

# Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods

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## 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.<sup>1-3</sup> 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.<sup>4</sup>



The original sample preparation techniques use butyl esterification (i.e., derivatized) of amino acids and acylcarnitines in dried blood spots (DBS) to take advantage of the increased sensitivity that derivatization provides. 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 technological improvement 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 benefits the analysis of analytes that ionize poorly.

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

## Experimental

### Sample preparation

Sets of stable 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.

**Table 1. Daily working internal standard concentrations**

Internal standard	Concentration (µmol/L)
Alanine- <i>d</i> <sub>4</sub>	2.50
Arginine- <sup>13</sup> C- <i>d</i> <sub>4</sub>	2.50
Aspartic acid- <i>d</i> <sub>3</sub>	2.50
Citrulline- <i>d</i> <sub>2</sub>	2.50
Glutamic acid- <i>d</i> <sub>3</sub>	2.50
Glycine- <sup>13</sup> C- <sup>15</sup> N	12.50
Leucine- <i>d</i> <sub>3</sub>	2.50
Methionine- <i>d</i> <sub>3</sub>	2.50
Ornithine- <i>d</i> <sub>2</sub>	2.50
Phenylalanine- <sup>13</sup> C <sub>6</sub>	2.50
Tyrosine- <sup>13</sup> C <sub>6</sub>	2.50
Valine- <i>d</i> <sub>8</sub>	2.50
Succinylacetone- <sup>13</sup> C <sub>5</sub>	2.50
C0-Carnitine- <i>d</i> <sub>9</sub>	0.76
C2-Carnitine- <i>d</i> <sub>3</sub>	0.19
C3-Carnitine- <i>d</i> <sub>3</sub>	0.04
C4-Carnitine- <i>d</i> <sub>3</sub>	0.04
C5-Carnitine- <i>d</i> <sub>9</sub>	0.04
C5DC-Carnitine- <i>d</i> <sub>3</sub>	0.08
C5OH-Carnitine- <i>d</i> <sub>3</sub>	0.04
C8-Carnitine- <i>d</i> <sub>3</sub>	0.04
C12-Carnitine- <i>d</i> <sub>9</sub>	0.04
C14-Carnitine- <i>d</i> <sub>9</sub>	0.04
C16-Carnitine- <i>d</i> <sub>3</sub>	0.08
C18-Carnitine- <i>d</i> <sub>3</sub>	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

Parameter	Value
Pump	Thermo Scientific™ Dionex™ UltiMate™ HPG-3200 RS Pump
Autosampler	Thermo Scientific™ Dionex™ UltiMate™ WPS-3000 TRS Autosampler
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:

Parameter	Value
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 (part 1). SRM parameters (derivatized)

Analyte	Precursor ion (m/z)	Product ion (m/z)	Internal standard	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF lens (V)
Alanine	146.20	44.20	Alanine- <i>d</i> <sub>4</sub>	150.20	48.18	17	62
Arginine	231.28	70.13	Arginine- <sup>13</sup> C- <i>d</i> <sub>4</sub>	236.28	75.13	30	87
Aspartic acid	246.18	144.13	Aspartic acid- <i>d</i> <sub>3</sub>	249.25	147.13	15	103
Citrulline	232.28	113.13	Citrulline- <i>d</i> <sub>2</sub>	234.28	115.18	20	85
Glutamic acid	260.28	157.93	Glutamic acid- <i>d</i> <sub>3</sub>	263.33	161.13	16	94
Glycine	131.80	76.05	Glycine- <sup>13</sup> C- <sup>15</sup> N	134.20	78.10	8	55
Leucine	188.25	86.10	Leucine- <i>d</i> <sub>3</sub>	191.25	89.18	15	75
Methionine	206.23	104.13	Methionine- <i>d</i> <sub>3</sub>	209.20	107.23	16	81
Ornithine	189.25	70.10	Ornithine- <i>d</i> <sub>2</sub>	191.18	72.13	24	79
Phenylalanine	222.25	120.13	Phenylalanine- <sup>13</sup> C <sub>6</sub>	228.33	126.18	19	105
Tyrosine	238.30	136.13	Tyrosine- <sup>13</sup> C <sub>6</sub>	244.28	142.15	18	93
Valine	174.25	72.13	Valine- <i>d</i> <sub>8</sub>	182.23	80.18	16	73
SUAC	211.18	137.05	SUAC- <sup>13</sup> C <sub>5</sub>	216.18	142.05	12	91
C0-Carnitine	218.28	85.05	C0-Carnitine- <i>d</i> <sub>9</sub>	227.33	85.05	28	104
C2-Carnitine	260.30	85.05	C2-Carnitine- <i>d</i> <sub>3</sub>	263.30	85.05	25	113
C3-Carnitine	274.33	85.05	C3-Carnitine- <i>d</i> <sub>3</sub>	277.33	85.05	25	121
C3DC-Carnitine	360.33	85.05					

Table 3 (part 2). SRM parameters (derivatized)

Analyte	Precursor ion (m/z)	Product ion (m/z)	Internal standard	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF lens (V)
C4-Carnitine	288.33	85.05	C4-Carnitine-d <sub>3</sub>	291.33	85.05	27	117
C4OH-Carnitine	304.33	85.05					
C5-Carnitine	302.33	85.05	C5-Carnitine-d <sub>9</sub>	311.38	85.05	30	113
C6-Carnitine	316.35	85.05					
C5DC-Carnitine	388.35	85.05	C5DC-Carnitine-d <sub>3</sub>	391.35	85.05	31	138
C5OH-Carnitine	318.38	85.05	C5OH-Carnitine-d <sub>3</sub>	321.38	85.05	31	138
C8-Carnitine	344.38	85.05	C8-Carnitine-d <sub>3</sub>	347.38	85.05	32	141
C10-Carnitine	372.40	85.05					
C12-Carnitine	400.43	85.05	C12-Carnitine-d <sub>9</sub>	409.43	85.05	36	184
C14-Carnitine	428.48	85.05	C14-Carnitine-d <sub>9</sub>	437.48	85.05	35	193
C16-Carnitine	456.55	85.05	C16-Carnitine-d <sub>3</sub>	459.55	85.05	37	183
C16OH-Carnitine	472.55	85.05					
C18-Carnitine	484.55	85.05	C18-Carnitine-d <sub>3</sub>	487.55	85.05	38	215
C18OH-Carnitine	500.55	85.05					

Table 4. SRM parameters (nonderivatized)

Analyte	Precursor ion (m/z)	Product ion (m/z)	Internal standard	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF lens (V)
Alanine	90.15	44.20	Alanine-d <sub>4</sub>	94.15	48.20	13	45
Arginine	175.23	70.15	Arginine- <sup>13</sup> C-d <sub>4</sub>	180.23	75.15	24	92
Aspartic acid	134.20	116.13	Aspartic acid-d <sub>3</sub>	137.20	119.13	6	54
Citrulline	176.20	113.13	Citrulline-d <sub>2</sub>	178.20	115.13	18	59
Glutamic acid	148.15	130.08	Glutamic acid-d <sub>3</sub>	151.15	133.08	9	62
Glycine	76.08	30.25	Glycine- <sup>13</sup> C- <sup>15</sup> N	78.08	32.25	13	43
Leucine	132.25	86.13	Leucine-d <sub>3</sub>	135.25	89.13	11	56
Methionine	150.18	133.08	Methionine-d <sub>3</sub>	153.18	136.08	9	60
Ornithine	133.15	70.15	Ornithine-d <sub>2</sub>	135.15	72.15	19	63
Phenylalanine	166.20	120.15	Phenylalanine- <sup>13</sup> C <sub>6</sub>	172.20	126.15	16	69
Tyrosine	182.15	136.18	Tyrosine- <sup>13</sup> C <sub>6</sub>	188.15	142.18	15	71
Valine	118.23	72.15	Valine-d <sub>8</sub>	126.23	80.15	13	53
SUAC	155.18	109.12	SUAC- <sup>13</sup> C <sub>5</sub>	160.18	114.12	22	63
C0-Carnitine	162.23	85.05	C0-Carnitine-d <sub>9</sub>	171.23	85.05	23	69
C2-Carnitine	204.23	85.05	C2-Carnitine-d <sub>3</sub>	207.23	85.05	21	96
C3-Carnitine	218.23	85.05	C3-Carnitine-d <sub>3</sub>	221.23	85.05	23	91
C3DC-Carnitine	248.23	85.05					
C4-Carnitine	232.18	85.05	C4-Carnitine-d <sub>3</sub>	235.18	85.05	21	78
C4OH-Carnitine	248.25	85.05					
C5-Carnitine	246.30	85.05	C5-Carnitine-d <sub>9</sub>	255.30	85.05	25	96
C6-Carnitine	260.30	85.05					
C5DC-Carnitine	276.30	85.05	C5DC-Carnitine-d <sub>3</sub>	279.30	85.05	25	96
C5OH-Carnitine	262.30	85.05	C5OH-Carnitine-d <sub>3</sub>	265.30	85.05	25	96
C8-Carnitine	288.33	85.05	C8-Carnitine-d <sub>3</sub>	291.33	85.05	26	108
C10-Carnitine	316.33	85.05					
C12-Carnitine	344.45	85.05	C12-Carnitine-d <sub>9</sub>	353.45	85.05	39	152
C14-Carnitine	372.45	85.05	C14-Carnitine-d <sub>9</sub>	381.45	85.05	39	152
C16-Carnitine	400.45	85.05	C16-Carnitine-d <sub>3</sub>	403.45	85.05	36	185
C16OH-Carnitine	416.45	85.05					
C18-Carnitine	428.45	85.05	C18-Carnitine-d <sub>3</sub>	431.45	85.05	36	185
C18OH-Carnitine	444.45	85.05					

## Data processing

Tandem MS data were analyzed using a meta-calculation software, iRC PRO (2Next srl, Prato, Italy). This off-line, automated analysis tool can process peak areas and formulas as well as quantify target analytes from the ion ratios (unlabeled:labeled) of SRM data (see Figure 1).<sup>6</sup>

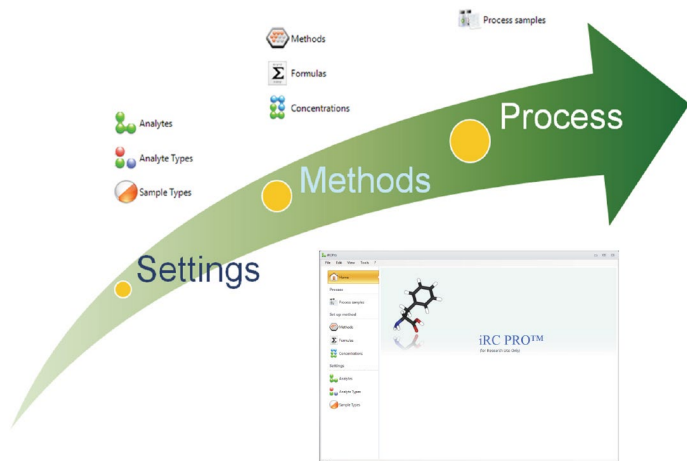


Figure 1. iRC PRO intuitive workflow – icon-based user interface

## 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 and discussion

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). Figures 2 and 3 show full-scan spectra of derivatized and nonderivatized internal standards, respectively.

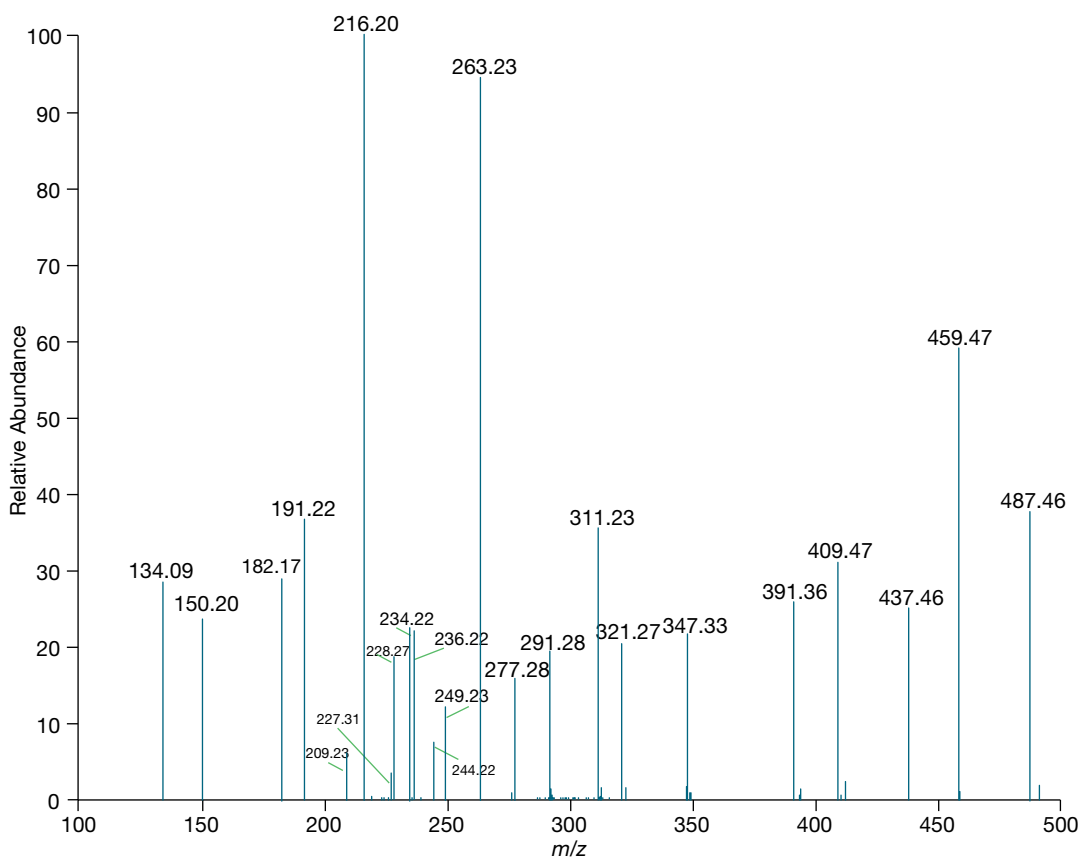


Figure 2. Full-scan spectra of derivatized internal standards

Internal standard	m/z
Alanine- <i>d</i> <sub>4</sub>	150.20
Arginine- <sup>13</sup> C- <i>d</i> <sub>4</sub>	236.22
Aspartic acid- <i>d</i> <sub>3</sub>	249.23
Citrulline- <i>d</i> <sub>2</sub>	234.22
Glutamic acid- <i>d</i> <sub>3</sub>	263.23
Glycine- <sup>13</sup> C- <sup>15</sup> N	134.09
Leucine- <i>d</i> <sub>3</sub>	191.22
Methionine- <i>d</i> <sub>3</sub>	209.23
Ornithine- <i>d</i> <sub>2</sub>	191.22
Phenylalanine- <sup>13</sup> C <sub>6</sub>	228.27
Tyrosine- <sup>13</sup> C <sub>6</sub>	244.22
Valine- <i>d</i> <sub>8</sub>	182.17
Succinylacetone- <sup>13</sup> C <sub>5</sub>	216.20
C0-Carnitine- <i>d</i> <sub>9</sub>	227.31
C2-Carnitine- <i>d</i> <sub>3</sub>	263.23
C3-Carnitine- <i>d</i> <sub>3</sub>	277.28
C4-Carnitine- <i>d</i> <sub>3</sub>	291.28
C5-Carnitine- <i>d</i> <sub>9</sub>	311.23
C5DC-Carnitine- <i>d</i> <sub>3</sub>	391.36
C5OH-Carnitine- <i>d</i> <sub>3</sub>	321.27
C8-Carnitine- <i>d</i> <sub>3</sub>	347.33
C12-Carnitine- <i>d</i> <sub>9</sub>	409.47
C14-Carnitine- <i>d</i> <sub>9</sub>	437.46
C16-Carnitine- <i>d</i> <sub>3</sub>	459.47
C18-Carnitine- <i>d</i> <sub>3</sub>	487.46

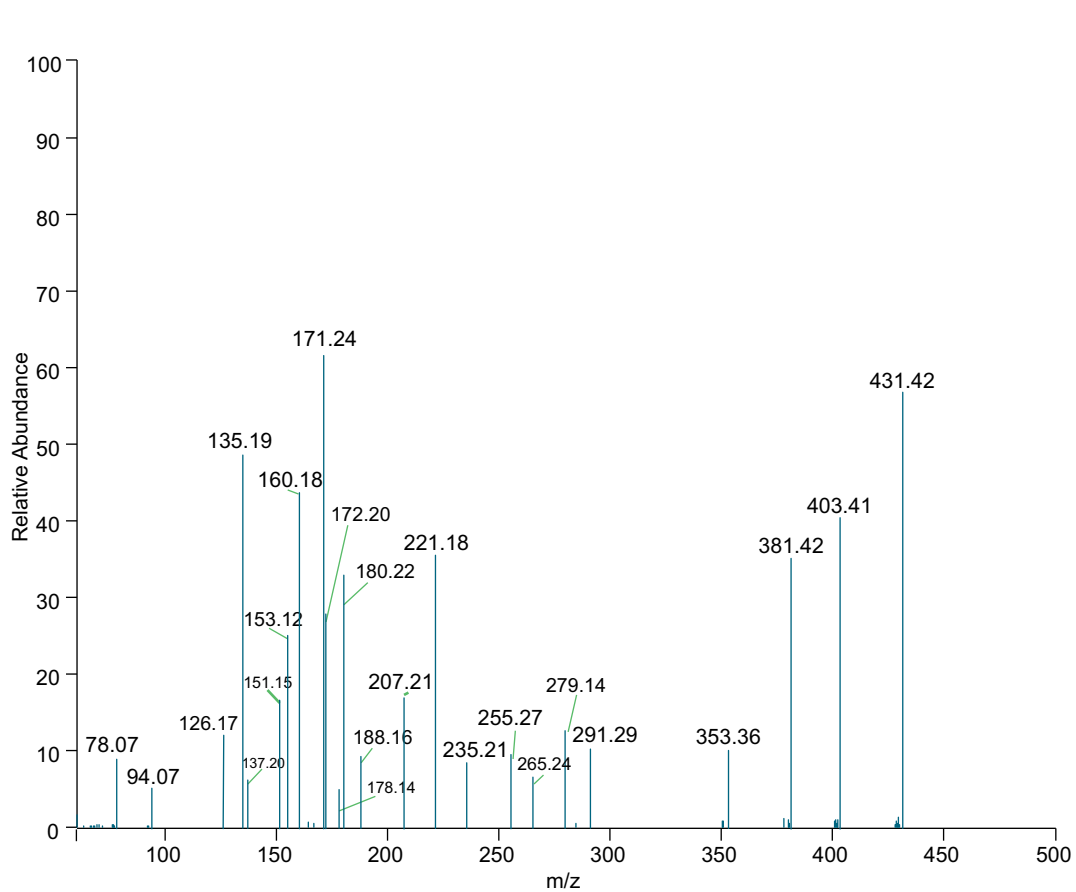


Figure 3. Full-scan spectra of nonderivatized internal standards

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
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

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 (Figures 4 and 5).

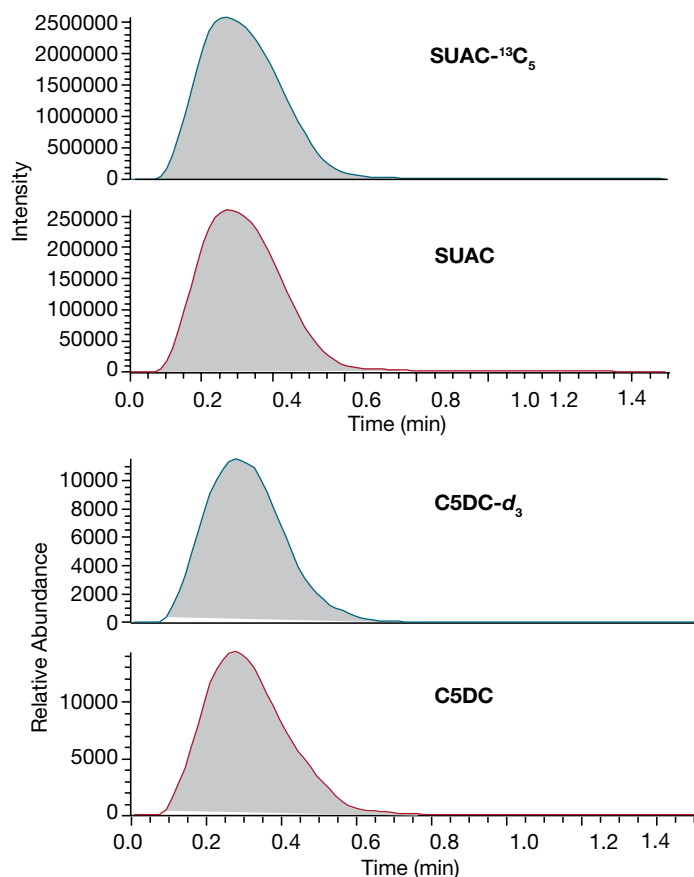


Figure 4. Flow injection analysis (FIA) profiles of SUAC-<sup>13</sup>C<sub>5</sub>, SUAC and C5DC-*d*<sub>3</sub>, C5DC using derivatized method

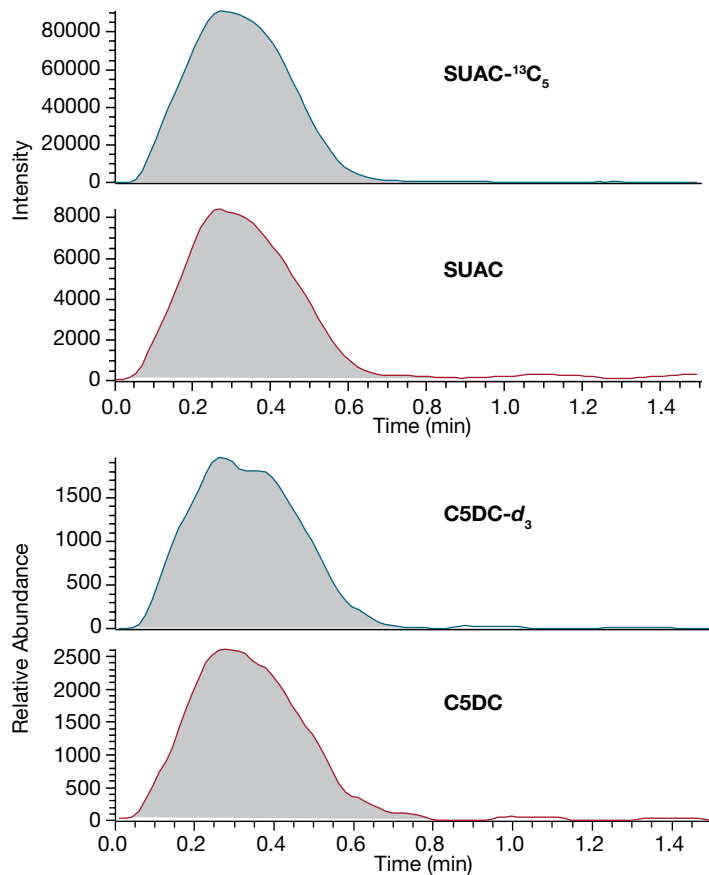


Figure 5. Flow injection analysis (FIA) profiles of SUAC-<sup>13</sup>C<sub>5</sub>, SUAC and C5DC-*d*<sub>3</sub>, C5DC using nonderivatized method

### 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).

**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.



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 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).

**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.

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

Analyte	Coefficient of variation (%)			Concentrations in $\mu\text{mol/L}$		
	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.<sup>5</sup>

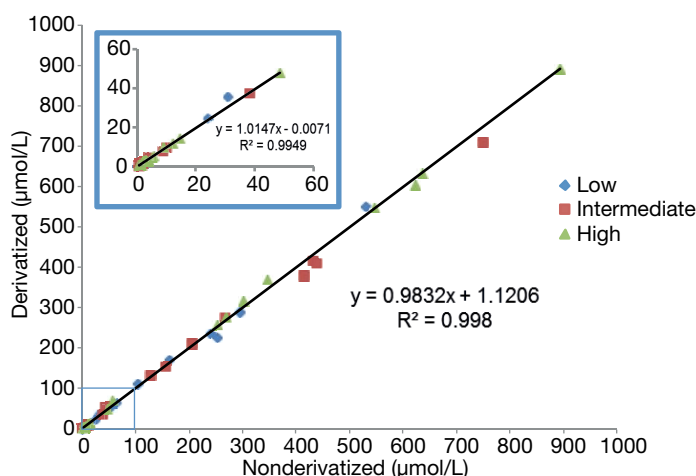


Figure 6. Comparisons between quantitative values of 12 AA, SUAC, and 18 AC resulting from nonderivatized and derivatized methods

### 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 the TSQ Endura triple quadrupole MS with a run time of 1.5 min.
- SRM data acquisition mode was optimized for each analyte, and the 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.

### References

- 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.
- 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.
- 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. Inher. Metab. Dis.*, **1990**, *13*(3), 321–4.
- 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.
- 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), 68–9.
- Wang, J. et al., Thermo Fisher Scientific PN64229, The Use of A New Meta-calculation Software for Automated Data Processing of Tandem MS for Inborn Error Metabolism Research. <https://assets.thermofisher.com/TFS-Assets/CMD/posters/PN-64229-MSACL-EU-IRCPro-Endura-Newborn-PN64229-EN.pdf>

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