# Acyl-carnitines analysis

## Analysis summary

The assay is intended to profile 30 acyl-carnitine species in plasma, serum, or tissues. Analysis is performed by liquid chromatography – mass spectroscopy. Absolute quantitation (µM concentrations of analytes) is obtained using appropriate internal standards, data are normalized to original sample weight or volume. Assay coefficient of variation is usually within 15%.

## Acyl-carnitines internal standards

Table 1. Acyl-carnitines isotope-labeled internal standards solution stock (IS), Labeled Carnitines Set B, Cambridge Isotopes catalog# NSK-B1

|  |  |
| --- | --- |
| Reference Standard | Concentration, nmol/mL |
| d9-carnitine (free carnitine, CN) | 152 |
| d3-acetylcarnitine (C2) | 38 |
| d3-propionylcarnitine (C3) | 7.6 |
| d3-butyrylcarnitine (C4) | 7.6 |
| d9-isovalerylcarnitine (C5) | 7.6 |
| d3-octanoylcarnitine (C8) | 7.6 |
| d9-myristoylcarnitine (C14) | 7.6 |
| d3-palmitoylcarnitine | 15.2 |

## Analyte extraction and sample preparation

Extraction solvent - methanol : chloroform : water 8 : 1 : 1, 0.25% v/v of Acyl-carnitines isotope-labeled internal standards solution (IS).

Reconstitution solvent - methanol : water 9 : 1.

### Blood plasma or serum extraction

* Create pooled sample by combining 10µL aliquots of each individual extract.
* Transfer 20µL of the sample into a pre-labeled micro-centrifuge tube, add 80µL of extraction solvent, vortex.
* Leave on ice for 5min, vortex, keep on ice for another 5min, vortex again.
* Centrifuge for 5min at 4oC, 15,000rpm.
* Transfer supernatant into a pre-labeled auto-sampler vial.
* Dry all samples and reconstitute in 60 µL of reconstitution solvent.

### Tissue extraction

* Homogenize tissue samples using the liquid nitrogen chilled homogenizer (Bullet Blender Gold) or probe sonicator, depending on tissue type.
* Weigh ~30mg of pulverized tissue in pre-labeled micro-centrifuge tube.
* Add 1mL of extraction solvent, vortex, sonicate for 10-20 seconds using the probe sonicator at 20% output, level 4.
* Incubate for 10min at 4oC, vortex again.
* Centrifuge for 10min at 4oC, 15,000g.
* Transfer 120µL of supernatant to an auto-sampler vial with glass insert, store samples at -20oC until LC-MS.
* Create pooled sample by combining 10µL aliquots of each individual extract.

### Cell culture extraction (samples supplied on culture plates)

* Place the sample plates and extraction solvent on dry ice.
* Clean cell scraper with paper tissue soaked in methanol.
* 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.
* Transfer cell suspension to a pre-labeled 2mL micro-centrifuge tube, place the tube on dry ice.
* Centrifuge for 10 min at 4°C, 15,000g.
* Transfer 600µL of supernatant to glass auto-sampler vials, store samples at -20oC until LC-MS.
* Create pooled sample by combining 10µL aliquots of each individual extract.

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

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

## LC-MS

* Chromatographic column - ACQUITY UPLC RP18, 2.1mm X 50mm (Waters).
* LC gradient
  + Phase A: 5mM ammonium acetate in water, pH 9.9 (adjusted using LC-MS grade ammonium hydroxide).
  + Phase B: 100% acetonitrile
  + timetable – listed in table 2 below
* Auto-sampler temperature 4°C.
* Injection volume 10 µL.
* Mass-spectrometer parameters
  + Instrument - Agilent 6410 QQQ
  + Mode – ESI positive.
* Monitored MRM transitions for individual ceramides are listed in table 3 below.

The specific LC-MS method details are provided in supplementary material (QM-004-Xbridg2mm\_ACar+\_MRM-Insert\_LC\_PARAMS.xml and QM-004-Xbridg2mm\_ACar+\_MRM-Insert\_MS\_PARAMS.xml files).

Table 2. LC gradient timetable

|  |  |  |
| --- | --- | --- |
| Time, min | %B | Flow, ml/min |
| 0 | 0 | 0.25 |
| 7 | 80 | 0.25 |
| 7.1 | 100 | 0.25 |
| 10 | 100 | 0.25 |
| 10.1 | 0 | 0.25 |
| 15.9 | 0 | 0.25 |

Table 3 MRM transitions for individual acyl-carnitines

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Name | MS1 M/Z | MS2 M/Z | Dwell time | Fragmentor, V | Collision energy, V | Polarity |
| C5:0-DC | 276.2 | 85 | 50 | 380 | 20 | Positive |
| IS C5:0 | 255.2 | 85 | 50 | 380 | 20 | Positive |
| C5:0 | 246.2 | 85 | 50 | 380 | 20 | Positive |
| IS C4 | 235.2 | 85 | 50 | 380 | 18 | Positive |
| C4 | 232.2 | 85 | 50 | 380 | 18 | Positive |
| IS C3 | 221.1 | 85 | 50 | 380 | 18 | Positive |
| C3 | 218.2 | 85 | 50 | 380 | 18 | Positive |
| IS C2 | 207.1 | 85 | 50 | 380 | 16 | Positive |
| C2+1 | 205.1 | 85 | 50 | 380 | 16 | Positive |
| C2 | 204.1 | 85 | 50 | 380 | 16 | Positive |
| IS L\_car | 171.1 | 85 | 50 | 380 | 21 | Positive |
| L-Carnitine | 162.1 | 85 | 50 | 380 | 21 | Positive |

## References

[Donald H. Chace](http://www.clinchem.org/search?author1=Donald+H.+Chace&sortspec=date&submit=Submit), [James C. DiPerna](http://www.clinchem.org/search?author1=James+C.+DiPerna&sortspec=date&submit=Submit), [Brenda L. Mitchell](http://www.clinchem.org/search?author1=Brenda+L.+Mitchell&sortspec=date&submit=Submit), [Bethany Sgroi](http://www.clinchem.org/search?author1=Bethany+Sgroi&sortspec=date&submit=Submit), [Lindsay F. Hofman](http://www.clinchem.org/search?author1=Lindsay+F.+Hofman&sortspec=date&submit=Submit) and [Edwin W. Naylor](http://www.clinchem.org/search?author1=Edwin+W.+Naylor&sortspec=date&submit=Submit) (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 Chemistry4(7): 1166-1182.