Evaluation and Comparison of Nonderivatization and Derivatization Tandem Mass Spectrometry Methods for Multianalyte Analysis in Dried Blood Spots

Xiaolei Xie, Marta Kozak
Thermo Fisher Scientific, San Jose, CA, USA

Poster Note 64422

Overview

Purpose:

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

Methods:

Analytes were extracted from DBS samples using internal standards containing solvent followed by nonderivatization or derivatization process. Resuspended samples were directly injected into a tandem mass spectrometer without LC column separation. Acquired MS/MS data were processed using streamlined iRC PRO™ software.

Results:

▪Both nonderivatization and derivatization methods were capable of accurately quantifying 12 AAs, 18 ACs, and SUAC on a 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 nonderivatization and derivatization methods was minor (<15%) for the majority of analytes.

Introduction

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

Methods

The method workflow is described in Figure 1.

FIGURE 1. Workflow of flow injection tandem mass spectrometry analysis.

Sample Preparation

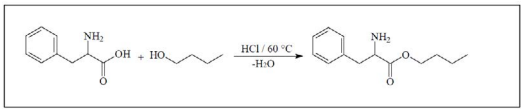
The following steps worked for both methods. However, step 6 was for the derivatization method only.

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.

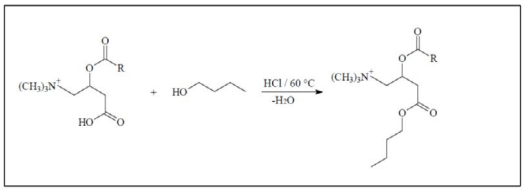
Thermo SCIENTIFIC

The derivatization processes for phenylalanine and acylcarnitines are described in Figure 2.

FIGURE 2. Derivatization of amino acids and acylcarnitines.



Derivatization of phenylalanine to phenylalanine butyl ester



Derivatization of acylcarnitines to acylcarnitine butyl esters

Liquid Chromatography

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

Autosampler: UltiMate™ Open Autosampler OAS-3300TXRS

HPLC column: None

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

Gradient: See Table 1

TABLE 1. 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

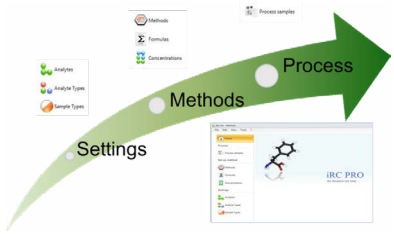
Flow injection MS/MS analysis was performed on a TSQ Endura mass spectrometer equipped with a Thermo Scientific™ Ion Max NG source and heated electrospray ionization (HESI) probe (Figure 3). SRM was used to acquire MS/MS data.

FIGURE 3. UltiMate 3000 RSLC system and TSQ Endura MS.

Data Analysis

Tandem MS data were processed using a meta-calculation software, iRC PRO (2Next srl, Prato, Italy, Figure 4). The concentrations of target analytes were calculated by using ion intensity ratios against internal standards. The software eliminates the manual calculation process and removes transcription errors in the post-analytical phase.

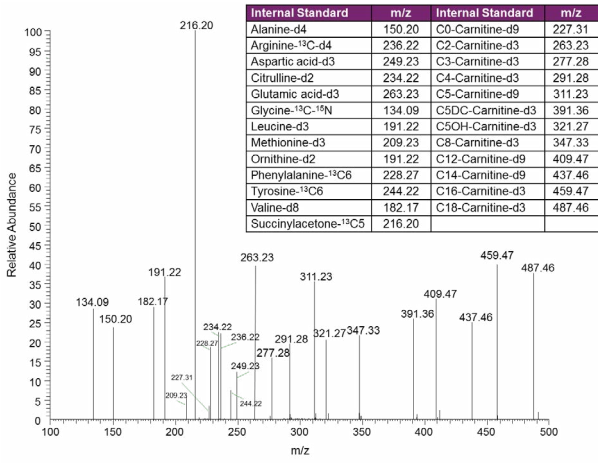
FIGURE 4. iRC PRO intuitive workflow – icon based user interface.



Results

Results are shown in Figures 5 through 7 and Table 2.

FIGURE 5. Full-scan spectra of derivatized internal standards.



Internal Standard	m/z	Internal Standard	m/z
Alanine-d4	150.20	C0-Carnitine-d9	227.31
Arginine-^{13}C-d4	236.22	C2-Carnitine-d3	263.23
Aspartic acid-d3	249.23	C3-Carnitine-d3	277.28
Citrulline-d2	234.22	C4-Carnitine-d3	291.28
Glutamic acid-d3	263.23	C5-Carnitine-d9	311.23
Glycine-^{13}C-^{15}N	134.09	C5DC-Carnitine-d3	391.36
Leucine-d3	191.22	C5OH-Carnitine-d3	321.27
Methionine-d3	209.23	C8-Carnitine-d3	347.33
Ornithine-d2	191.22	C12-Carnitine-d9	409.47
Phenylalanine-^{13}C6	228.27	C14-Carnitine-d9	437.46
Tyrosine-^{13}C6	244.22	C16-Carnitine-d3	459.47
Valine-d8	182.17	C18-Carnitine-d3	487.46
Succinylacetone-^{13}C5	216.20		

FIGURE 6. Flow injection analysis profiles of SUAC-^{13}C5, SUAC and C5DC-d3, C5DC.

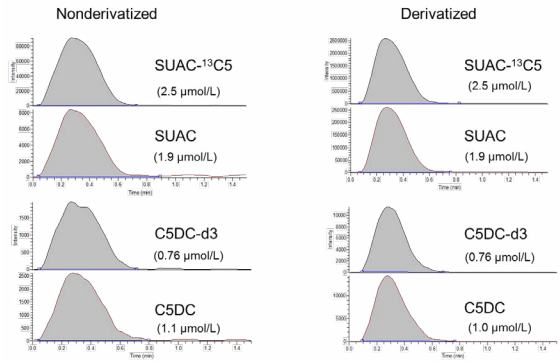
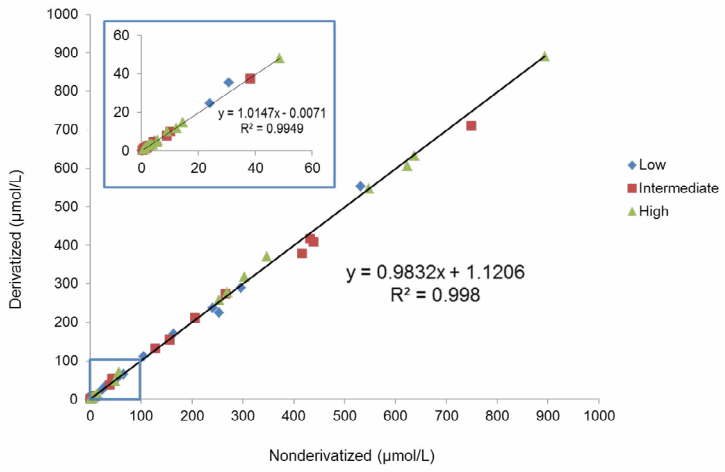


TABLE 2. Method within-run (n=10) and run-to-run imprecisions (n=70) at three concentrations (low, medium, and high) for nonderivatization (top) and derivatization method (bottom).

Nonderivatized	Within-run Imprecision (CV, %)			Run-to-run Imprecision (CV, %)		
	Low	Medium	High	Low	Medium	High
Alanine	4.7	7.2	11.7	20.0	16.1	15.6
Arginine	6.1	7.2	9.7	12.0	11.3	12.2
Aspartic acid	13.0	13.7	15.1	13.4	17.5	18.1
Citrulline	4.4	7.8	8.0	10.7	11.4	9.7
Glutamic acid	8.0	3.8	7.3	10.4	9.1	10.6
Glycine	8.6	9.7	10.6	13.4	13.7	14.8
Leucine	5.5	6.3	9.2	10.8	9.7	10.2
Methionine	8.1	4.8	9.7	18.8	17.5	20.2
Ornithine	5.4	7.7	9.4	8.6	8.8	8.8
Phenylalanine	4.9	5.7	9.4	7.7	8.7	11.2
Tyrosine	5.2	5.9	7.5	8.1	10.0	10.8
Valine	5.1	6.3	10.1	9.1	9.3	10.1
SUAC	10.5	14.1	13.0	18.1	21.0	13.7
C0-Carnitine	5.6	6	6.6	12.5	11.3	12.0
C2-Carnitine	6.7	5.4	6.8	10.3	10.0	10.9
C3-Carnitine	8.7	3.9	8.9	9.8	9.7	11.8
C3DC-Carnitine	6.9	6.5	5.9	12.4	11.8	9.1
C4-Carnitine	9.6	5.2	8.5	10.3	10.8	11.6
C4OH-Carnitine	5.2	5.5	7.3	11.3	10.5	10.6
C5-Carnitine	7.8	7.3	9	11.2	11.6	11.6
C6-Carnitine	6.3	6.8	10.8	16.9	16.5	12.7
C5DC-Carnitine	8.7	7.1	10.3	11.3	9.1	10.1
C5OH-Carnitine	10.1	7.8	10.3	12.8	11.3	12.3
C8-Carnitine	8.3	5.2	7.8	9.9	8.6	10.7
C10-Carnitine	9.6	6.8	9.2	18.4	13.5	13.2
C12-Carnitine	6.7	4.6	6.5	12.2	8.7	9.8
C14-Carnitine	5.8	8.2	5.9	11.3	8.0	10.0
C16-Carnitine	7.8	4	5.5	10.9	8.4	12.2
C18-Carnitine	7.1	3.4	9.5	12.1	7.8	11.6

Derivatized	Within-run Imprecision (CV, %)			Run-to-run Imprecision (CV, %)		
	Low	Medium	High	Low	Medium	High
Alanine	9.5	8.9	11.5	12.2	9.6	10.3
Arginine	5.4	9.6	7.6	17.1	16.6	18.6
Aspartic acid	9.0	6.5	8.0	11.2	10.7	7.9
Citrulline	6.8	4.0	5.6	17.0	14.8	12.5
Glutamic acid	10.1	5.9	4.7	13.0	10.8	10.4
Glycine	8.8	8.2	6.8	10.3	12.2	10.4
Leucine	8.5	8.4	6.8	12.2	12.2	12.1
Methionine	7.7	6.4	8.7	13.2	11.6	11.5
Ornithine	8.4	12.3	8.4	17.2	15.4	17.5
Phenylalanine	7.5	8.5	5.5	11.8	12.8	12.5
Tyrosine	7.8	10.8	8.6	13.6	12.5	14.0
Valine	9.6	8.3	8.1	11.4	12.8	11.5
SUAC	8.2	7.2	9.4	13.0	13.4	9.4
C0-Carnitine	12.2	5.0	6.8	15.7	15.1	13.4
C2-Carnitine	10.8	8.6	7.6	13.8	14.1	15.1
C3-Carnitine	11.6	12.7	11.7	16.3	15.3	16.6
C3DC-Carnitine	7.4	6.9	9.0	13.8	14.1	16.1
C4-Carnitine	6.7	6.5	10.6	16.3	13.4	17.5
C4OH-Carnitine	7.1	6.1	8.6	16.2	18.4	15.5
C5-Carnitine	6.9	5.4	10.6	15.1	14.6	16.6
C6-Carnitine	8.1	5.7	5.9	14.9	12.3	14.2
C5DC-Carnitine	4.7	8.6	8.3	13.7	15.6	15.2
C5OH-Carnitine	8.4	7.3	9.1	13.4	16.2	14.7
C8-Carnitine	9.0	9.5	4.0	15.4	13.8	16.6
C10-Carnitine	7.4	6.8	6.9	17.4	16.9	18.0
C12-Carnitine	6.1	6.7	8.8	15.4	17.1	17.3
C14-Carnitine	8.9	10.8	8.7	14.5	14.9	17.1
C16-Carnitine	10.7	10.9	10.8	14.7	16.1	16.2
C18-Carnitine	10.2	7.4	13.6	14.7	18.2	15.5

FIGURE 7. Comparison between quantitative values of 12 AA, SUAC, and 18 AC resulting from nonderivatization and derivatization methods.



Conclusion

- Flow injection-tandem mass spectrometry methods were developed to simultaneously detect and quantify amino acids, acylcarnitines, and succinylacetone in a single extraction process in dried blood spots for research. Rapid data processing was performed using iRC PRO metacalculation software.
- Both nonderivatization and derivatization sample preparation methods were capable of accurately quantifying AA/AC/SUAC on a TSQ Endura triple quadrupole MS with a run time of 1.5 min.
- The TSQ Endura MS can provide within-run imprecision (n=10) at three enriched concentrations of less than 10% and run-to-run precision (n=70) of less than 15% for both nonderivatization and derivatization methods.
- The method difference between quantitative values resulting from nonderivatization and derivatization methods was minor and both methods are highly correlated.

References

1. Chace, D.H.; Kalas, T.A.; Naylor, E.W. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. *Clin. Chem.*, **2003**, *49*(11), 1797-817.
2. Chace, D.H., et al. Rapid diagnosis of phenylketonuria by quantitative analysis for phenylalanine and tyrosine in neonatal blood spots by tandem mass spectrometry. *Clin. Chem.*, **1993**, *39*(1), 66-71.
3. Millington, D.S., et al. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. *J. Inherit. Metab. Dis.*, **1990**, *13*(3), 321-4.
4. Dhillon, K.S., et al. Improved tandem mass spectrometry (MS/MS) derivatized method for the detection of tyrosinemia type I, amino acids and acylcarnitine disorders using a single extraction process. *Clin. Chim. Acta*, **2011**, *412*(11-12), 873-9.
5. De Jesus, V.R., et al. Comparison of amino acids and acylcarnitines assay methods used in newborn screening assays by tandem mass spectrometry. *Clin. Chim. Acta*, **2010**, *411*(9-10), 684-9.

www.thermofisher.com

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 10 3292 200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 45 453 9100
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Spain +34 914 845 965
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

PN64422-EN 0217S