Simultaneous Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots for Research Using Nonderivatized and Derivatized Methods

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Application Note 623

Key Words
Amino acids, acylcarnitines, succinylacetone, dried blood spot, nonderivatized, derivatized, TSQ Endura

Goal

To develop flow injection analysis-tandem mass spectrometry (FIA-MS/MS) research methods for simultaneous detection and quantification of 12 amino acids, 18 acylcarnitines, and succinylacetone in dried blood spots and compare derivatization and nonderivatization sample preparation methods.

Introduction

Flow injection tandem mass spectrometry (FIA-MS/MS) has been frequently used for analysis of amino acids (AA) and acylcarnitines (AC) in dried blood spots for inborn errors of metabolism research.[1-3] Established methods for detecting succinylacetone (SUAC) can be laborious because they require additional extraction due to its insolubility in the first extraction solution for AA and AC. In this application note, a single extraction step was used to simultaneously analyze AA, AC, and SUAC in dried blood spots on the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer.[4]

The original sample preparation techniques use butyl esterification (i.e., derivatized) of amino acids and acylcarnitines in dried blood spots (DBS) due to the increased sensitivity that derivatization provides.

Key		
Alanine (Ala)	Valine (Val)	Hydroxyisovalerylcarnitine (C5OH)
Arginine (Arg)	Succinylacetone (SUAC)	Hexanoylcarnitine (C6)
Aspartic acid (Asp)	Free carnitine (C0)	Octanoylcarnitine (C8)
Citrulline (Cit)	Acetylcarnitine (C2)	Decanoylcarnitine (C10)
Glutamic acid (Glu)	Propionylcarnitine (C3)	Dodecanoylcarnitine (C12)
Glycine (Gly)	Malonylcarnitine (C3DC)	Myristoylcarnitine (C14)
Leucine (Leu)	Butyrylcarnitine (C4)	Palmitoylcarnitine (C16)
Methionine (Met)	Hydroxybutyrylcarnitine (C4OH)	Hydroxypalmitoylcarnitine (C16OH)
Ornithine (Orn)	Isovalerylcarnitine (C5)	Stearoylcarnitine (C18)
Phenylalanine (Phe)	Glytarylcarnitine (C5DC)	Hydroxystearoylcarnitine (C18OH)
Tyrosine (Tyr)		

However, with the improved sensitivity of new mass spectrometry technologies, it is possible to detect both amino acids and acylcarnitines as their native free acids (i.e., nonderivatized). This simplifies analytical operation and minimizes the use of corrosive chemicals. In this application note, both nonderivatization and derivatization sample preparation methods were compared.

Many previous FIA-MS/MS studies on dried blood spots deployed neutral-loss scan mode (acylcarnitines) and precursor ion scan mode (some amino acids) for fast method development. In this application note, selected-reaction monitoring (SRM) was used for all AA, AC, and SUAC data acquisition. The advantage of SRM is that it accurately quantifies analytes and ensures both high selectivity and sensitivity, which especially benefited analysis of analytes that ionize poorly.

Method

Sample Preparation

Sets of isotope-labeled internal standards of amino acids (NSK-A), acylcarnitines (NSK-B and NSK-B-G), and succinylacetone (NSK-T) were purchased from Cambridge Isotope Laboratories, Inc. The daily working internal standard concentration is listed in Table 1. Hydrazine, 1-butanol, and acetyl chloride were purchased from Sigma-Aldrich®. The other reagents were from Thermo Fisher Scientific.

The DBS quality control (QC) samples were kindly provided by the United States Centers for Disease Control and Prevention (CDC) for research purposes. The QC samples contained enriched analytes at three concentrations: low, intermediate, and high.

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Table 1. Daily working internal standard concentrations.

Internal Standard	Concentrations (µmol/L)
Alanine-d_4	2.50
Arginine-^{13}C-d_4	2.50
Aspartic acid-d_3	2.50
Citrulline-d_2	2.50
Glutamic acid-d_3	2.50
Glycine-^{13}C-^{15}N	12.50
Leucine-d_3	2.50
Methionine-d_3	2.50
Ornithine-d_2	2.50
Phenylalanine-$^{13}C_6$	2.50
Tyrosine-$^{13}C_6$	2.50
Valine-d_8	2.50
Succinylacetone-$^{13}C_5$	2.50
C0-Carnitine-d_9	0.76
C2-Carnitine-d_3	0.19
C3-Carnitine-d_3	0.04
C4-Carnitine-d_3	0.04
C5-Carnitine-d_9	0.04
C5DC-Carnitine-d_3	0.08
C5OH-Carnitine-d_3	0.04
C8-Carnitine-d_3	0.04
C12-Carnitine-d_9	0.04
C14-Carnitine-d_9	0.04
C16-Carnitine-d_3	0.08
C18-Carnitine-d_3	0.08

The following protocols were used to prepare the DBS samples:

Derivatized

1. Punch one 1/8 inch diameter disc from DBS sample into a 96-well plate.
2. Add 100 µL of working internal standard solution (containing internal standards of 12 amino acids, 12 acylcarnitines, and SUAC) to each well.
3. Shake the well plate for 45 min at 45 °C.
4. Transfer the eluates to another well 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 50:50:0.02 acetonitrile/water/formic acid.

Nonderivatized

1. Punch one 1/8 inch diameter disc from DBS sample into a 96-well plate.
2. Add 100 µL of working internal standard solution (containing internal standards of 12 amino acids, 12 acylcarnitines, and SUAC) to each well.
3. Shake the well plate for 45 min at 45 °C.
4. Transfer the eluates to another well 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. Reconstitute each sample well with 100 µL of 50:50:0.02 acetonitrile/water/formic acid.

Liquid Chromatography

Pump	Thermo Scientific™ Dionex™ UltiMate™ HPG-3200 RS
Autosampler	UltiMate WPS-3000 TRS
HPLC column	None
Mobile phase	50:50:0.02 acetonitrile/water/formic acid
LC flow gradient	Refer to Table 2

Table 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

Flow injection MS/MS analysis was performed on a TSQ Endura triple quadrupole mass spectrometer. The mass spectrometer conditions were as follows:

Ionization	Heated electrospray ionization (HESI)
Spray voltage	Positive, 3500 V
Sheath gas	50 Arb
Aux gas	7 Arb
Sweep gas	0 Arb
Ion transfer tube temperature	350 °C
Vaporizer temperature	200 °C
Data acquisition mode	Selected-reaction monitoring (SRM)
Cycle time	1 s
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
CID gas	1.5 mTorr
Source fragmentation	0 V
Chrom filter	3 s
SRM parameters	Refer to Table 3 (Derivatized) and Table 4 (Nonderivatized)

Table 3. SRM parameters (derivatized).

Analyte	Precursor Ion	Product Ion	Internal Standard	Precursor Ion	Product Ion	Collision Energy (V)	RF Lens (V)
Alanine	146.20	44.20	Alanine-d_4	150.20	48.18	17	62
Arginine	231.28	70.13	Arginine-^{13}C-d_4	236.28	75.13	30	87
Aspartic acid	246.18	144.13	Aspartic acid-d_3	249.25	147.13	15	103
Citrulline	232.28	113.13	Citrulline-d_2	234.28	115.18	20	85
Glutamic acid	260.28	157.93	Glutamic acid-d_3	263.33	161.13	16	94
Glycine	131.80	76.05	Glycine-^{13}C-^{15}N	134.20	78.10	8	55
Leucine	188.25	86.10	Leucine-d_3	191.25	89.18	15	75
Methionine	206.23	104.13	Methionine-d_3	209.20	107.23	16	81
Ornithine	189.25	70.10	Ornithine-d_2	191.18	72.13	24	79
Phenylalanine	222.25	120.13	Phenylalanine-$^{13}C_6$	228.33	126.18	19	105
Tyrosine	238.30	136.13	Tyrosine-$^{13}C_6$	244.28	142.15	18	93
Valine	174.25	72.13	Valine-d_8	182.23	80.18	16	73
SUAC	211.18	137.05	SUAC-$^{13}C_5$	216.18	142.05	12	91
C0-Carnitine	218.28	85.05	C0-Carnitine-d_9	227.33	85.05	28	104
C2-Carnitine	260.30	85.05	C2-Carnitine-d_3	263.30	85.05	25	113
C3-Carnitine	274.33	85.05	C3-Carnitine-d_3	277.33	85.05	25	121
C3DC-Carnitine	360.33	85.05					
C4-Carnitine	288.33	85.05	C4-Carnitine-d_3	291.33	85.05	27	117
C4OH-Carnitine	304.33	85.05					
C5-Carnitine	302.33	85.05	C5-Carnitine-d_9	311.38	85.05	30	113
C6-Carnitine	316.35	85.05					
C5DC-Carnitine	388.35	85.05	C5DC-Carnitine-d_3	391.35	85.05	31	138
C5OH-Carnitine	318.38	85.05	C5OH-Carnitine-d_3	321.38	85.05	31	138
C8-Carnitine	344.38	85.05	C8-Carnitine-d_3	347.38	85.05	32	141
C10-Carnitine	372.40	85.05					
C12-Carnitine	400.43	85.05	C12-Carnitine-d_9	409.43	85.05	36	184
C14-Carnitine	428.48	85.05	C14-Carnitine-d_9	437.48	85.05	35	193
C16-Carnitine	456.55	85.05	C16-Carnitine-d_3	459.55	85.05	37	183
C16OH-Carnitine	472.55	85.05					
C18-Carnitine	484.55	85.05	C18-Carnitine-d_3	487.55	85.05	38	215
C18OH-Carnitine	500.55	85.05					

Table 4. SRM parameters (nonderivatized).

Analyte	Precursor Ion	Product Ion	Internal Standard	Precursor Ion	Product Ion	Collision Energy (V)	RF Lens (V)
Alanine	90.15	44.20	Alanine-d_4	94.15	48.20	13	45
Arginine	175.23	70.15	Arginine-^{13}C-d_4	180.23	75.15	24	92
Aspartic acid	134.20	116.13	Aspartic acid-d_3	137.20	119.13	6	54
Citrulline	176.20	113.13	Citrulline-d_2	178.20	115.13	18	59
Glutamic acid	148.15	130.08	Glutamic acid-d_3	151.15	133.08	9	62
Glycine	76.08	30.25	Glycine-^{13}C-^{15}N	78.08	32.25	13	43
Leucine	132.25	86.13	Leucine-d_3	135.25	89.13	11	56
Methionine	150.18	133.08	Methionine-d_3	153.18	136.08	9	60
Ornithine	133.15	70.15	Ornithine-d_2	135.15	72.15	19	63
Phenylalanine	166.20	120.15	Phenylalanine-$^{13}C_6$	172.20	126.15	16	69
Tyrosine	182.15	136.18	Tyrosine-$^{13}C_6$	188.15	142.18	15	71
Valine	118.23	72.15	Valine-d_8	126.23	80.15	13	53
SUAC	155.18	109.12	SUAC-$^{13}C_5$	160.18	114.12	22	63
C0-Carnitine	162.23	85.05	C0-Carnitine-d_9	171.23	85.05	23	69
C2-Carnitine	204.23	85.05	C2-Carnitine-d_3	207.23	85.05	21	96
C3-Carnitine	218.23	85.05	C3-Carnitine-d_3	221.23	85.05	23	91
C3DC-Carnitine	248.23	85.05					
C4-Carnitine	232.18	85.05	C4-Carnitine-d_3	235.18	85.05	21	78
C4OH-Carnitine	248.25	85.05					
C5-Carnitine	246.30	85.05	C5-Carnitine-d_9	255.30	85.05	25	96
C6-Carnitine	260.30	85.05					
C5DC-Carnitine	276.30	85.05	C5DC-Carnitine-d_3	279.30	85.05	25	96
C5OH-Carnitine	262.30	85.05	C5OH-Carnitine-d_3	265.30	85.05	25	96
C8-Carnitine	288.33	85.05	C8-Carnitine-d_3	291.33	85.05	26	108
C10-Carnitine	316.33	85.05					
C12-Carnitine	344.45	85.05	C12-Carnitine-d_9	353.45	85.05	39	152
C14-Carnitine	372.45	85.05	C14-Carnitine-d_9	381.45	85.05	39	152
C16-Carnitine	400.45	85.05	C16-Carnitine-d_3	403.45	85.05	36	185
C16OH-Carnitine	416.45	85.05					
C18-Carnitine	428.45	85.05	C18-Carnitine-d_3	431.45	85.05	36	185
C18OH-Carnitine	444.45	85.05					

Data Processing

~~Tandem MS data were processed using a new meta-calculation software, iRC PRO (2Next srl, Prato, Italy). This off-line automated data processing tool can process peak area, concentration, and user-defined formulas (Figure 1).~~

The metacalculation software 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.

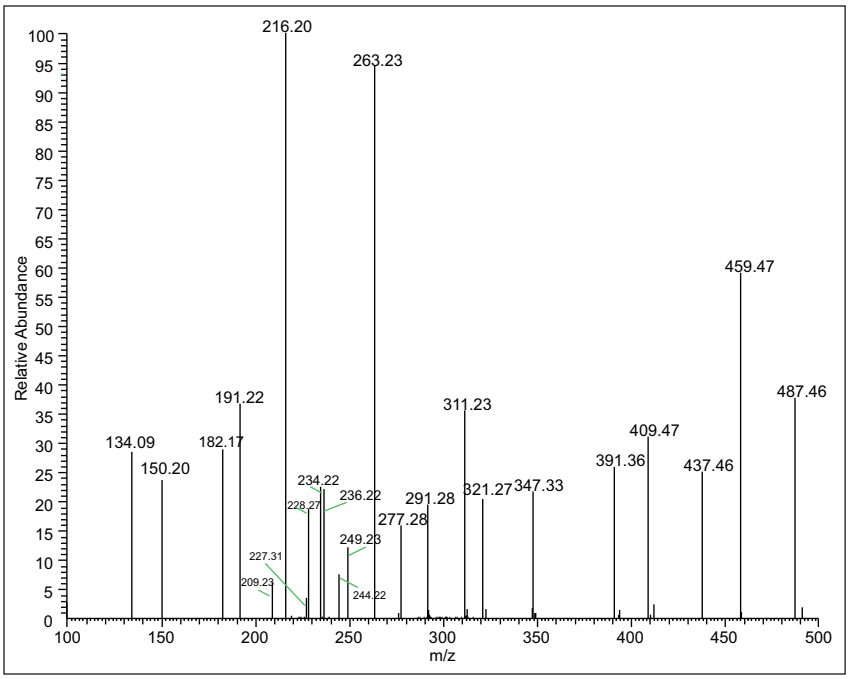
Figure 1. iRC PRO intuitive workflow – icon-based user interface.

Assay Validation

The intra-assay precision was determined at three concentrations by means of ten successive, independent measurement of DBS samples (n=10). The inter-assay precision was determined at three concentrations by means of ten independent measurement of DBS samples in seven different test series (n=70).

Results

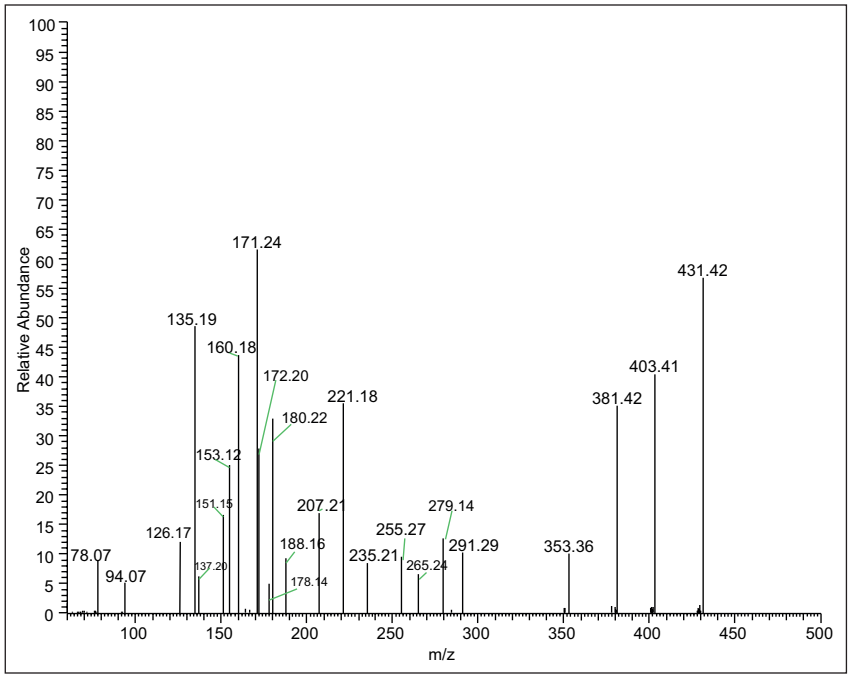
The derivatization process using butanol converted free amino acids and acylcarnitines into the butyric esters and added a mass of 56 (except for aspartic acid, glutamic acid, and C5DC, in which a mass of 112 was added). Figure 2 and 3 show full-scan spectra of derivatized and nonderivatized internal standards respectively.



Internal Standard	*m/z*
Alanine-d_4	150.20
Arginine-^{13}C-d_4	236.22
Aspartic acid-d_3	249.23
Citrulline-d_2	234.22
Glutamic acid-d_3	263.23
Glycine-^{13}C-^{15}N	134.09
Leucine-d_3	191.22
Methionine-d_3	209.23
Ornithine-d_2	191.22
Phenylalanine-$^{13}C_6$	228.27
Tyrosine-$^{13}C_6$	244.22
Valine-d_8	182.17
Succinylacetone-$^{13}C_5$	216.20

Internal Standard	*m/z*
C0-Carnitine-d_9	227.31
C2-Carnitine-d_3	263.23
C3-Carnitine-d_3	277.28
C4-Carnitine-d_3	291.28
C5-Carnitine-d_9	311.23
C5DC-Carnitine-d_3	391.36
C5OH-Carnitine-d_3	321.27
C8-Carnitine-d_3	347.33
C12-Carnitine-d_9	409.47
C14-Carnitine-d_9	437.46
C16-Carnitine-d_3	459.47
C18-Carnitine-d_3	487.46

Figure 2. Full-scan spectra of derivatized internal standards.



Internal Standard	*m/z*
Alanine-d_4	94.07
Arginine-^{13}C-d_4	180.22
Aspartic acid-d_3	137.20
Citrulline-d_2	178.14
Glutamic acid-d_3	151.15
Glycine-^{13}C-^{15}N	78.07
Leucine-d_3	135.19
Methionine-d_3	153.12
Ornithine-d_2	135.19
Phenylalanine-$^{13}C_6$	172.20
Tyrosine-$^{13}C_6$	188.16
Valine-d_8	126.17
Succinylacetone-$^{13}C_5$	160.18

Internal Standard	*m/z*
C0-Carnitine-d_9	171.24
C2-Carnitine-d_3	207.21
C3-Carnitine-d_3	221.18
C4-Carnitine-d_3	235.21
C5-Carnitine-d_9	255.27
C5DC-Carnitine-d_3	279.14
C5OH-Carnitine-d_3	265.24
C8-Carnitine-d_3	291.29
C12-Carnitine-d_9	353.36
C14-Carnitine-d_9	381.42
C16-Carnitine-d_3	403.41
C18-Carnitine-d_3	431.42

Figure 3. Full-scan spectra of nonderivatized internal standards.

SRM was used to acquire MS/MS data for all the analytes. Collision energy and RF lens parameters were optimized for each target and internal standard to ensure maximum selectivity and sensitivity. 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 4 and 5).

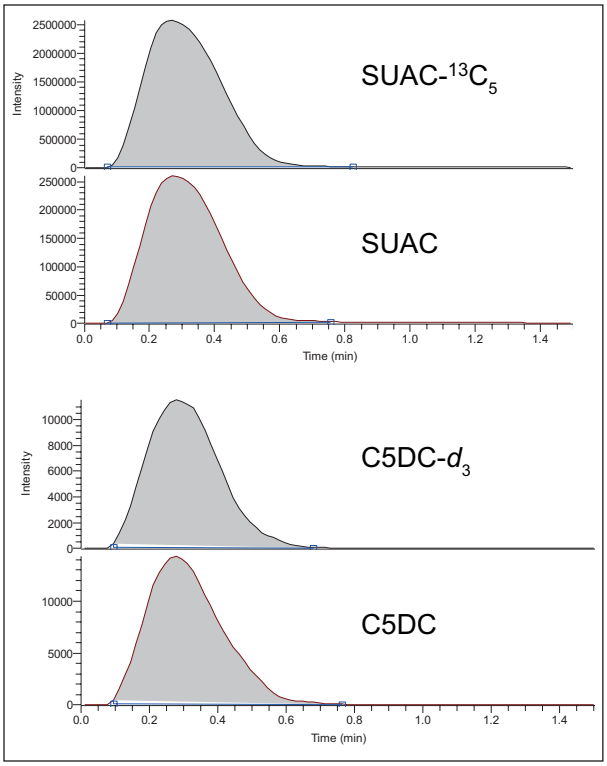


Figure 4. Flow injection analysis (FIA) profiles of SUAC-^{13}C5, SUAC and C5DC-D3, C5DC using derivatized method.

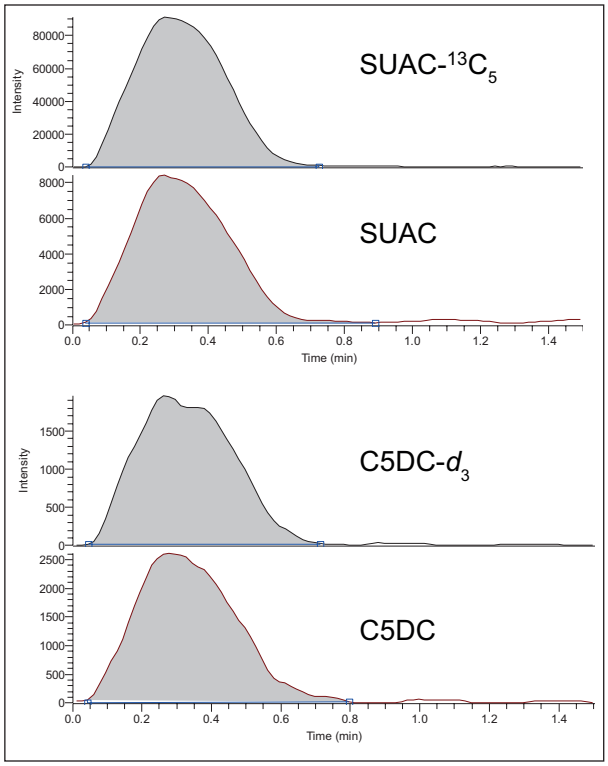


Figure 5. Flow injection analysis (FIA) profiles of SUAC-^{13}C5, SUAC and C5DC-D3, C5DC using nonderivatized method.

Intra-assay Precision

For the derivatized method, the average intra-assay precisions (n=10) for 12 AA and SUAC at three concentrations were 7.9% (low), 8.0% (intermediate), and 8.0% (high). The average intra-assay precisions for 18 AC at three concentrations were 8.9% (low), 8.3% (intermediate), and 9.0% (high) (Table 5).

For the nonderivatized method, the average intra-assay precisions (n=10) for AA and SUAC at three concentrations were 6.1% (low), 7.2% (intermediate), and 9.8% (high). The average intra-assay precisions for AC at three concentrations were 7.6% (low), 6.2% (intermediate), and 8.2% (high) (Table 6).

Table 5. Derivatized method intra-assay precision at three concentrations (low, intermediate, and high). n=10.

Analyte	Coefficient of Variation (%)			Concentrations in µmol/L		
	Low	Intermediate	High	Low	Intermediate	High
Alanine	9.5	8.9	11.5	552.3	710.2	890.3
Arginine	5.4	9.6	7.6	111.2	211.4	317.8
Aspartic acid	9.0	6.5	8.0	N/A	N/A	N/A
Citrulline	6.8	4.0	5.6	55.8	131.6	277.4
Glutamic acid	10.1	5.9	4.7	N/A	N/A	N/A
Glycine	8.8	8.2	6.8	N/A	N/A	N/A
Leucine	8.5	8.4	6.8	225.1	378.8	633.0
Methionine	7.7	6.4	8.7	65.1	154.6	257.5
Ornithine	8.4	12.3	8.4	N/A	N/A	N/A
Phenylalanine	7.5	8.5	5.5	169.8	274.9	369.7
Tyrosine	7.8	10.8	8.6	236.6	416.5	605.0
Valine	9.6	8.3	8.1	289.5	410.0	547.8
SUAC	8.2	7.2	9.4	1.9	4.6	9.9
C0-Carnitine	12.2	5.0	6.8	35.3	54.4	70.6
C2-Carnitine	10.8	8.6	7.6	24.6	37.6	47.9
C3-Carnitine	11.6	12.7	11.7	4.9	10.1	14.6
C3DC-Carnitine	7.4	6.9	9.0	0.2	0.6	1.1
C4-Carnitine	6.7	6.5	10.6	1.1	2.8	5.0
C4OH-Carnitine	7.1	6.1	8.6	0.3	0.6	1.4
C5-Carnitine	6.9	5.4	10.6	0.6	1.7	3.2
C6-Carnitine	8.1	5.7	5.9	0.7	1.3	3.3
C5DC-Carnitine	4.7	8.6	8.3	1.0	2.2	3.1
C5OH-Carnitine	8.4	7.3	9.1	0.6	1.2	2.8
C8-Carnitine	9.0	9.5	4.0	0.6	1.2	2.7
C10-Carnitine	7.4	6.8	6.9	0.6	1.2	3.1
C12-Carnitine	6.1	6.7	8.8	0.7	1.3	2.8
C14-Carnitine	8.9	10.8	8.7	0.6	1.5	2.9
C16-Carnitine	10.7	10.9	10.8	3.5	7.8	11.8
C16OH-Carnitine	12.6	13.0	12.6	0.1	0.4	0.7
C18-Carnitine	10.2	7.4	13.6	1.6	2.6	5.6
C18OH-Carnitine	10.9	11.1	8.8	0.4	0.7	1.1

N/A, the analytes were not enriched in QC samples.

Table 6. Nonderivatized method intra-assay precision at three concentrations (low, intermediate, and high). n=10.

Analyte	Coefficient of Variation (%)			Concentrations in µmol/L		
	Low	Intermediate	High	Low	Intermediate	High
Alanine	4.7	7.2	11.7	531.2	749.2	894.0
Arginine	6.1	7.2	9.7	104.8	206.2	303.3
Aspartic acid	13.0	13.7	15.1	N/A	N/A	N/A
Citrulline	4.4	7.8	8.0	54.0	127.3	269.0
Glutamic acid	8.0	3.8	7.3	N/A	N/A	N/A
Glycine	8.6	9.7	10.6	N/A	N/A	N/A
Leucine	5.5	6.3	9.2	252.9	416.0	637.4
Methionine	8.1	4.8	9.7	65.0	155.7	253.7
Ornithine	5.4	7.7	9.4	N/A	N/A	N/A
Phenylalanine	4.9	5.7	9.4	164.1	266.2	347.7
Tyrosine	5.2	5.9	7.5	240.4	431.7	623.5
Valine	5.1	6.3	10.1	295.9	439.5	547.3
SUAC	10.5	14.1	13.0	1.9	4.2	9.1
C0-Carnitine	5.6	6.0	6.6	30.8	43.4	56.9
C2-Carnitine	6.7	5.4	6.8	24.1	38.3	48.5
C3-Carnitine	8.7	3.9	8.9	4.6	10.1	14.6
C3DC-Carnitine	6.9	6.5	5.9	0.3	0.6	1.4
C4-Carnitine	9.6	5.2	8.5	1.1	2.8	5.3
C4OH-Carnitine	5.2	5.5	7.3	0.3	0.7	1.4
C5-Carnitine	7.8	7.3	9.0	0.6	1.7	3.2
C6-Carnitine	6.3	6.8	10.8	0.7	1.4	3.3
C5DC-Carnitine	8.7	7.1	10.3	1.1	1.9	2.7
C5OH-Carnitine	10.1	7.8	10.3	0.5	1.0	2.3
C8-Carnitine	8.3	5.2	7.8	0.6	1.1	2.7
C10-Carnitine	9.6	6.8	9.2	0.8	1.6	3.9
C12-Carnitine	6.7	4.6	6.5	0.4	0.9	2.1
C14-Carnitine	5.8	8.2	5.9	0.5	1.4	2.6
C16-Carnitine	7.8	4.0	5.5	3.7	8.9	12.2
C16OH-Carnitine	5.9	8.3	8.7	0.1	0.4	0.7
C18-Carnitine	7.1	3.4	9.5	1.7	2.7	5.6
C18OH-Carnitine	9.6	10.1	10.4	0.4	0.8	1.4

N/A, the analytes were not enriched in QC samples.

Inter-assay Precision

For the derivatized method, the average inter-assay precisions (n=70) for 12 AA and SUAC at three concentrations were 13.5% (low), 12.9% (intermediate), and 12.5% (high). The average inter-assay precisions for 18 AC at three concentrations were 15.0% (low), 15.6% (intermediate), and 16.1% (high) (Table 7).

For the nonderivatized method, the average inter-assay precisions (n=70) for AA and SUAC at three concentrations were 12.8% (low), 12.8% (intermediate), and 12.6% (high). The average inter-assay precisions for AC at three concentrations were 12.7% (low), 10.5% (intermediate), and 11.8% (high) (Table 8).

Table 7. Derivatized method inter-assay precision at three concentrations (low, intermediate, and high). n=70.

Analyte	Coefficient of Variation (%)			Concentrations in µmol/L		
	Low	Intermediate	High	Low	Intermediate	High
Alanine	12.2	9.6	10.3	538.1	711.4	882.2
Arginine	17.1	16.6	18.6	123.8	222.5	326.0
Aspartic acid	11.2	10.7	7.9	N/A	N/A	N/A
Citrulline	17.0	14.8	12.5	58.9	132.6	285.3
Glutamic acid	13.0	10.8	10.4	N/A	N/A	N/A
Glycine	10.3	12.2	10.4	N/A	N/A	N/A
Leucine	12.2	12.2	12.1	224.2	381.2	640.3
Methionine	13.2	11.6	11.5	66.2	154.5	246.3
Ornithine	17.2	15.4	17.5	N/A	N/A	N/A
Phenylalanine	11.8	12.8	12.5	172.1	271.6	362.2
Tyrosine	13.6	12.5	14.0	232.9	400.9	604.1
Valine	11.4	12.8	11.5	290.4	409.3	539.3
SUAC	13.0	13.4	9.4	2.0	4.7	9.9
C0-Carnitine	15.7	15.1	13.4	36.4	51.7	71.8
C2-Carnitine	13.8	14.1	15.1	24.2	37.1	49.4
C3-Carnitine	16.3	15.3	16.6	4.8	9.5	14.2
C3DC-Carnitine	13.8	14.1	16.1	0.2	0.5	1
C4-Carnitine	16.3	13.4	17.5	1.0	2.5	4.7
C4OH-Carnitine	16.2	18.4	15.5	0.3	0.6	1.3
C5-Carnitine	15.1	14.6	16.6	0.6	1.5	2.9
C6-Carnitine	14.9	12.3	14.2	0.7	1.3	3.1
C5DC-Carnitine	13.7	15.6	15.2	1.1	2.1	3.1
C5OH-Carnitine	13.4	16.2	14.7	0.6	1.1	2.7
C8-Carnitine	15.4	13.8	16.6	0.6	1.1	2.6
C10-Carnitine	17.4	16.9	18.0	0.6	1.1	2.8
C12-Carnitine	15.4	17.1	17.3	0.7	1.2	2.6
C14-Carnitine	14.5	14.9	17.1	0.6	1.4	2.7
C16-Carnitine	14.7	16.1	16.2	3.7	7.9	11.5
C16OH-Carnitine	13.0	17.6	18.3	0.1	0.4	0.7
C18-Carnitine	14.7	18.2	15.5	1.7	2.5	5.1
C18OH-Carnitine	15.2	17.1	16.5	0.4	0.7	1.1

N/A, the analytes were not enriched in QC samples.

Table 8. Nonderivatized method inter-assay precision at three concentrations (low, intermediate, and high). n=70.

Analyte	Coefficient of Variation (%)			Concentrations in µmol/L		
	Low	Intermediate	High	Low	Intermediate	High
Alanine	20.0	16.1	15.6	632.1	863.2	1002.4
Arginine	12.0	11.3	12.2	103.3	204.2	303.2
Aspartic acid	13.4	17.5	18.1	N/A	N/A	N/A
Citrulline	10.7	11.4	9.7	54.3	129.0	263.3
Glutamic acid	10.4	9.1	10.6	N/A	N/A	N/A
Glycine	13.4	13.7	14.8	N/A	N/A	N/A
Leucine	10.8	9.7	10.2	260.6	414.2	646.0
Methionine	18.8	17.5	20.2	53.8	129.2	200.1
Ornithine	8.6	8.8	8.8	N/A	N/A	N/A
Phenylalanine	7.7	8.7	11.2	162.9	268.8	351.3
Tyrosine	8.1	10.0	10.8	239.7	435.9	615.8
Valine	9.1	9.3	10.1	300.1	432.9	539.5
SUAC	18.1	21.0	13.7	2.7	4.9	9.3
C0-Carnitine	12.5	11.3	12.0	29.4	40.3	51.9
C2-Carnitine	10.3	10.0	10.9	24.1	38.1	47.8
C3-Carnitine	9.8	9.7	11.8	4.5	9.9	14.3
C3DC-Carnitine	12.4	11.8	9.1	0.3	0.6	1.3
C4-Carnitine	10.3	10.8	11.6	1.1	2.7	5.2
C4OH-Carnitine	11.3	10.5	10.6	0.3	0.6	1.4
C5-Carnitine	11.2	11.6	11.6	0.6	1.7	3.2
C6-Carnitine	16.9	16.5	12.7	0.7	1.4	3.2
C5DC-Carnitine	11.3	9.1	10.1	1.0	1.9	2.6
C5OH-Carnitine	12.8	11.3	12.3	0.5	1.0	2.4
C8-Carnitine	9.9	8.6	10.7	0.6	1.1	2.6
C10-Carnitine	18.4	13.5	13.2	0.8	1.6	4.0
C12-Carnitine	12.2	8.7	9.8	0.4	0.9	2.1
C14-Carnitine	11.3	8.0	10.0	0.5	1.4	2.7
C16-Carnitine	10.9	8.4	12.2	3.6	8.7	12.2
C16OH-Carnitine	14.3	9.6	13.9	0.1	0.4	0.7
C18-Carnitine	12.1	7.8	11.6	1.7	2.7	5.7
C18OH-Carnitine	21.3	11.2	17.8	0.4	0.9	1.5

N/A, the analytes were not enriched in QC samples.

Method Comparison

The concentration of analytes obtained from nonderivatized and derivatized methods were compared. The average method differences of 12 AA and SUAC between quantitative values resulting from derivatization and nonderivatization methods at three concentrations were 3.8% (low), 4.8% (intermediate), and 3.2% (high). The average method differences of 18 AC at three concentrations were 14.2% (low), 11.4% (intermediate), and 10.5% (high) (Figure 6). Therefore the two methods were highly correlated. Our data are consistent with the reported results from a comprehensive empirical analysis.[5]

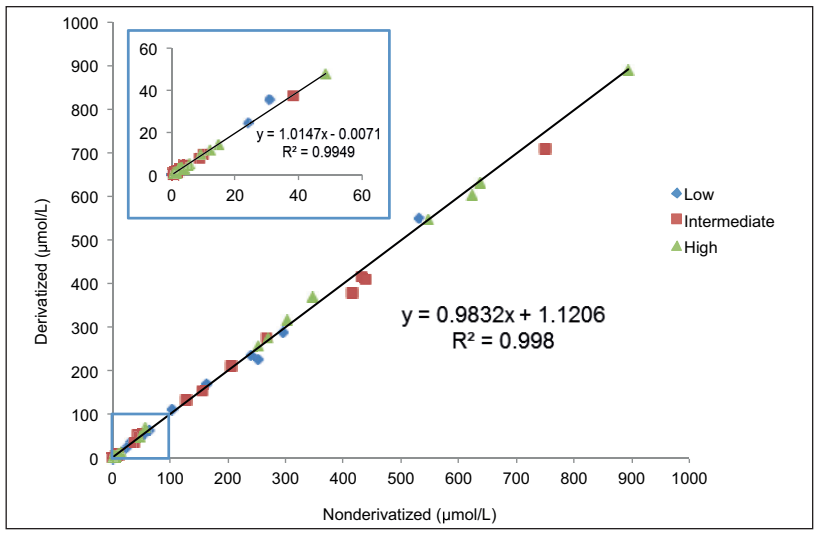


Figure 6. Comparisons between quantitative values of 12 AA, SUAC, and 18 AC resulting from nonderivatized and derivatized methods.

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 derivatization and nonderivatization sample preparation methods were capable of accurately quantifying AA/AC/SUAC on TSQ Endura triple quadrupole MS with a run time of 1.5 min.
- SRM data acquisition mode optimized for each analyte and internal standard guarantees both high sensitivity and high selectivity.
- The TSQ Endura MS system can provide average intra-assay precision (n=10) at three enriched concentrations of less than 10% and average inter-assay precision (n=70) of less than 15% for both nonderivatized and derivatized methods.
- The method difference between quantitative values resulting from nonderivatized and derivatized methods was minor and both methods are highly correlated.

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