AcylCarnitine Profile
Service Code: Acar

Summary: Profile 30 acylcarnitine species in plasma, serum, or tissues by solvent protein crash. Separated by RPLC in a 20 min cycle. All analytes and Internal Standards are measured by ESI+ on a LC-QQQ mass spectrometer using MRM methods and reported as uM normalized to wet tissue weight. CV's are generally 10%.

Container: Eppendorf Tube or equivalent
Normal Volume: Plasma (100 ul) Tissue (50-100 mgs); Cells (2E7).
Minimal Volume: Plasma (50 uL)Tissue (30 mg); Cells (~5E6)
Special Handling: If human or primate, note any known presence of infectious agents.
Sample Collection: Snap freeze by liquid nitrogen. For tissues, resect and snap-freeze as soon as practical in tared centrifuge tube. Provide both sample weight and tared vial weight on sample submission

Reference: Donald H. Chace, James C. DiPerna, Brenda L. Mitchell, Bethany Sgroi, Lindsay F. Hofman and Edwin W. Naylor (2001) "Electrospray Tandem Mass Spectrometry for Analysis of Acylcarnitines in Dried Postmortem Blood Specimens Collected at Autopsy from Infants with Unexplained Cause of Death" *Clinical Chemistry* 4(7): 1166-1182.

Table I: Analytes (30) reported. Others on special request:

Analyte	Abbr.	Mol Formula	PubCHEM	Typical(b) or (u) (uM)	Transition	LOQ(uM)
L-carnitine	Carn	C7H15NO4	10917	30(b)	162.1->85	0.05
Acetyl-	C2	C9H17NO4	1	5.5(b)	204.1 -> 85.0	0.05
Propionyl-	C3	C10H19NO4	107738	0.35(b)	218.2 -> 85.0	0.05
Butyryl-	C4	C11H21NO4	439829	0.26(b)	235.2 -> 85.0	0.05
Valeryl-	C5	C12H23NO4	6426903	0.14(b)	246.2 -> 85.0	0.05
Glutaryl-	C5DC	C12H21NO6	53481622		276.2 -> 85.0	0.05
Hexanoyl- (caproyl-)	C6	C13H25NO4	6426853	0.08(b)	260.2 -> 85.0	0.05
Octanoyl-	C8	C15H29NO4	11953814	0.23(b)	288.2 -> 85.0	0.05
trans-2-Octenoyl-	C8:1	C15H27NO4	71464472	0.20(b)	286.2 -> 85.0	0.05
Decanoyl-	C10	C17H33NO4	11953821	0.26(b)	316.2 -> 85.0	0.05
cis-4-Decenoyl-	C10:1	C17H31NO4	71464497	0.17(b)	314.2 -> 85.0	0.05
Lauroyl-	C12	C19H37NO4	168381	0.10(b)	344.2 -> 85.0	0.05
trans-2-Dodecenoyl-	C12:1	C19H35NO4	53481671		342.2 -> 85.0	0.05
3-Hydroxy-dodecanoyl-	C12-OH	C19H37NO5			360.2 -> 85.0	0.05
Myristoyl-	C14	C21H41NO4	53477791	0.04(b)	372.3 -> 85.0	0.05

cis-5-Tetradecenoyl-	C14:1	C21H39NO4	22833575	0.06(b)	370.3 -> 85.0	0.05
Tetradecadienoyl-	C14:2	C21H37NO4	53481681		368.3 -> 85.0	0.05
3-Hydroxymyristoyl-	C14-OH	C21H41NO5	71464541		388.3 -> 85.0	0.05
Palmitoyl-	C16	C23H45NO4	461	0.11(b)	400.3 -> 85.0	0.05
Palmitoleoyl-	C16:1	C23H43NO4	53481653		398.3 -> 85.0	0.05
3-hydroxyhexadecanoyl-	C16-OH	C23H45NO5	53481691		416.3 -> 85.0	0.05
Stearoyl-	C18	C25H49NO4	6426855	0.04(b)	428.3 -> 85.0	0.05
Oleoyl- (Elaidic-, Vaccenyl-)	C18:1	C25H47NO4	6441392		426.3 -> 85.0	0.05
Linoleyl- (linoelaidyl-)	C18:2	C25H45NO4	51000598		424.3 -> 85.0	0.05
3-Hydroxy-linoleyl-	C18:2-OH	C25H45NO5	71464556		442.3 -> 85.0	0.05
Arachidoyl-	C20	C27H53NO4	533477833		454.3 -> 85.0	0.05
cis-11-Eicosenoyl-	C20:1	C27H51NO4	71464507		452.3 -> 85.0	0.05
11*cis*,14*cis*-Eicosadienoyl-	C20:2	C27H49NO4	71464509		450.3 -> 85.0	0.05
Eicosatrienoyl-	C20:3	C27H47NO4			448.3 -> 85.0	0.05
Arachidonoyl-	C20:4	C27H45NO4	53477832		456.3 -> 85.0	0.05

Table II: Internal standards and corresponding analytes

Internal Standards	**Source**	**Cat#**	**Analytes quantified**	uM
Labeled Carnitine Stds Set B	Cambridge Isotopes	NSK-B1	**all**	
		Including:	L-carnitine-d_9	152
		Including:	C2-carnitine-d_3	38
		Including:	C3,C4,C5,C8,C14-carnitines; all -d_3)	7.6
		Including:	C16-carnitine- d_3	15.2

Materials

1. Acylcarnitine authentic standards and stable-isotope labeled internal standards (see Tables I & II)
2. LC/MS grade water, acetonitrile (ACN), isopropanol (iPOH)
3. ACS grade methanol, chloroform, ammonium acetate, ammonium hydroxide
4. Benchtop Refrigerated Centrifuge capable of 13,000g with eppendorf tube rotor
5. Eppendorf Vacufuge
6. Waterbath sonicator
7. Accurate pipettors (1 uL-1000 uL)
8. Microbalance
9. Vortex mixer
10. Agilent 6410 triple quad mass spectrometer

11. Agilent 1260 LC System

PROCEDURES:

Extraction solvent preparation:

1. Solvent 1: Prepare 20 mL of 8:1:1 methanol:water:chloroform (16 mL methanol, 2 mL water, 2 mL chloroform).
2. Solvent 2: 18952.5 uL of 8:1:1, + 47.5 uL of isotope labeled Acar IS.

Plasma/Serum Sample Preparation

1. Carefully pipette 20 uL of each plasma/serum sample into a labeled eppendorf tube.
2. Add 80 uL of extraction solvent to all tubes, then vortex to mix.
3. Let sit 5 minutes in refrigerator, then vortex again. Let sit 5 more minutes, then vortex again.
4. Centrifuge 5 minutes at 15,000 xg at 4 C.
5. Transfer supernatant into a clean, labeled autosampler vial (no insert).
6. Dry all samples and reconstitute in 60 uL of 90% of H2O and ready for analysis

Tissue Sample Preparation

1. Weight out 20 mg ±5 of tissue
2. Pre-chill extraction solvent 2.
3. Keep sample tubes on dry ice until all samples are sonicated.
4. To each tube to be extracted, add 500 uL extraction solvent 2.
5. Rinse probe sonicator with methanol and wipe off with a kimwipe.
6. Keep the tube on ice while using the probe sonicator to sonicate sample at 40% output power, 20% duty cycle for 10 seconds or until tissue is homogenized. Keep the tip near the bottom of the tube to avoid ejection of solvent from tube.
7. Repeat steps 5-6 for all samples to be homogenized.
8. Vortex tubes for 10 seconds, then let sit on ice for 10 minutes.
9. Transfer 100 uL of supernatant to an autosampler vial (with insert)
10. Create a pooled sample by taking an appropriate amount of each supernatant to yield at least 100 uL for LC-MS analysis.
11. Analyze acylcarnitines by LC-QQQ.

Cell culture extraction (samples supplied on culture plates)

1. Place the sample plates and extraction solvent on dry ice.
2. Clean cell scraper with paper tissue soaked in methanol.
3. One plate at a time, move each plate on regular ice, add 1.5 mL of extraction solvent, and scrape cells, then scrape cell suspension to the side of the tilted plate.
4. Transfer cell suspension to a pre-labeled 2mL micro-centrifuge tube, place the tube on dry ice.
5. Centrifuge for 10 min at 4°C, 15,000g.
6. Transfer 600µL of supernatant to glass auto-sampler vials, store samples at -20^{o}C until LC-MS.

7. Create pooled sample by combining 10µL aliquots of each individual extract.

Cell culture extraction (samples supplied as precipitated cells in micro-centrifuge tubes)

1. Add 300µL of extraction solvent to each cell sample, vortex to completely re-suspend the pellet.
2. Sonicate at 40% output power, 20% duty cycle for 20 seconds, keep samples on ice throughout the procedure.
3. Leave for 5 minutes at 4°C or on ice, vortex.
4. Centrifuge for 5 min at 4°C, 14,000rpm.
5. Transfer 100µL of supernatant to auto-sampler vial with glass insert for LC-MS analysis.
6. Create pooled sample by combining 10µL aliquots of each individual extract.

LC-MS procedure

1. LC column: Waters X BridgeC18 1mm x 50mm; 40 °C
2. Mobile phase A: 5 mM ammonium acetate in water, adjust to pH 9.9 with ammonium hydroxide
3. Mobile phase B: acetonitrile (ACN)
4. Gradient: 0min, 0%B; 7 min 80%B, 701 min 100%B, 10min, 100%B; 16min, 0%B; flow rate: 0. 250 mL/min
5. Autosampler: 4°C, 1 uL injection (or greater if initial runs show low signal.)
6. Agilent 6410 QQQ: ESI^+, Method: QM-004-xbridg2mm_Acar+_MRM.m or equivalent
7. Relative quantitation: 0.05 ml IS to the sample, 0.1ug IS to the sample, so normalize: tissue: 0.1ug* ratio/mass, cell: 0.1ug*ratio/0.5ml