Supporting Information

Quantitation of Urinary Acylcarnitines by DMS-MS/MS Uncovers the Effects of Total Body Irradiation in Cancer Patients

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Supporting Information

Table S1.

Quantified Acylcarnitine	Slope of Acylcarnitine Calibration Curves	Stable Isotope Internal Standard	Calibration Curve Reference
Acetylcarnitine (C2:0-CN)	0.1282x	Deuterated acetylcarnitine (d_3-2:0-CN)	Acetylcarnitine (C2:0-CN)
Propionylcarnitine (C3:0-CN)	0.0611x	Deuterated acetylcarnitine (d_3-2:0-CN)	Propionylcarnitine (C3:0-CN)
Butyrylcarnitine (C4:0-CN)	0.1017x	Deuterated butyrylcarnitine (d_3-4:0-CN)	Butyrylcarnitine (C4:0-CN)
Valerylcarnitine (C5:0-CN)	0.1912x	Deuterated butyrylcarnitine (d_3-4:0-CN)	Valerylcarnitine (C5:0-CN)
Hexanoylcarnitine (C6:0-CN)	0.1116x	Deuterated butyrylcarnitine (d_3-4:0-CN)	Hexanoylcarnitine (C6:0-CN)
Heptanoylcarnitine (C7:0-CN)	0.1116x	Deuterated butyrylcarnitine (d_3-4:0-CN)	Hexanoylcarnitine (C6:0-CN)
Octanoylcarnitine (C8:0-CN)	0.1053x	Deuterated octanylcarnitine (d_3-8:0-CN)	Octanoylcarnitine (C8:0-CN)
Octenoylcarnitine (C8:1-CN)	0.1053x	Deuterated octanylcarnitine (d_3-8:0-CN)	Octanoylcarnitine (C8:0-CN)
Nonanoylcarnitine (C9:0-CN)	0.1072x	Deuterated decanoylcarnitine (d_3-10:0-CN)	Decanoylcarnitine (C10:0-CN)

Table S1. Quantitation of human urinary acylcarnitines. List of human urinary acylcarnitine species, along with their corresponding internal standards and calibration curves used for quantitation. Slopes of each acylcarnitine calibration curve are also provided.

Table S2.

Common Name	Synonym	Q1	Q3	COV (V)
Acetylcarnitine	C2:0-CN	204.2	85.1	-50
Propionylcarnitine	C3:0-CN	218.2	85.1	-47
Butyrylcarnitine	C4:0-CN	232.2	85.1	-42
Valerylcarnitine	C5:0-CN	246.2	85.1	-38
Hexanoylcarnitine	C6:0-CN	260.2	85.1	-36
Heptanoylcarnitine	C7:0-CN	274.2	85.1	-32
Octanoylcarnitine	C8:0-CN	288.2	85.1	-30
Octenoylcarnitine	C8:1-CN	286.2	85.1	-29
Nonanoylcarnitine	C9:0-CN	302.2	85.1	-27
Decanoylcarnitine	C10:0-CN	316.2	85.1	-25
Decenoylcarnitine	C10:1-CN	314.2	85.1	-23
Undecanoylcarnitine	C11:0-CN	330.2	85.1	-19
Tetradecanoylcarnitine	C14:0-CN	372.2	85.1	-16
Palmitoylcarnitine	C16:0-CN	400.2	85.1	-13
Deuterated Acetylcarnitine	C2:0-CN-d3	207.2	85.1	-50
Deuterated Butyrylcarnitine	C4:0-CN-d3	235.2	85.1	-42
Deuterated Octanoylcarnitine	C8:0-CN-d3	291.2	85.1	-30
Deuterated Decanoylcarnitine	C10:0-CN-d3	319.2	85.1	-25
Deuterated Palmitoylcarnitine	C16:0- CN-d3	403.2	85.1	-16

Table S2. Acylcarnitine MRM transitions. List of acylcarnitine species found in the DMS-MS/MS method. Acylcarnitine common names, along with their corresponding synonyms, are provided. Each acylcarnitine MRM transition is listed, along with its corresponding optimized COV value. Additional DMS parameters include: SV = 3500; DMS modifier = ethyl acetate. It is important to note that acylcarnitines are no longer zwitterionic following resuspension in 70% methanol, thereby leading to protonation of the carboxylate group, and ultimately a positively charged center. Consequently, COV values are not altered due to the zwitterionic nature of acylcarnitines.

Table S3.

	m/z	COV	COV peak width at max height
C2:0-CN	204.2	-50	5
C3:0-CN	218.2	-47	4
C4:0-CN	232.2	-42	4
C5:0-CN	246.2	-38	5
C6:0-CN	260.2	-36	3
C8:0-CN	288.2	-30	3
C10:0-CN	316.2	-25	4
C10:1-CN	314.2	-23	5
C14:0-CN	372.2	-16	3
C16:0-CN	400.2	-13	4

Table S3. Optimal acylcarnitine COV's and their corresponding peak widths at maximum height. List of acylcarnitine species that were used to generate a 3D-bubble plot (**Figure 1**) with m/z on the x-axis, optimal COV on the y-axis, and the bubble size indicating the COV peak width at max height.

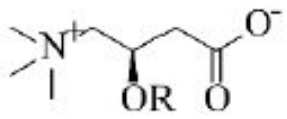
Table S4.

Acylcarnitine	Sample ID	Theor. Conc. (μM)	Exp. Conc. (μM)	Exp./Theor.
C3:0-CN	1	20	21.70	1.09
	2	2	0.92	0.46
	11	60	75.11	1.25
C6:0-CN	3	60	66.03	1.10
	4	20	20.86	1.04
	11	2	0.64	0.32
C8:0-CN	5	6	1.59	0.27
	6	2	-0.30	-0.15
	11	20	22.69	1.13
C10:1-CN	7	60	83.33	1.39
	8	6	8.30	1.38
	11	6	8.54	1.42
C14:0-CN	9	20	19.33	0.97
	10	6	4.65	0.78
	11	6	4.68	0.78

Table S4. Blind FIA-DMS-MS/MS urine analyses of male Sprague Dawley rat urine spiked with five acylcarnitine species at various concentrations. Theoretical and

experimental acylcarnitine concentrations are listed for each urine sample, along with the corresponding ratio taken from the experimental values/theoretical values.

Structures S1-S10.



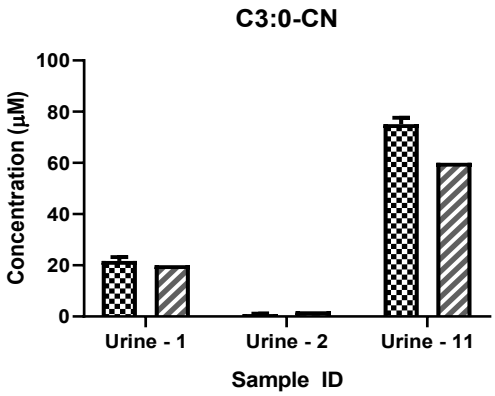
Acylcarnitine
R = acyl group

[1]

Common Name	"R"
Acetylcarnitine	CH_3-
Propionylcarnitine	CH_3-CH_2
Butyrylcarnitine	CH_3-$(CH_2)_2$
Valerylcarnitine	CH_3-$(CH_2)_3$
Hexanoylcarnitine	CH_3-$(CH_2)_4$
Octanoylcarnitine	CH_3-$(CH_2)_6$
Decanoylcarnitine	CH_3-$(CH_2)_8$
Decenoylcarnitine	CH_2=CH-($CH_2)_7$
Tetradecanoylcarnitine	CH_3-$(CH_2)_{12}$
Palmitoylcarnitine	CH_3-$(CH_2)_{14}$

Structures S1-S10. Acylcarnitine structures. "R" groups for each acylcarnitine are provided. Calibration curves were prepared for each of the listed compounds for validation purposes.

Figure S1.



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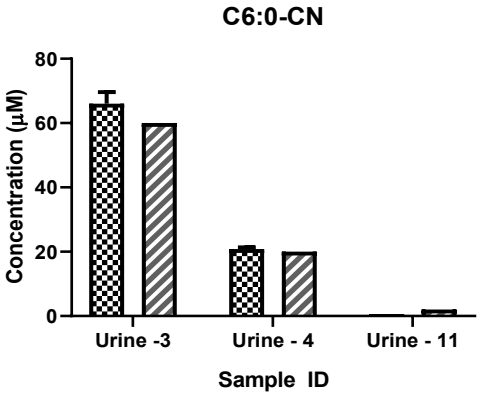

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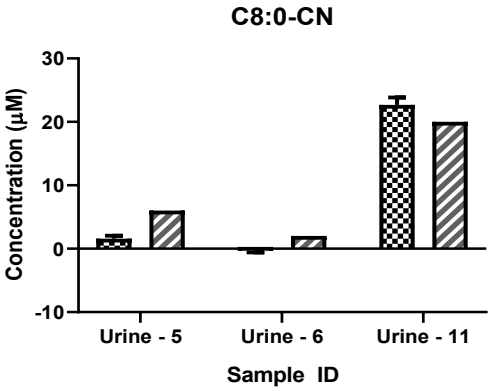

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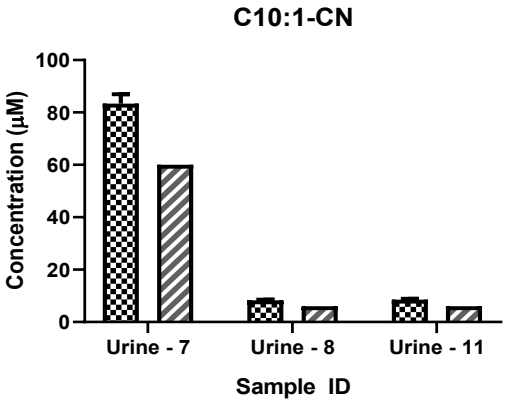

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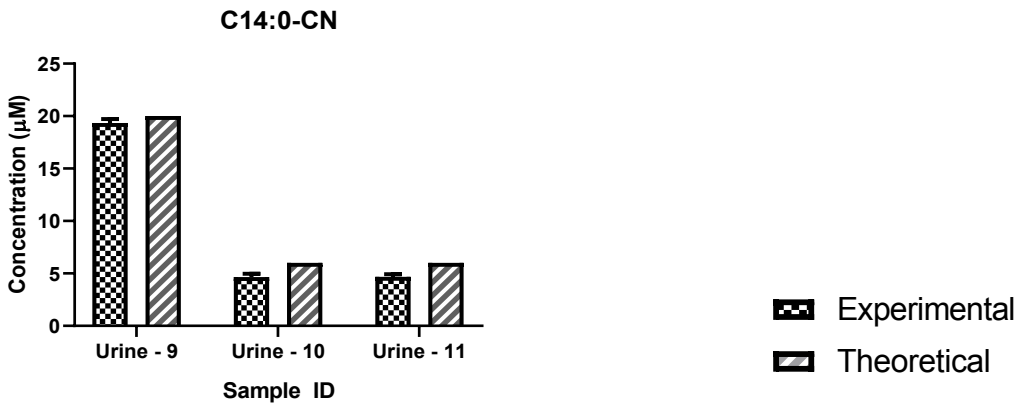
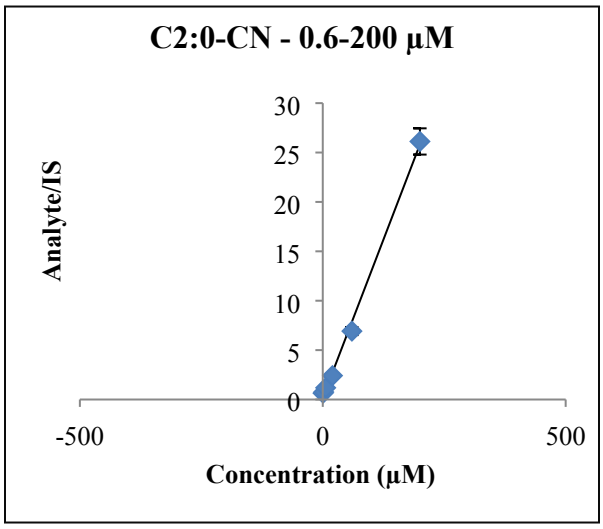


(e)

Fig. S1. Blind FIA-DMS-MS/MS urine analyses of male Sprague Dawley rat urine spiked with five acylcarnitine species at various concentrations (a-e). Acylcarnitine experimental mean values are compared with the corresponding theoretical values. Error bars for the experimental values represent the standard error of the mean (SEM) for each technical replicate

Figure S2.



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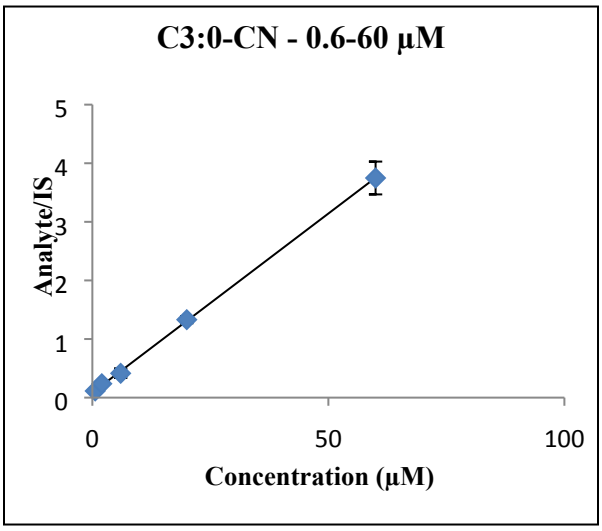

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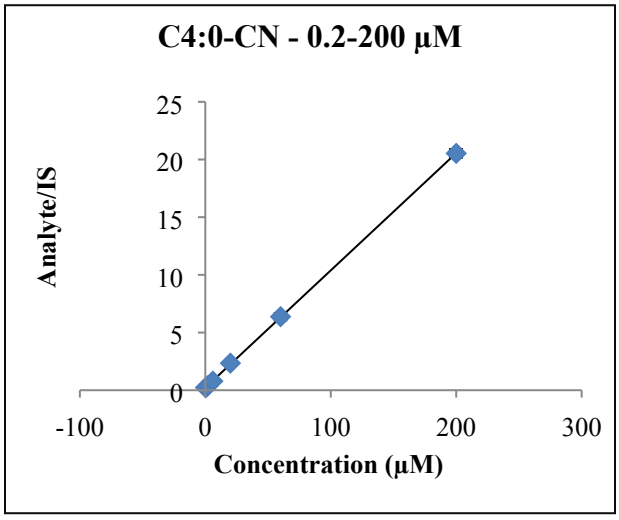

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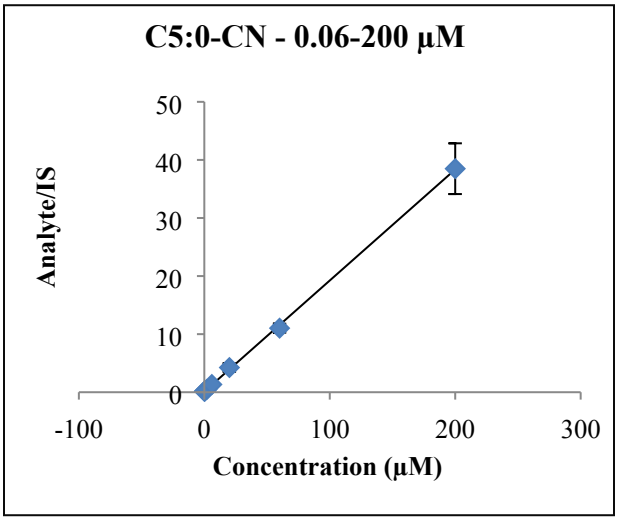

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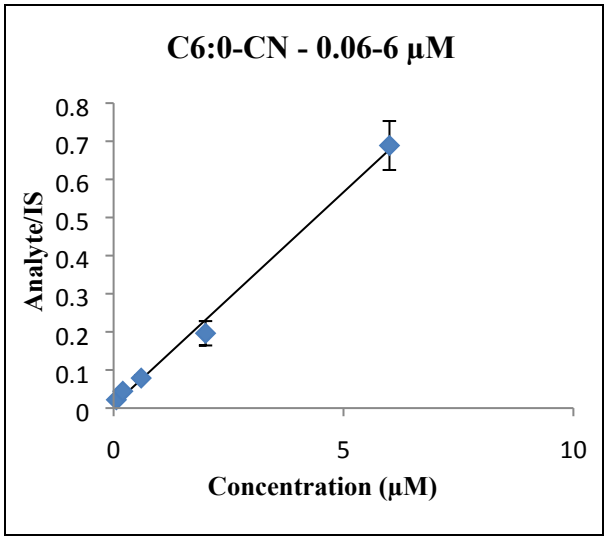

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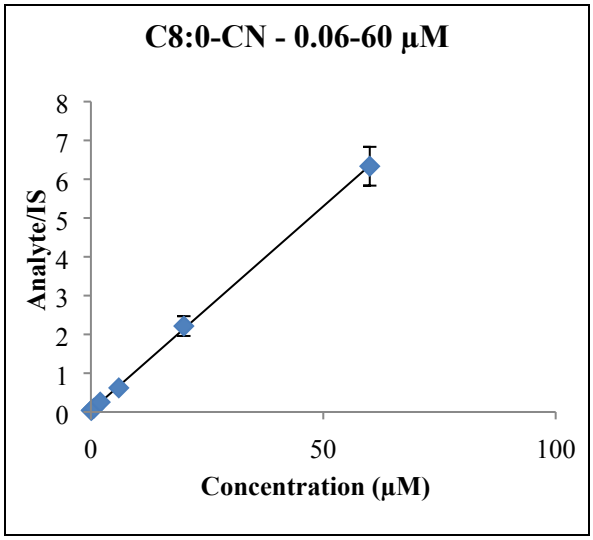

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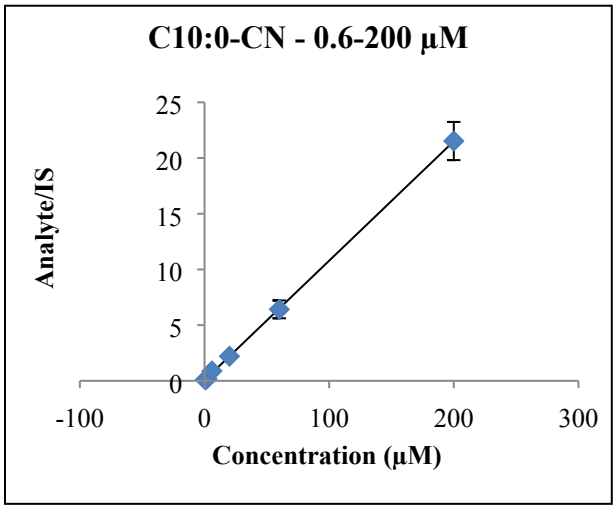
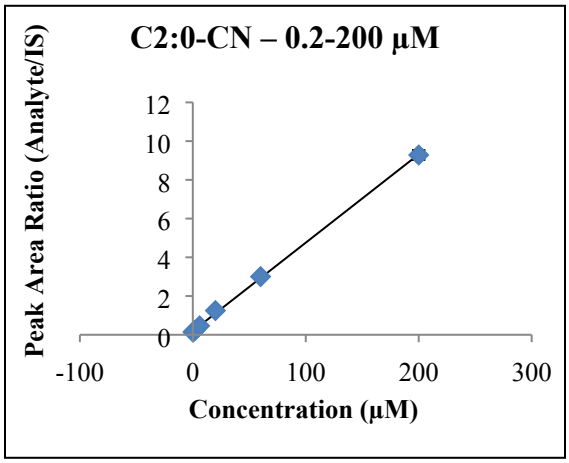


(g)

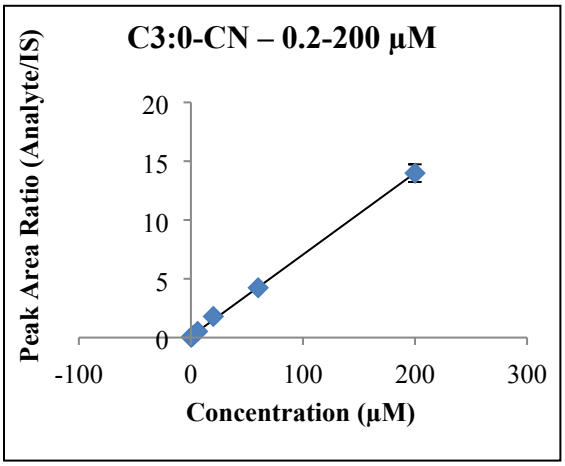
Fig. S2. Acylcarnitine calibration curves spiked into pooled human urine samples and analyzed by FIA-DMS-MS/MS. Plots that capture the linear dynamic range for each acylcarnitine have been generated. **(a)** C2:0-CN, 0.6-200 µM, with 6 calibration curve points in total; **(b)** C3:0-CN, 0.6-60 µM, with 5 calibration curve points in total; (**c**) C4:0-

CN, 0.2-200 μM, with 7 calibration curve points in total; **(d)** C5:0-CN, 0.06-200 μM, with 8 calibration curve points in total; **(e)** C6:0-CN, 0.06-6 μM, with 5 calibration curve points in total; **(f)** C8:0-CN, 0.06-60 μM, with 7 calibration curve points in total; **(g)** C10:0-CN, 0.6-200 μM, with 6 calibration curve points in total. All calibration curves were run in triplicate. Error bars represent standard error of the mean (SEM)

Figure S3.



(A)



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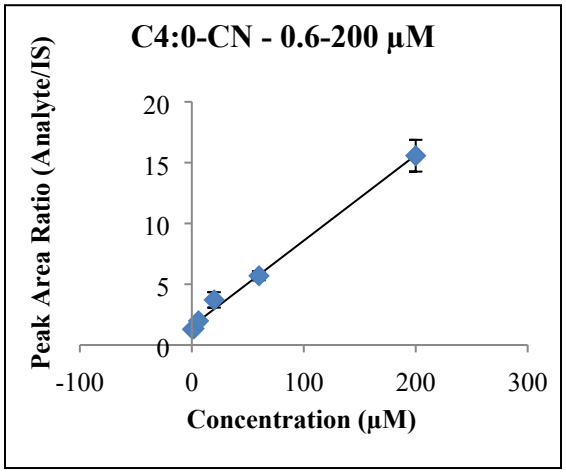

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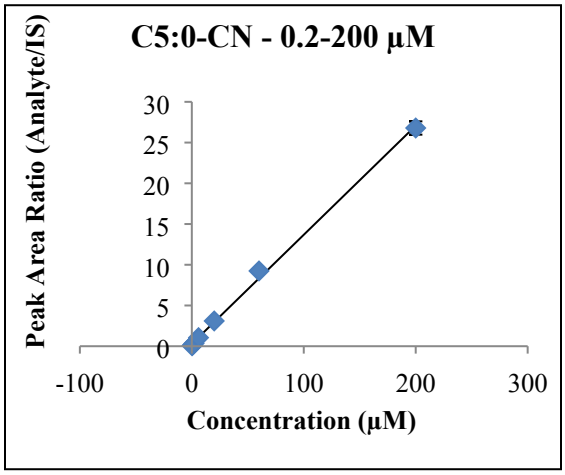

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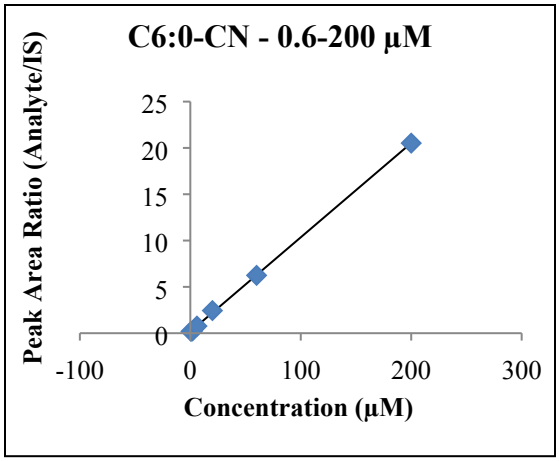
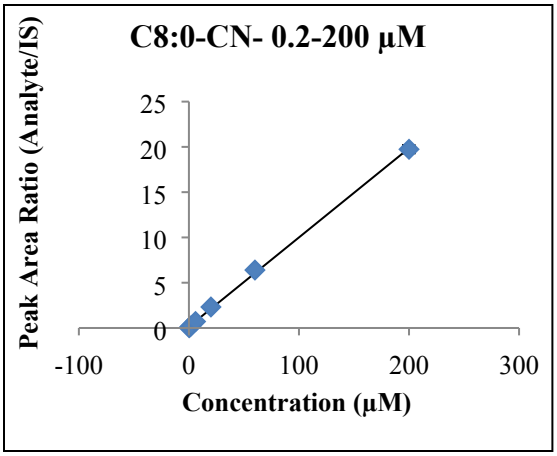
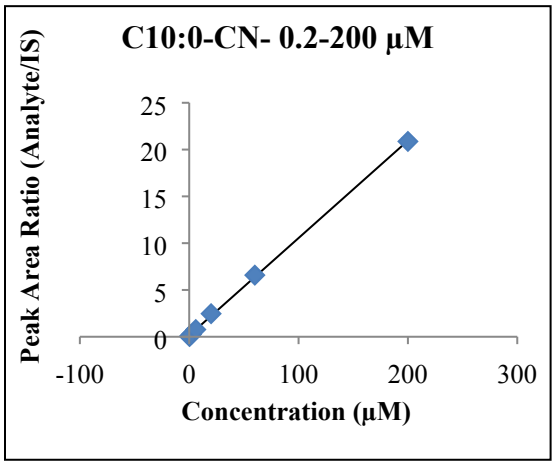

C6:0-CN - 0.6-200 µM
Peak Area Ratio (Analyte/IS)
25
20
15
10
5
0
-100
0
100
200
300
Concentration (µM)

(E)


C8:0-CN- 0.2-200 µM
Peak Area Ratio (Analyte/IS)
25
20
15
10
5
0
-100
0
100
200
300
Concentration (µM)

(F)


C10:0-CN- 0.2-200 µM
Peak Area Ratio (Analyte/IS)
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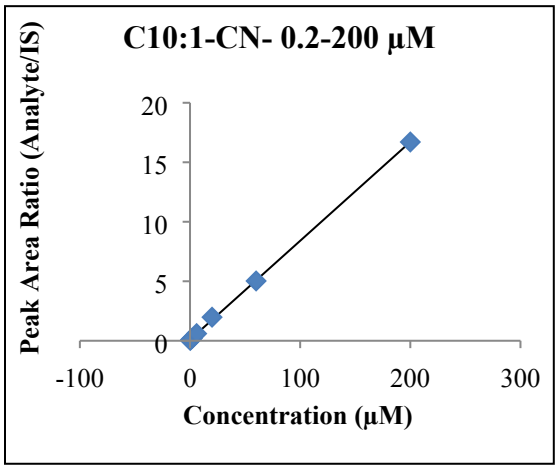
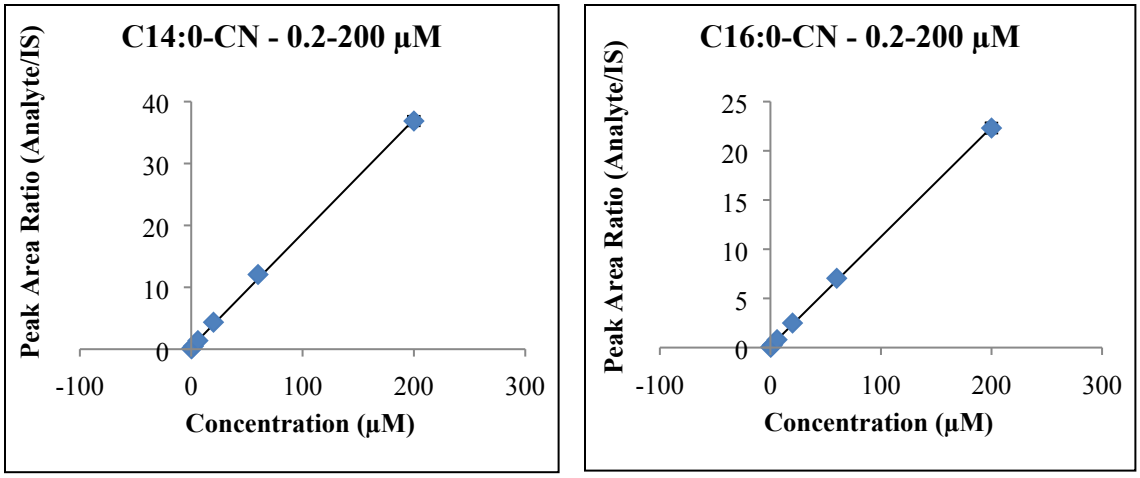
20
15
10
5
0
-100
0
100
200
300
Concentration (µM)

(G)


C10:1-CN- 0.2-200 µM
Peak Area Ratio (Analyte/IS)
20
15
10
5
0
-100
0
100
200
300
Concentration (µM)

(H)



(I) (J)

Figure S3. **Acylcarnitine calibration curves spiked into rat urine samples and analyzed by FIA-DMS-MS/MS.** Each plot captures the linear dynamic range for the associated acylcarnitine. (A) C2:0-CN, 0.2-200 µM; (B) C3:0-CN, 0.2-200 µM; (C) C4:0-CN, 0.6-200 µM; (D) C5:0-CN, 0.2-200 µM; (E) C6:0-CN, 0.6-200 µM; (F) C8:0-CN, 0.2-200 µM; (G) C10:0-CN, 0.2-200 µM; (H) C10:1-CN, 0.2-200 µM; (I) C14:0-CN, 0.2-200 µM; (J) C16:0-CN, 0.2-200 µM. All calibration curves were run in triplicate. Error bars represent standard error of the mean (SEM).

REFERENCES:

1. Li S, Gao D, Jiang Y: **Function, Detection and Alteration of Acylcarnitine Metabolism in Hepatocellular Carcinoma**. *Metabolites* 2019, **9**(2).