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MetaboQuan-R for Acylcarnitines in Human Serum

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Abstract

This application note demonstrates a high-throughput UPLC-MS/MS research method for the quantitative analysis of acylcarnitines in human serum samples, without the need for derivitization.

A rapid UPLC-MS/MS methodology has been developed for the research analysis of acylcarnitines. This method has been demonstrated to be suitable for the analysis of physiologically relevant levels of these analytes in human serum. This method utilizes a generic LC-MS platform that can be used for various compound classes (including metabolomics, lipidomics, and proteomics), meaning it can be applied as part of a suite of analyses that are run subsequently as part of a targeted multi-omics workflow.

Benefits

- Simultaneous analysis of 20 acylcarnitines in a single analytical run that is under three minutes
- High throughput analysis means larger sample sets can be analyzed rapidly
- A simple analytical workflow without derivitization
- Use of a generic LC-MS configuration yields versatility for switching from one compound class to another

Introduction

Acylcarnitines are important intermediate metabolites generated during lipid metabolism (see Figure 1). The analysis of these important biomarkers is generally performed using derivitization, followed by flow injection analysis – tandem mass spectrometry (FIA-MS/MS). This method however cannot distinguish isobaric species resulting in limited information acquired from these types of analyses. Here we demonstrate a high-throughput UPLC-MS/MS research method for the quantitative analysis of acylcarnitines in human serum samples, without the need for derivitization. This application note is also part of a MetaboQuan-R method package.

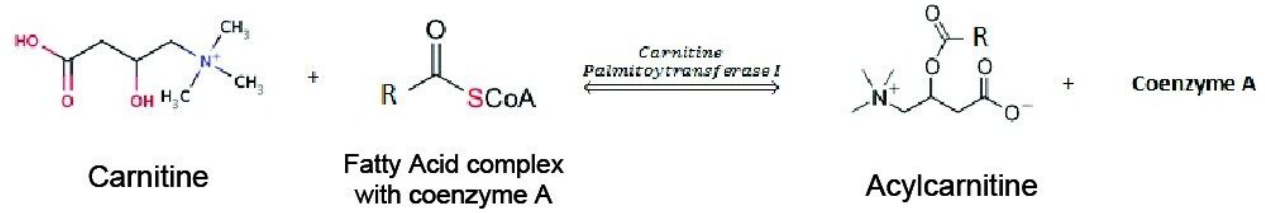

Figure 1. Schematic of fatty acid metabolism to produce acylcarnitines.

Experimental

Human serum sample preparation

100 µL of human serum was protein precipitated with 400 µL of methanol and centrifuged for three minutes at 25,000 g. Of the resulting supernatant, 100 µL was diluted with 100 µL of deionized water and mixed. 0.5 µL of this was then injected onto the UPLC-MS/MS system.

LC conditions

UPLC separation was performed with an ACQUITY UPLC I-Class System (fixed loop), equipped with a CORTECS T3, 2.7 µm (2.1 × 30 mm) analytical column. A sample of 0.5 µL was injected at a flow rate of 1.3 mL/min. Mobile phase A was 0.01% formic acid (aq) containing 0.2 mM Ammonium Formate and mobile phase B was 50% isopropanol in acetonitrile containing 0.01% formic acid and 0.2 mM Ammonium Formate. After an initial 0.1 minute hold at 2% Mobile phase B, the acylcarnitines were eluted from the column and separated with a gradient of 2–98% Mobile phase B over 0.7 minutes, followed by a 0.9 minute column wash at 98% Mobile phase B. The column was then re-equilibrated to initial conditions. The analytical column temperature was maintained at 60 °C.

MS conditions

Multiple Reaction Monitoring (MRM) analyses were performed using a Xevo TQ-S micro Mass Spectrometer. All experiments were performed in positive electrospray ionization (ESI+) mode. The ion source temperature and capillary voltage were kept constant and set to 150 °C and 2.0 kV respectively. The cone gas flow rate was 50 L/hr and desolvation temperature was 650 °C.

Informatics

Method information was imported onto the LC-MS system using the Quanpedia functionality within MassLynx. This extendable and searchable database produces LC and MS methods as well as processing methods for use in TargetLynx for compound quantification.

Results and Discussion

The 20 acylcarnitines detailed in Table 1 were separated and detected using the LC-MS platform and extraction protocol described herein. Some of the unsaturated acylcarnitines shown give multiple peaks in their MRM trace. This is most probably due to isomeric forms of these analytes. Figures 2 and 3 show example chromatograms for the separation achieved using the UPLC method detailed above.

Acylcarnitine	MRM transition	RT (mins)	Cone voltage (V)	Collision energy (eV)
C2	204.10>85.00	0.07	32	18
C3	218.10>85.00	0.09	32	18
C4	232.10>85.00	0.14	34	20
C5	246.10>85.00	0.26	38	22
C5:1	244.20>85.00	0.23	38	22
C6	260.20>85.00	0.32	54	20
C8	288.20>85.00	0.41	42	22
C8:1	286.20>85.00	0.37/0.39 (Multiple peaks)	42	22
C10	316.20>85.00	0.49	42	24
C10:1	314.20>85.00	0.46/0.47 (Multiple peaks)	42	24
C12	344.30>85.00	0.55	46	24
C12:1	342.30>85.00	0.52–0.53 (Multiple peaks)	46	24
C14	372.30>85.00	0.60	46	26
C14:1	370.30>85.00	0.57	46	26
C14:2	368.30>85.00	0.54–0.56 (Multiple peaks)	46	26
C16	400.30>85.00	0.65	50	26
C16:1	398.30>85.00	0.62	50	26
C18:0	428.40>85.00	0.69	50	28
C18:1	426.40>85.00	0.66	50	28
C18:2	424.30>85.00	0.63	50	28

Table 1. List of MS/MS conditions and retention times for acylcarnitines.

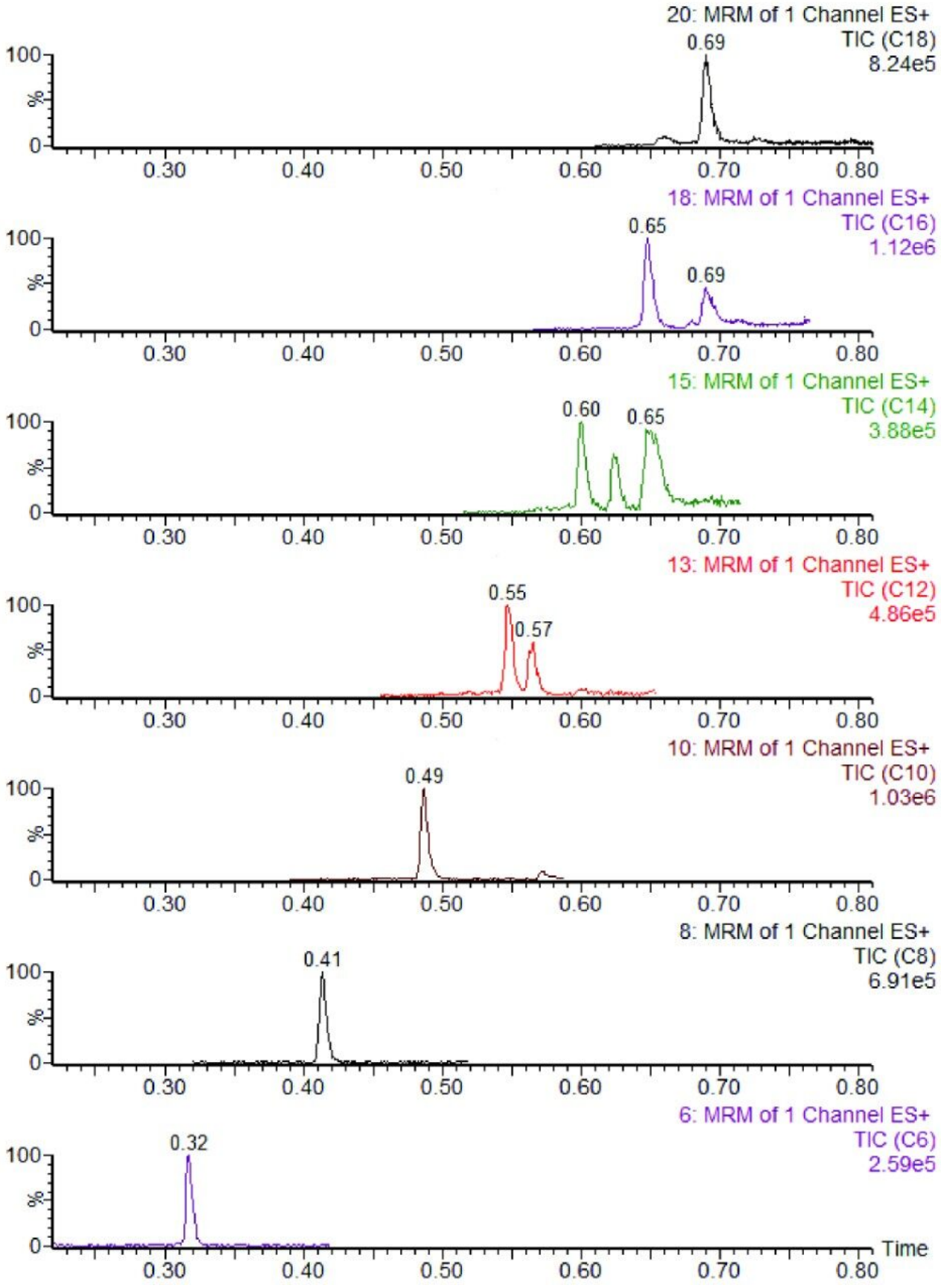
20: MRM of 1 Channel ES+
TIC (C18)
8.24e5
0.69
18: MRM of 1 Channel ES+
TIC (C16)
1.12e6
0.65
0.69
15: MRM of 1 Channel ES+
TIC (C14)
3.88e5
0.60
0.65
13: MRM of 1 Channel ES+
TIC (C12)
4.86e5
0.55
0.57
10: MRM of 1 Channel ES+
TIC (C10)
1.03e6
0.49
8: MRM of 1 Channel ES+
TIC (C8)
6.91e5
0.41
6: MRM of 1 Channel ES+
TIC (C6)
2.59e5
0.32
100
%
0
0.30
0.40
0.50
0.60
0.70
0.80
Time

Figure 2. Rapid UPLC separation achieved for saturated acylcarnitines in human serum.

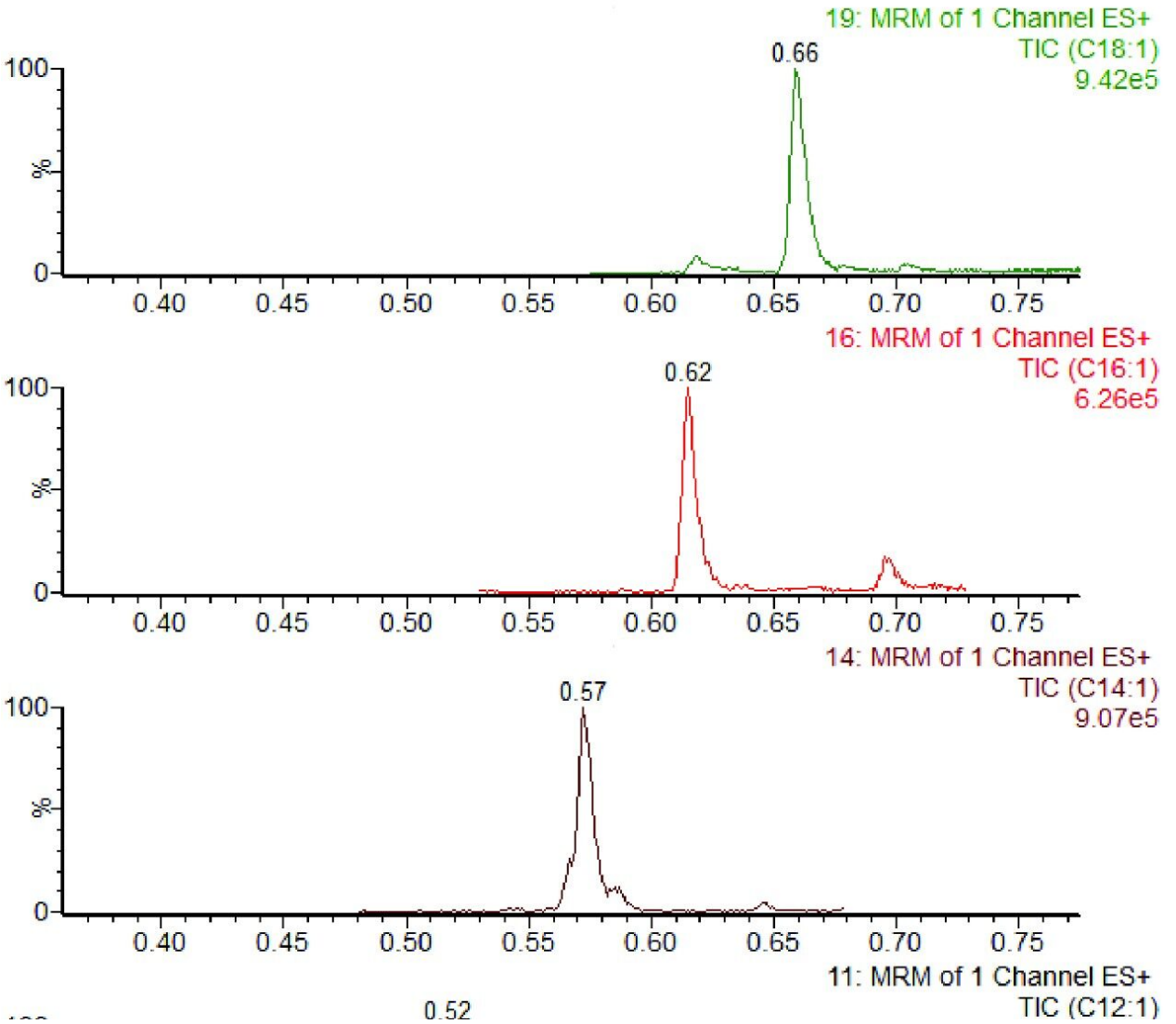

Figure 3. Rapid UPLC separation achieved for unsaturated acylcarnitines in human serum.

Conclusion

A rapid UPLC-MS/MS methodology has been developed for the research analysis of acylcarnitines. This method has been demonstrated to be suitable for the analysis of physiologically relevant levels of these

analytes in human serum. This method utilizes a generic LC-MS platform that can be used for various compound classes (including metabolomics, lipidomics, and proteomics), meaning it can be applied as part of a suite of analyses that are run subsequently as part of a targeted multi-omics workflow.

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ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <https://www.waters.com/134798856>

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Quanpedia <https://www.waters.com/10148049>

MetaboQuan-R <https://www.waters.com/134977273>

Available for purchase online:

CORTECS T3 Column, 120Å, 2.7 µm, 2.1 mm X 30 mm <
https://www.waters.com/waters/partDetail.htm?partNumber=186008481>

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