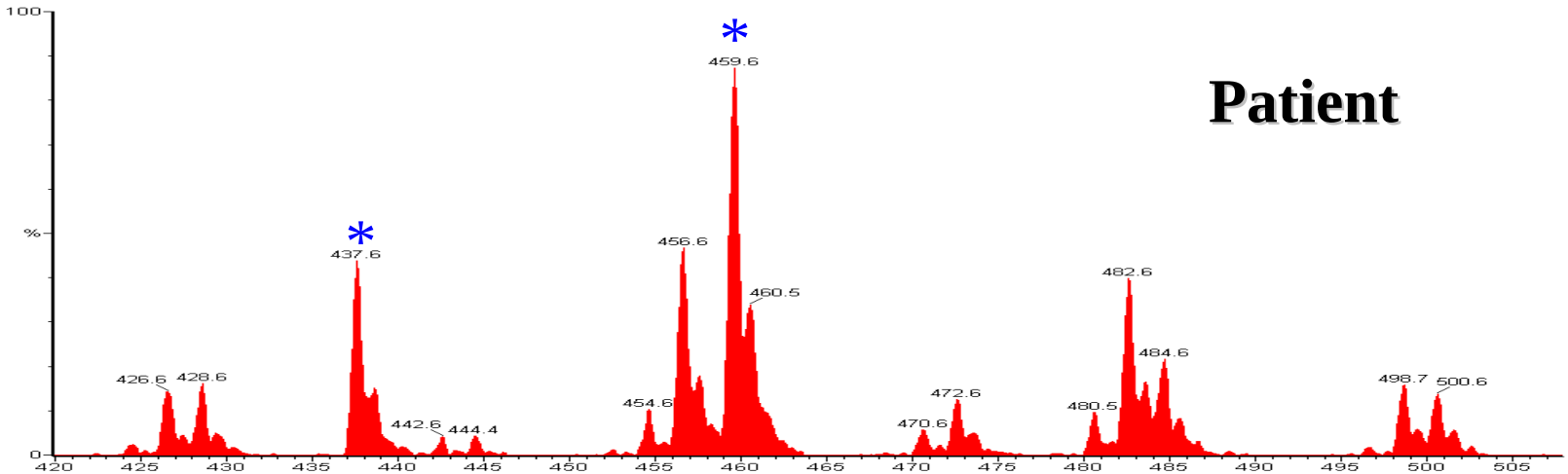
LCHADD



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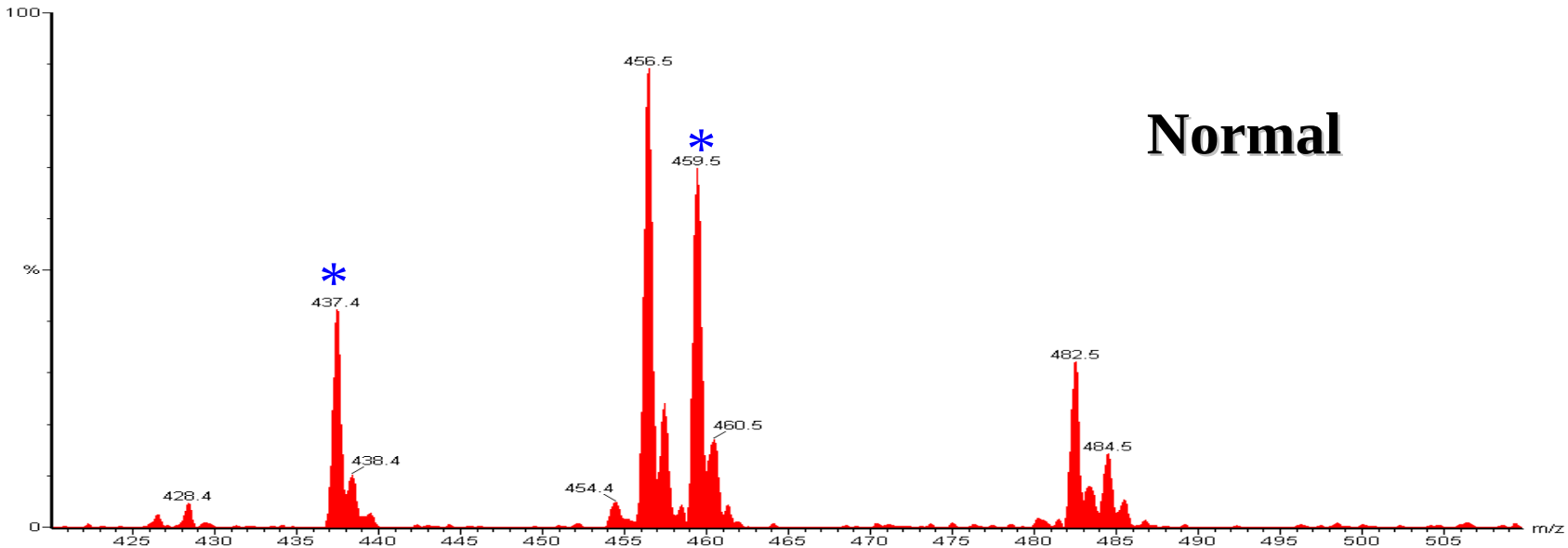
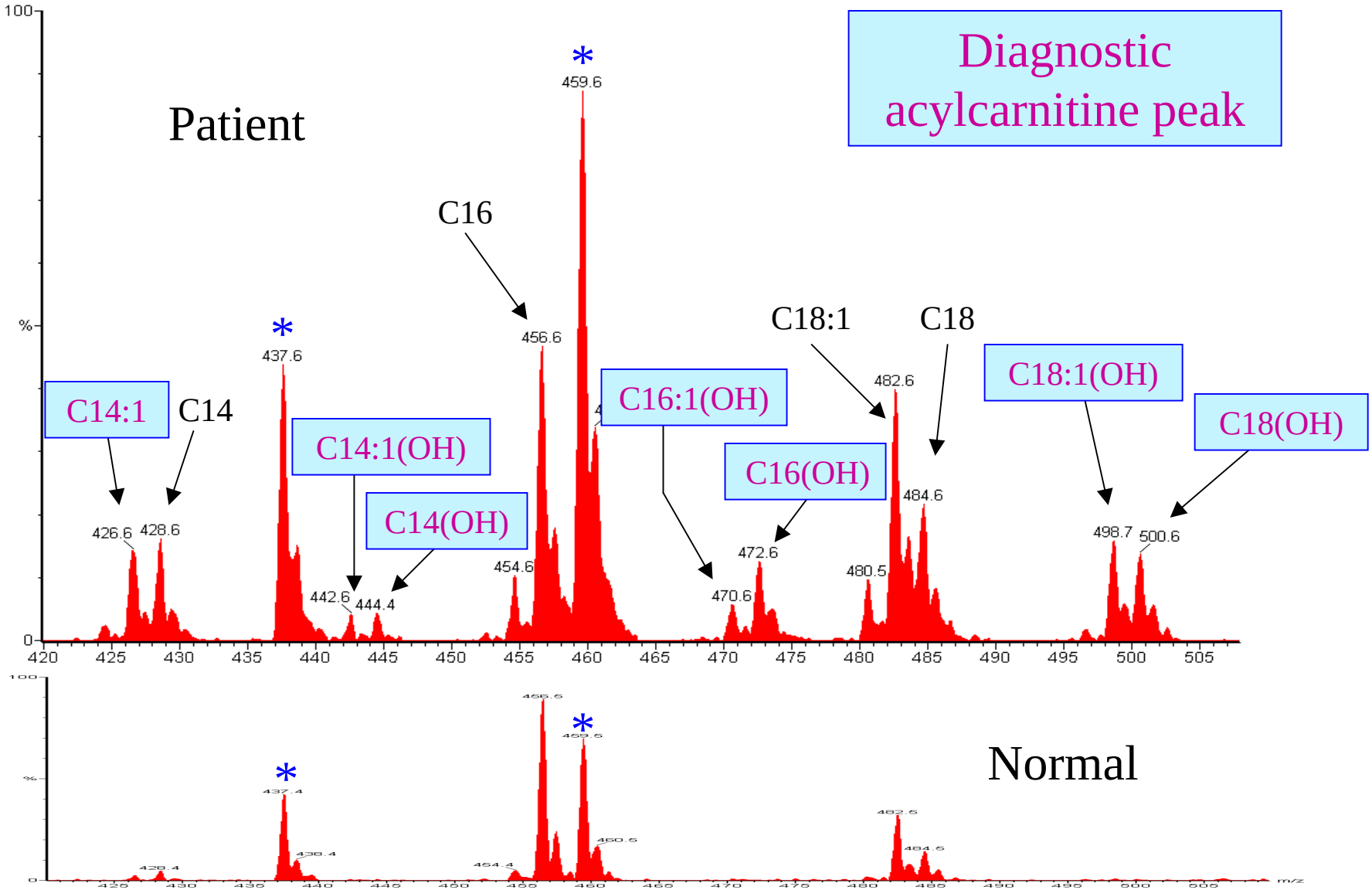


Normal

LCHADD


Patient
Diagnostic acylcarnitine peak
C14:1
C14
C14:1(OH)
C14(OH)
C16
C16:1(OH)
C16(OH)
C18:1
C18
C18:1(OH)
C18(OH)
426.6
428.6
437.6
442.6
444.4
454.6
456.6
459.6
470.6
472.6
480.5
482.6
484.6
498.7
500.6
Normal

Very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD)

- Enzyme catalyses initial rate-limiting step in mitochondrial long-chain fatty acid ß-oxidation
- Autosomal recessive inheritance
- Clinically heterogeneous – 3 phenotypes:
  - severe childhood form (early onset, high mortality & cardiomyopathy)
  - milder childhood form (hypoketotic hypoglycaemic)
  - adult form (isolated skeletal muscle, rhabdomyolysis triggered by exercise)

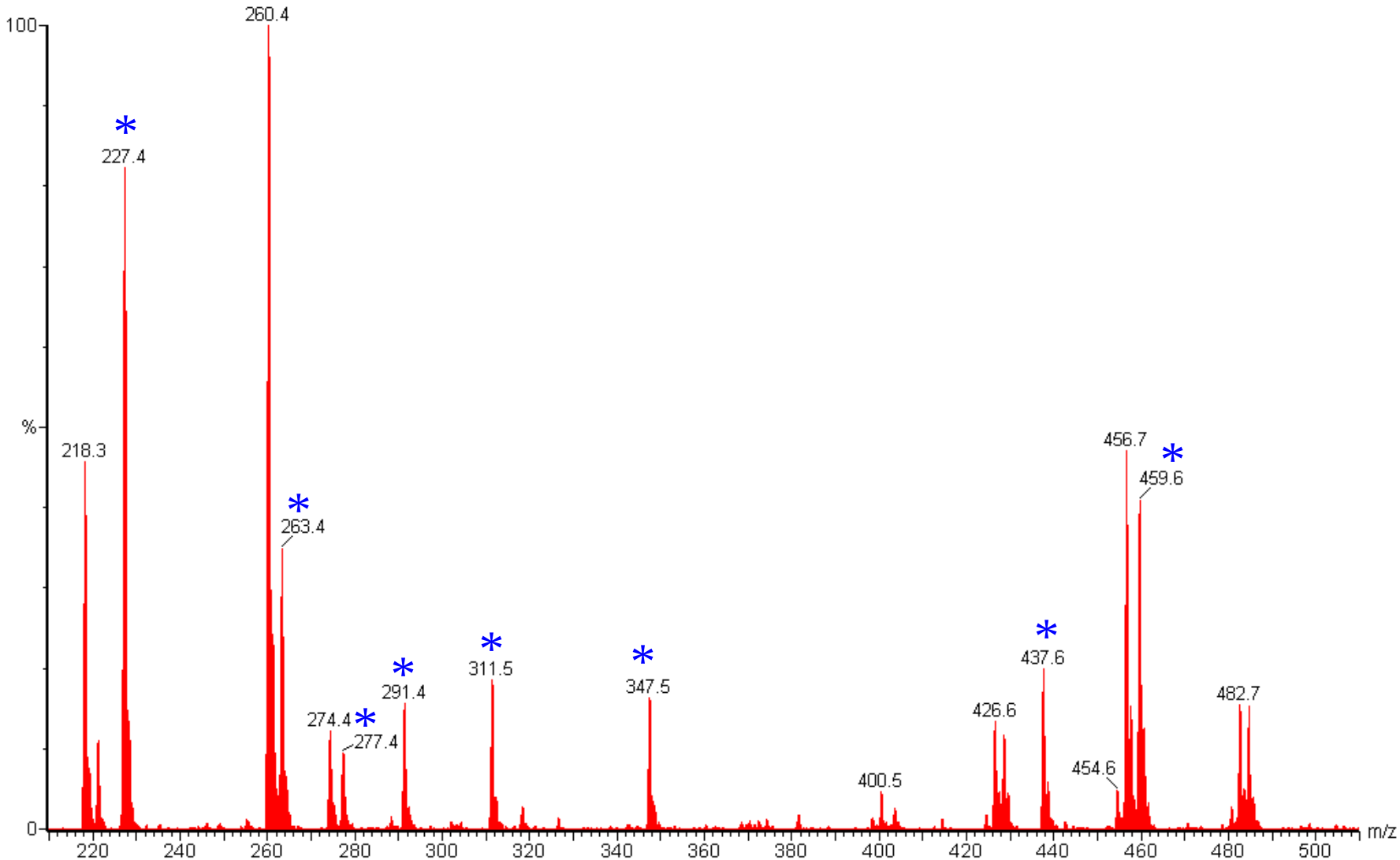
VLCADD

- Presentation:
  - hypoketotic hypoglycemia
  - hepatomegaly
  - myopathy & cardiomyopathy
- Urine organic acids:
  - medium to long-chain dicarboxylic & 3-hydroxy-dicarboxylic acids

VLCADD

- Acylcarnitine profile:
  - Elevated C14:1 (possibly C16:1, C14, C12)
- Treatment:
  - avoid prolonged fasting
  - low-fat, high carbohydrate diet
  - MCT & cornstarch supplementation
  - avoid long chain fatty acids in diet
  - carnitine supplementation

VLCADD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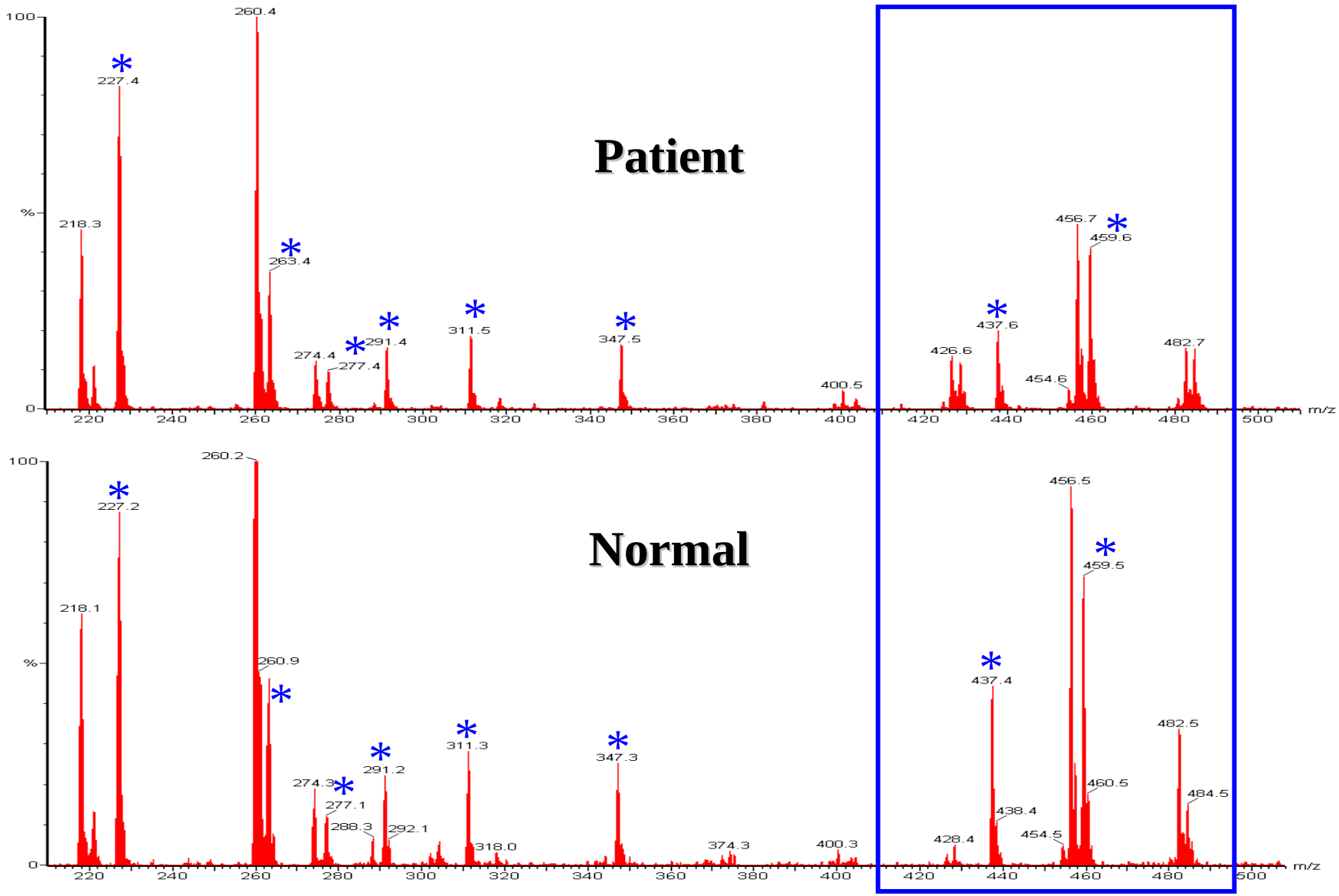
VLCADD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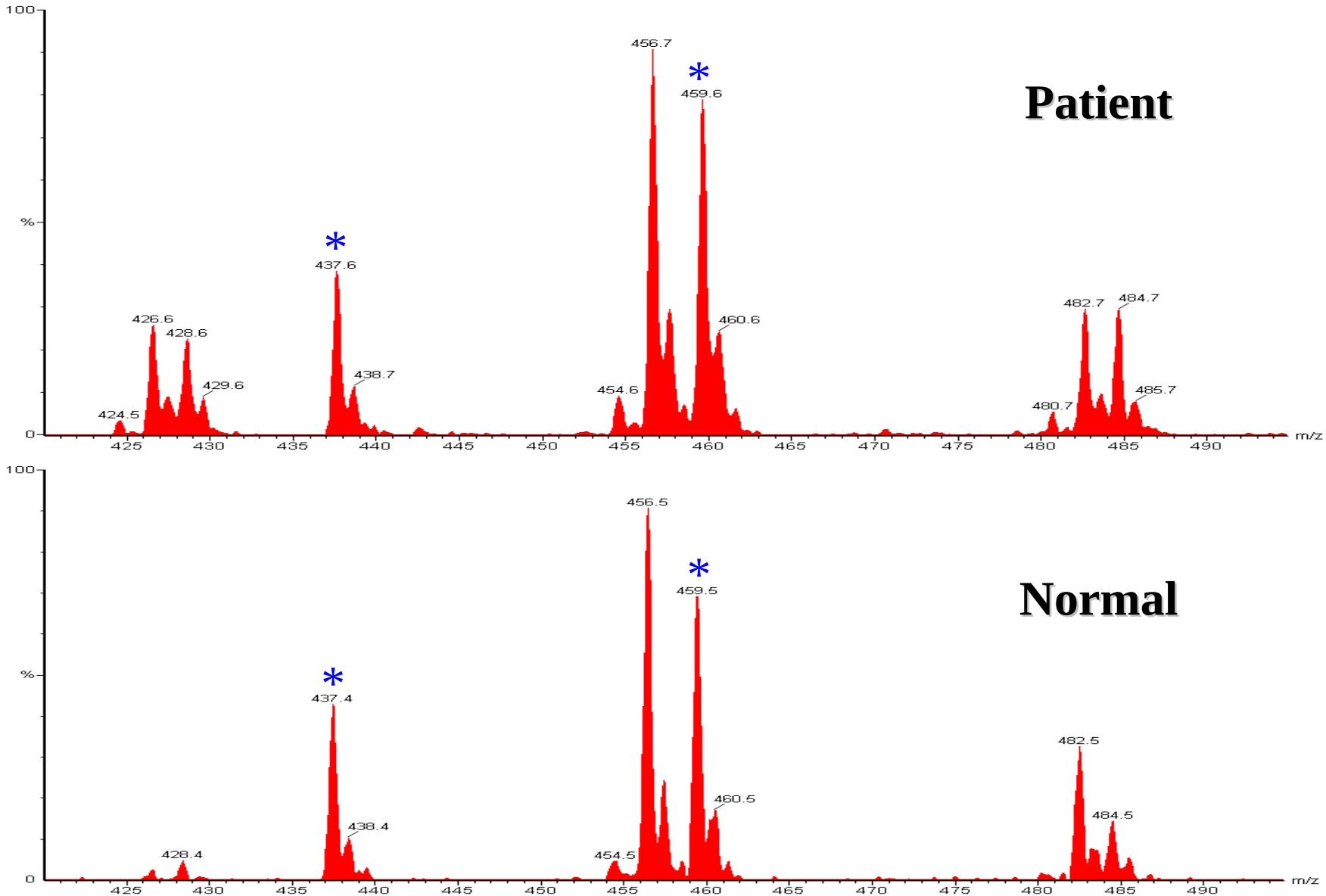
VLCADD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470
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485
490
m/z
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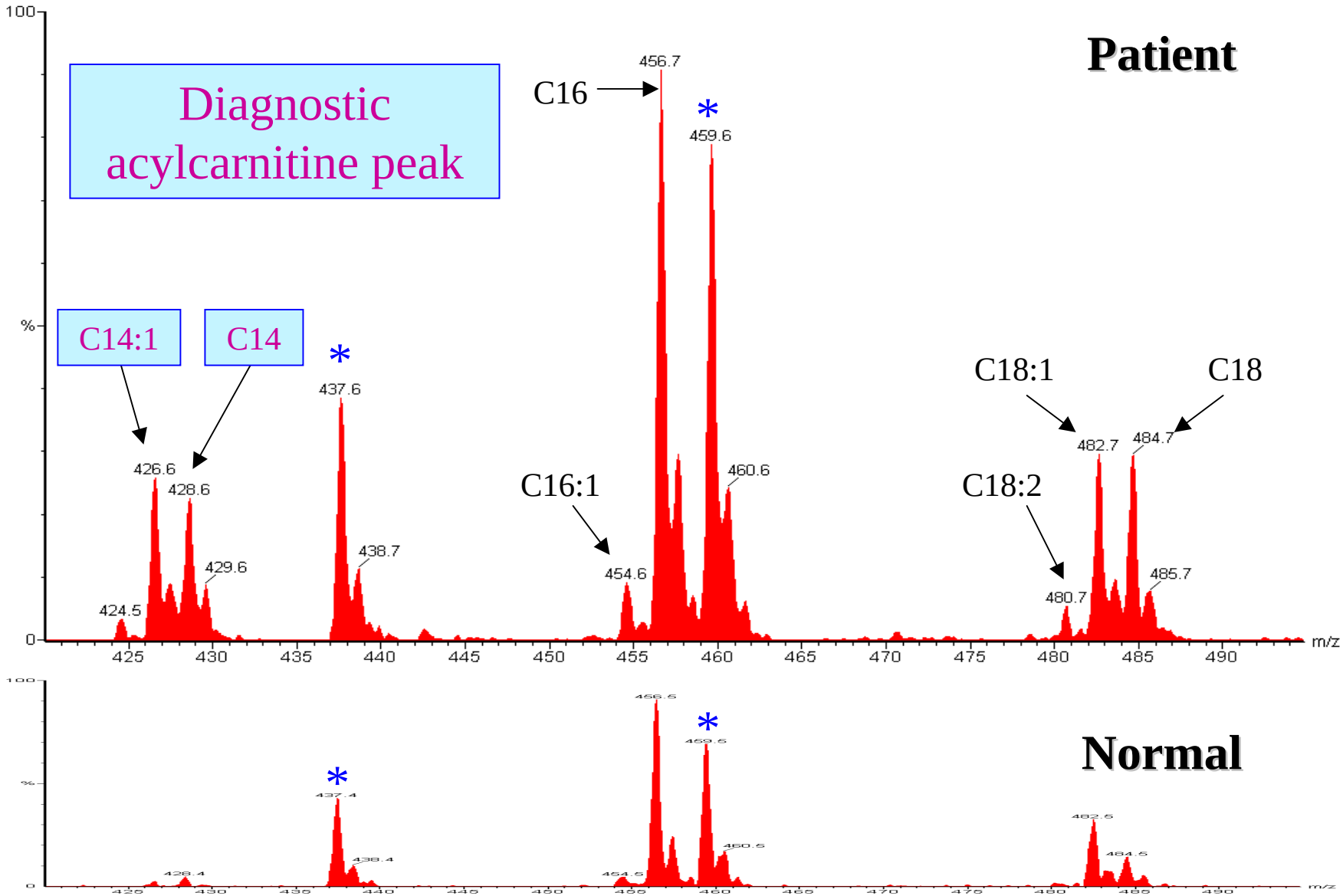
460.5
482.5
484.5
425
430
435
440
445
450
455
460
465
470
475
480
485
490
m/z

VLCADD


Patient
Diagnostic acylcarnitine peak
C14:1
C14
C16
C16:1
C18:1
C18
C18:2
*
456.7
459.6
437.6
426.6
428.6
429.6
424.5
438.7
454.6
460.6
480.7
482.7
484.7
485.7
100
%
0
m/z
425
430
435
440
445
450
455
460
465
470
475
480
485
490
Normal

Glutaric aciduria type 1 (GA-I)

- Defect: Glutaryl-CoA dehydrogenase deficiency
- Pathways affected: lysine, hydroxylysine and tryptophan
- Presentation:
  - macrocephaly
  - neurodegeneration
  - dystonia
  - ataxia and dyskinesia
  - seizures
  - frontotemporal atrophy on MRI & CT
  - hypotonia
  - death due to Reye-like syndrome

GA-I

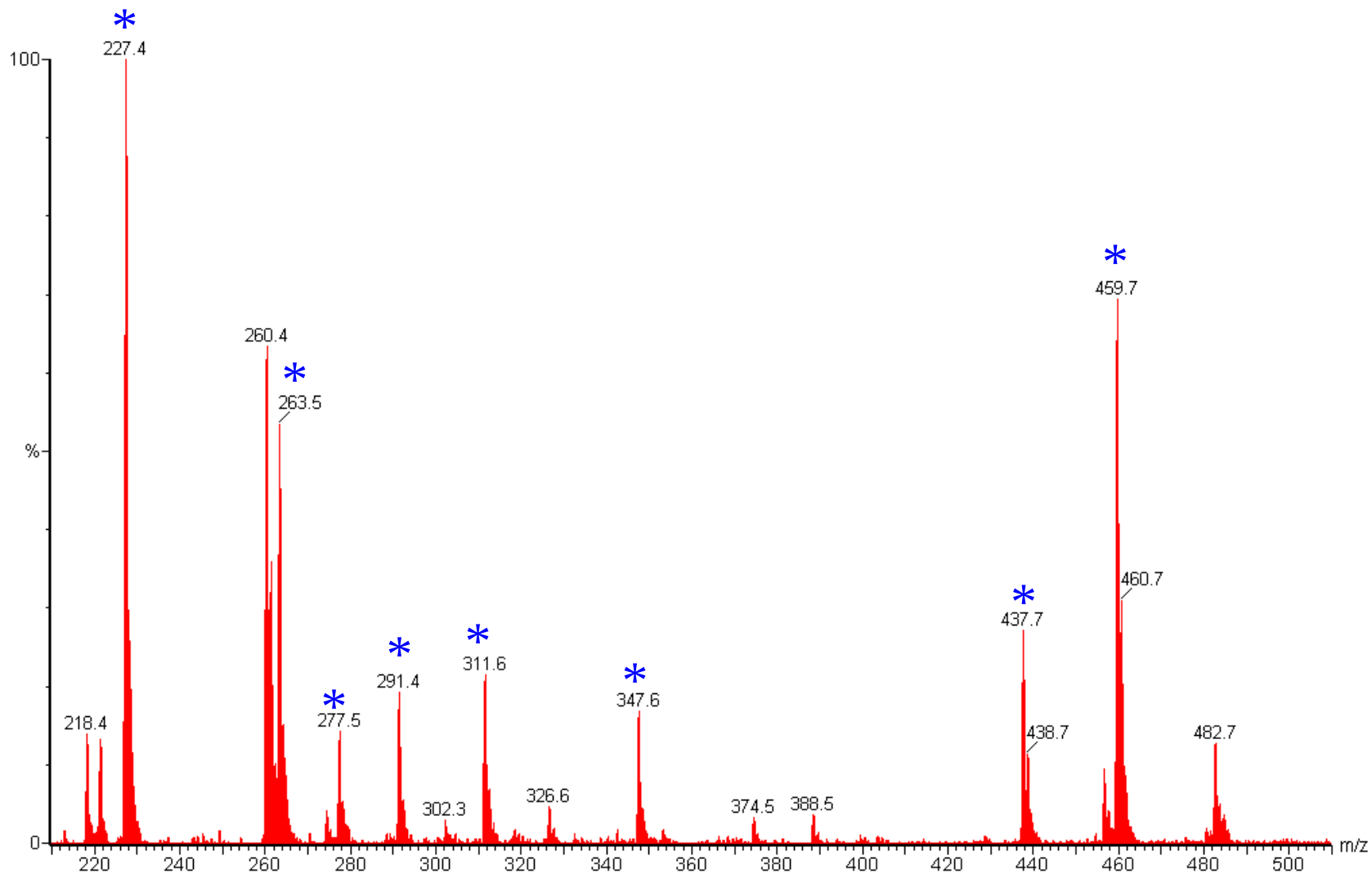
- Urine organic acids:
  - increased glutarate
  - 3-hydroxyglutarate
  - glutaconate
- Acylcarnitine profile:
  - elevated C5-DC (glutaryl carnitine)

- NB Metabolites not always reliably increased

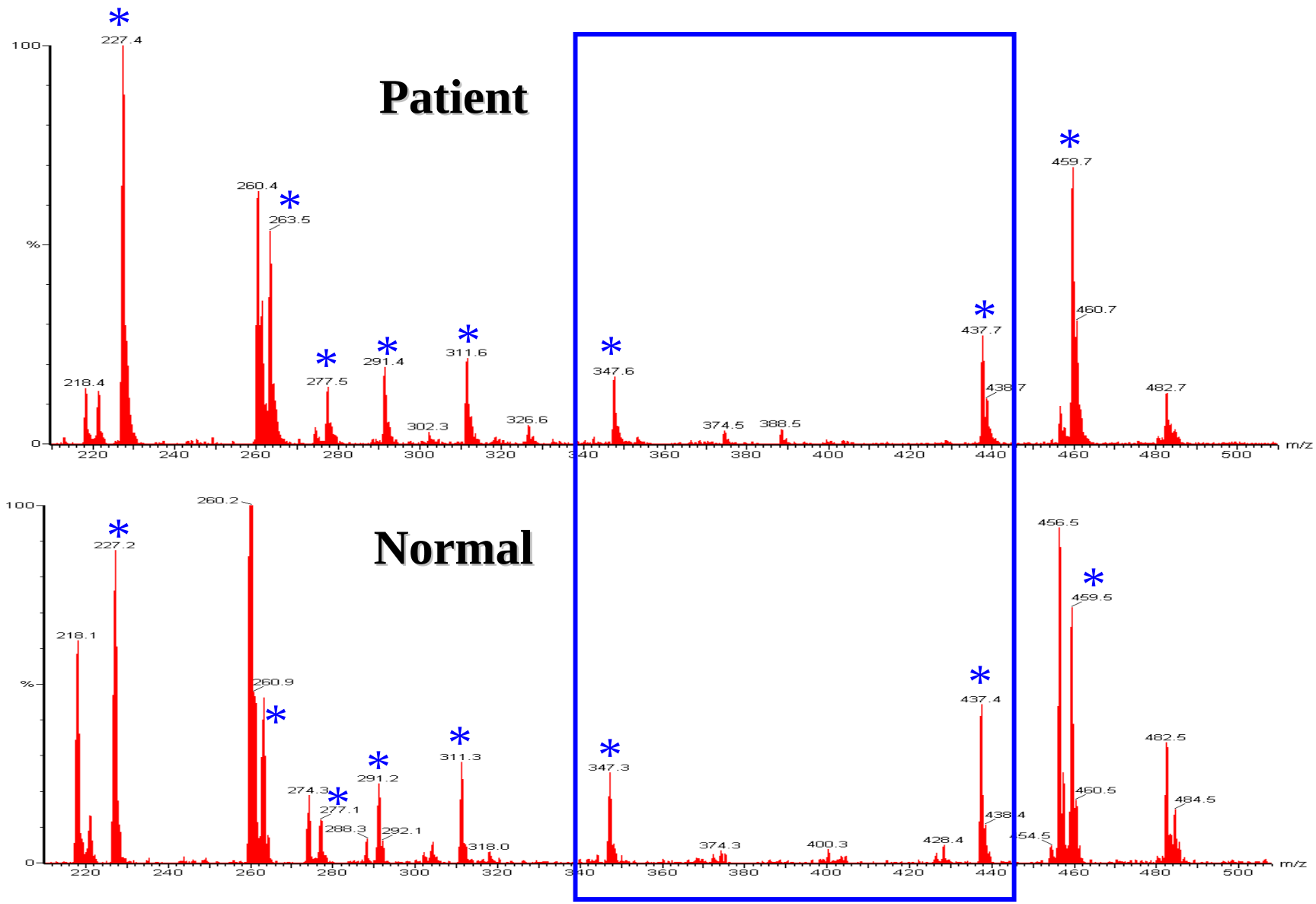
GA-I

- Treatment:
  - lysine and tryptophan restricted diet
  - riboflavin supplementation
  - carnitine supplementation
  - i.v. glucose during acute illness

GA-I


100
%
0
227.4
260.4
263.5
277.5
291.4
311.6
347.6
437.7
459.7
218.4
302.3
326.6
374.5
388.5
438.7
460.7
482.7
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

GA-I


Patient
100
%
0
227.4
260.4
263.5
218.4
277.5
291.4
311.6
302.3
326.6
347.6
374.5
388.5
437.7
438.7
459.7
460.7
482.7
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
Normal
100
%
0
227.2
260.2
218.1
260.9
274.3
277.1
288.3
291.2
292.1
311.3
318.0
347.3
374.3
400.3
428.4
437.4
438.4
454.5
456.5
459.5
460.5
482.5
484.5
220
240
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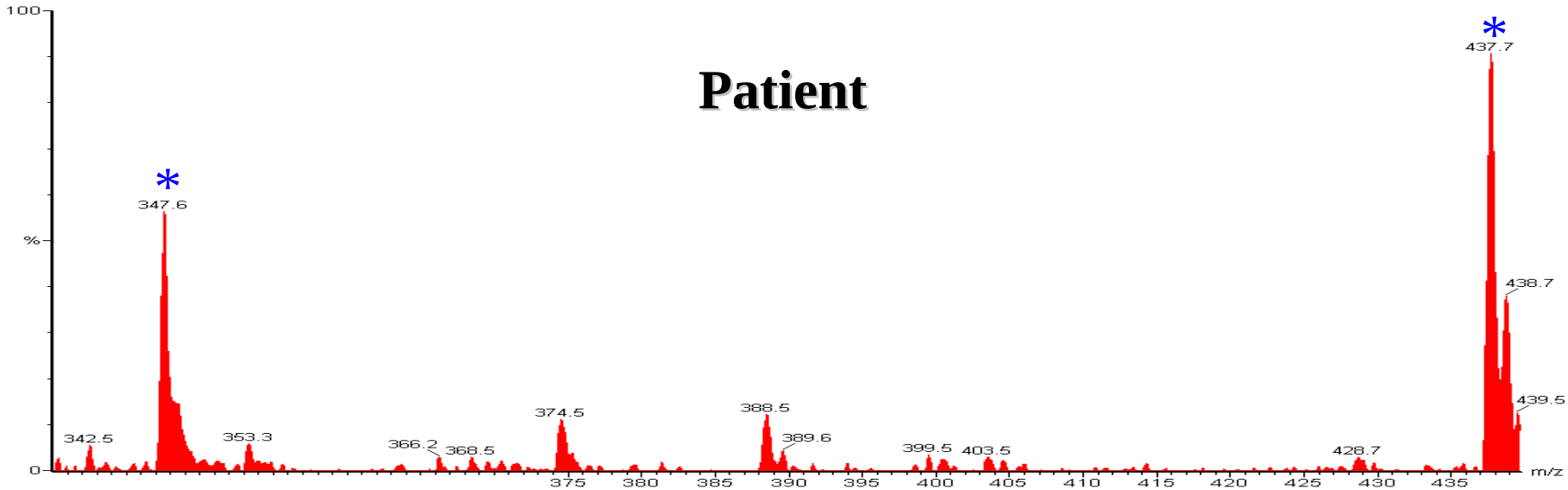
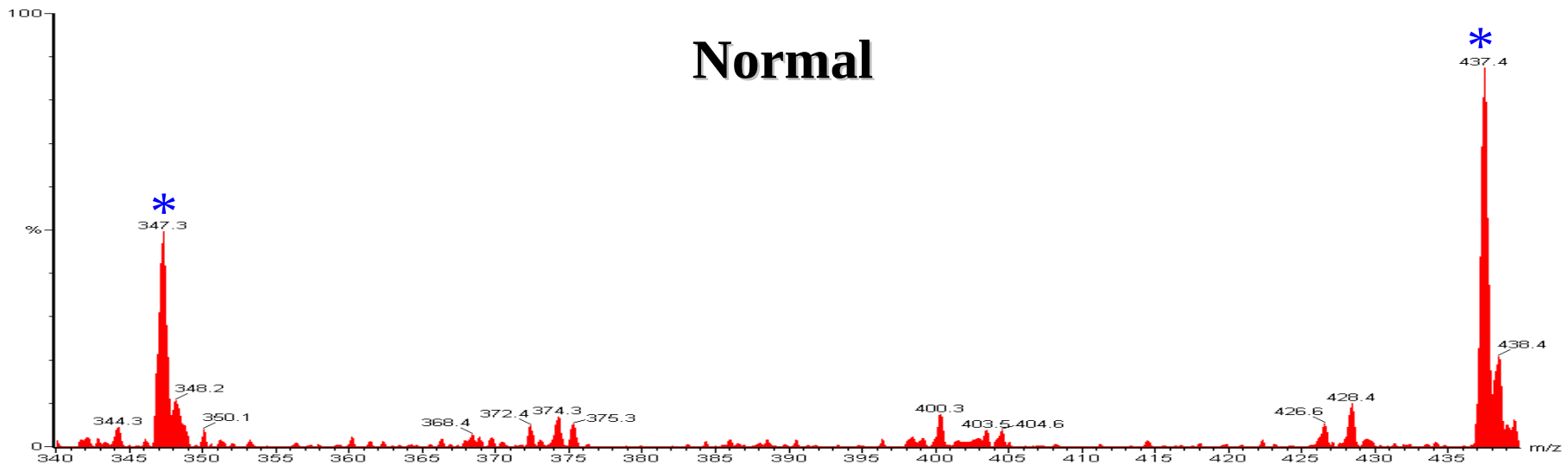
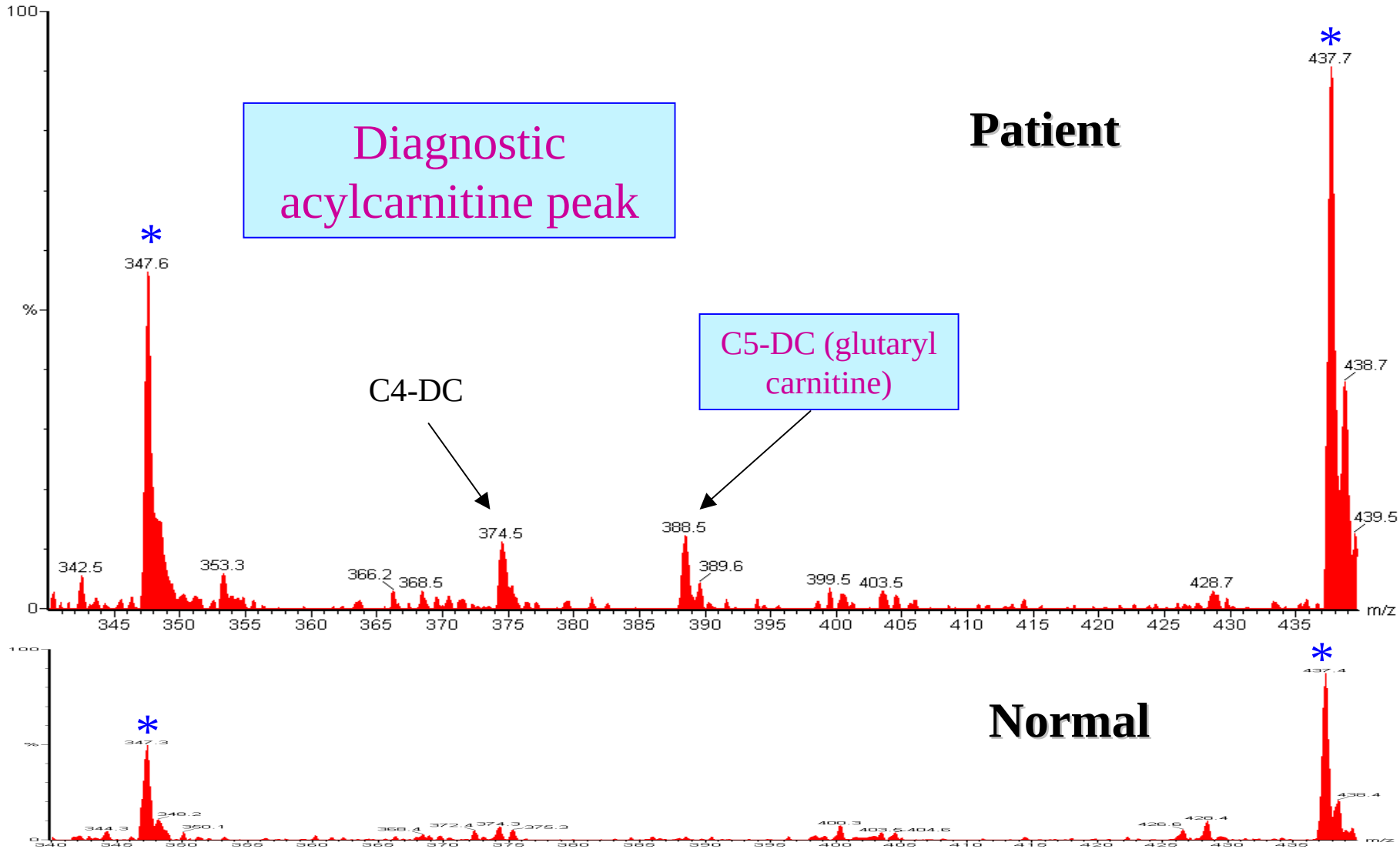
400
420
440
460
480
500
m/z

GA-I


Patient
100
%
0
347.6
437.7
342.5
353.3
366.2
368.5
374.5
388.5
389.6
399.5
403.5
428.7
438.7
439.5
375
380
385
390
395
400
405
410
415
420
425
430
435
m/z


Normal
100
%
0
347.3
437.4
344.3
348.2
350.1
368.4
372.4
374.3
375.3
400.3
403.5
404.6
426.6
428.4
438.4
340
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
m/z

GA-I


Diagnostic acylcarnitine peak
Patient
C4-DC
C5-DC (glutaryl carnitine)
100
%
0
347.6
437.7
438.7
439.5
342.5
353.3
366.2
368.5
374.5
388.5
389.6
399.5
403.5
428.7
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
m/z
Normal
347.3
437.4
438.4
344.3
348.2
350.1
368.4
372.4
374.3
375.3
400.3
404.6
426.6
428.4

Glutaric aciduria Type II (GA-II)

- Also termed Multiple acyl-CoA dehydrogenase deficiency (MADD)
- Autosomal recessive inheritance
- Defect is in mitochondrial transport of electrons from acyl-CoAs to ubiquinone
- Affects all of the fatty-acid acyl-CoA dehydrogenase enzyme systems

- Catabolism of branched-chain amino acids also affected

GA-II

- Phenotypes:
- Neonatal onset
  - with/without congenital anomalies
    - severe nonketotic hypoglycaemia
    - hyperammonaemia
    - abnormal odour
    - hypotonia
    - hepatomegaly
    - severe metabolic acidosis
    - dysplastic kidneys
  - often fatal within first week of life

GA-II

- Mild or Late onset
  - hypotonia
  - hepatomnegaly
  - metabolic acidosis
  - hypoketotic hypoglycaemia
- mild patients show broad disease spectrum
- Some patients are riboflavin-responsive

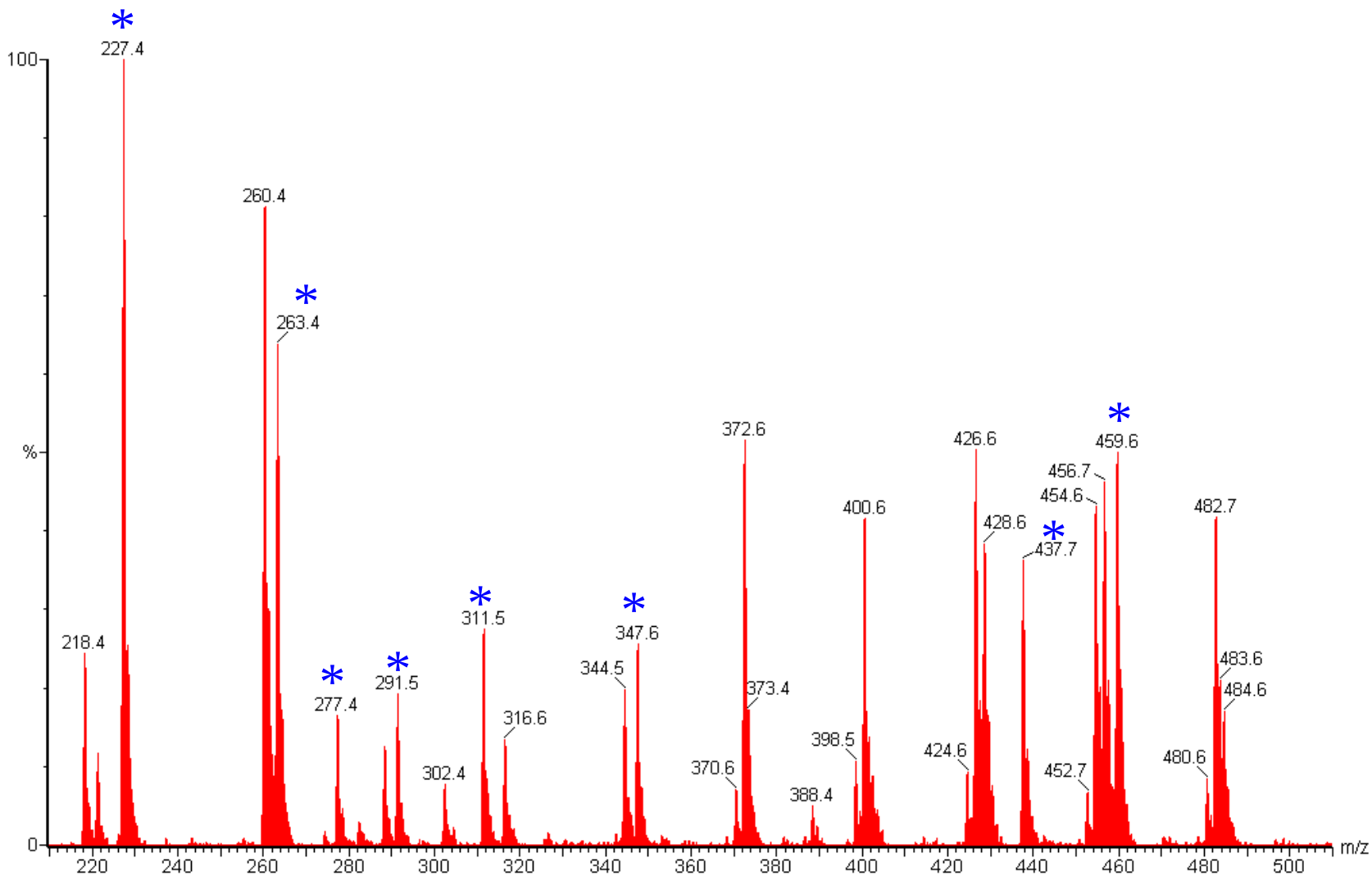
GA-II

- Urine organic acids:
  - prominent glutaric & lactic acidurias
  - increased medium-chain dicarboxylic acids (C6-C12)
  - hexanoylglycine (suberylglycine)
  - butyrylglycine
  - ethymalonate
  - isovalerylglycine
  - methylsuccinate
  - 2-OH glutaric aciduria can distinguish between GA I and GA II

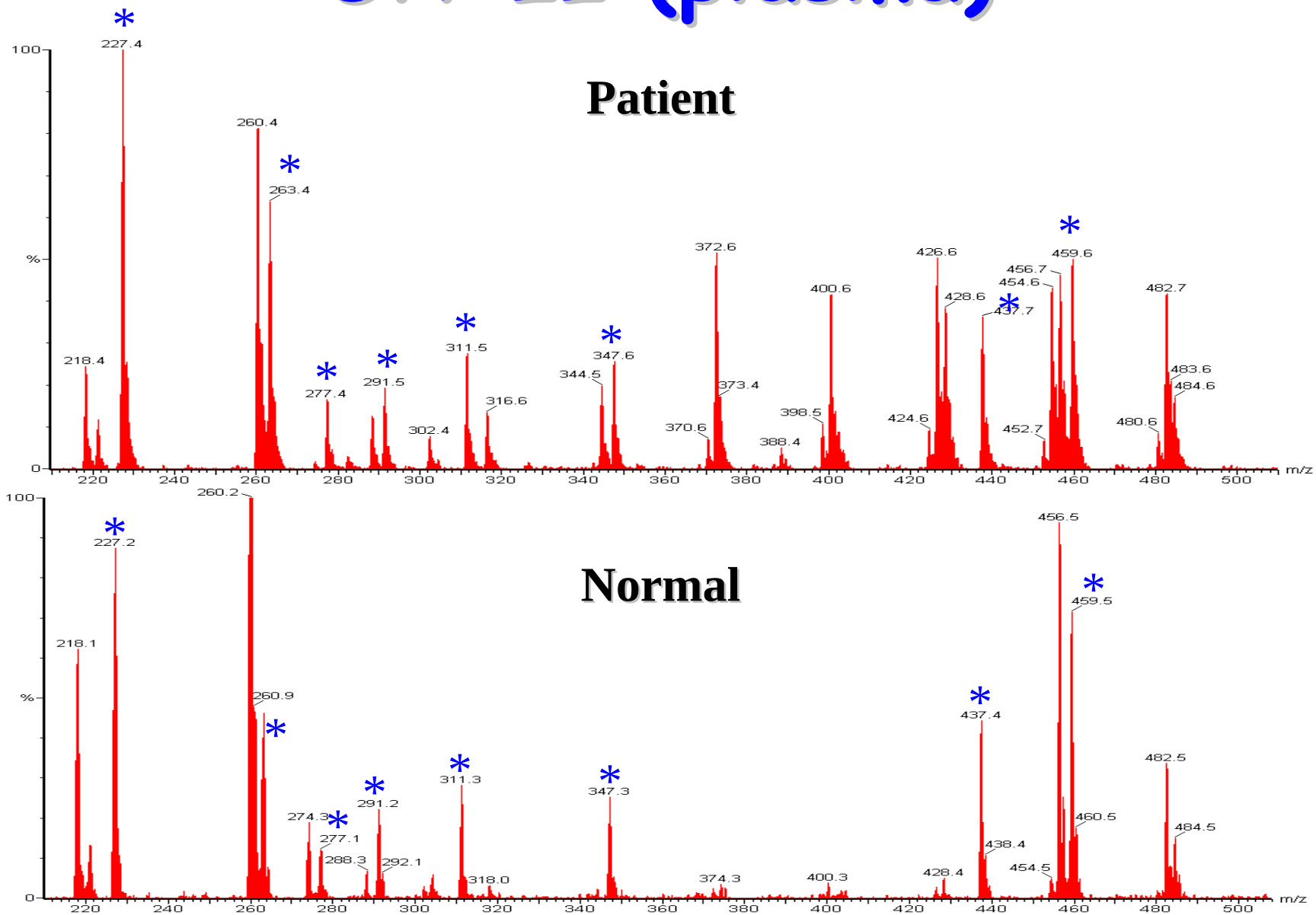
GA-II

- Acylcarnitine profile:
  - C5-DC
  - elevated C4, C5, C6, C8, C10, C12, C14, C14:1, C16:2, C16:2, C18 & C18:1
- Treatment:
  - in severe neonatal cases: not effective
  - avoid prolonged fasting
  - a diet low in fat & protein and high in carbohydrate
  - 3-hydroxybutyrate
  - mild cases - Riboflavin supplementation
  - supplements of glycine and L-carnitine

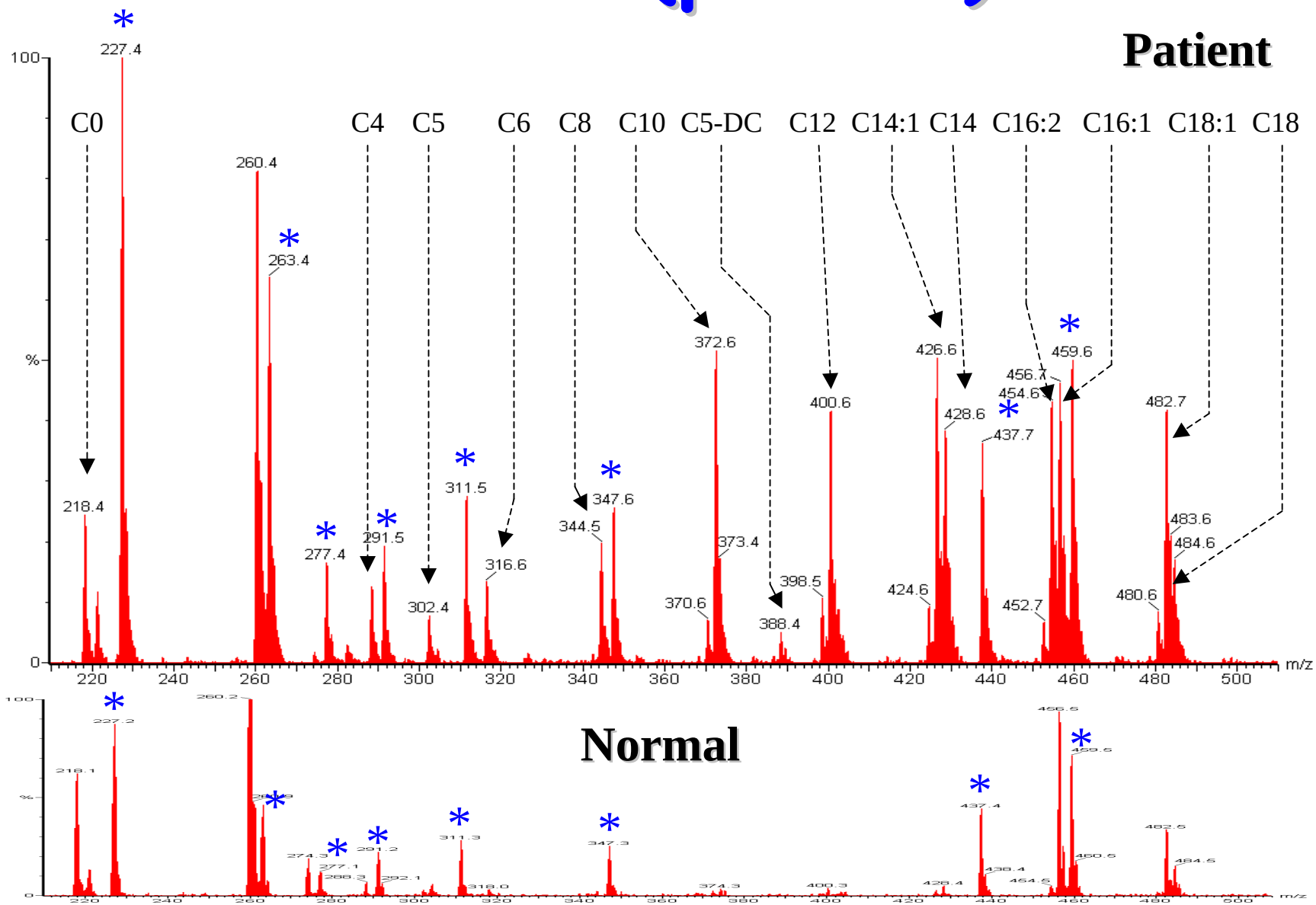
GA-II (plasma)


100
%
0
227.4
260.4
263.4
277.4
291.5
302.4
311.5
316.6
344.5
347.6
370.6
372.6
373.4
388.4
398.5
400.6
424.6
426.6
428.6
437.7
452.7
454.6
456.7
459.6
480.6
482.7
483.6
484.6
218.4
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

GA-II (plasma)


Patient
100
%
0
227.4
260.4
263.4
218.4
277.4
291.5
311.5
316.6
302.4
344.5
347.6
372.6
373.4
370.6
388.4
398.5
400.6
424.6
426.6
428.6
437.7
452.7
454.6
456.7
459.6
480.6
482.7
483.6
484.6
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
Normal
100
%
0
227.2
260.2
260.9
218.1
274.3
277.1
288.3
291.2
292.1
311.3
318.0
347.3
374.3
400.3
428.4
437.4
438.4
454.5
456.5
459.5
460.5
482.5
484.5
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

GA-II (plasma)


Patient
C0
C4
C5
C6
C8
C10
C5-DC
C12
C14:1
C14
C16:2
C16:1
C18:1
C18
227.4
260.4
263.4
218.4
277.4
291.5
302.4
311.5
316.6
344.5
347.6
370.6
372.6
373.4
388.4
398.5
400.6
424.6
426.6
428.6
437.7
452.7
454.6
456.7
459.6
480.6
482.7
483.6
484.6
100
%
0
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z
Normal

Carnitine palmitoyltranserase-II deficiency (CPT-II)

- Catalyses trans-esterification of acyl-carnitine to acyl-CoA on inner mitochondrial membrane
- >25 mutations known
- 3 Phenotypes
  - Late onset (mild)
    - muscle pain & stiffness after exercise or in extremes of temperature
  - Severe infantile (intermediate)
    - liver, heart and skeletal muscle involvement
    - hypoketotic hypoglycaemia

CPT-II

- Lethal neonatal form
  - hypoketotic hypoglycaemia
  - liver disease
  - hypotonia
  - cardiomyopathy
  - congenital abnormalities

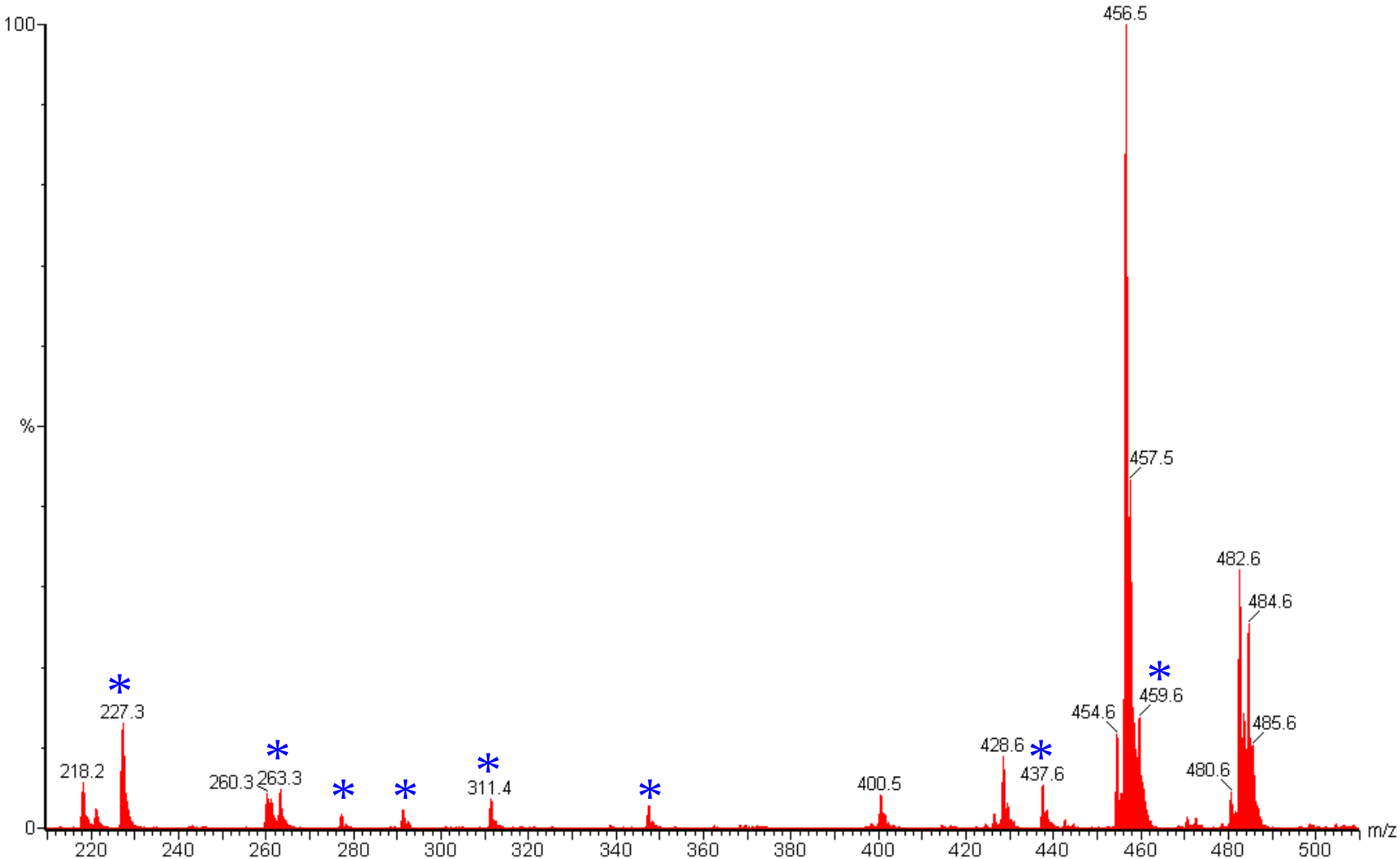
CPT-II

- Characteristics include:
  - low plasma carnitine
  - raised long-chain acylcarnitines
  - raised CK levels & rhabdomyolysis
- Acylcarnitine Profile:
  - raised (C12, C14) C16, C18, C18:1 & C18:2
  - raised plasma (C16+C18:1)/C2 ratio

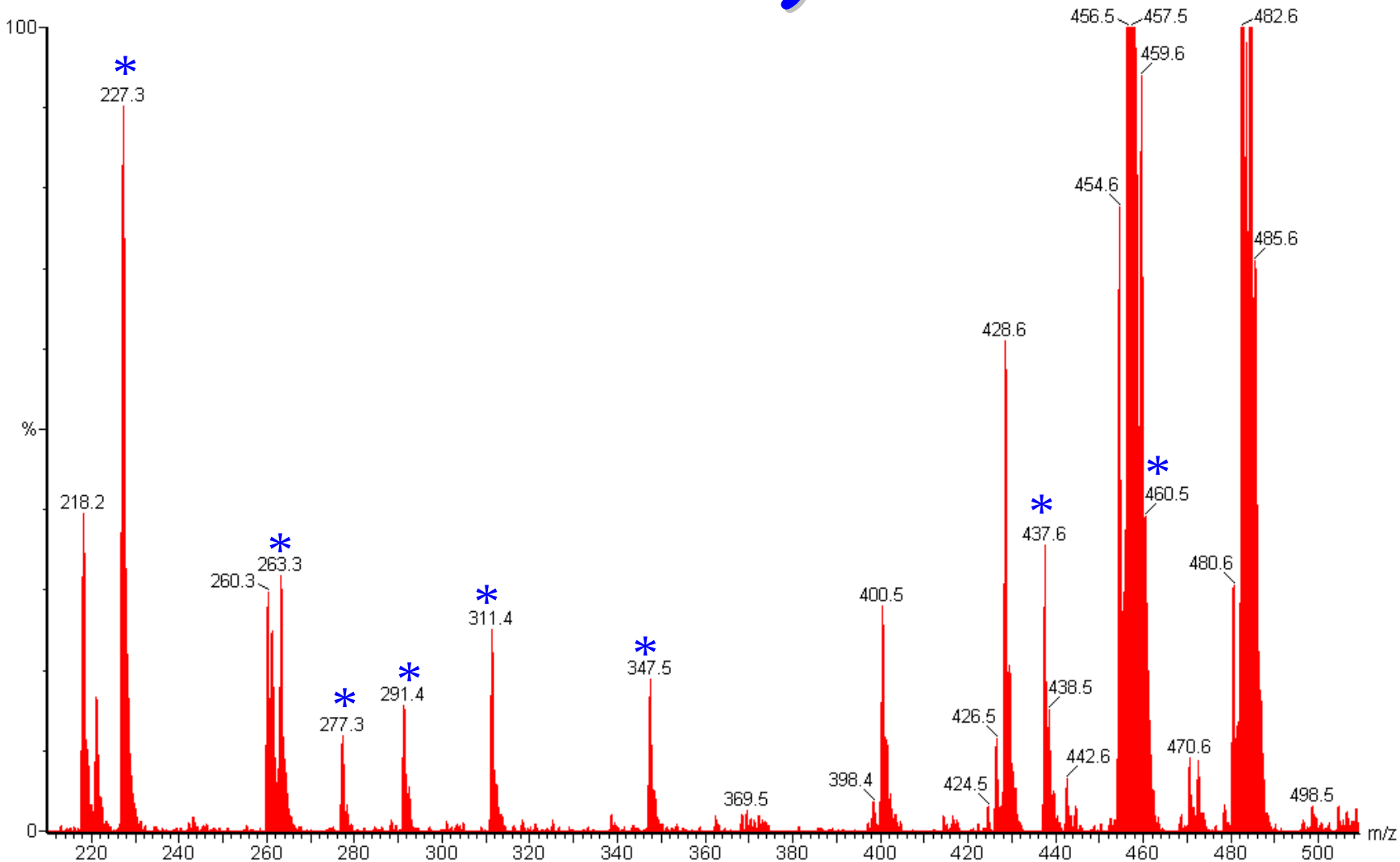
CPT-II

- Treatment:
  - avoid prolonged fasting
  - low-fat, high carbohydrate diet
  - MCT & cornstarch supplementation
  - carnitine supplementation
  - i.v. glucose during acute episodes

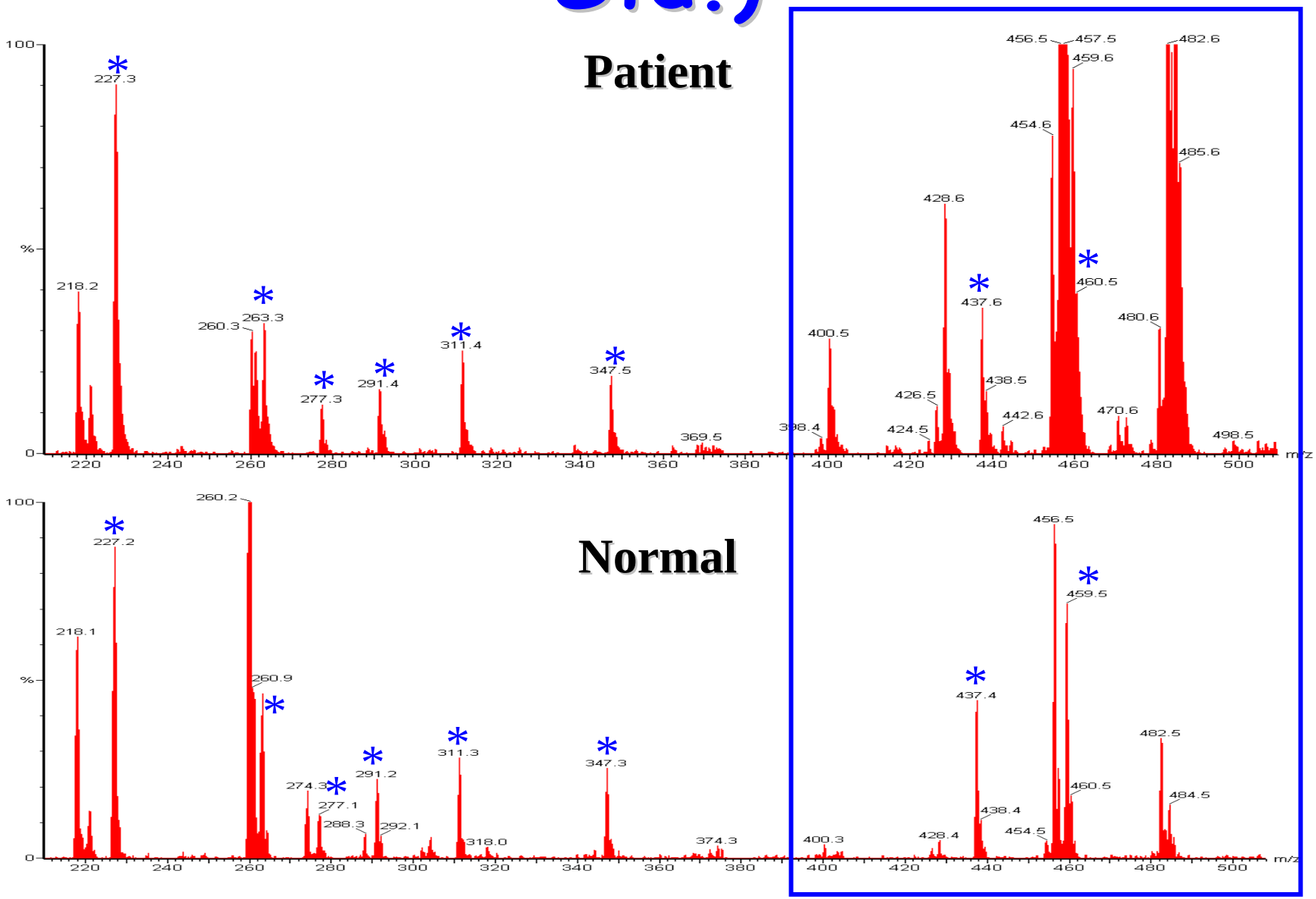
CPT-II (severe)



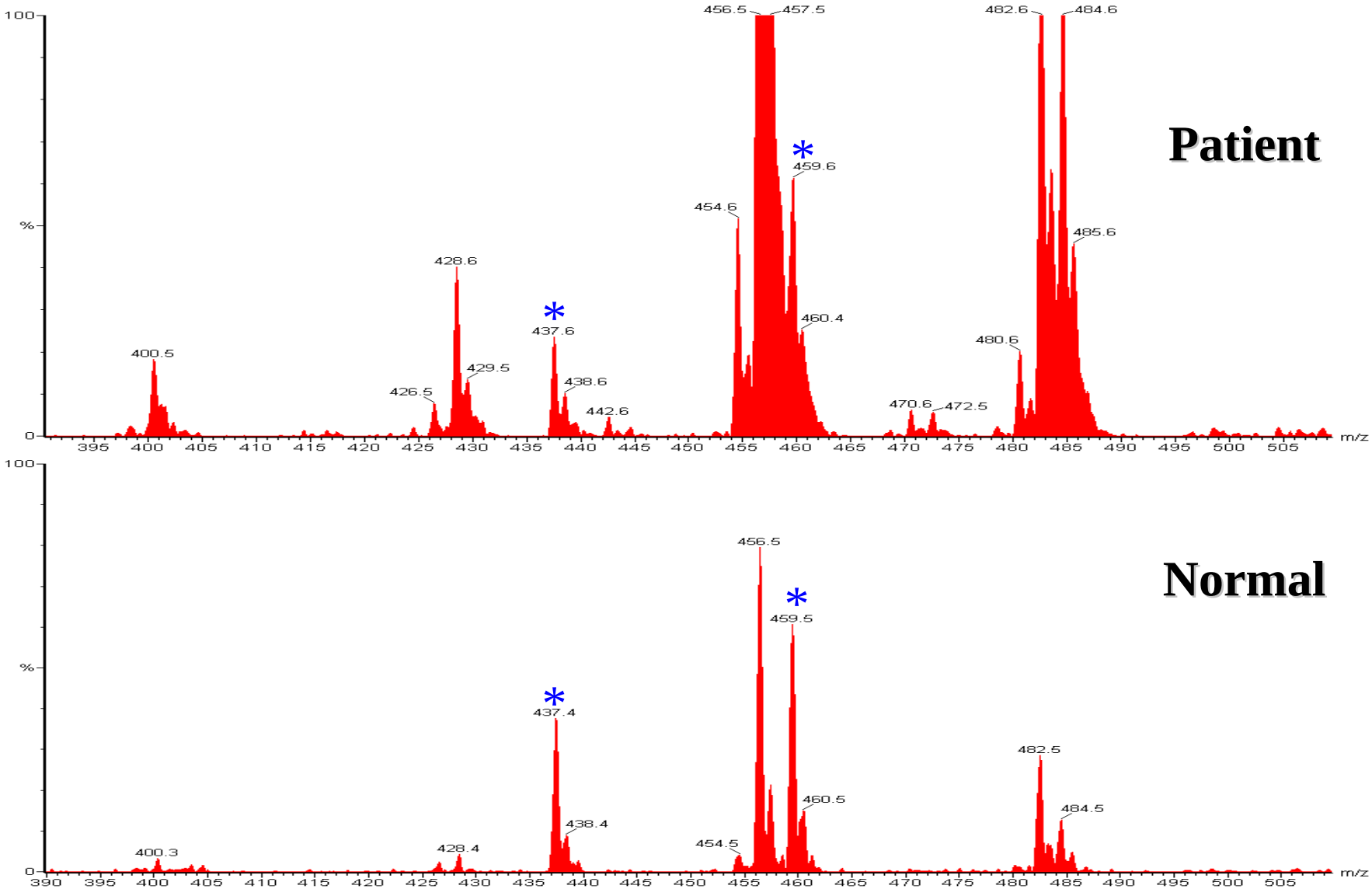
CPT-II (scaled to CO Int. Std.)



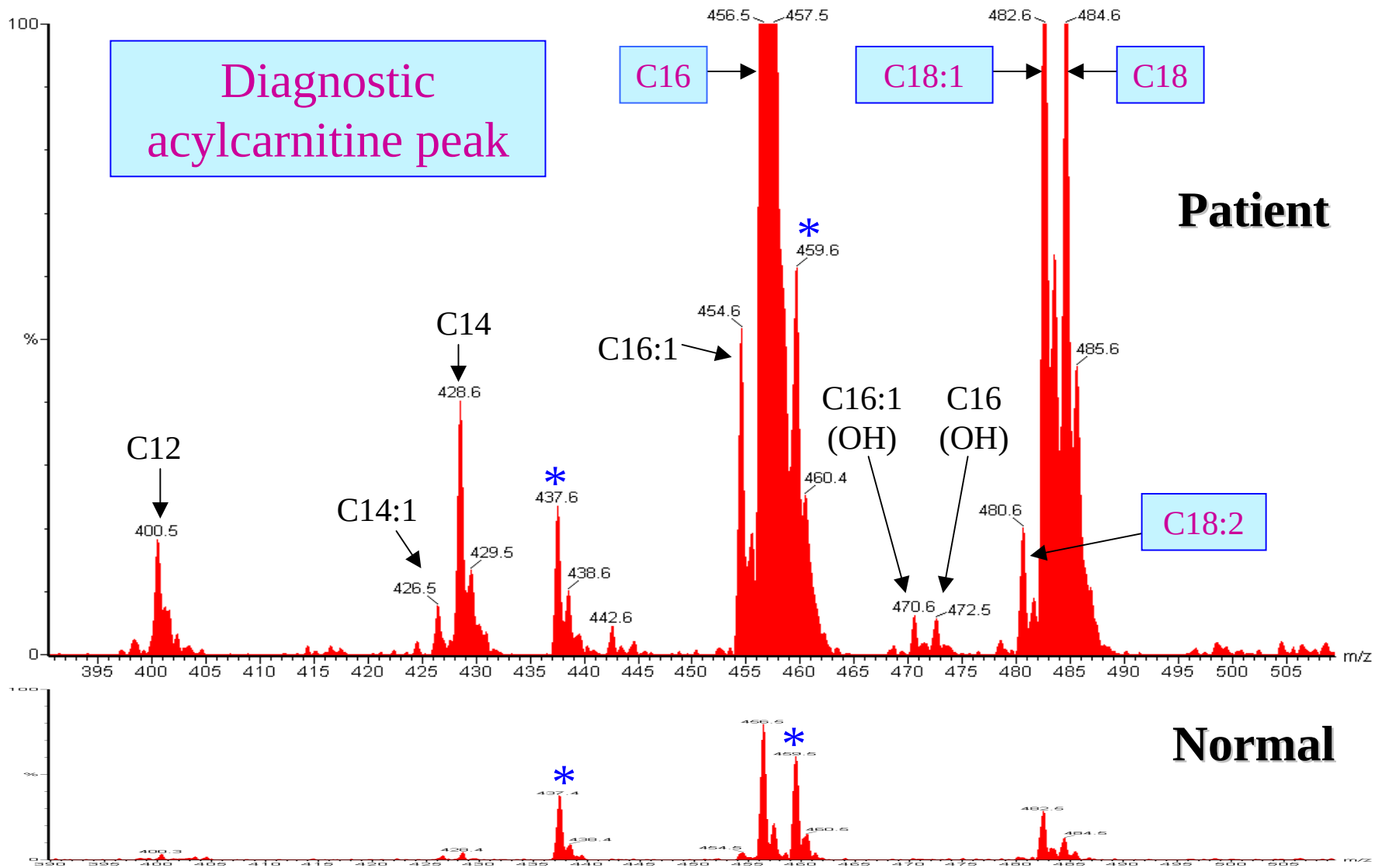
CPT-II (scaled to CO Int. Std.)



CPT-II


Patient
100
%
0
456.5
457.5
482.6
484.6
459.6
454.6
485.6
428.6
460.4
437.6
480.6
400.5
429.5
426.5
438.6
470.6
472.5
442.6
395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505
m/z
Normal
100
%
0
456.5
459.5
437.4
482.5
460.5
484.5
438.4
454.5
400.3
428.4
390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505
m/z

CPT-II


Diagnostic acylcarnitine peak
C16
C18:1
C18
Patient
C12
C14
C14:1
C16:1
C16:1 (OH)
C16 (OH)
C18:2
Normal
100
%
0
m/z

ß-Ketothiolase deficiency

- Defect: deficiency in enzyme that converts 2-methylacetoacetyl-CoA to propionyl-CoA and acetyl-CoA
  - ß-Ketothiolase – sixth step of isoleucine pathway
- Autosomal recessive inheritance
- Neonatal presentation is rare
- Clinical heterogeneity in presentation:
  - recurrent, severe metabolic acidosis with ketosis
  - vomiting and diarrhoea
  - lethargy

ß-Ketothiolase deficiency

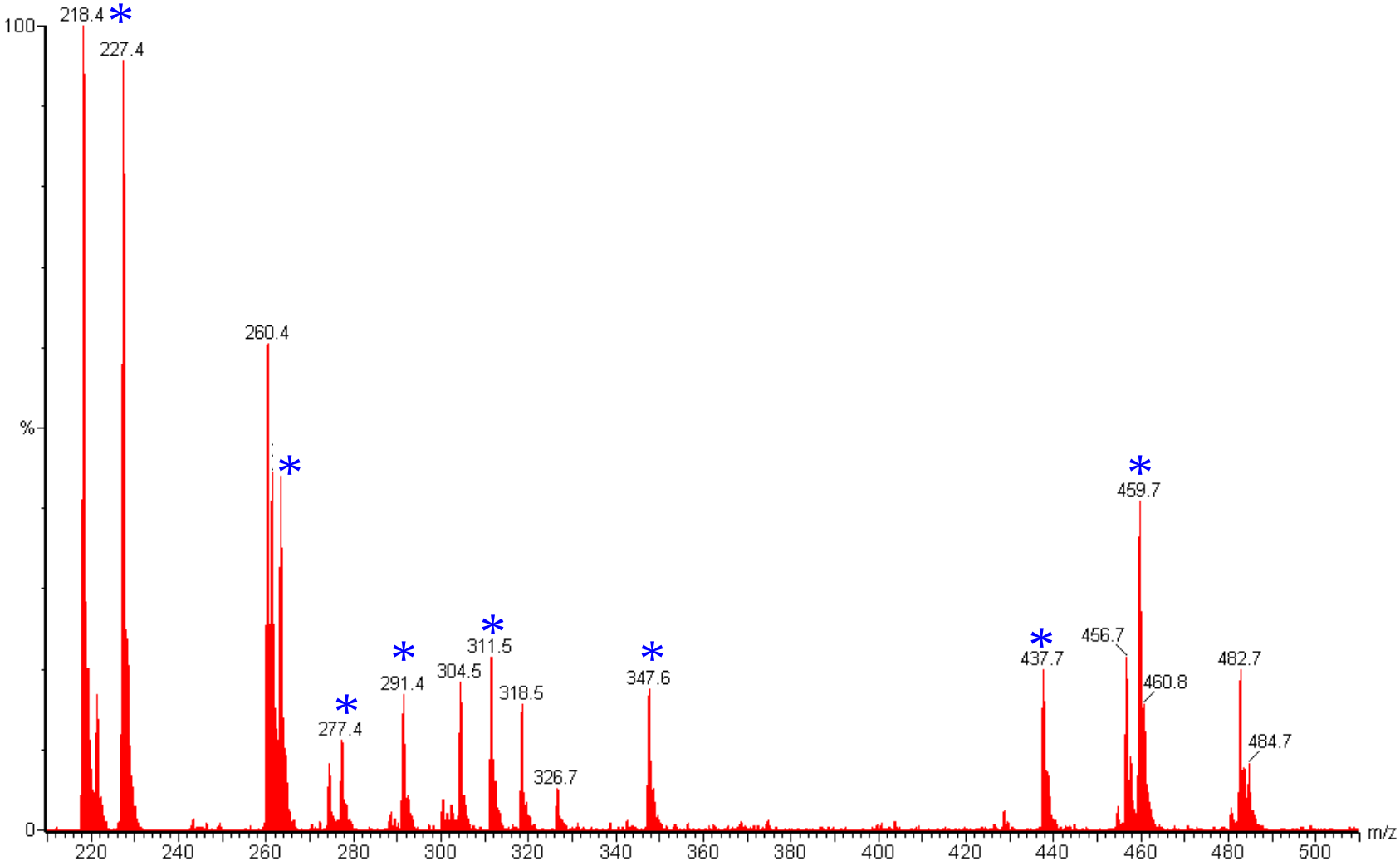
- Urine organic acids:
  - raised 2-methyl-3-hydroxybutyrate
  - 2-methylacetoacetate
  - tiglylglycine
  - ketone bodies
- Acylcarnitine Profile
  - raised C5(OH) (2-Methyl-3-hydroxy-butyrylcarnitine), C5:1 (tiglylcarnitine)

ß-Ketothiolase deficiency

- Treatment:
  - avoid prolonged fasting
  - restricted isoleucine intake
  - bicarbonate therapy and i.v. glucose during acute crises
  - carnitine supplementation.

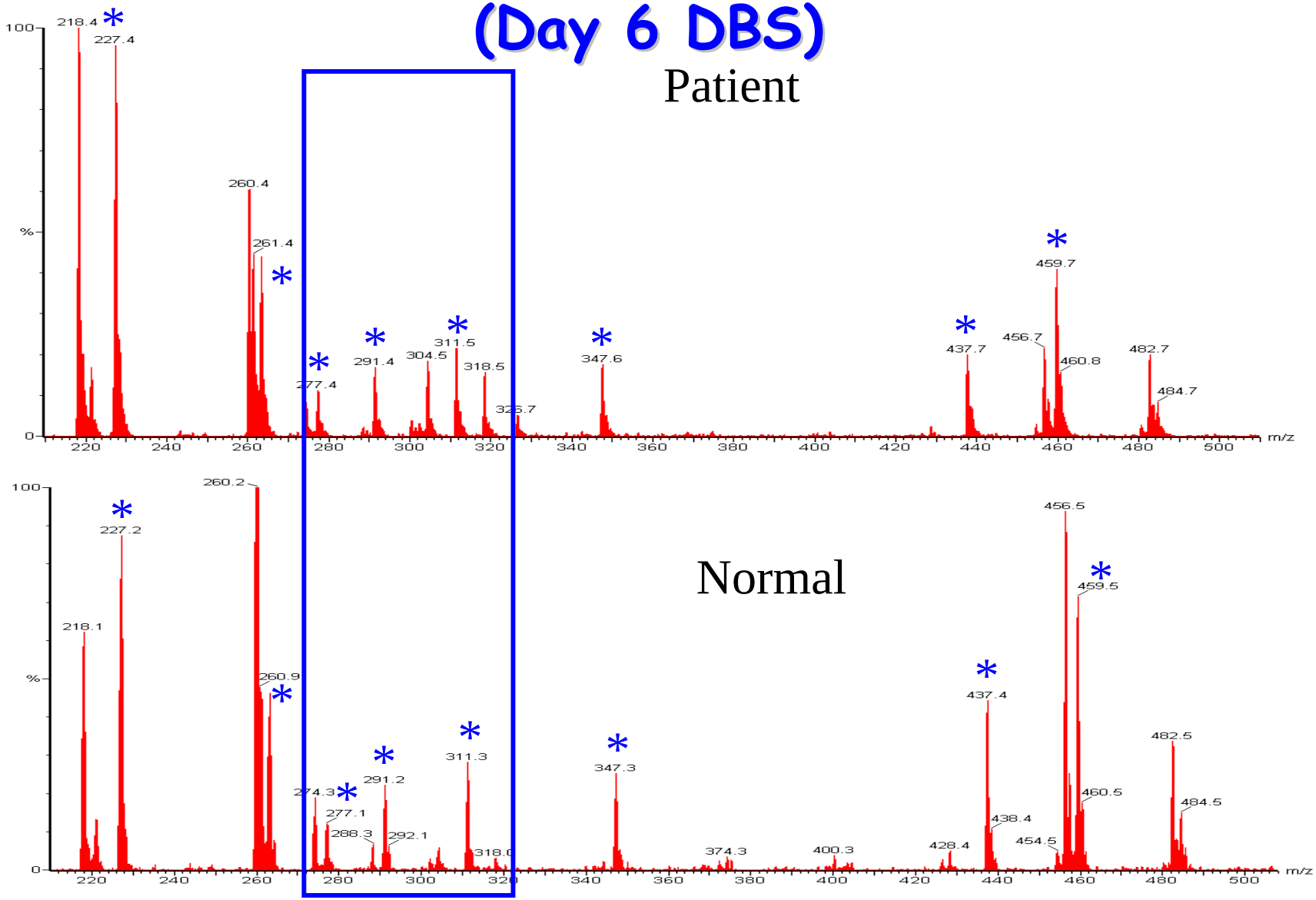
ß-Ketothiolase deficiency

(Day 6 DBS)



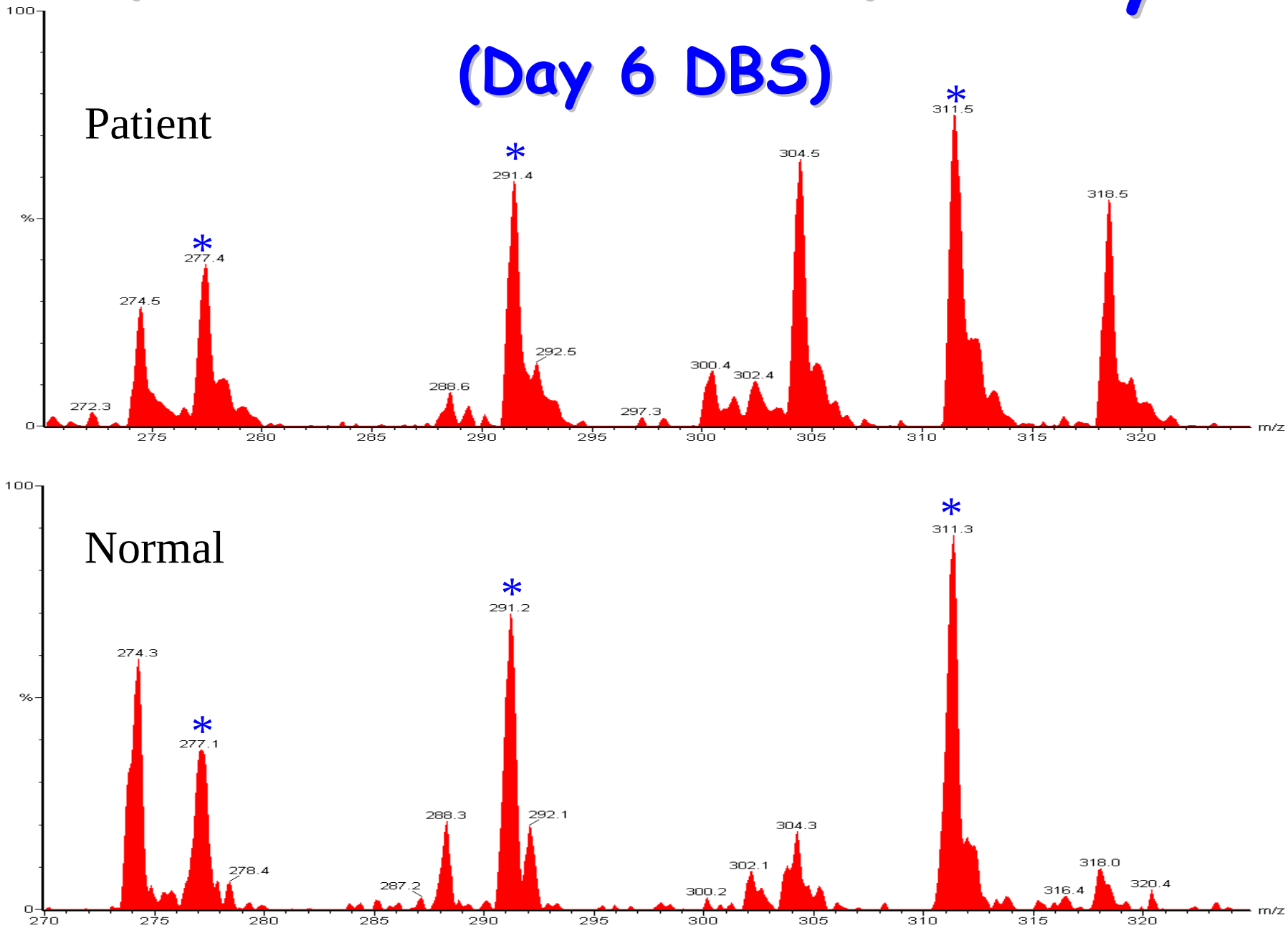
ß-Ketothiolase deficiency

(Day 6 DBS)



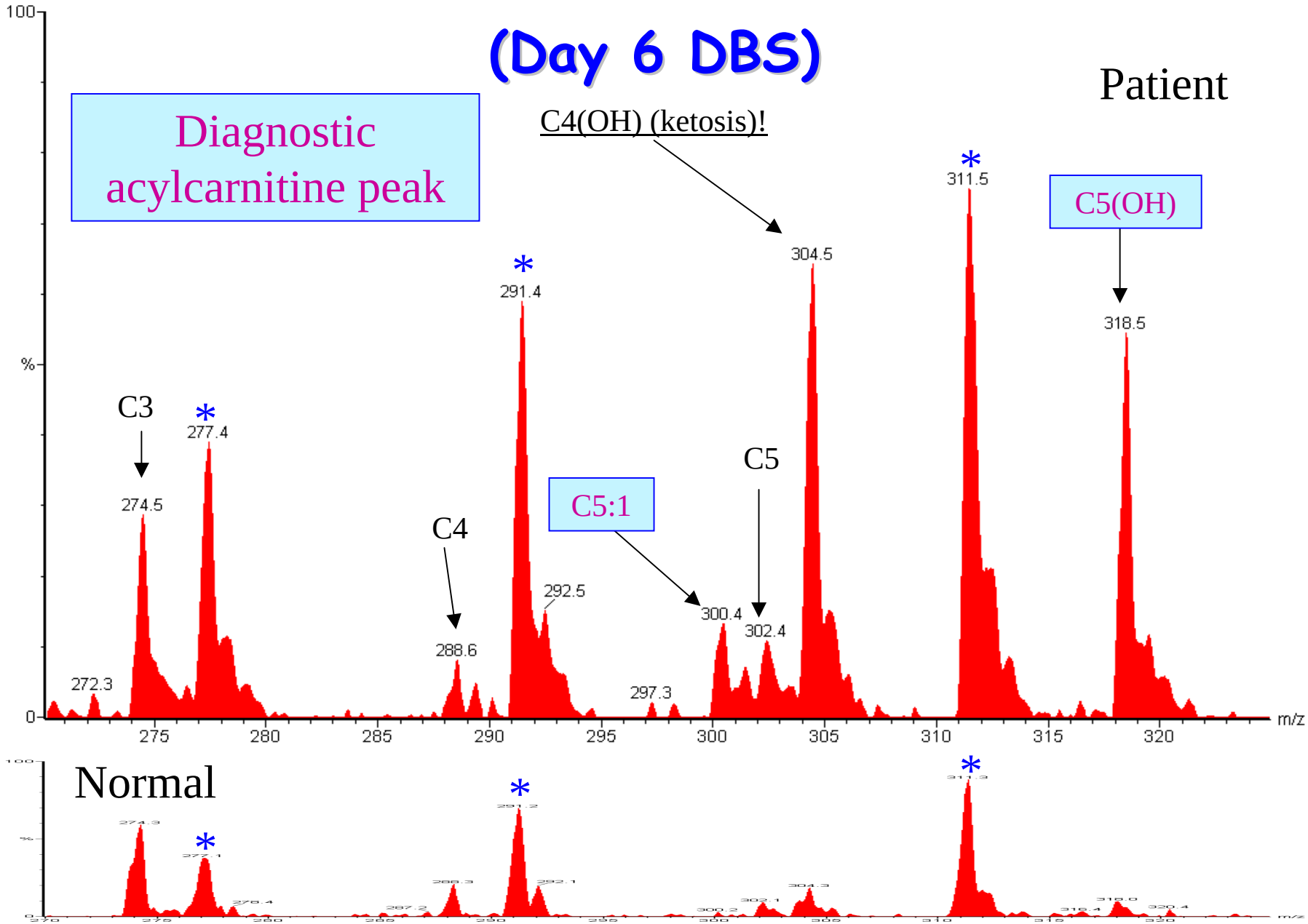
ß-Ketothiolase deficiency

(Day 6 DBS)



ß-Ketothiolase deficiency

(Day 6 DBS)



Methylmalonic aciduria (MMA)

- Enzyme: methylmalonyl CoA mutase
  - catalyses formation of succinyl CoA from methylmalonyl CoA in branched chain amino-acid catabolism pathway
  - enzyme requires Vitamin B12 as a co-factor
- Autosomal recessive inheritance
- Various forms including Vit B12 responsive & non-responsive

MMA

- Wide clinical spectrum
- Presentation:
  - gross ketosis
  - metabolic acidosis
  - recurrent vomiting → dehydration
  - Failure to thrive
  - hyperammonaemia → mental retardation
  - characteristic facial features (eg low set ears, high forehead broad nasal bridge etc)
  - hypotonia
  - death if not treated

MMA

- Urine organic acids: Raised
  - Methylmalonate
  - Methylcitrate
  - 3-OH-propionate
- Acylcarnitine profile:
  - Raised C3 propionyl carnitine

MMA

- Treatment:
  - protein-restricted diet (nb isoleucine, threonine etc are essential amino acids for normal growth & development)
  - Vitamin B12 injections
  - carnitine supplementation (replace intracellular stores)
  - oral antibiotic therapy (decrease gut propionate production)

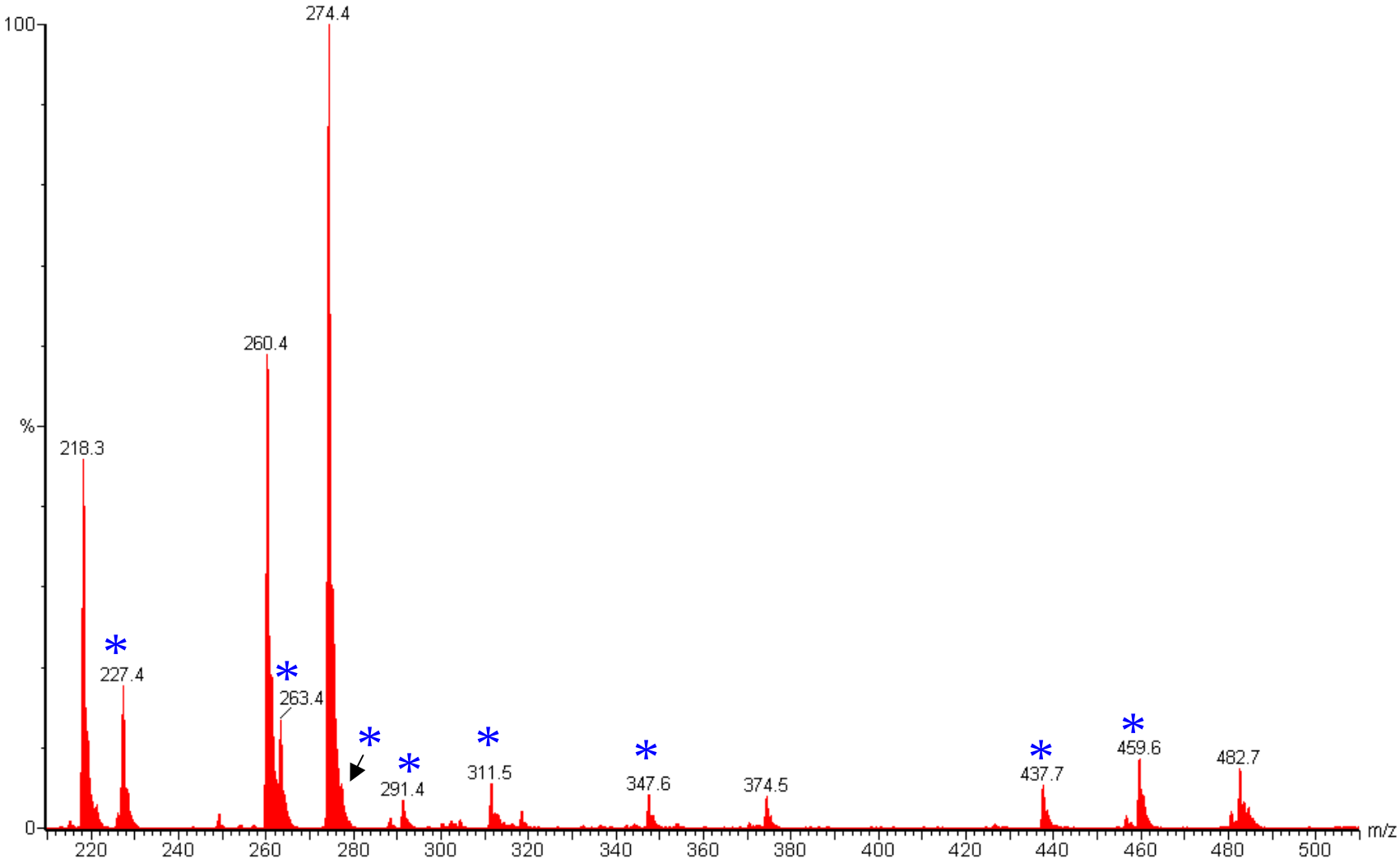
Propionic aciduria (PA)

- Defect – deficiency of enzyme Propionyl CoA carboxylase
  - catalyses formation of methylmalonyl CoA from Propionyl-CoA in branched-chain amino acid catabolism
  - biotin-dependent enzyme
- Autosomal recessive inheritance
- Similar presentation to MMA (one stage upstream in metabolic pathway from MMA)

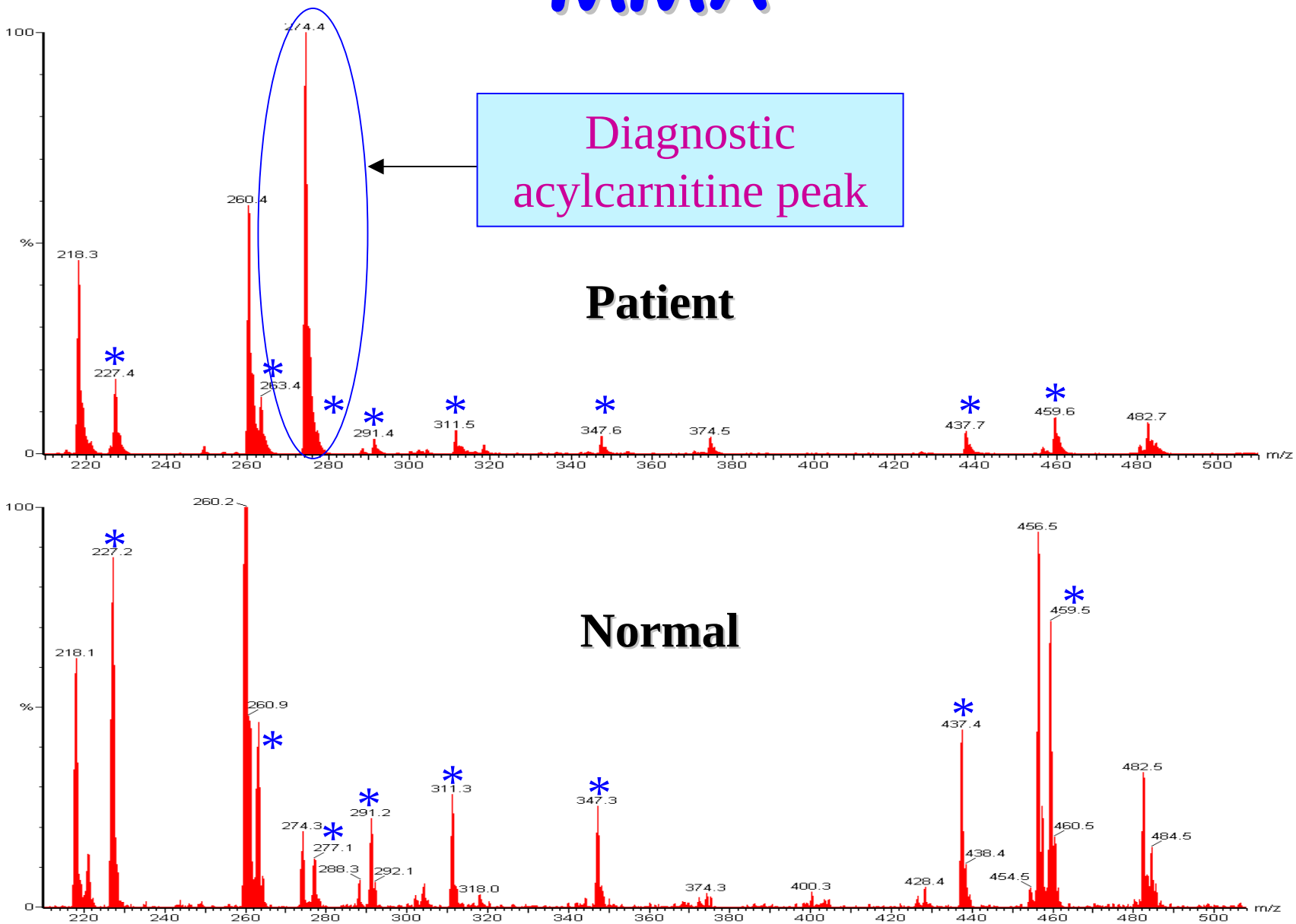
PA

- Urine organic acids: Raised
  - 3-OH-propionate
  - propionate
  - methyl citrate
  - propionylglycine & tiglylglycine
- Acylcarnitine profile:
  - raised C3 propionyl carnitine
- Treatment:
  - protein-restricted diet
  - carnitine supplementation (replace intracellular stores)
  - oral antibiotic therapy (decrease gut propionate production)

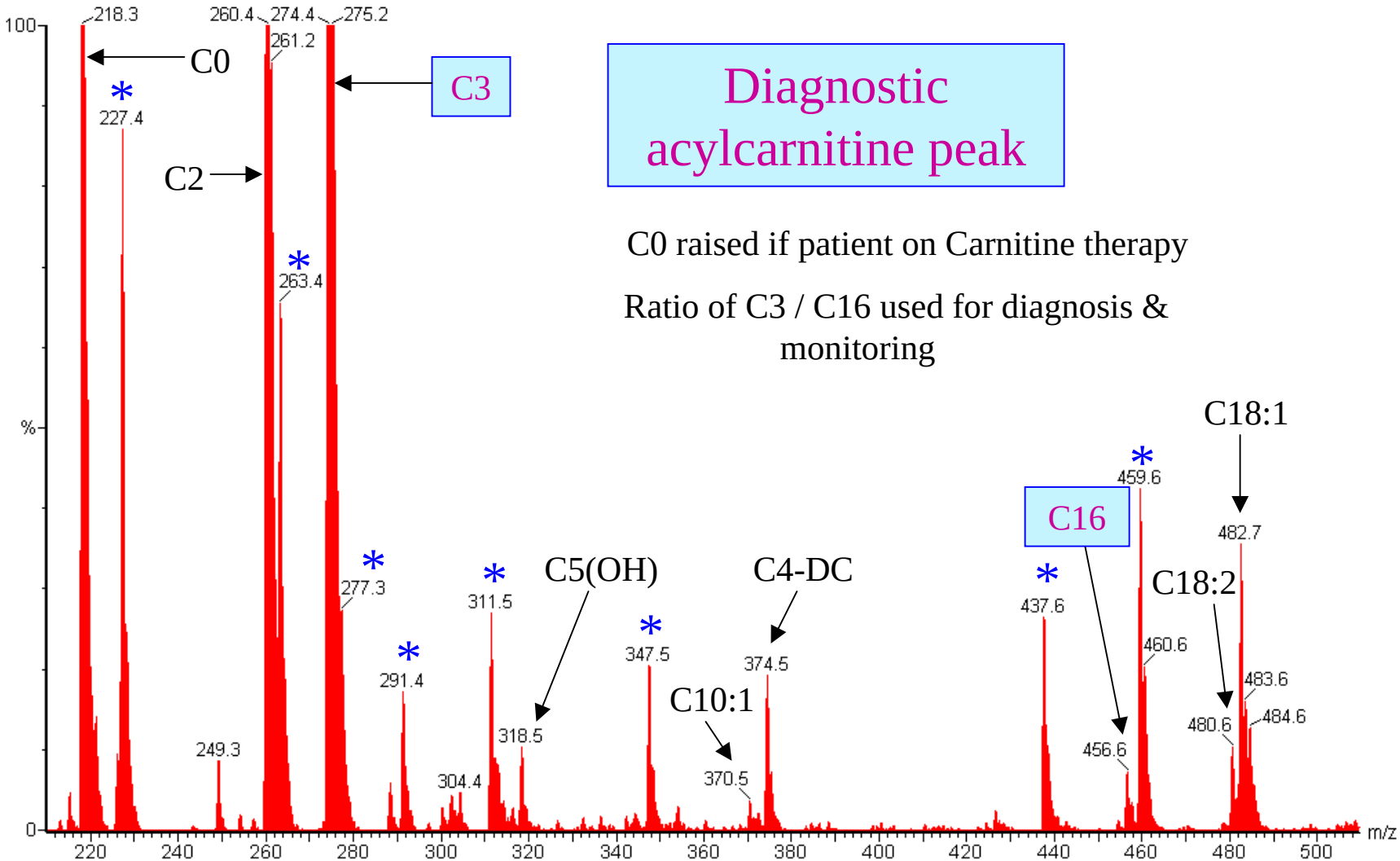
MMA


100
%
0
274.4
260.4
218.3
227.4
263.4
291.4
311.5
347.6
374.5
437.7
459.6
482.7
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

MMA


Diagnostic acylcarnitine peak
Patient
Normal
100
%
0
m/z
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
274.4
260.4
218.3
227.4
263.4
291.4
311.5
347.6
374.5
437.7
459.6
482.7
260.2
227.2
218.1
260.9
274.3
277.1
288.3
291.2
292.1
311.3
318.0
347.3
374.3
400.3
428.4
437.4
438.4
454.5
456.5
459.5
460.5
482.5
484.5

MMA (scaled to C0 Int. Std.)



Diagnostic acylcarnitine peak

C0 raised if patient on Carnitine therapy

Ratio of C3 / C16 used for diagnosis & monitoring

Isovaleric aciduria (IVA)

- Defect: Isovaleryl-CoA dehydrogenase deficiency
  - catalyses formation of 3-methyl-crotonyl-CoA from Isovaleryl-CoA during leucine catabolism
- Autosomal recessive inheritance

IVA

- Presentation includes:
  - vomiting
  - metabolic acidosis & ketosis
  - characteristic odour 'sweaty feet'
  - failure to thrive
  - hypotonia
  - encephalopathy

IVA

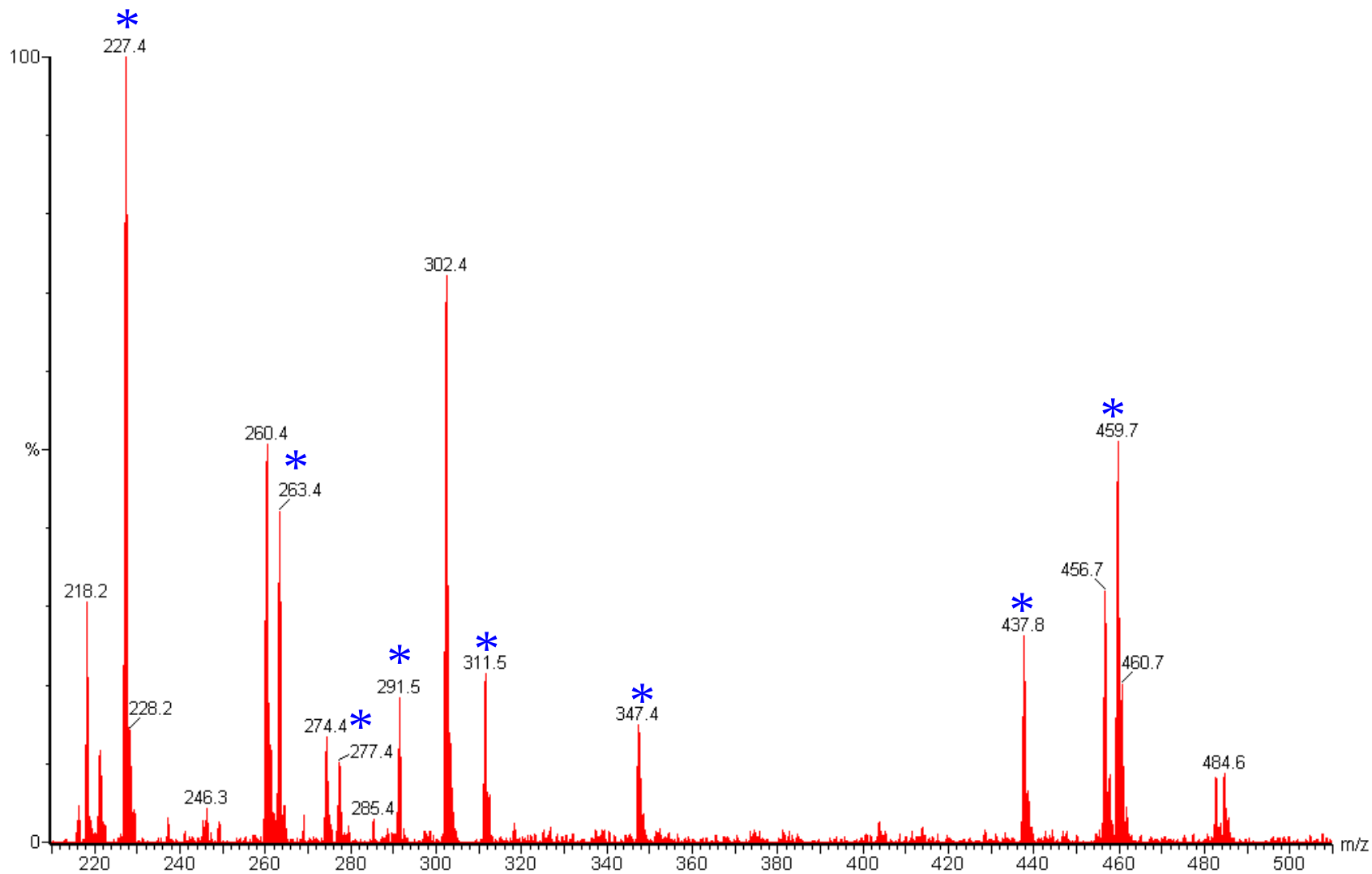
- Urine organic acids: Raised
  - 4-hydroxyisovaleric acid
  - isovaleryl glycine
  - 3-hydroxyisovalerate
  - Methylsuccinate
  - isovalerylglucuronide

- Acylcarnititne profile:
  - Raised C5 (isovaleryl carnitine)
  - NB Pivoxilsulbactam antibiotics form m/z 302 peak (pivaloylcarnitine butyl ester)

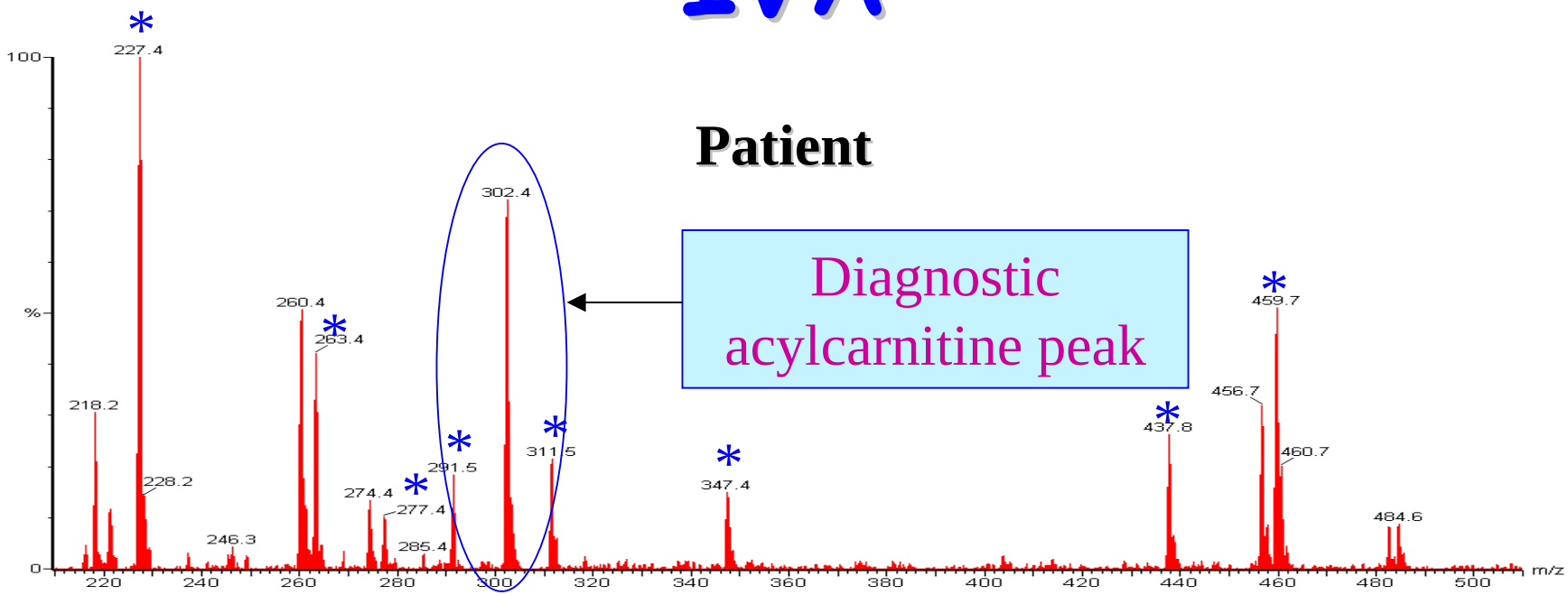
Isovaleric aciduria (IVA)

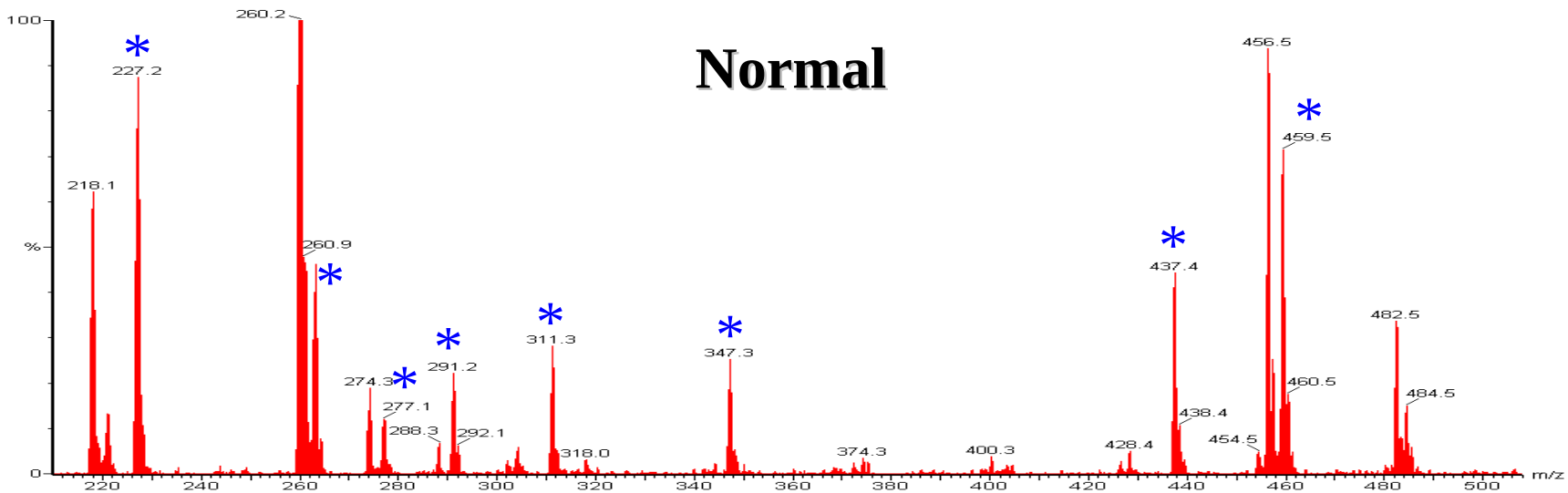
- Treatment:
  - low protein/restricted leucine diet
  - glycine supplementation (conjugates toxic metabolites)
  - carnitine supplementation

IVA

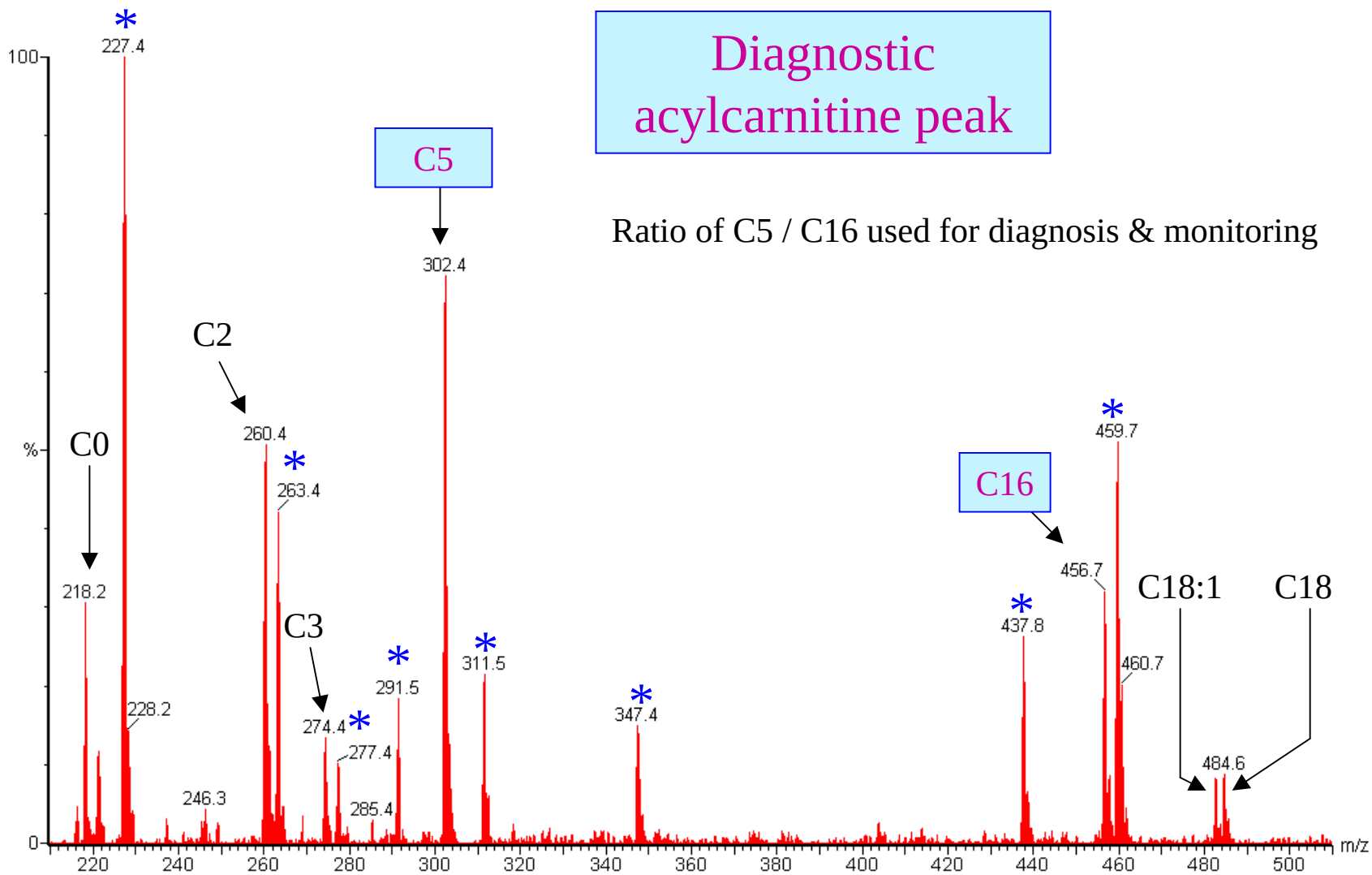

100
%
0
227.4
218.2
228.2
246.3
260.4
263.4
274.4
277.4
285.4
291.5
302.4
311.5
347.4
437.8
456.7
459.7
460.7
484.6
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

IVA


Patient
Diagnostic acylcarnitine peak
100
%
0
227.4
218.2
228.2
246.3
260.4
263.4
274.4
277.4
285.4
291.5
302.4
311.5
347.4
437.8
456.7
459.7
460.7
484.6
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z


Normal
100
%
0
227.2
218.1
260.2
260.9
274.3
277.1
288.3
291.2
292.1
311.3
318.0
347.3
374.3
400.3
428.4
437.4
438.4
454.5
456.5
459.5
460.5
482.5
484.5
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

IVA


Diagnostic acylcarnitine peak
Ratio of C5 / C16 used for diagnosis & monitoring
C0
C2
C3
C5
C16
C18:1
C18
100
%
0
227.4
218.2
228.2
246.3
260.4
263.4
274.4
277.4
285.4
291.5
302.4
311.5
347.4
437.8
456.7
459.7
460.7
484.6
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
m/z

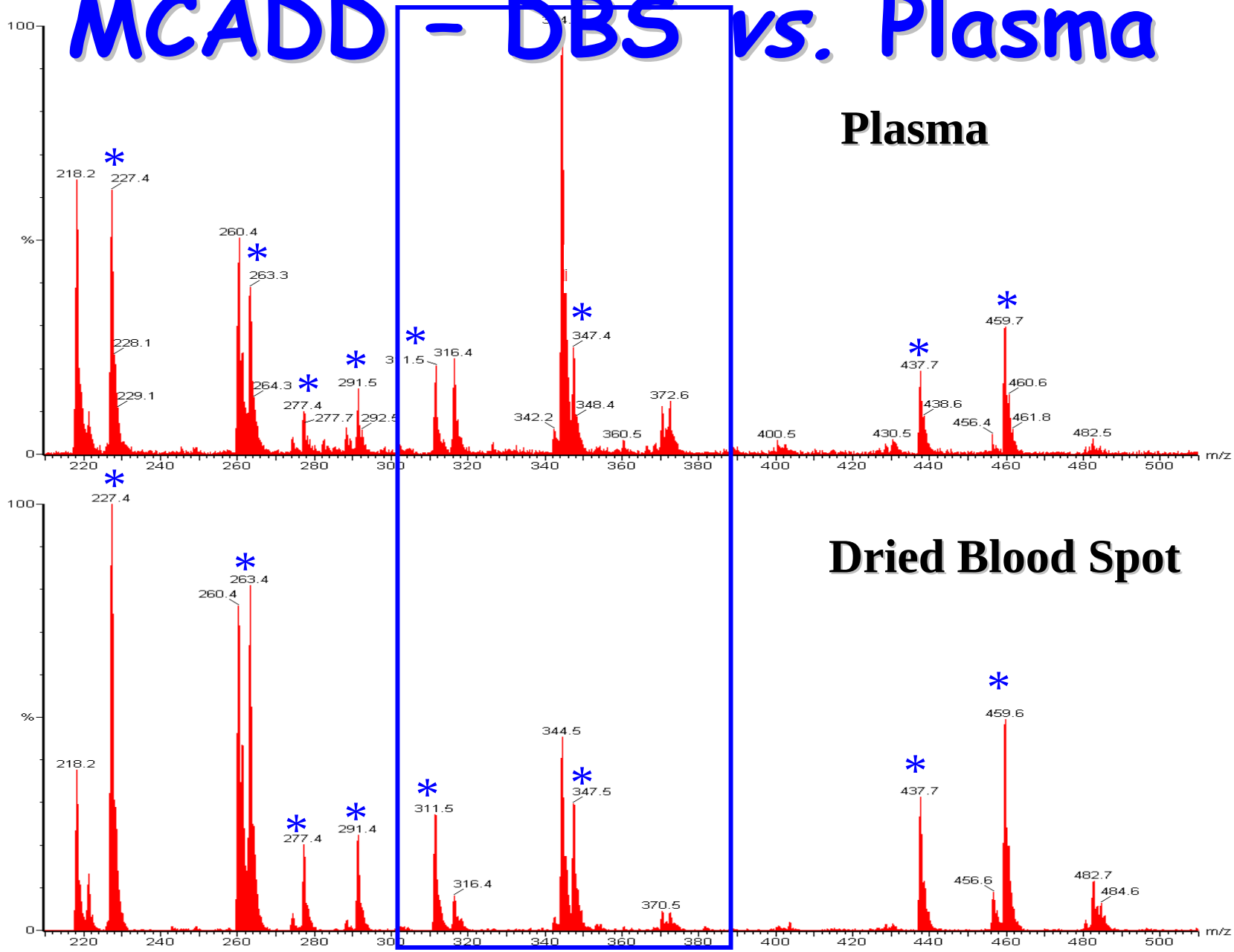
Sample type – plasma or DBS?

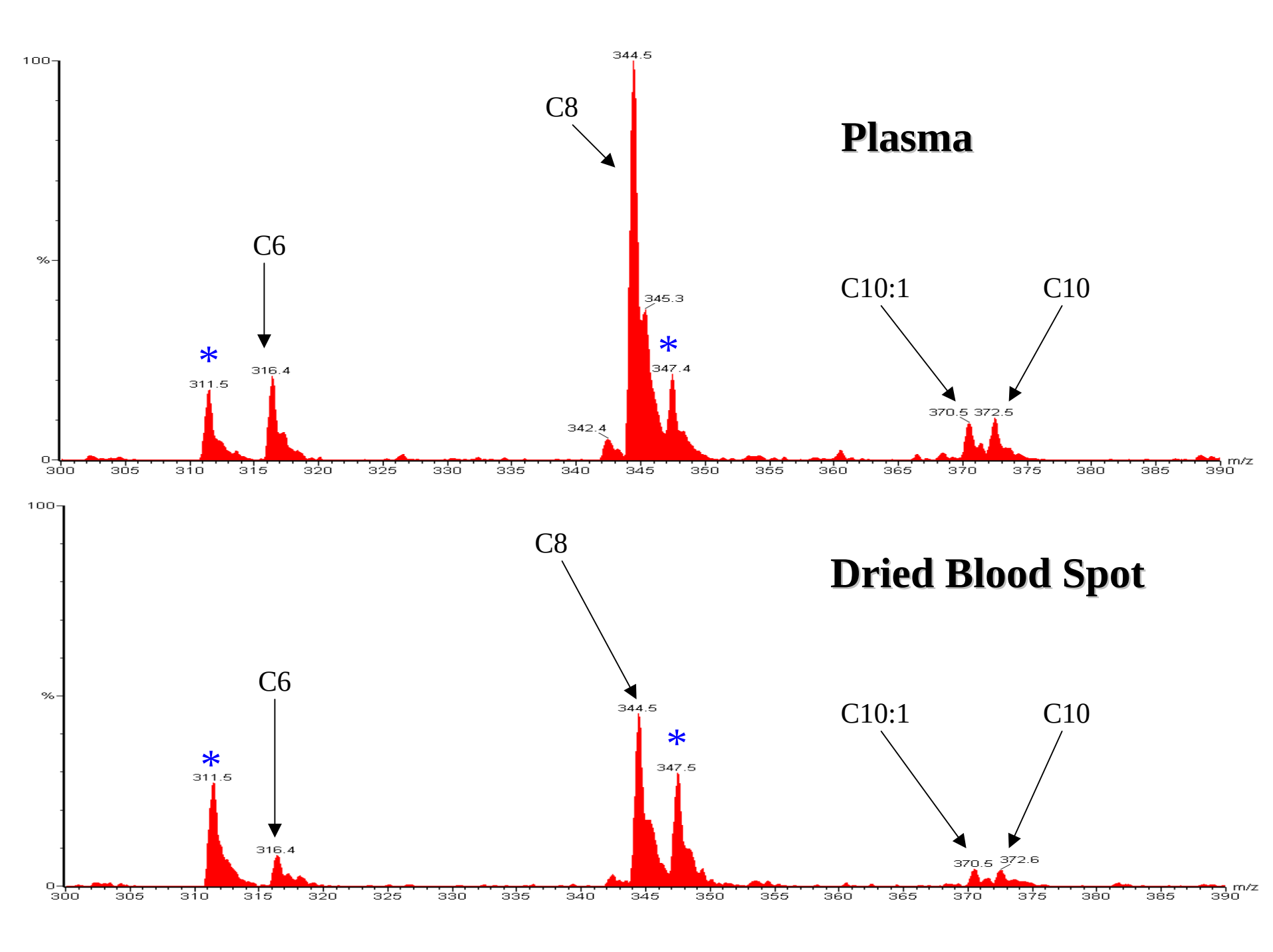
- Traditional isotope-dilution methods require liquid samples for quantitation
- Advantages of DBS
  - easy to transport (ie post to lab)
  - easy to store
  - in UK all babies have DBS taken at 6 days → a useful retrospective sample bank

Sample type – plasma or DBS?

- Disadvantages of DBS
  - requires elution from DBS (→ slower than plasma)
  - ?recovery during elution
    - ?use of ratios instead of absolute values
  - ?volume of blood per DBS - ?depends on haematocrit
- Differences between DBS & plasma
  - Altered profile – long-chain acylcarnitines reside within red cell non-polar lipid-bilayer
    - Reference ranges not directly comparable
  - Plasma maybe more representative of disease state

MCADD - DBS *vs.* Plasma




Plasma
C8
C6
C10:1
C10
344.5
345.3
347.4
316.4
311.5
342.4
370.5
372.5
100
%
0
m/z
300
305
310
315
320
325
330
335
340
345
350
355
360
365
370
375
380
385
390
Dried Blood Spot
C8
C6
C10:1
C10
344.5
347.5
316.4
311.5
370.5
372.6
100
%
0
m/z

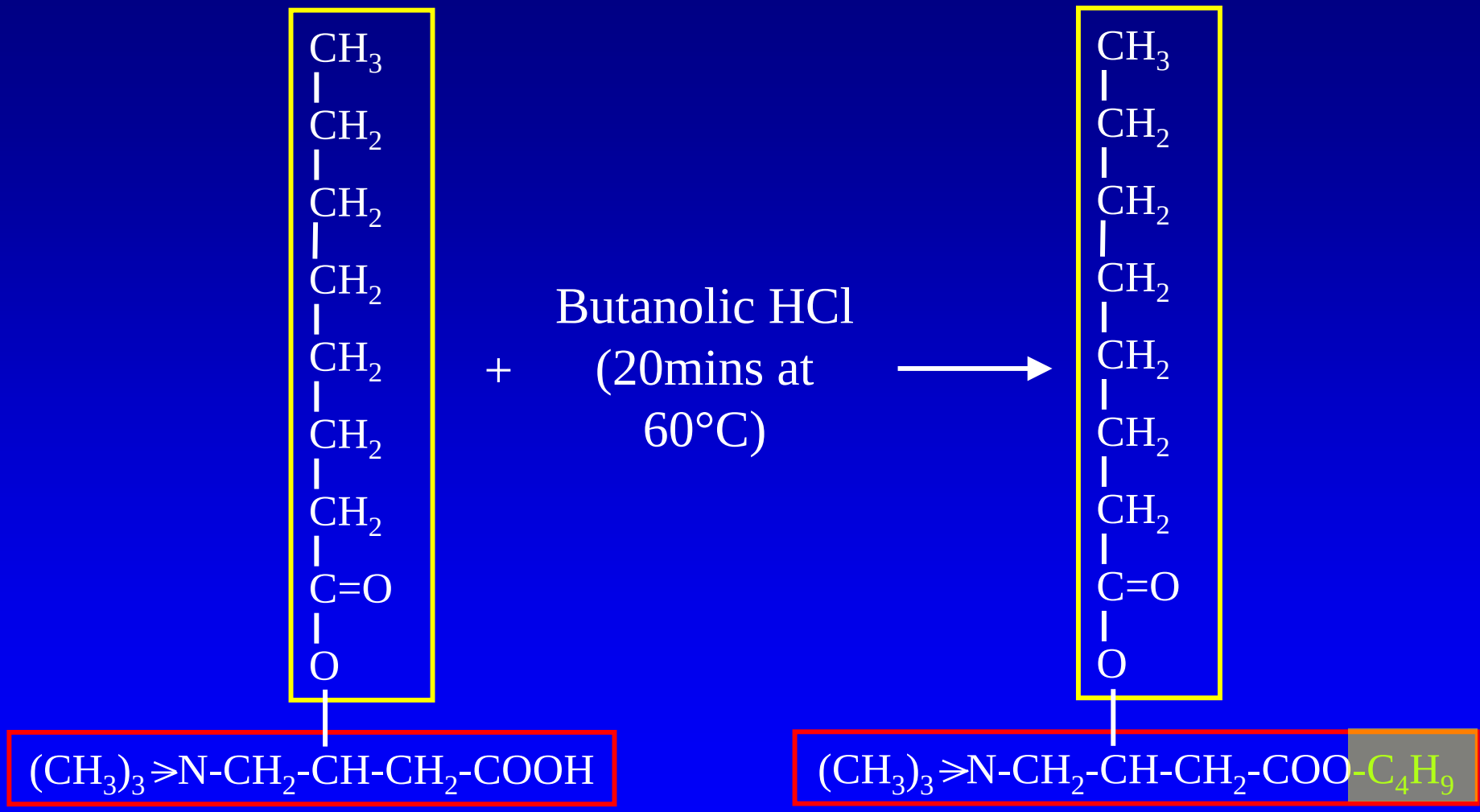
Derivatisation

- Formation of butyl esters using butanol/hydrochloric acid
- Advantages:
  - optimise ionisation & increase sensitivity
  - increased mass of derivatives reduces effect of low mass-contaminants
  - reduction of interference & ability to differentiate isobaric compounds (eg m/z 248)
  - harmonisation between centres (eg more published studies use butyl ester derivatives)

Derivatisation

- Disadvantages:
  - use of HCl - corrosive reagents
  - sample preparation for large batches more time consuming
  - possibility of acylcarnitine hydrolysis during process (→ spurious free- and acyl-carnitine levels)
  - more complicated methodology

Derivatisation



Mass (m/z) = 288

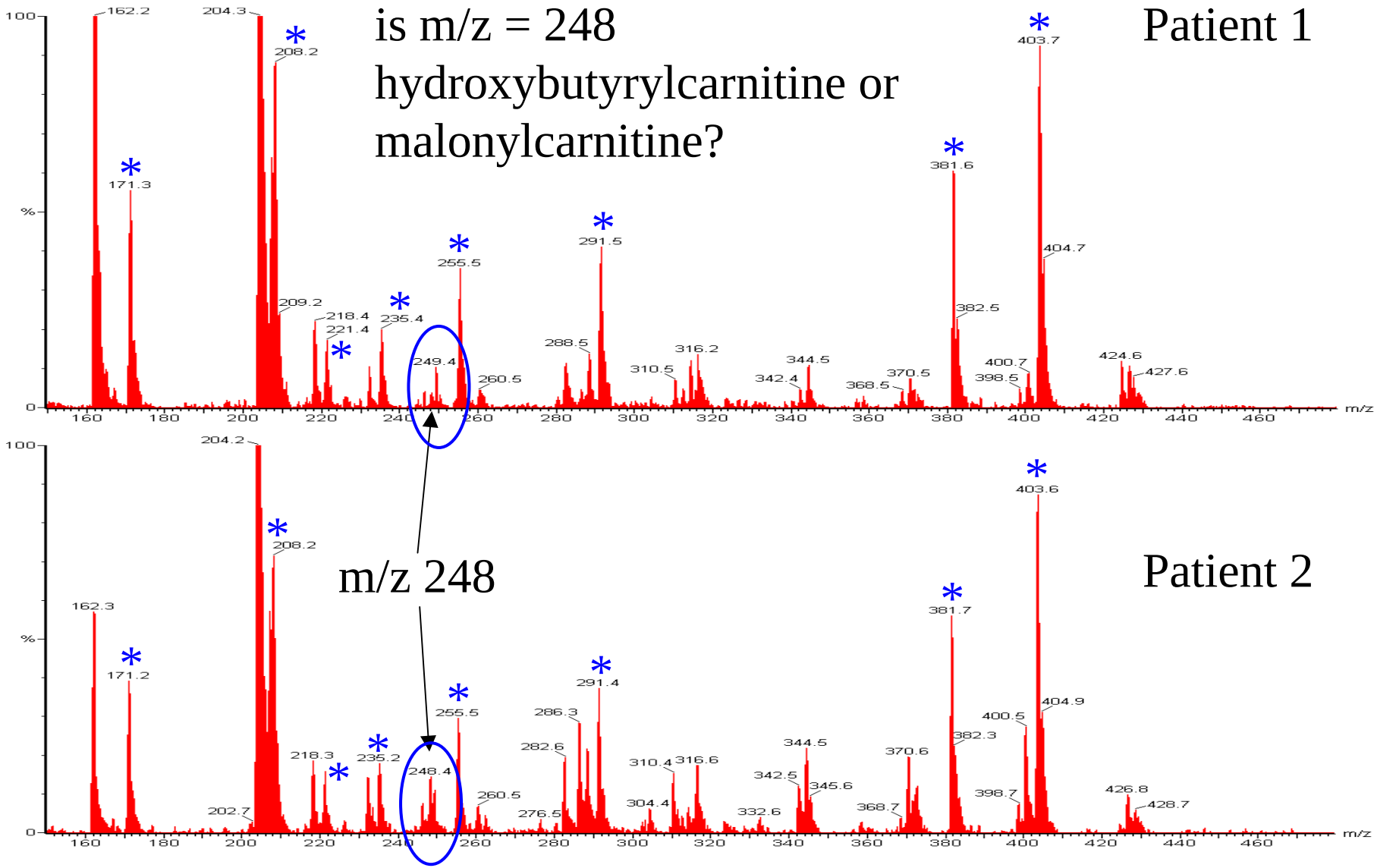
Mass (m/z) = 344

Derivatisation to distinguish between C4-OH & malonylcarnitine

- When underivatised, both have m/z = 248
  - has diagnostic implications
  - requirement to distinguish between acylcarnitine species

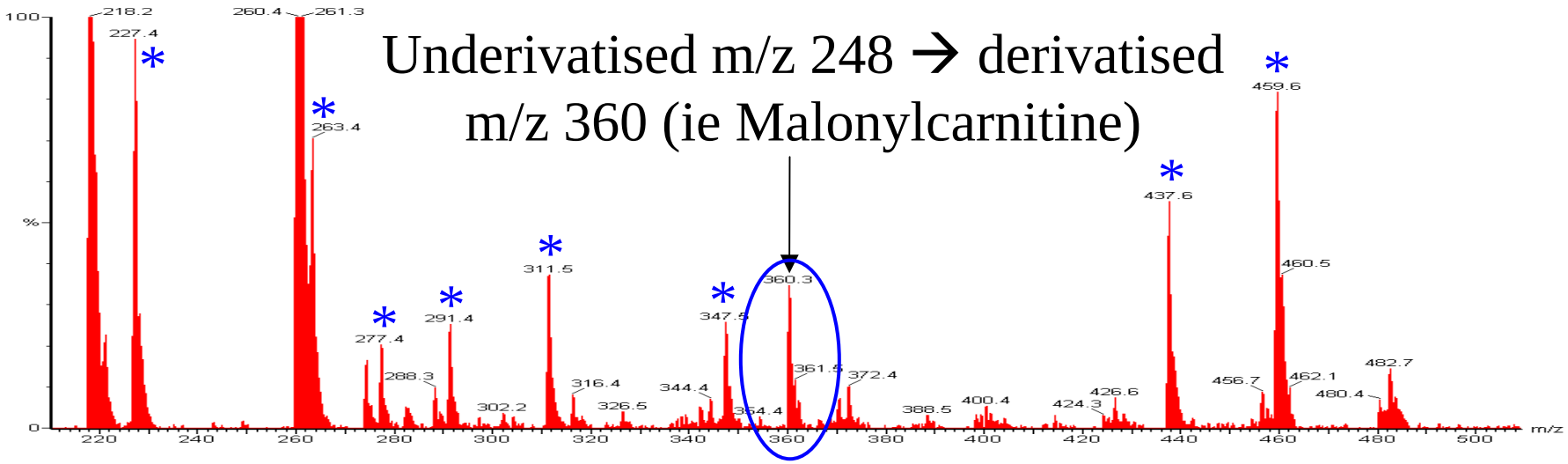
- Derivatisation by butylation → butyl-esters with different m/z values
  - can distinguish between C4-OH & malonylcarnitine

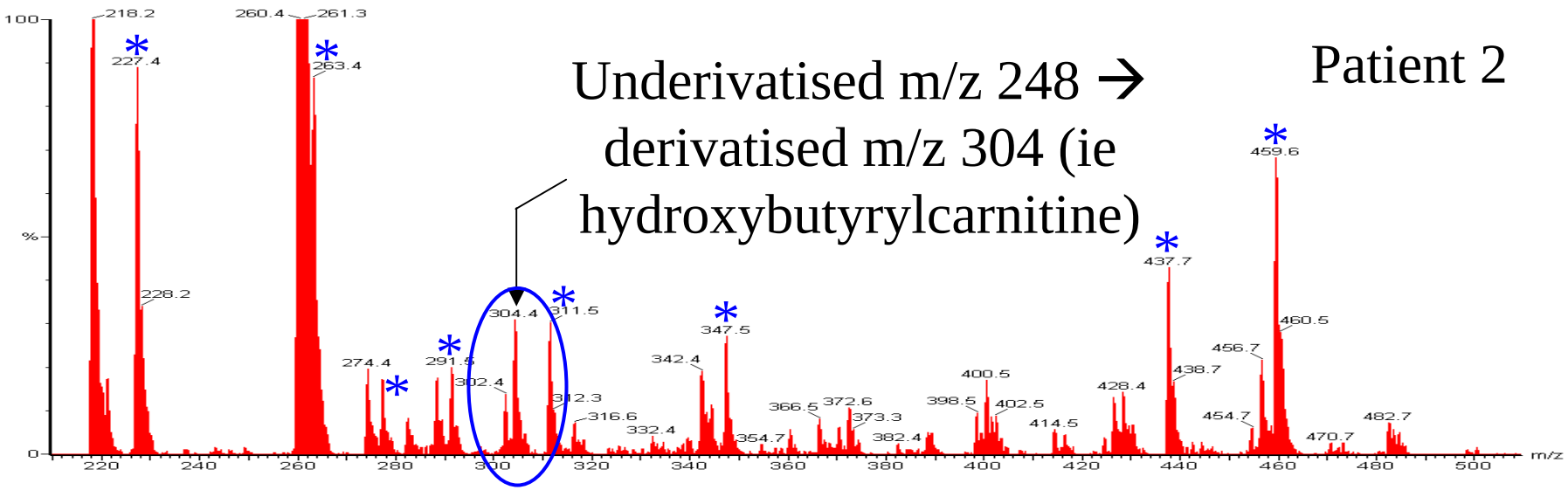
Underivatised plasma sample



Derivatised plasma sample

Patient 1





Current approach in SCH

- Underivatised
  - newborn screening
  - urgent plasma analysis
  - confirmation for routine investigation (pseudo-glutarylcarnitinaemia)
- Derivatised
  - re-run for confirmation
  - routine investigation