Poster Reprint

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Quantification of underivatized acylcarnitines and carnitine intermediates using RP chromatography and ion funnel triple quadrupole in fecal samples.

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Introduction

Acylcarnitines Panel

Acylcarnitines are intermediate metabolites of the mitochondria and serve a significant role in fatty acid oxidation and amino acid metabolism in various biological systems.

Currently, acylcarnitine high-throughput analysis does not provide differentiation and quantification of isobaric and isomeric compounds.

Furthermore, isobaric matrix interferences can lead to an overestimation of metabolite concentrations.

Figure 1. Agilent Infinity II 1290 Bio LC (left), and detection with the new Agilent 6495D LC/TQ (right).

To address many issues inherent to such analysis, a new comprehensive LC-MS/MS method was developed to quantify 32 acylcarnitine species, with acyl-chain lengths from C0 to C20 in commercial fecal samples.

Experimental

Commercial Samples Extraction

Approximately 120 mg of certified human fecal sample (Medix Biochemica, Maryland Heights, MO) is weighed into 2 mL bead tubes containing ceramic beads and extracted with ice-cold methanol:water (8:2, v/v) containing internal standards to a final concentration of 100 mg/mL. Samples were homogenized, and the slurries were incubated overnight at -80°C. Slurries were vortexed, centrifuged for 20 minutes (20,000 x g, at 4°C), and 100 µL of the supernatant was dried. Samples were reconstituted in water:methanol (8:2,v/v) and transferred to injection vials.

Experimental

One LC-MS/MS Method – Wide Coverage

Method development was achieved using the new MH 12.2 software built-in with Compound and Source Optimizer.

Table 1. LC conditions.

LC Conditions	
Column	Agilent Zorbax Eclipse Plus C18, 2.1x100mm, 1.8 µm
Column temp	15°C / Autosampler Temp: 4°C
Needle washes	S1. 5s ACN:MeOH:H_2O (1:1:1, v/v) S2. 5s Milli-Q water
Mobile phase	A: 15 mM ammonium acetate + 0.3 % formic acid B: MeOH:H_2O (95:5, v/v) + 15 mM ammonium acetate + 0.3 % formic acid
Flow rate	0.4 mL/min
Gradient program	0.0 – 1.5 min (2%B), 1.6 – 3.0 min (6.5%B), 3.1 – 7.0 min (7.5%B), 7.1 – 13.0 min (15%B), 13.1 – 15.0 min (85 – 100%B), 15.0 – 18.0 min (100%B), return initial conditions.
Total run time	21 min

Table 2. 6495D Mass Spectrometer Parameters.

6495D MS Conditions	
Sheath Gas Temperature	300 ºC
Sheath Gas Flow	11.0 L/min
Gas Temperature	250 ºC
Gas Flow	18.0 L/min
Nebulizer	35.0 psi
Capillary	3000 V (+)
Nozzle Voltage	0 V (+)
iFunnel Mode	Fragile
Detector Gain	2

Results and Discussion

Chromatographic Method Overview and Instrument Response.

Targeted acylcarnitines analysis allows the detection of 32 analytes, covering short-, medium-, and long-chain acylcarnitines in one RP method.

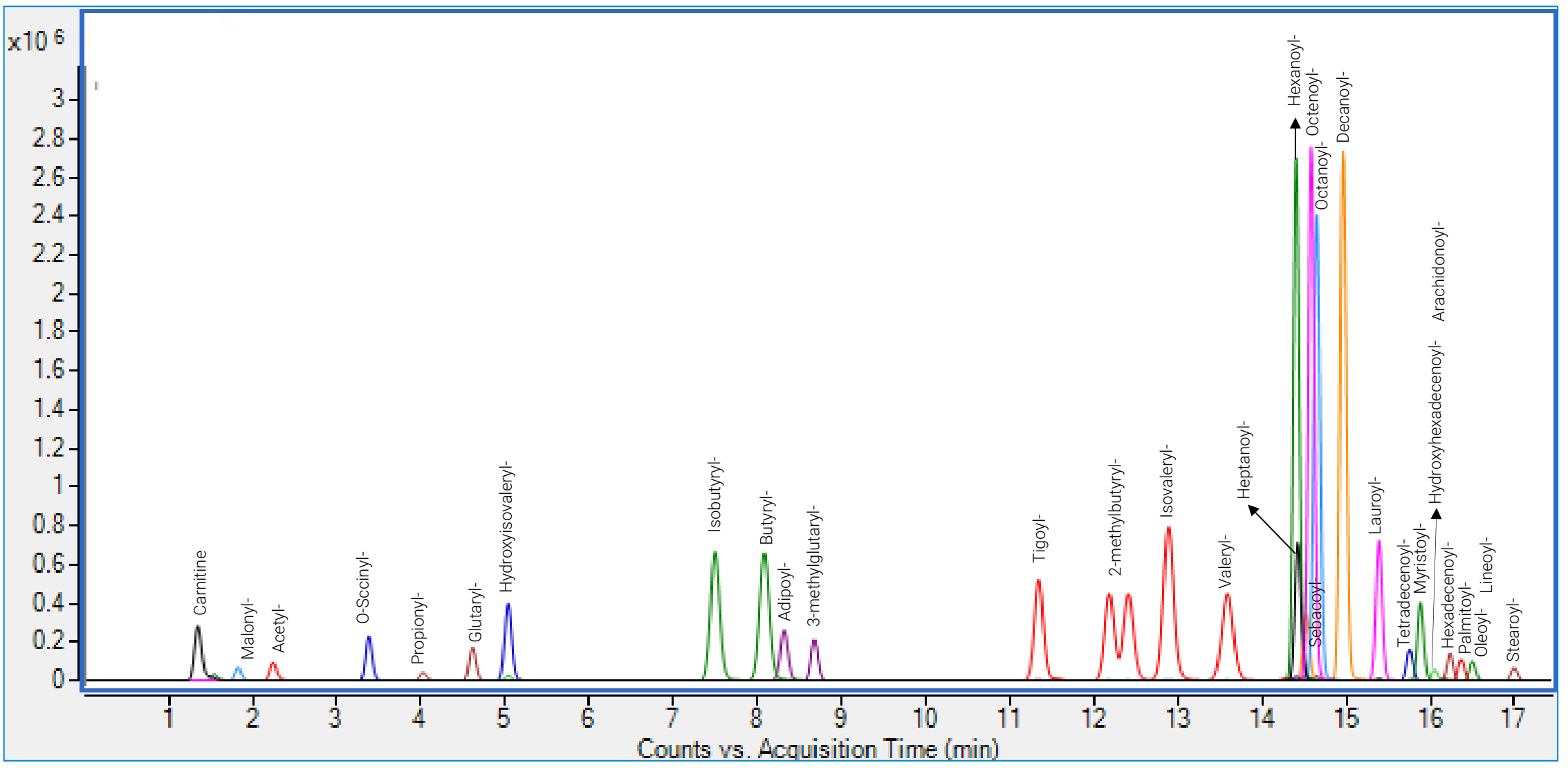

Figure 2. Chromatographic separation using the ZORBAX Eclipse Plus C18 column for all 32 acylcarnitines.

A robust chromatography separation for the main acylcarnitine isomers was achieved using a unique reverse phase method (Figure 2). Isomers of C4, C5, and C6 were separated as shown in Figure 3. For each analyte, two transitions were monitored, and one transition for the respective internal standard. The product ion 85.1 was used as the quantifier ion for all compounds, except for L-carnitine (43.1 *m/z*) and deoxycarnitine (45.1 *m/z*).

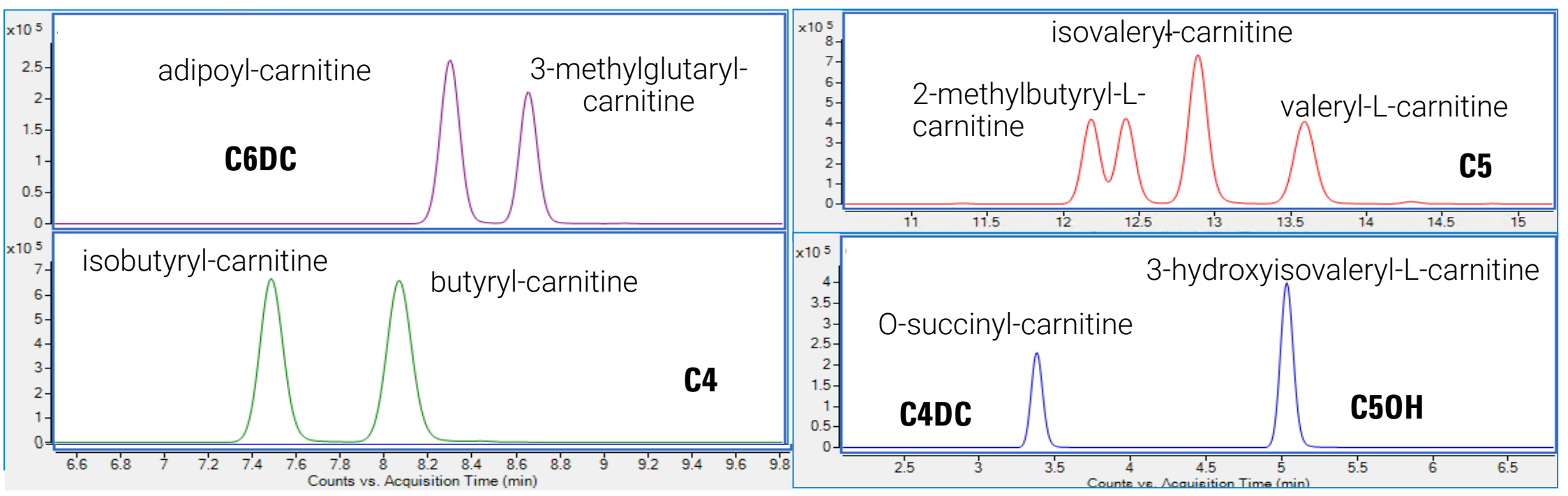

Figure 3. Main isomers separation.

Results and Discussion

Calibration Curves

All calibration curves showed an excellent linearity (R^2 > 0.99) in the concentration range of 0.1 to 500 nmol/L. Figure 4 depicts four acylcarnitine examples. The analytes tested with matching labeled internal standards showed very low RSD (<10%). And the recovery tests showed an excellent recovery for all the analytes (80.4-121.9%). MassHunter Quantitative Analysis 12.0 generates calibration-at-a-glace figures allowing for quick processing of all calibration curves and customizable visualization to fit any view and subset of compounds (Figure 4).

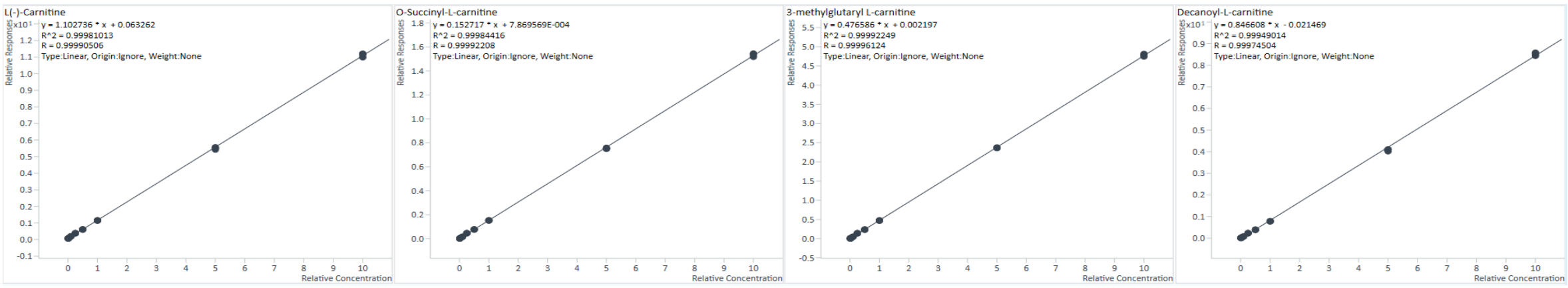

Figure 4. Calibration-at-a-glace of the acylcarnitines.

Analysis of biological samples

Using this targeted analytical method, we profiled acylcarnitines in a small cohort of inflammatory bowel disease (IBD) samples obtained from Medix Biochemica. The dataset included 5 controls, 5 Crohn's disease samples, and 5 ulcerative colitis samples. Figure 5 shows a trend towards decreased levels of L-carnitine and various acylcarnitines in the IBD samples. However, due to the limited sample size, statistical significance could not be assessed.

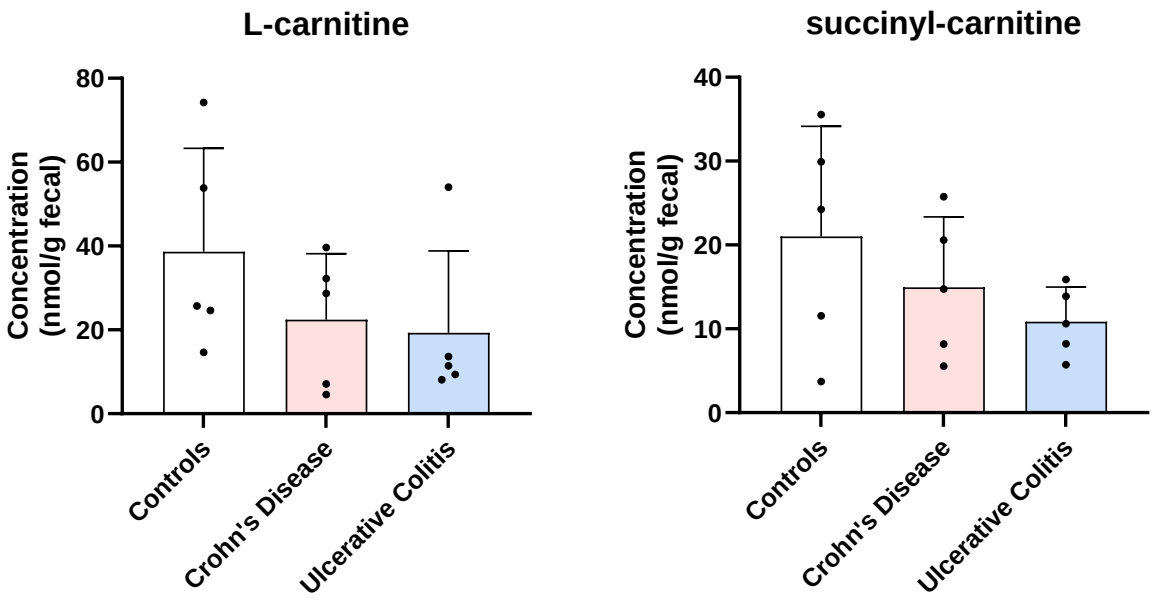

Figure 5. MassHunter Quantitative Analysis 12.0 was used to analyze and quantify the acylcarnitines in the study samples. Concentrations were plotted using GraphPad Prism software.

Conclusions

- **Comprehensive coverage:** This method provides broad and reliable detection of fecal acylcarnitines.
- **High-throughput:** The total run time of 19 minutes (including conditioning) enables efficient sample processing.
- **User-friendly workflow:** No derivatization required – easily implemented within standardized metabolomics platforms.
- **Excellent performance:** The method delivers strong reproducibility and recovery with a simple, one-step extraction.
- **Biological insights:** Fecal acylcarnitine profiles hold the potential to identify biomarkers and metabolic signatures in various health conditions, including inflammatory bowel disease (IBD).

References

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