Application Note 635

Use of a Tandem Mass Spectrometry Research Method for the Analysis of Amino Acids and Acylcarnitines in Dried Blood Spots

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Keywords
Amino acids, acylcarnitines, dried blood spots, tandem mass spectrometry, TSQ Quantum Access MAX

Goal
To develop a research method for simultaneous analysis of derivatized amino acids and acylcarnitines in dried blood spots using the Thermo Scientific™ TSQ Quantum Access MAX™ triple quadrupole mass spectrometer.

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] In this application note, a FIA-MS/MS method was used to simultaneously analyze derivatized AA and AC in dried blood spots on the TSQ Quantum Access MAX triple quadrupole mass spectrometer (Figure 1).

Experimental
Sample Preparation
Sets of isotope-labeled internal standards of amino acids (NSK-A and 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. Acetyl chloride and 1-butanol were purchased from Sigma-Aldrich®. The other reagents were from Thermo Fisher Scientific.

The DBS quality control (QC) filter paper samples (Lot# 1462, 1463, 1464, 1422, 1423, 1424) were kindly provided by the United States Centers for Disease Control and Prevention (CDC) for research purpose. The QC samples containing enriched analytes of various concentrations were labeled as low, intermediate, and high.

Figure 1. UltiMate 3000 RSLC system and TSQ Quantum Access MAX mass spectrometer.

The following protocol was used to prepare the test samples:

1. Punch one 1/8 inch diameter disc from DBS QC filter paper sample.
2. Add 100 µL of working internal standard solution (containing internal standards of 12 amino acids and 12 acylcarnitines) to each well.
3. Shake the plate for 45 min at 45 °C.
4. Transfer the eluates into another plate and evaporate at 50 °C under nitrogen flow.
5. 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.
6. Reconstitute each sample well with 100 µL of 50:50:0.02 acetonitrile/water/formic acid.

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Table 1. Daily working internal standard concentrations.

Internal Standard	Concentration (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
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

Liquid Chromatography

The Thermo Scientific™ Dionex™ UltiMate™ RSLC system (Figure 1) was utilized in this study under the following conditions:

Pump	Thermo Scientific Dionex UltiMate HPG-3200 RS
Autosampler	UltiMate 3000 XRS Open Autosampler OAS-3300TXRS
HPLC Column	None
Mobile Phase	50:50:0.02 acetonitrile/water/formic acid
LC Flow Gradient	Refer to Table 2

Table 2. LC flow time event table.

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 Quantum Access MAX triple quadrupole mass spectrometer. The mass spectrometer conditions were as follows:

Ionization	Heated electrospray ionization (HESI)
Spray voltage	Positive, 3500 V
Sheath gas	30 Arb
Aux gas	5 Arb
Sweep gas	0 Arb
Capillary temperature	270 °C
Vaporizer temperature	75 °C
Data acquisition mode	Selected reaction monitoring (SRM)
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
CID gas	1.5 mTorr
SRM parameters	Refer to Table 3

Table 3. SRM parameters.

Analyte	Precursor Ion	Product Ion	Internal Standard	Precursor Ion	Product Ion	Collision Energy (V)	RF Lens (V)
Alanine	146.2	44.2	Alanine-d_4	150.2	48.2	12	87
Arginine	231.2	70.1	Arginine-^{13}C-d_4	236.2	75.1	33	99
Aspartic acid	246.2	144.1	Aspartic acid-d_3	249.2	147.1	13	105
Citrulline	232.2	113.2	Citrulline-d_2	234.2	115.2	17	96
Glutamic acid	260.2	158.1	Glutamic acid-d_3	263.2	161.1	14	95
Glycine	132.2	76.1	Glycine-^{13}C-^{15}N	134.2	78.1	6	95
Leucine	188.2	86.2	Leucine-d_3	191.2	89.2	13	94
Methionine	206.2	104.2	Methionine-d_3	209.2	107.2	13	97
Ornithine	189.2	70.1	Ornithine-d_2	191.2	72.1	19	93
Phenylalanine	222.2	120.1	Phenylalanine-$^{13}C_6$	228.2	126.2	14	102
Tyrosine	238.2	136.2	Tyrosine-$^{13}C_6$	244.2	142.2	15	107
Valine	174.2	72.2	Valine-d_8	182.2	80.2	13	104
C0-Carnitine	218.2	85.1	C0-Carnitine-d_9	227.2	85.1	25	103
C2-Carnitine	260.2	85.1	C2-Carnitine-d_3	263.2	85.1	21	102
C3-Carnitine	274.2	85.1	C3-Carnitine-d_3	277.2	85.1	22	99
C3DC-Carnitine	360.2	85.1					
C4-Carnitine	288.2	85.1	C4-Carnitine-d_3	291.2	85.1	20	99
C4OH-Carnitine	304.2	85.1					
C5-Carnitine	302.3	85.1	C5-Carnitine-d_9	311.3	85.1	24	107
C6-Carnitine	316.3	85.1					
C5DC-Carnitine	388.3	85.1	C5DC- Carnitine-d_3	391.3	85.1	24	107
C5OH-Carnitine	318.3	85.1	C5OH- Carnitine-d_3	321.3	85.1	24	107
C8-Carnitine	344.3	85.1	C8-Carnitine-d_3	347.3	85.1	24	115
C10-Carnitine	372.4	85.1					
C12-Carnitine	400.4	85.1	C12-Carnitine-d_9	409.4	85.1	26	117
C14-Carnitine	428.4	85.1	C14-Carnitine-d_9	437.4	85.1	26	117
C16-Carnitine	456.4	85.1	C16- Carnitine-d_3	459.4	85.1	28	118
C16OH-Carnitine	472.4	85.1					
C18-Carnitine	484.5	85.1	C18- Carnitine-d_3	487.5	85.1	28	118
C18OH-Carnitine	500.5	85.1					

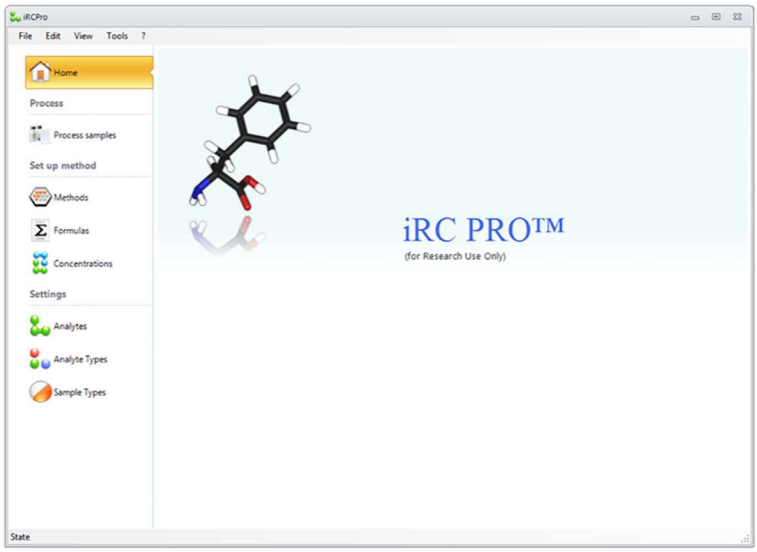

Figure 2. iRC PRO intuitive workflow – icon-based user interface.

Data Processing

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

Method Precision

The within-run precision was determined at three concentrations by means of five successive, independent measurements of dried blood spot (DBS) samples (n=5). The run-to-run precision was determined at three concentrations by means of five independent measurements of DBS samples in five different test series (n=25) (five replicates per run times five runs).

Results

Selected reaction monitoring (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 provides a specific transition of precursor ion to product ion for each analyte, so that it will be able to identify and quantify the particular analyte in a mixture without chromatographic separation. C5DC is known to have poor ionization. As shown in Figure 3, C5DC showed high peak area counts with good signal to noise using SRM parameters defined in Table 3.

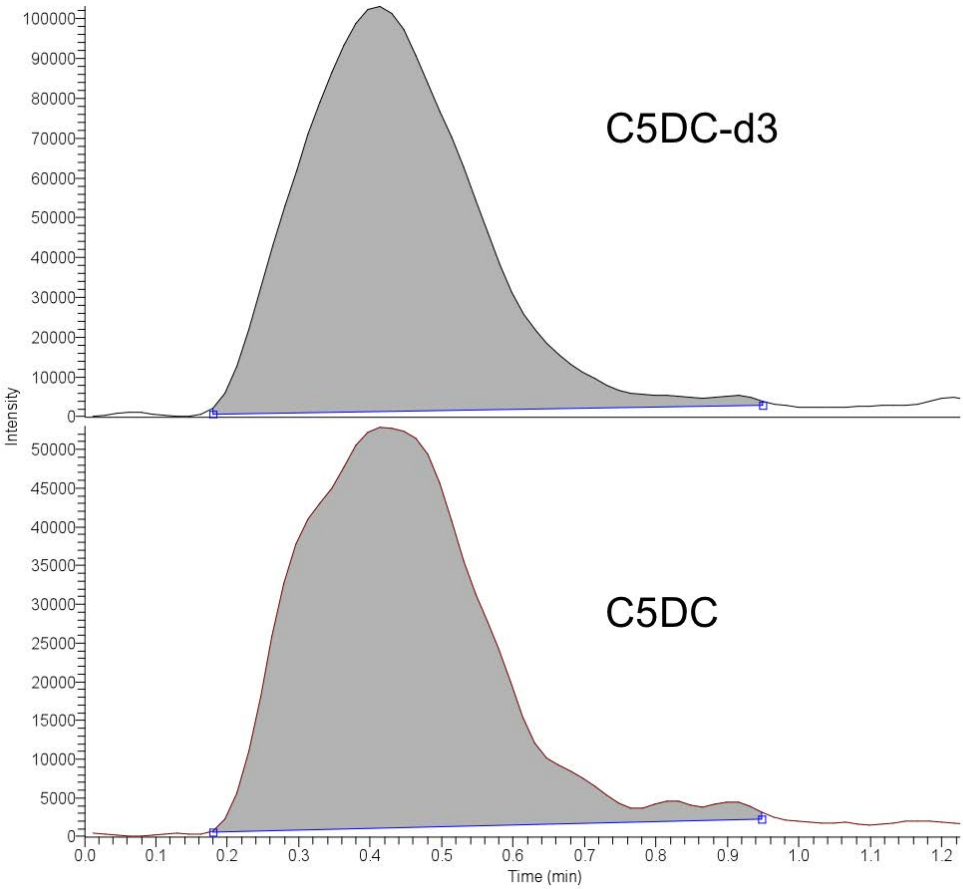

Figure 3. Flow injection analysis (FIA) profile of C5DC-d3 (0.76 µmol/L) and C5DC (0.6 µmol/L blood).

Within-Run Precision

The within-run precisions (n=5) at three concentrations ranged from 1.0% to 12.6% (low), 0.9% to 12.2% (intermediate), and 2.6% to 10.7% (high). (Table 4).

Table 4. Within-run precision at three concentrations (low, intermediate, and high). n=5.

Analyte	Coefficient of variation (%)			Concentrations in µmol/L		
	Low	Intermediate	High	Low	Intermediate	High
Alanine	8.1	10.7	7.3	482.6	668.3	777.0
Arginine	1.0	11.3	4.3	100.9	203.5	275.3
Citrulline	5.9	8.7	10.7	59.4	143.7	292.6
Leucine	1.7	9.6	4.9	204.8	364.4	558.6
Methionine	7.2	9.1	6.7	71.1	177.4	269.7
Phenylalanine	3.5	9.9	2.6	153.1	258.7	334.8
Tyrosine	1.2	9.2	5.4	246.6	448.5	607.0
Valine	10.5	11.7	5.4	294.7	465.5	519.1
C0-Carnitine	4.1	3.4	7.3	31.6	51.4	65.0
C2-Carnitine	5.6	3.7	6.1	23.2	36.6	46.3
C3-Carnitine	8.7	2.3	5.1	4.6	10.4	15.8
C3DC-Carnitine	7.2	12.2	6.8	0.3	1.0	1.9
C4-Carnitine	4.4	4.9	8.1	1.1	2.9	5.2
C4OH-Carnitine	4.8	6.8	6.6	0.4	0.8	1.8
C5-Carnitine	2.6	4.4	5.6	0.6	1.7	3.1
C5DC-Carnitine	5.9	3.4	9.2	0.7	1.4	3.4
C5OH-Carnitine	7.1	6.1	9.3	1.2	2.4	3.3
C6-Carnitine	4.8	4.2	5.6	0.4	1.0	2.3
C8-Carnitine	2.6	0.9	4.6	0.6	1.2	2.8
C10-Carnitine	10.0	4.4	8.4	0.6	1.1	2.7
C12-Carnitine	12.6	5.1	6.3	0.6	1.4	2.9
C14-Carnitine	3.9	3.3	6.5	0.6	1.7	3.3
C16-Carnitine	7.1	1.7	8.3	3.6	8.9	12.4
C16OH-Carnitine	4.0	6.8	9.8	0.1	0.4	0.9
C18-Carnitine	2.8	5.5	9.0	1.7	2.9	5.9
C18OH-Carnitine	7.3	4.3	9.1	0.3	0.7	1.2

Run-to-Run Precision

The run-to-run precisions (n=25) at three concentrations ranged from 4.5% to 16.3% (low), 3.9% to 15.1% (intermediate), and 5.1% to 14.5% (high). (Table 5).

Table 5. Run-to-run precision at three concentrations (low, intermediate, and high). n=25.

Analyte	Coefficient of variation (%)			Concentrations in µmol/L		
	Low	Intermediate	High	Low	Intermediate	High
Alanine	5.2	10.9	6.4	474.9	671.6	747.7
Arginine	7.6	9.6	9.8	101.0	193.5	282.5
Citrulline	9.2	11.8	8.0	59.1	139.7	292.3
Leucine	5.5	12.3	5.6	210.8	359.3	556.3
Methionine	13.3	13.5	8.5	71.5	164.1	261.6
Phenylalanine	4.5	10.8	5.1	153.8	258.3	336.3
Tyrosine	4.9	12.2	5.8	241.5	445.7	616.2
Valine	7.6	15.1	9.0	304.4	438.3	530.6
C0-Carnitine	5.8	5.8	8.0	31.4	49.3	61.1
C2-Carnitine	4.6	4.1	5.6	23.5	36.5	45.1
C3-Carnitine	6.8	6.6	8.1	4.5	10.5	15.7
C3DC-Carnitine	14.0	9.5	10.5	0.3	1.0	1.9
C4-Carnitine	6.8	6.5	9.0	1.0	2.9	5.2
C4OH-Carnitine	8.4	10.8	9.4	0.4	0.8	1.8
C5-Carnitine	7.4	6.1	6.7	0.6	1.7	3.1
C5DC-Carnitine	8.2	5.7	7.6	0.6	1.4	3.3
C5OH-Carnitine	8.6	5.9	7.7	1.1	2.4	3.3
C6-Carnitine	7.1	5.5	6.4	0.4	1.0	2.3
C8-Carnitine	8.6	5.1	9.5	0.5	1.2	2.8
C10-Carnitine	11.1	7.1	10.2	0.5	1.1	2.7
C12-Carnitine	11.5	6.4	6.6	0.6	1.4	2.9
C14-Carnitine	7.4	5.3	12.5	0.6	1.7	3.4
C16-Carnitine	7.5	3.9	6.8	3.6	9.1	12.3
C16OH-Carnitine	16.3	7.4	14.5	0.1	0.4	0.8
C18-Carnitine	10.9	5.7	11.3	1.7	2.9	5.7
C18OH-Carnitine	15.7	7.4	12.7	0.3	0.7	1.3

Conclusion

- A flow injection-tandem mass spectrometry research method was developed to simultaneously detect and quantify amino acids and acylcarnitines in dried blood spots. Rapid data processing was performed using iRC Pro meta-calculation software.
- The derivatized sample preparation method was capable of accurately quantifying AA and AC on an affordable TSQ Quantum Access MAX triple quadrupole MS with a run time of 1.5 min.
- The TSQ Quantum Access MAX MS can provide within-run precision (n=5) at three enriched concentrations in the range of 0.9-12.6% and run-to-run precision (n=25) in the range of 3.9-16.3%.

Abbreviations

Alanine (Ala), arginine (Arg), aspartic acid (Asp), citrulline (Cit), glutamic acid (Glu), glycine (Gly), leucine (Leu), methionine (Met), ornithine (Orn), phenylalanine (Phe), tyrosine (Tyr), valine (Val)

Free carnitine (C0), acetylcarnitine (C2), propionylcarnitine (C3), malonylcarnitine (C3DC), butyrylcarnitine (C4), hydroxybutyrylcarnitine (C4OH), isovalerylcarnitine (C5), glutarylcarnitine (C5DC), hydroxyisovalerylcarnitine (C5OH), hexanoylcarnitine (C6), octanoylcarnitine (C8), decanoylcarnitine (C10), dodecanoylcarnitine (C12), myristoylcarnitine (C14), palmitoylcarnitine (C16), hydroxypalmitoylcarnitine (C16OH), octadecanoylcarnitine (C18), Hydroxyoctadecanoylcarnitine (C18OH)

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