Poster Note 64379

Comparison of Non-derivatization and Derivatization Tandem Mass Spectrometry Research Methods for Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots

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Overview

Purpose:

To compare non-derivatization and derivatization research methods for analysis of amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC) in dried blood spots (DBS) using Thermo Scientific™ TSQ Endura™ mass spectrometer.

Methods:

FIGURE 1. Workflow of flow injection tandem mass spectrometry analysis

Results:

- Both non-derivatization and derivatization methods were capable of accurately quantifying 12 AAs, 18 ACs, and SUAC on TSQ Endura MS with a run time of 1.5 min.
- Both methods had excellent analytical precision performance. The within-run imprecision (n=10) was less than 10% and run-to-run imprecision (n=70) was less than 15%.
- The quantitative value difference between non-derivatization and derivatization methods was minor (<15%) for the majority of analytes.

Introduction

Original FIA-MS/MS sample preparation techniques detect butyl esterification (i.e., derivatized) of AAs, ACs, and SUAC. However, with improved sensitivity of MS instruments, it is possible to detect AAs, ACs, and SUAC as their native free acids (i.e., non-derivatized). This simplifies analytical operation and minimizes the use of corrosive chemicals.

Methods

Sample Preparation

The following steps worked for both methods. However, step 6 was for derivatization method only.

1. Punch one 1/8 inch diameter disc from DBS sample and put into 96-well plate.
2. Add 100 µL of working internal standard solution to each well.
3. Shake the plate for 45 min at 45°C.
4. Transfer the eluates to another plate and evaporate at 50°C under nitrogen flow.
5. Pipet 50 µL of methanol into each sample well and evaporate under nitrogen flow.
6. **Pipet 50 µL of 3 N butanol HCl into each sample well and incubate at 65 °C for 20 min. Then evaporate under nitrogen flow.**
7. Reconstitute each sample well with 100 µL of mobile phase.

Liquid Chromatography

LC pump: Thermo Scientific™ Dionex™ Ultimate™ HPG-3200 RS

Autosampler: Thermo Scientific™ Ultimate WPS-3000 TRS

HPLC column: None

Mobile phase: 50:50:0.02 acetonitrile/water/formic acid

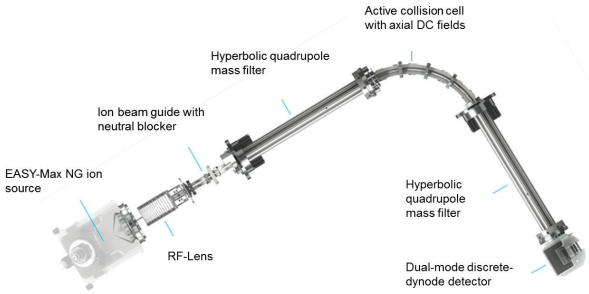
FIGURE 2. LC flow gradient

Time (min)	Flow rate (mL/min)	%A (mobile phase)
0.00	0.09	100
1.23	0.09	100
1.25	0.30	100
1.50	0.09	100

Mass Spectrometry

With best in class sensitivity, unprecedented usability, and exceptional robustness, the TSQ Endura triple quadrupole mass spectrometer delivers exceptional value. SRM was used to acquire MS/MS data.

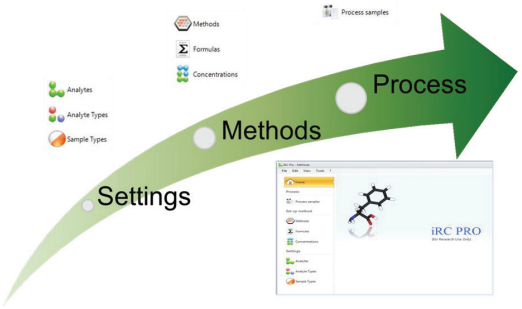
FIGURE 3. Schematic diagram of TSQ Endura MS



Data Analysis

iRC PRO software (2Next srl, Prato, Italy) can process peak area, concentration and user-defined formulas. It improves time effectiveness by eliminating the manual calculation process and removing transcription errors in the post-analytical phase. The processing time is reduced from hours to minutes.

FIGURE 4. iRC PRO intuitive workflow – icon based user interface



Results

SRM allowed acquisition of peaks with good signal-to-noise ratios even for analytes with poor ionization such as SUAC and C5DC regardless of whether derivatization was used.

FIGURE 5. Flow injection analysis profiles of SUAC, C5DC and their IS

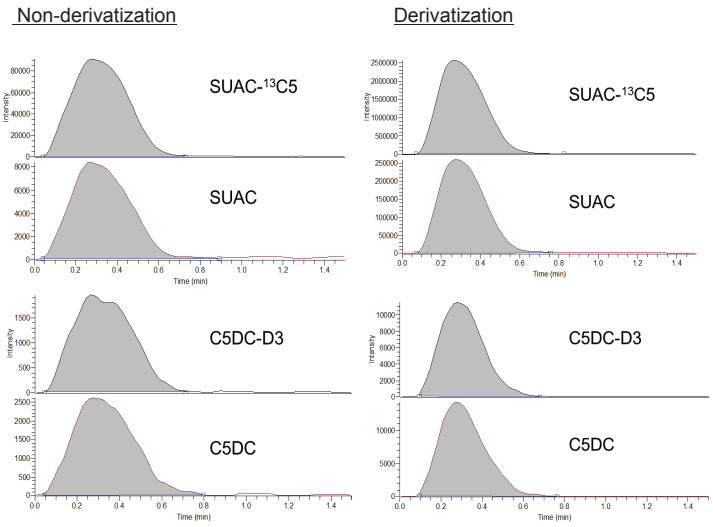
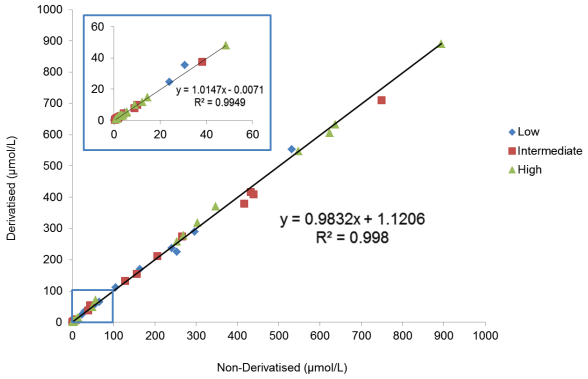


FIGURE 6. Within-run (n=10) and run-to-run (n=70) imprecision (CV, %) for Non-derivatization (Non-deriv.) and derivatization (Deriv.) methods

Target	Non-deriv. (within-run)	Non-deriv. (run-to-run)	Deriv. (within-run)	Deriv. (run-to-run)
Alanine	7.9	17.2	10.0	10.7
Arginine	7.7	11.8	7.5	17.4
Aspartic acid	13.9	16.3	7.8	9.9
Citrulline	6.7	10.6	5.5	14.8
Glutamic acid	6.4	10.0	6.9	11.4
Glycine	9.6	14.0	7.9	11.0
Leucine	7.0	10.2	7.9	12.2
Methionine	7.5	18.8	7.6	12.1
Ornithine	7.5	8.7	9.7	16.7
Phenylalanine	6.7	9.2	7.2	12.4
Tyrosine	6.2	9.6	9.1	13.4
Valine	7.2	9.5	8.7	11.9
Succinylacetone	12.5	17.6	8.3	11.9
Carnitine	6.1	11.9	8.0	14.7
C2-Carnitine	6.3	10.4	9.0	14.3
C3-Carnitine	7.2	10.4	12.0	16.1
C3DC-Carnitine	6.4	11.1	7.8	14.7
C4-Carnitine	7.8	10.9	7.9	15.7
C4OH-Carnitine	6.0	10.8	7.3	16.7
C5-Carnitine	8.0	11.5	7.6	15.4
C5DC-Carnitine	8.0	15.4	6.6	13.8
C5OH-Carnitine	8.7	10.2	7.2	14.8
C6-Carnitine	9.4	12.1	8.3	14.8
C8-Carnitine	7.1	9.7	7.5	15.3
C10-Carnitine	8.5	15.0	7.0	17.4
C12-Carnitine	5.9	10.2	7.2	16.6
C14-Carnitine	6.6	9.8	9.5	15.5
C16-Carnitine	5.8	10.5	10.8	15.7
C16OH-Carnitine	7.6	12.6	12.7	16.3
C18-Carnitine	6.7	10.5	10.4	16.1
C18OH-Carnitine	10.0	16.8	10.3	16.3
Average	**7.7**	**12.1**	**8.4**	**14.4**

FIGURE 7. Comparisons between non-derivatization and derivatization methods



Conclusions

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