

# Chapter 2

## Measurement of Plasma/Serum Acylcarnitines Using Tandem Mass Spectrometry

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### Abstract

Acylcarnitine analysis using tandem mass spectrometry has become a powerful tool in the investigation of pediatric patients presenting with clinical signs and symptoms suggestive of fatty acid oxidation defects. These signs are diverse and include failure to thrive, feeding difficulties, and cardiomyopathy. Because the signs and symptoms are nonspecific, the identification of acylcarnitines characteristic of these inherited diseases is necessary for diagnosis. We describe a method for the analysis of acylcarnitines in plasma or serum samples using electrospray ionization tandem mass spectrometry.

**Key words:** Fatty acid oxidation disorders, organic acidemia, medium-chain acyl CoA dehydrogenase deficiency, inborn errors of metabolism, tandem mass spectrometry, MSMS.

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### 1. Introduction

Although organic aciduria characterizes a variety of metabolic disorders, many patients who have a fatty acid oxidation defect present with nonspecific organic aciduria or with an uninformative organic acid profile during asymptomatic periods (1). For this reason, the analyses of carnitines and acylcarnitines have become essential for the diagnosis of fatty acid oxidation defects (FAOD). The FAOD defects include at least 11 disease states characterized by deficiencies of specific enzymes, which are associated with decreased concentrations of carnitine, as well as with the identification of specific combinations of abnormal acylcarnitines (2–7).

The development of tandem mass spectrometry (MSMS) methods for the measurement of acylcarnitines is considered one of the most important laboratory advances in the management of metabolic diseases. In the method that follows, a derivatized

plasma or serum extract, containing deuterated acylcarnitine internal standards, is introduced into the MSMS using electrospray ionization (ESI). A combination of the specific primary mass and the unique secondary MS fragment ion is used to selectively monitor the compound to be quantified.

## 2. Materials

### 2.1. Specimens

1. Serum or heparinized plasma is used for analysis (*see Note 1*). EDTA specimens are stable when stored at  $-20^{\circ}\text{C}$  until analysis.

### 2.2. Reagents

1. Reconstituting solution: Prepare an 80% acetonitrile solution in deionized water. Stable for 1 month when stored at room temperature. Cover with vented aluminum foil and sonicate for 20 min before use to degas.
2. 10% acetonitrile in deionized water. Stable for 1 month when stored at room temperature. Cover with vented aluminum foil and sonicate for 20 min before use to degas.
3. Derivatizing reagent: 3 N HCl in *n*-butanol.
4. L-Carnitine (C0) (Sigma-Aldrich, St. Louis, MO). **Table 2.1** contains a description of L-Carnitine. Protect from light and moisture. Store desiccated at room temperature.

**Table 2.1**  
**Description of acylcarnitines–C0**

Acylcarnitines: unlabelled	C-nomenclature	Chemical formula	Formula weight	Parent ion mass
L-Carnitine	C0	$\text{C}_7\text{H}_{15}\text{NO}_3$	161.20	221.2

### 2.3. Acylcarnitine Standards (*see Note 2*)

1. Acylcarnitine standards (*see Note 2*): **Table 2.2** contains a listing of the acylcarnitine standards. Protect from light and moisture. Store desiccated at room temperature.
2. Prepare 15 mL of 2.0 mmol/L solutions for short- and medium-chain acylcarnitines (C0–C12) in deionized water. Stable for 1 year when stored at  $2-8^{\circ}\text{C}$ .
3. Prepare 15 mL of 2.0 mmol/L solutions for long-chain acylcarnitines (C14, C16, C18) in methanol. Stable for 1 year when stored at  $2-8^{\circ}\text{C}$ .

**Table 2.2**  
**Description of acylcarnitines–deuterated and non-deuterated**

Acylcarnitines: Labelled (Deuterated)	C-nomenclature	Formula weight
[methyl-d3]-L-Carnitine.HCl	D3-C0	200.686
[d3] Acetyl-L-Carnitine.HCl	D3-C2	242.72
[d3] Propionyl-L-Carnitine.HCl	D3-C3	256.75
[d3] Butyryl-L-Carnitine.HCl	D3-C4	270.78
[d9] Isovaleryl-L-Carnitine.HCl	D9-C5	290.85
[d3] Octanoyl-L-Carnitine.HCl	D3-C8	326.88
[d3] Tetradecanoyl-L-Carnitine.HCl	D3-C14	411.045
[d3] Hexadecanoyl-L-Carnitine.HCl	D3-C16	439.099
Acylcarnitines: Non-deuterated	C-nomenclature	Formula weight
Acetyl-L-Carnitine.HCl	C2	239.699
Propionyl-L-Carnitine.HCl	C3	253.73
Butyryl-L-Carnitine.HCl	C4	267.75
Isobutyryl-L-Carnitine.HCl	Iso-C4	267.75
Isovaleryl-L-Carnitine.HCl	C5	281.78
Hexanoyl-L-Carnitine.HCl	C6	295.81
Phenylacetyl-L-Carnitine.HCl	C7-Ar*	315.80
Octanoyl-L-Carnitine.HCl	C8	323.86
Decanoyl-L-Carnitine.HCl	C10	351.91
Dodecanoyl-L-Carnitine.HCl	C12	379.97
Tetradecanoyl-L-Carnitine.HCl	C14	408.02
Hexadecanoyl-L-Carnitine.HCl	C16	436.08
Octadecanoyl-L-Carnitine.HCl	C18	464.13

Ar\*-Aromatic

- Working standard solutions: Prepare 2 mL of 100  $\mu\text{mol/L}$  acylcarnitine (grouped) for C0, C2, (C3+C4+C5), (C6+C7+C8+C10), and (C12+C14+C16+C18) in methanol. Stable for 1 year when stored at 2–8°C.
- Dilute an aliquot of each of the above acylcarnitine solutions to provide 2.0 mL of 1  $\mu\text{mol/L}$  in methanol. Stable for 1 year when stored at 2–8°C.

6. Using 100  $\mu\text{mol/L}$  and 1  $\mu\text{mol/L}$  solutions prepare 200  $\mu\text{L}$  of each calibrator as follows, using methanol as diluent:
  - a. (C0) 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 50.0  $\mu\text{mol/L}$
  - b. (C2) 0.25, 0.5, 1.0, 2.5, 5.0, 10.0  $\mu\text{mol/L}$
  - c. (C3+C4+C5) 0.02, 0.05, 0.10, 0.25, 0.5, 1.0  $\mu\text{mol/L}$
  - d. (C6+C8+C10) 0.02, 0.05, 0.10, 0.25, 0.5, 1.0  $\mu\text{mol/L}$
  - e. (C12+C14+C16+C18) 0.02, 0.05, 0.10, 0.25, 0.5, 1.0  $\mu\text{mol/L}$

#### 2.4. Internal Standard Working Solutions

1. d5-phenylalanine Internal Standard (Cambridge Isotope Laboratories, Andover, MA). Protect from light and moisture. Store desiccated at room temperature. Prepare 2.0 mmol/L d5-phenylalanine in deionized water. Stable for 1 year at 4°C.
2. D-Acylcarnitines (*see* **Note 2** and **Table 2.2**): Prepare 2.0 mmol/L solutions of deuterated acylcarnitine standards in methanol. Stable for 1 year at  $-20^{\circ}\text{C}$ . Dilute an aliquot of each acylcarnitine deuterated standard in methanol to 0.2 mmol/L. Stable for 1 year at  $-20^{\circ}\text{C}$ .
3. Working deuterated internal standard solution: Prepare by adding the volumes of d5-phenylalanine and acylcarnitines seen in **Table 2.3** to a 50 mL volumetric flask. Bring to volume using methanol. Stable for 1 month at  $-20^{\circ}\text{C}$ .

**Table 2.3**  
Preparation of working deuterated internal standard. Final volume is 50 mL, using methanol as diluent

Deuterated amino acid/ acylcarnitine	Concentration of stock solution used (mmol/L)	Final concentration of internal standard in solution ( $\mu\text{mol/L}$ )	Volume ( $\mu\text{L}$ )
d5-Phe	2.0	200	250
d3-C0	0.2	30.0	375
d3-C2	0.2	15.0	187.5
d3-C3	0.2	5.0	62.5
d3-C4	0.2	5.0	62.5
d3-C5	0.2	5.0	62.5
d3-C8	0.2	3.0	37.5
d3-C14	0.2	6.0	75
d3-C16	0.2	6.0	75

**2.5. Quality Control**

1. Pool previously analyzed patient plasma found to have acylcarnitine concentrations within the reference range. Centrifuge to remove particulate matter and filter using a 0.22  $\mu\text{m}$  filter. Divide into three portions.
2. Normal control: Aliquot a portion to serve as the normal control.
3. Mid-control: Add acylcarnitine stock standards to concentrations that reflect the upper level of the reference range.
4. High control: Add acylcarnitine stock standards to high concentrations reflecting concentrations observed in fatty acid oxidation defects.
5. The controls are stable up to 1 year when stored at  $-20^{\circ}\text{C}$ .

**2.6. Instrumentation and Supplies**

1. Waters Corporation TQD Tandem Mass Spectrometer
2. Fume hood
3. Nitrogen evaporator and heating block
4. Microfuge centrifuge
5. 1.5 mL disposable polystyrene centrifuge tubes
6. 2 mL plastic transfer pipettes
7. 12  $\times$  75 mm glass culture tubes
8. 0.5 mL HPLC vials

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**3. Methods****3.1. Sample Preparation**

1. Appropriately label a microfuge tube (1.5 mL), 12  $\times$  75 mm glass tube, and 0.5 mL HPLC vial for each specimen.
2. Use distilled water as a blank sample to monitor background/contamination.
3. Mix and centrifuge specimens. Vortex internal standard working solution.
4. Pipet 20  $\mu\text{L}$  of each specimen into the appropriate microfuge tube.
5. Add 400  $\mu\text{L}$  of working deuterated internal standard solution to each.
6. Vortex samples for 10 sec.
7. Allow samples to equilibrate at room temperature for 5 min.
8. Centrifuge samples for 5 min at 1,4000  $\times g$ .
9. Using a plastic transfer pipet, transfer supernatant to 12  $\times$  75 mm glass tube. Take care not to transfer any precipitate.

10. Evaporate to dryness with gentle stream of nitrogen gas at 37°C.
11. Working in a fume hood, add 100 µL of 3 N HCl in *n*-Butanol to each tube.
12. Cap samples and vortex for 10 sec.
13. Incubate at 55°C for 20 min in heating block.
14. Remove tubes from heating block and evaporate to dryness under nitrogen gas at 37°C in a fume hood.
15. Reconstitute samples with 200 µL of the 80% acetonitrile reconstituting solution.
16. Vortex for 5 sec.
17. Transfer reconstituted samples to HPLC sample vials.

### 3.2. Tandem Mass Spectrometry Analysis

Purge lines using 100% methanol at a rate of 2.0 mL/min for 20 min. Follow with 80% acetonitrile (also used as the reconstituting solution) at 0.100 mL/min for 20 min to condition before analysis. Operating parameters are given in **Table 2.4**. Examples of three common profiles are shown in **Figs. 2.1, 2.2, and 2.3**.

**Table 2.4**  
**LC tandem MS operating parameters**

Source	
Source: ion mode	Electron spray positive (ES+)
Capillary (kV)	3.50
Cone (V)	40
Extractor (V)	3.00
RF lens (V)	0.0
Collision energy (V)	25
Source temperature (°C)	120
Desolvation temperature (°C)	350
Cone gas flow (L/Hr)	50
Desolvation gas flow (L/Hr)	600
Analyzer	
Time (minutes)	Start 0.08; end 1.8
Scan duration (seconds)	3.8
Mass ( $m/z$ ) for parents of 85	Start 210; end 580

(continued)

Table 2.4 (continued)

LM 1 resolution	14.2
HM 1 resolution	14.2
Ion energy 1	0.5
Entrance	−2
Collision	25
Exit	1
LM 2 resolution	14.2
HM 2 resolution	14.2
Ion energy 2	0.5
Multiplier (V)	570
Syringe pump flow (μL/min)	10.0
Inject volume (μL)	7.5
Gradient run time (min)	2.5
Solvent	80% acetonitrile
Gradient flow (mL/min) (initial)	0.100
Gradient flow (mL/min) (@0.30 min)	0.015
Gradient flow (mL/min) (@1.20 min)	0.100
Gradient flow (mL/min) (@1.55 min)	0.500
Gradient flow (mL/min) (@1.85 min)	0.100

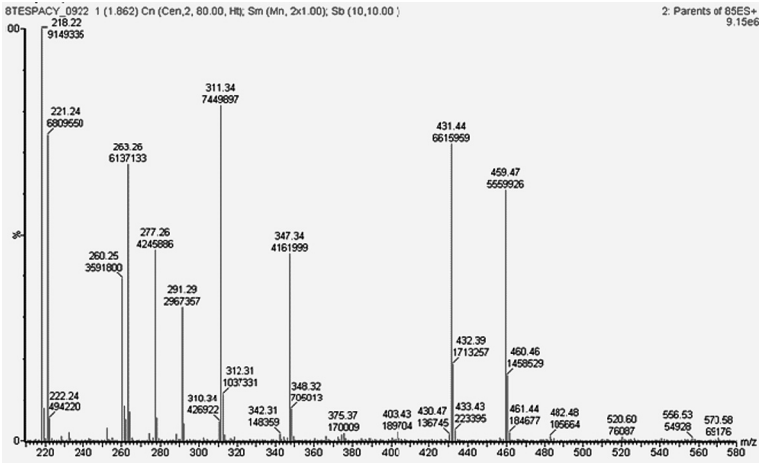


Fig. 2.1. Profile not consistent with inborn errors of metabolism.

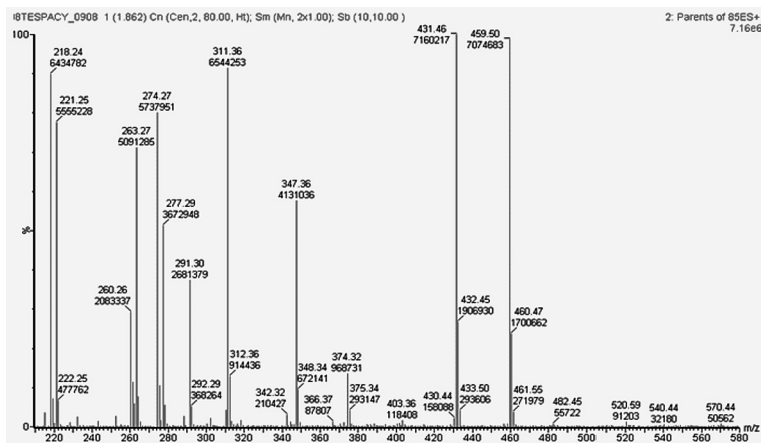


Fig. 2.2. Profile consistent with methylmalonic acidemia.

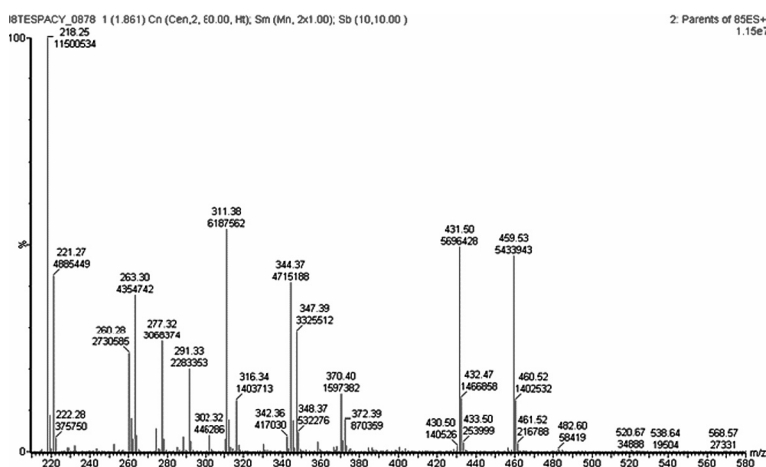


Fig. 2.3. Profile consistent with MCAD.

### 3.3. Processing Results

Analyze each sample using multiple reaction monitoring of two channels for the d5-phenylalanine internal standard such as the ion transition ( $m/z$  227.2  $\rightarrow$   $m/z$  125.2) and primary ions of  $m/z$  85 (see **Note 3**). Acylcarnitines are identified by monitoring transitions of primary ions of  $m/z$  85, and acylcarnitine ratios are used for calculation of final concentration (**Table 2.5**).

### 3.4. Evaluating Standard Curves and Calculating Response Factors

1. Acquire ion count intensity for each acylcarnitine and corresponding deuterated standard in each standard curve as listed in the right column of **Table 2.6**. For each sample, process the spectra and record the intensity of peaks after a background, smoothing process, and peak centering.
2. Concentration of deuterated standard in standard curves (**Table 2.7**): The absolute amount of deuterated standard is derivatized and dried under nitrogen. The sample is then



**Table 2.5**  
**Transitions for the acylcarnitines and acylcarnitine ratios used for calculation of final concentration**

Acylcarnitine	Deuterated standard	Transition ( <i>m/z</i> ) for		Non-deuterated/ Deuterated ratio used for final concentration
		Acylcarnitine	Deuterated standard	
C2 (acetyl)	D3-C2 ([d3]acetyl)	260.2→85	263.2→85	C2/d3-C2 “/”>>
C3:1 (propenyl)	D3-C3 ([3,3,3-d3] propionyl)	272.2→85	277.2→85	C3 / d3-C3
C3 (propionyl)	D3-C3 ([3,3,3-d3] propionyl)	274.2→85	277.2→85	C3 / d3-C3
C4 (butyryl)	D3-C4 ([4,4,4-d3] butyryl)	288.2→85	291.2→85	C4 / d3-C4
C5 (isovaleryl)	D9-C5 ([d9] isovaleryl)	302.2→85	311.3→85	C5 / d9-C5
C5:1 (tiglyl)	D9-C5 ([d9] isovaleryl)	300.2→85	311.3→85	C5 / d9-C5
C4-OH (3OH-butyryl)	D3-C4 ([4,4,4-d3] butyryl)	304.2→85	291.2→85	C4 / d3-C4
C6 (hexanoyl)	D9-C5 ([d9] isovaleryl)	316.3→85	311.3→85	C6 / d9-C5
C5-OH/2Me3OH butyryl	D9-C5 ([d9] isovaleryl)	318.2→85	311.3→85	C5 / d9-C5
Benzoyl	D3-C8 ([8,8,8-d3] octanoyl)	322.3→85	347.3→85	C8 / d3-C8
C6-OH (3OH hexanoyl)	D9-C5 ([d9] isovaleryl)	332.3→85	311.3→85	C6 / d9-C5
Phenylacetyl (C7*Ar)	D3-C8 ([8,8,8-d3] octanoyl)	336.2→85	347.3→85	Phenacet/d3-C8
C8:1 (octenoyl)	D3-C8 ([8,8,8-d3] octanoyl)	342.3→85	347.3→85	C8 / d3-C8
C8 (octanoyl)	D3-C8 ([8,8,8-d3] octanoyl)	344.3→85	347.3→85	C8 / d3-C8
C3DC (malonyl)	D3-C8 ([8,8,8-d3] octanoyl)	360.3→85	347.3→85	C8 / d3-C8
C10:3 (decatrienoyl)	D3-C8 ([8,8,8-d3] octanoyl)	366.3→85	347.3→85	C10 / d3-C8

(continued)

**Table 2.5 (continued)**

		Transition ( <i>m/z</i> ) for		Non-deuterated/ Deuterated ratio used for final concentration
Acylcarnitine	Deuterated standard	Acylcarnitine	Deuterated standard	
C10:2 (decadienoyl)	D3-C8 ([8,8,8-d3] octanoyl)	368.3→85	347.3→85	C10 / d3-C8
C10:1 (decenoyl)	D3-C8 ([8,8,8-d3] octanoyl)	370.3→85	347.3→85	C10 / d3-C8
C10 (decanoyl)	D3-C8 ([8,8,8-d3] octanoyl)	372.3→85	347.3→85	C10 / d3-C8
C4DC (McMalonyl/ suc-cinyl)	D3-C8 ([8,8,8-d3] octanoyl)	374.3→85	347.3→85	C10 / d3-C8
C5DC (glutaryl)/ 3OHC10	D3-C8 ([8,8,8-d3] octanoyl)	388.3→85	347.3→85	C10 / d3-C8
C12:1 (dodecenoyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	398.4→85	431.4→85	C12 / d3-C14
C12 (dodecanoyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	400.4→85	431.4→85	C12 / d3-C14
C6DC/3MeGlu- taryl	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	402.3→85	431.4→85	C12 / d3-C14
C12-OH (3OHDodecanoyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	416.4→85	431.4→85	C12 / d3-C14
C14:2 (tetradecadienoyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	424.3→85	431.4→85	C14 / d3-C14
C14:1 (tetradecenoyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	426.4→85	431.4→85	C14 / d3-C14
C14 (tetradecanoyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	428.4→85	431.4→85	C14 / d3-C14
C8DC (suberyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	430.4→85	431.4→85	C14 / d3-C14

(continued)

**Table 2.5 (continued)**

Acylcarnitine	Deuterated standard	Transition ( <i>m/z</i> ) for		Non-deuterated/ Deuterated ratio used for final concentration
		Acylcarnitine	Deuterated standard	
C14:1-OH (3OH tetradecenoyl)	D3-C14 ([14, 14, 14-d3] tetradecanoyl)	442.4→85	431.4→85	C14 / d3-C14
C14-OH (3OH tetradecanoyl)	d3-C14 ([14, 14, 14-d3] tetradecanoyl)	444.4→85	431.4→85	C14 / d3-C14
C16:1 (palmitoleyl)	d3-C16 ([16, 16, 16-d3] hexadecanoyl)	454.4→85	459.4→85	C16 / d3-C16
C16 (palmitoyl)	d3-C16 ([16, 16, 16-d3] hexadecanoyl)	456.4→85	459.4→85	C16 / d3-C16
C10DC (sebacyl)	d3-C16 ([16, 16, 16-d3] hexadecanoyl)	458.4→85	459.4→85	C16 / d3-C16
C16:1-OH (3OH palmitoleyl)	d3-C16 ([16, 16, 16-3] hexadecanoyl)	470.4→85	459.4→85	C16 / d3-C16
C16-OH (3OH palmitoyl)	d3-C16 ([16, 16, 16-d3] hexadecanoyl)	472.4→85	459.4→85	C16 / d3-C16
C18:2 (linoleyl)	d3-C16 ([16, 16, 16-d3] hexadecanoyl)	480.4→85	459.4→85	C18 / d3-C16
C18:1 (oleyl)	D3-C16 ([16, 16, 16-d3] hexadecanoyl)	482.4→85	459.4→85	C18 / d3-C16
C18 (stearoyl)	D3-C16 ([16, 16, 16-d3] hexadecanoyl)	484.4→85	459.4→85	C18 / d3-C16
C18:2-OH (3OH linoleyl)	D3-C16 ([16, 16, 16-d3] hexadecanoyl)	496.4→85	459.4→85	C18 / d3-C16
C18:1-OH (3OH oleyl)	D3-C16 ([16, 16, 16-d3] hexadecanoyl)	498.4→85	459.4→85	C18 / d3-C16

(continued)

Table 2.5 (continued)

Acylcarnitine	Deuterated standard	Transition ( <i>m/z</i> ) for		Non-deuterated/ Deuterated ratio used for final concentration
		Acylcarnitine	Deuterated standard	
C18-OH (3OH stearoyl)	D3-C16 ([16, 16, 16-d3] hexadecanoyl)	500.4→85	459.4→85	C18 / d3-C16
C16DC	D3-C16 ([16, 16, 16-d3] hexadecanoyl)	542.4→85	459.4→85	C18 / d3-C16
C18:1 DC	D3-C16 ([16, 16, 16-d3] hexadecanoyl)	568.5→85	459.4→85	C18 / d3-C16

Table 2.6  
Concentration of deuterated standard in standard curves

Deuterated acylcarnitine	Final concentration internal standard in standard curve (μmol/L)	Deuterated acylcarnitine	Final concentration internal standard in standard curve (μmol/L)
d3-C0	3.0	d3-C5	0.5
d3-C2	1.5	d3-C8	0.3
d3-C3	0.5	d3-C14	0.6
d3-C4	0.5	d3-C16	0.6

reconstituted with 200 μL 80% acetonitrile. However, in routine analysis, the internal standard is added into 20 μL of plasma. Therefore, the final concentrations of deuterated standards are ten times lower than for routine samples.

3. For each acylcarnitine standard curve, use the theoretical ratio data (**T** = concentration analyte/concentration internal standard) and the actual ratio (**A** = ion count intensity analyte peak/ion count intensity internal standard peak) data to calculate the response factor.

$$RF = \frac{1}{(A/T)} = \frac{T}{A}$$

Calculate the average response factor for each analyte.

**Table 2.7**  
**Mean levels and coefficients of variation (CV) for the three quality control materials used during a 6-month period**

	C0	C2	C3	C4	C5	C6	C8	C10	C12	C14	C16	C18
Level 1 (μmol/L)												
Mean	36.7	9.21	0.4	0.2	0.11	0.04	0.09	0.15	0.09	0.03	0.07	0.03
CV (%)	8.5	19.4	11.5	18.2	14.4	20.3	16.4	13.8	18.2	29.4	21.6	30.5
Level 2 (μmol/L)												
Mean	84.1	41.5	2.65	0.97	0.87	0.55	0.61	1.2	0.58	0.6	0.87	0.6
CV (%)	10.0	6.0	11.5	15	9.6	11.3	11.9	11.7	12.3	11.2	12.7	14.0
Level 3 (μmol/L)												
Mean	95.9	57.3	9.3	4.7	5.1	2.5	5.1	2.8	2.9	2.8	2.9	3.0
CV (%)	12.5	9.4	10.6	12.9	10.2	11.5	11.0	11.1	10.6	11.7	9.9	11.1

**3.5. Calculation of Concentration for Quality Control and Unknown Samples**

Concentrations (μmol/L) of unknown specimens are calculated by the following equation:

$$\frac{\text{(Ion count intensity analyte} \div \text{Ion count intensity IS)}}{\text{(IS concentration)}} \text{(average RF)}$$

1. IS concentrations for routine samples are listed above.
2. Review each peak to check for proper integration and area calculation of the peak.
3. Report acylcarnitine concentrations as “>upper limit of linearity” of standard curve when applicable. Do not extrapolate results.
4. The method is precise and specific and is associated with the following coefficients of variation (CV) for the three quality control materials described above. Examples of expected precision for the various acylcarnitines are seen in **Table 2.7**.

**3.6. Reference Intervals**

Reference intervals, provided in **Table 2.8**, are based on local data and should be validated by each laboratory.

**3.7. Interpretation**

Most fatty acid oxidation defects and several organic acidurias will show typical abnormal patterns of acylcarnitines, as analyzed by tandem mass spectrometry. The results should be separated or grouped according to chain length: short-chain (C2, C4), medium-chain (C6, C8, C10, C10:1), long-chain (C12, C14, C14:1, C14:2, C16, C18, C18:1), 3-hydroxy long-chain (C14-

**Table 2.8**  
**Reference intervals of acylcarnitines**

Acylcarnitine	Reference intervals	Acylcarnitine	Reference intervals
C2 (acetyl)	3.86–23.48	C6DC/3-Meglutaryl	<0.13
C3:1 (propenyl)	<0.03	C12-OH (3OH dodecanoyl)	<0.06
C3 (propionyl)	<0.65	C14:2 (tetradecadienoyl)	<0.19
C4 (butyryl)	<0.52	C14:1 (tetradecenoyl)	<0.18
C5:1 (tiglyl)	<0.06	C14 (tetradecanoyl)	<0.13
C5 (isovaleryl)	<0.38	C8DC (suberyl)	<0.16
C4-OH (3OH-butyryl)	<0.33	C14:1-OH (3OH tetradecenyl)	<0.06
C6 (hexanoyl)	<0.13	C14-OH (3OH tetradecanoyl)	<0.06
C5-OH/2Me3OH butyryl	<0.13	C16:1 (palmitoleyl)	<0.08
C6-OH (3OH-hexanoyl)	<0.03	C16 (palmitoyl)	<0.23
C7*Ar (phenylacetyl)	<0.05	C10DC (sebacyl)	<0.07
C8:1 (octenoyl)	<0.60	C16:1-OH (3OH palmitoleyl)	<0.06
C8 (octanoyl)	<0.33	C16-OH (3OH palmitoyl)	<0.03
C3DC (malonyl)	<0.08	C18:2 (linoleyl)	<0.22
C10:3 (decatrienoyl)	<0.36	C18:1 (oleyl)	<0.21
C10:2 (decadienoyl)	<0.08	C18 (stearoyl)	<0.11
C10:1 (decenoyl)	<0.37	C18:2-OH (3OH linoleyl)	<0.07
C10 (decanoyl)	<0.27	C18:1-OH (3OH oleyl)	<0.06
C4DC (McMalonyl/succinyl)	<0.10	C18-OH (3OH stearoyl)	<0.05
C5DC (glutaryl)/3OHC10	<0.12	C16DC	<0.10
C12:1 (dodecenoyl)	<0.16	C18:1DC	<0.06
C12 (dodecanoyl)	<0.23		

OH, C16-OH, C18-OH, C18:1-OH), and others indicative of organic acidurias (such as C3, C4DC C5:1, C5-OH, C5DC, etc.). Generally, specific patterns are associated with specific diseases. For example, the expected pattern for medium-chain acyl

CoA dehydrogenase deficiency (MCAD), a common fatty acid oxidation disorder, is an increase in medium-chains C6, C8, C10, C10:1 (8). The reader is referred to recent publications for listing of typical patterns for other diseases (2, 8, 9). While interpreting data, it is important to evaluate data for interferences (*see* **Notes 5–7**).

## 4. Notes

1. Grossly hemolyzed samples are unsuitable for analysis to avoid inaccurate quantitative results.
2. Acylcarnitine standards were obtained from Dr. Herman J. ten Brink VU Medical Center Metabolic Laboratory, 0A082 P.O. Box 7057 1007 MB Amsterdam, The Netherlands.
3. For each sample, examine the chromatogram and total ion chromatogram (TIC) to determine whether the injection and flow rate are acceptable. An adequate flow is characterized by three main steps: The first is a sharp increase in the intensity ( $y$ -axis) reflecting proper ionization; the second is a constant intensity for the next 1.4 min; and the last is a return to baseline at the end of the analyzer time. **Figure 2.4** shows an adequate flow as monitored using d5-phenylalanine (MRM of two channels). A plugged and/or dirty electrospray probe tip will affect the chromatogram, resulting in a poor (unacceptable) flow rate such as that seen via **Fig. 2.5**.
4. Response factor information generated from these standards may be stored in a program such as NeoLynx and used to calculate patient concentrations for a period of up to 1 year before re-calibration (**Section 3.4**).

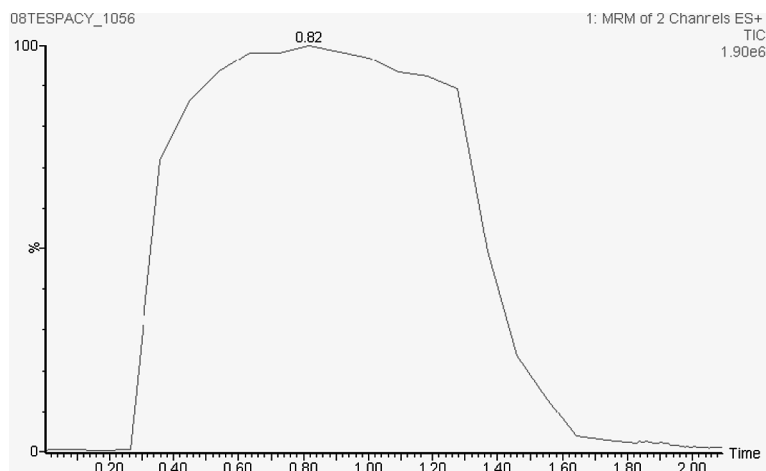


Fig. 2.4. Profile of adequate flow as monitored by d5-phenylalanine (MRM of two channels).

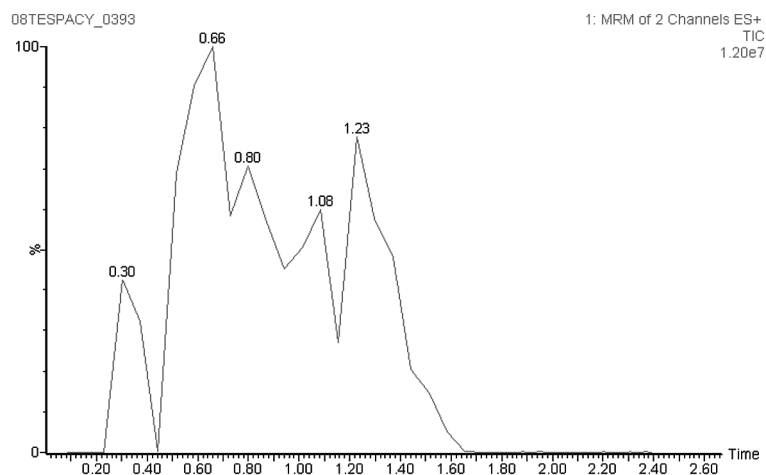


Fig. 2.5. Profile of inadequate flow as monitored by d5-phenylalanine (MRM of two channels).

5. There is potential interference from glutamate for the quantitation of C2 in the parent of 85 scans used to measure acylcarnitines, as both, having an  $m/z$  value of 260, produce a fragment ion at 85  $m/z$ .
6. Elevated pivaloylcarnitine concentrations in patients administered the antibiotic pivalic acid may result in falsely elevated isovalerylcarnitine levels, as pivaloylcarnitine is an isomer of isovalerylcarnitine.
7. Incomplete butylation of acylcarnitines in the 260–428  $m/z$  range may interfere with smaller chain acylcarnitines in the same mass range. An excess of 3.0 N HCl n-butanol is used in the assay to minimize this effect.

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