Poster Reprint

ASMS 2025
Poster number WP 370

Rapid Analysis of Acylcarnitines in Dried Blood Spots Using Fast Chromatography with Ion Mobility High Resolution Mass Spectrometry

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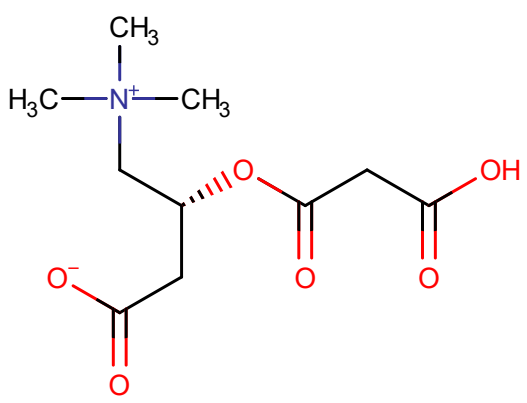
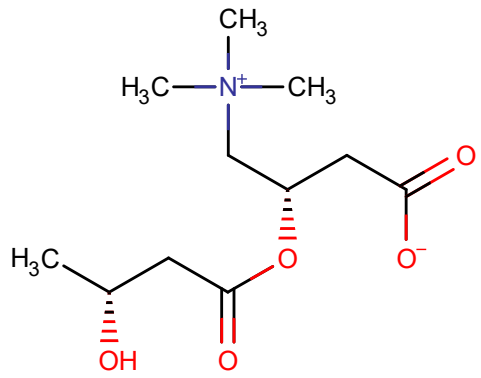
Introduction

Acylcarnitines play a key role in fatty acid metabolism, making their analysis vital for better understanding metabolic disorders. However, isobaric acylcarnitines, which share identical nominal masses but differ in structure, present a challenge in separation and identification. In this study, we introduce a 1-minute method for separating and identifying acylcarnitines in dried blood spots (DBS) using fast chromatography with ion mobility mass spectrometry. By leveraging ion mobility spectrometry as an additional dimension, we achieve separation of nominal isobaric acylcarnitines offering improved confidence in reporting and shorter analysis times.

This method enables rapid and accurate profiling of acylcarnitines, enhancing the potential for high-throughput metabolic investigations. It significantly reduces analysis time making it a promising tool for in-depth metabolic research and method development.





$C_{10}H_{17}NO_6$

(M+H)+	248.1129		0.1129

Malonyl Carnitine, C3DC

Theoretical CCS Value (Å2)=158.3 (Ref 1)

$C_{11}H_{21}NO_5$

(M+H)+	248.1492		0.1492

Isovaleryl Carnitine, C4OH

Theoretical CCS Value (Å2)=166.6 (ref 1)

Experimental

Sample Preparation

Methods

A simplified sample preparation protocol was used in which a 3 mm disc was punched from each dried blood spot (DBS) and incubated with 100 µL of SABG Internal Standard for 45 minutes with shaking. After centrifugation, the supernatant was collected directly for mass spectrometry analysis. No derivatization or drying down of extracts was performed.

The simplified protocol reduces hands-on time and enables faster throughput without compromising analytical performance.

A flow diagram of the workflow is shown in Figure 1.

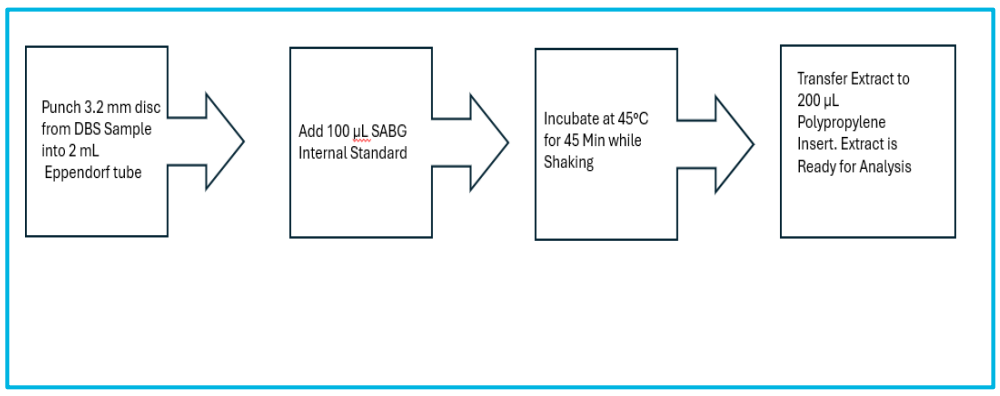


Figure 1. Flow Diagram of Sample Preparation

LC/IMQTOF Conditions

A short C18, 100mm column was used with a 1-minute gradient. The initial mobile solvent composition is 70:30 acetonitrile:water with ammonium .The gradient was ramped to 95:5 ACN:Water in 0.5 min. achieving rapid separation of acylcarnitines

Detection and separation were performed using an ion mobility QTOF mass spectrometer, enabling the differentiation of isobaric acylcarnitines based on their size and shape.

Results and Discussion

Preliminary Data

Among the isobaric species encountered in newborn screening (NBS), the most challenging to differentiate and quantitate are C3DC (malonylcarnitine) and C4OH (3-hydroxybutyrylcarnitine). These metabolites are critical biomarkers with nearly identical nominal masses and theoretical m/z values of 248.1134 (C3DC) and 248.1498 (C4OH). Their structural similarities and overlapping masses make them particularly difficult to distinguish using conventional mass spectrometry methods.

Derivatization is often employed to enhance ionization efficiency and improve sensitivity, but it does not resolve the issue of mass overlap. MS/MS analysis is generally insufficient for unambiguous identification, as both species yield similar fragmentation patterns dominated by the m/z 85 fragment, characteristic of acylcarnitines.

High-resolution mass spectrometry (HRMS) with the 6560 IMQTOF provides the mass resolution needed to separate these nominal isobars. C3DC and C4OH differ by 0.03 Da. Additionally, ion mobility spectrometry (IMS) coupled introduces an orthogonal dimension of separation. While the collisional cross section (CCS) values of C3DC and C4OH are close, the resolving power of the 6560 IM-QTOF is sufficient to achieve separation, enabling confident identification in complex biological matrices. This is especially useful in high-throughput workflows requiring analysis times under one minute.

The results for a DBS sample enriched with C3DC are shown in Figure 2. The concentration of C4OH is significantly lower than that of C3DC in this sample Despite the disparity in abundance, clear separation between these nominal isobars is observed in both accurate mass and drift time domains. This demonstrates the capability of high-resolution mass spectrometry combined with ion mobility to differentiate closely related isobaric species, even when present at different concentrations.

Discussion of Results

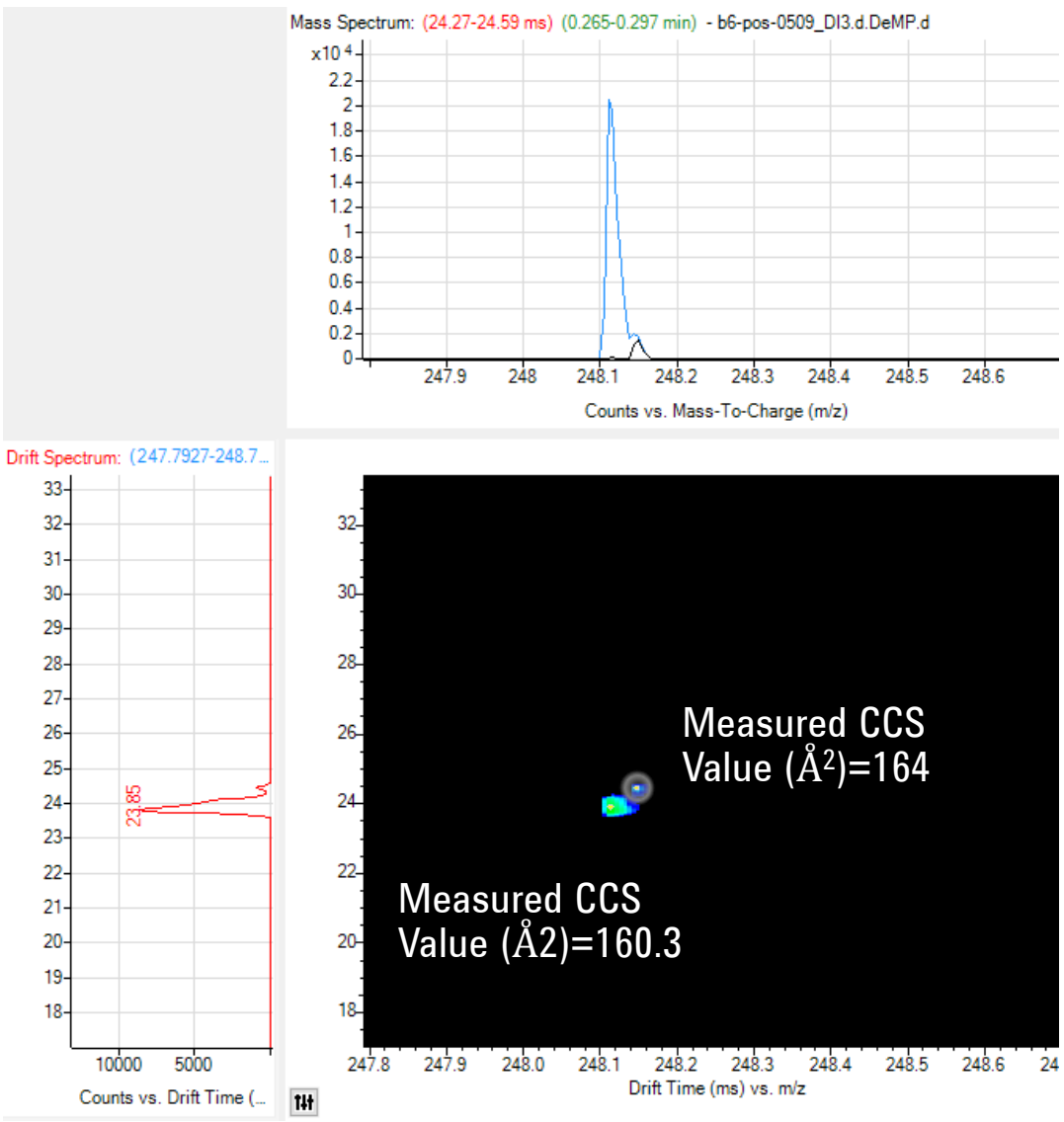


Figure 2. Separation in mass and drift time for C3DC and C4OH in DBS.

The ion mobility-mass spectrometry (IM-MS) data demonstr clear separation between C3DC and C4OH in dried blood spo (DBS), despite their similar m/z values. The observed differe drift time corresponds to a slight variation in their collision cr section (CCS), with a difference of approximately 4 Å². This s structural difference enables their resolution in the drift dime

Notably, even though the concentrations of the two compou differ, both components are distinctly detectable in the DBS highlighting the sensitivity and resolving power of the IM-MS technique.

Results and Discussion

Discussion of Results.

Extracted Ion Chromatograms for Sample B and Sample D are shown below in Figure 3. Skyline™ was used for extracting the Ion Mobility signals and building the calibration curves shown in Figures 4 and 5.

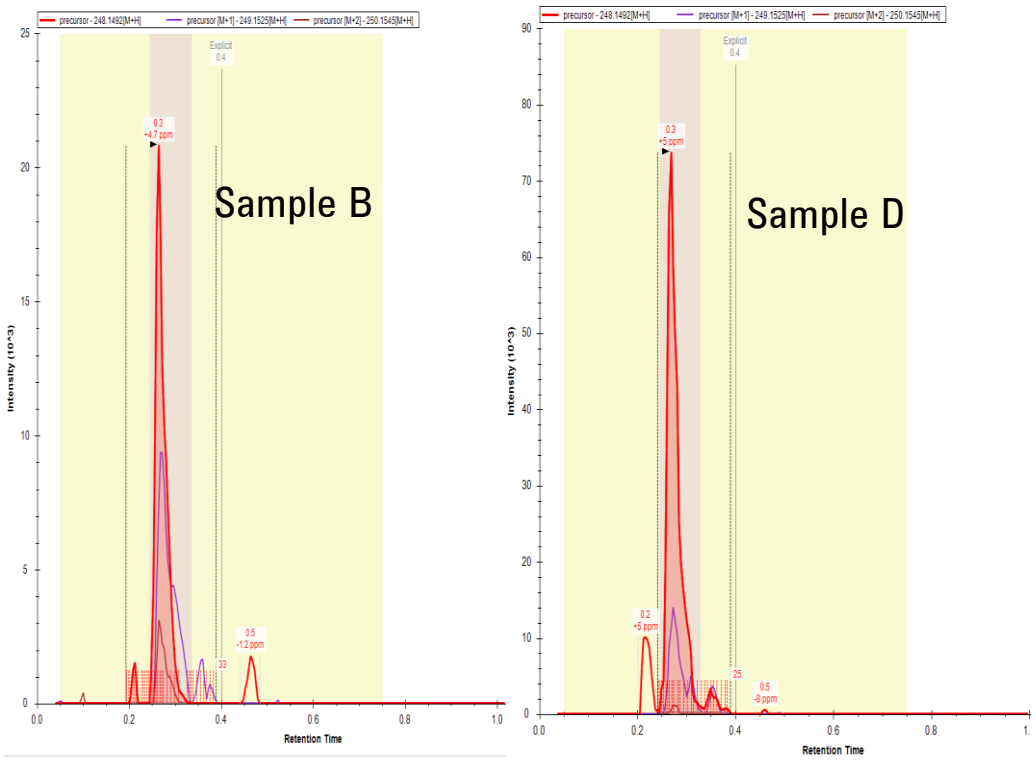


Figure 3. Extracted ion chromatograms for C4OH in DBS Sample B and Sample D.

Figure 3 shows the extracted ion chromatograms for the precursor and isotopic peaks using Skyline™, with Sample B and D analyzed to assess detection sensitivity. Ion Mobility filtering with a resolving power of 100 was used for extraction.

The clear and strong signal observed for the [M+H]+ ion at m/z 248.1492, along with distinguishable M+1 and M+2 isotopic peaks, indicates robust detection. Given the signal intensity and peak shape, particularly at the primary retention time (~0.3 min), it would be advisable to proceed with a 5 mm punch or two smaller punches. This approach maximizes analyte recovery and enhances signal clarity, supporting reliable quantitation in low-concentration DBS samples.

Results

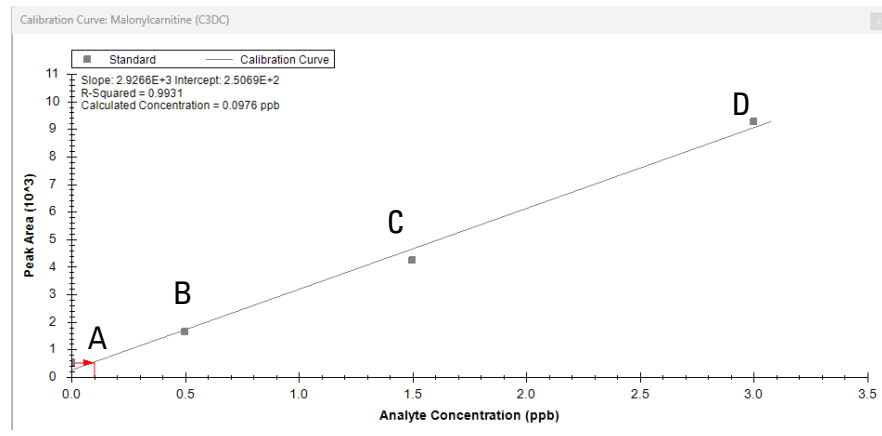


Figure 4. Calibration Curve for C3DC

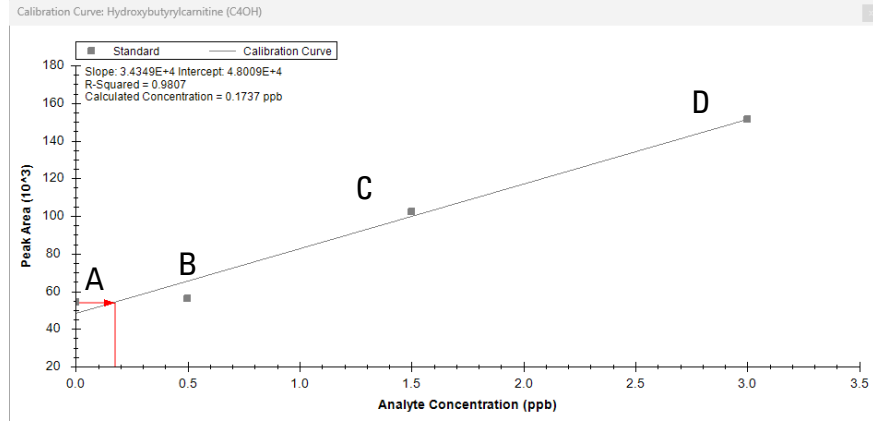


Figure 5Calibration Curve for C4OH.

Conclusions

In conclusion, this preliminary study demonstrates that ion mobility coupled with high-resolution mass spectrometry offers a powerful approach for differentiating isobaric acylcarnitine isomers, providing an additional dimension of separation via drift time. The method enables rapid and confident analysis directly from DBS samples. Future studies will focus on evaluating larger DBS spot sizes to enhance sensitivity and quantitation, optimizing sample input for more comprehensive metabolic investigations.

References

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Published in USA, May 15, 2025

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