Services Offered

1) Quantitative Acylcarnitines by HPLC-MS including Total Carnitine, Free Carnitine, and Butyrobetaine

2) Carnitine Alone (Total, Free, Butyrobetaine)

Sample Types (minimum amounts):
Plasma (50 µl)
Skeletal Muscle (5 mg)
Urine (350 µl)

Acylcarnitine Analysis

	HPLC-MS/MS	Tandem MS
Separation of Isomers	YES	NO
Synthesized Standards	YES	NO
Accurate Quantitation	YES	NO

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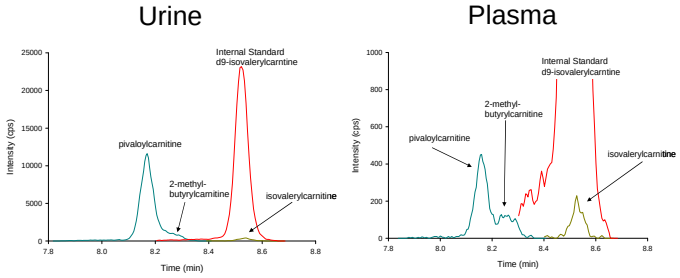
Plasma and Urine from Patient containing Pivaloylcarnitine

The concentration in urine is 5.63 µmol/g creatinine.
The concentration in plasma is 0.05 µmol/L



Among the nation's leading academic medical centers, University Hospitals Case Medical Center is the primary affiliate of Case Western Reserve University School of Medicine, a nationally recognized leader in medical research and education.

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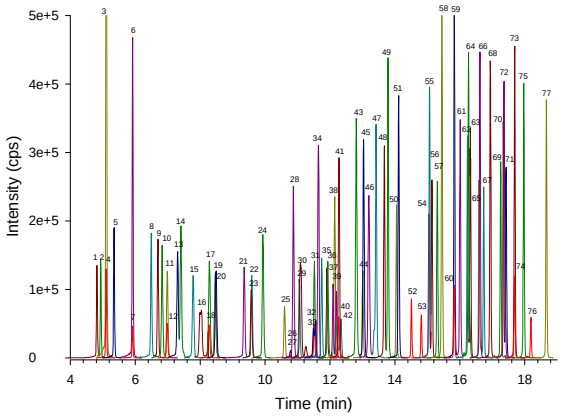
Carnitine and Acylcarnitine Analysis using HPLC-MS/MS



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BULLET GUIDE TO ACYLCARNITINE ANALYSIS BY HPLC-MS/MS

Follow-up to Newborn Screening by analogy to Amino Acid Analysis

Newborn Screening of Amino Acids

- Tandem MS
- Follow-up to Positive Samples
  - Reanalyze using chromatographic method
    - Accurate quantification
    - Resolution of isomers (e.g. leucine and isoleucine)
    - False positives identified

Newborn Screening of Acylcarnitines

- Tandem MS
- Follow-up to Positive Samples
  - Reanalyze using HPLC-MS/MS
    - Accurate quantification
    - Resolution of isomers (e.g. butyryl- and isobutyrylcarnitine)
    - False positives identified

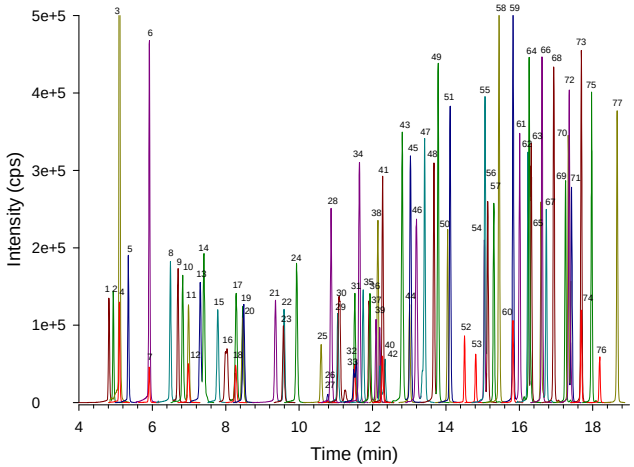
Carnitine and Acylcarnitine Analysis by HPLC-MS/MS

We developed a validated HPLC-MS/MS method for the quantitative determination of carnitine and acylcarnitines

- Chromatographically removes isobaric contaminants
- Chromatographically resolves isomeric compounds
- MRM triple quadrupole detection
- Rigorously quantitative
  - Standardized compounds
  - Internal standards
  - Multiple-point calibration curves

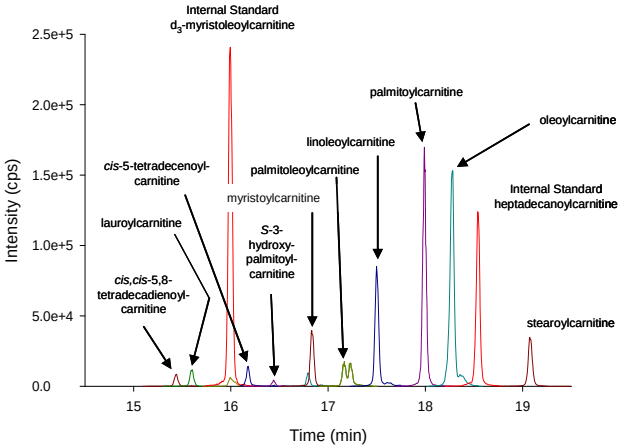
When should you use acylcarnitine analysis by HPLC-MS/MS?

- Follow-up to positive newborn screening results
- Patients with disease
  - False-negative newborn screening results
  - Not tested by newborn screening
  - Adolescent- or adult-onset disease
- Protocols for treatment and metabolism research
  - Accurate quantification of carnitine and acylcarnitine biomarkers
  - Selective, accurate, and precise to meet FDA standards



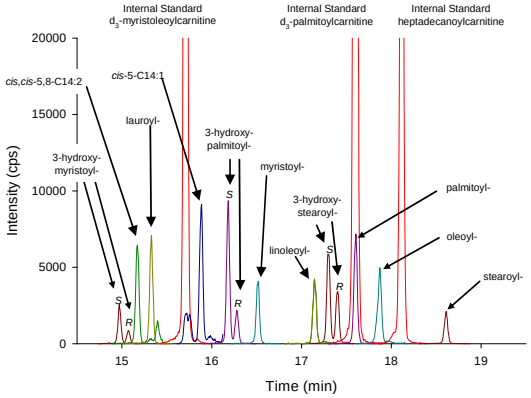
HPLC-MS/MS chromatogram of a calibration curve high point. Overlaid XICs of 77 transitions monitored for 65 compounds and 12 internal standards (internal standards are colored red).

Peak Identities: 1) *S*-3-hydroxy-butyryl-, 2) *R*-3-hydroxy-butyryl-, 3) acetyl-, 4) d6-acetyl-, 5) 3-hydroxy-isovaleryl-, 6) propionyl-, 7) d3-propionyl-, 8) *S*-3-hydroxy-hexanoyl-, 9) *R*-3-hydroxy-hexanoyl-, 10) isobutyryl-, 11) butyryl-, 12) d3-butyryl-, 13) tigloyl-, 14) 3-methyl-crotonyl-, 15) benzoyl-, 16) 2-methyl-butyryl-, 17) isovaleryl-, 18) d9-isovaleryl-, 19) valeryl-, 20) phenylacetyl-, 21) *S*-3-hydroxy-octanoyl-, 22) *R*-3-hydroxy-octanoyl-, 23) phenylpropionyl-, 24) hexanoyl-, 25) 4-phenyl-butyryl-, 26) malonyl-, 27) d3-malonyl-, 28) 4-methyl-hexanoyl-, 29) succinyl-, 30) *cis*-3,4-methylene-heptanoyl-, 31) glutaroyl-, 32) d3-glutaroyl-, 33) methyl-malonyl-, 34) valproyl-, 35) *S*-3-hydroxy-decanoyl-, 36) *R*-3-hydroxy-decanoyl-, 37) adipoyl-, 38) 5-decynoyl-, 39) 3-methyl-glutaroyl-, 40) ethyl-malonyl-, 41) octanoyl-, 42) d3-octanoyl-, 43) 2,6-dimethyl-heptanoyl-, 44) suberoyl-, 45) 4-methyl-octanoyl-, 46) *cis*-3,4-methylene-nonanoyl-, 47) *cis*-4-decenoyl-, 48) *S*-3-hydroxy-lauroyl-, 49) *R*-3-hydroxy-lauroyl-, 50) sebacoyl-, 51) decanoyl-, 52) undecanedioyl-, 53) undecanoyl-, 54) *S*-3-hydroxy-myristoyl-, 55) *trans*-2-dodecenoyl-, 56) *R*-3-hydroxy-myristoyl-, 57) *cis*,*cis*-5,8-tetradecadienoyl-, 58) lauroyl-, 59) myristoleoyl-, 60) d3-myristoleoyl-, 61) *cis*-5-tetradecenoyl-, 62) *S*-3-hydroxy-palmitoyl-, 63) *R*-3-hydroxy-palmitoyl-, 64) *trans*-2-tetradecenoyl-, 65) a-linolenoyl-, 66) myristoyl-, 67) g-linolenoyl-, 68) palmitoleoyl-, 69) linoleoyl-, 70) *S*-3-hydroxy-stearoyl-, 71) *R*-3-hydroxy-stearoyl-, 72) *trans*-2-hexadecenoyl-, 73) palmitoyl-, 74) d3-palmitoyl-, 75) oleoyl-, 76) heptadecanoyl-, 77) stearoyl-.



Skeletal Muscle from Patient with Elevated Long-Chains

All in nmol/g wet weight: *cis,cis*-5,8- tetra-decadienoyl- (2.76), lauroyl- (2.20), *cis*-5-tetradecenoyl- (2.74), *S*-3-hydroxy-palmitoyl- (1.19), myristoyl- (8.84), palmitoleoyl- (4.85), linoleoyl- (34.3), palmitoyl- (46.2), oleoyl- (75.2), stearoyl- (17.8)



Plasma from Patient with LCHAD Deficiency

All in µmol/l: *S*-3-hydroxy-myristoyl- (0.10), *R*-3-hydroxy-myristoyl- (<0.05), *cis,cis*-5,8- tetradecadienoyl- (0.26), lauroyl- (0.13), *cis*-5-tetradecenoyl- (0.25), *S*-3-hydroxy-palmitoyl- (0.25), *R*-3-hydroxy-palmitoyl- (0.06), myristoyl- (0.08), linoleoyl- (0.12), *S*-3-hydroxy-stearoyl- (0.15), *R*-3-hydroxy-stearoyl- (0.12), palmitoyl- (0.13), oleoyl- (0.12), stearoyl- (<0.05)