Results Evaluation of Derivatised and Non-derivatised Methods for Research Analysis of Amino Acids and Acylcarnitines in Dried Blood Spots Using a Novel Triple Quadrupole Mass Spectrometer

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Overview

Purpose: To evaluate derivatised and non-derivatised methods from two commercially available products for analysis of amino acid and acylcarnitines in dried blood spots (DBS) using a novel triple quadrupole mass spectrometer, Thermo Scientific ™ TSQ Endura ™ mass spectrometer.

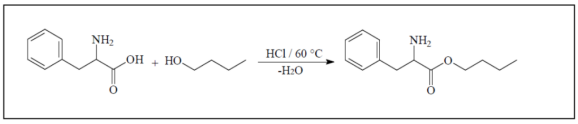
Methods: DBS QC samples obtained from Centers for Disease Control and Prevention (CDC) were prepared using two products, reagents from Chromsystems® kit and internal standards from Cambridge Isotope Laboratories (CIL). Two sample preparation methods, derivatised and non-derivatised, were evaluated for each product. Amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC) were extracted simultaneously followed by flow injection analysis (FIA) on TSQ Endura™ MS. Selected-reation monitoring (SRM) mode was set up for data acquisition for each target and internal standard. Automated data processing was performed using a new meta-calculation software, iRC PRO ™ (2Next srl, Prato, Italy).

Results: The exceptional sensitivity and selectivity of TSQ Endura MS SRM mode enabled us to analyze AA, AC, and SUAC simultaneously in 1.5 min on either derivatised or non-derivatised method from two products.

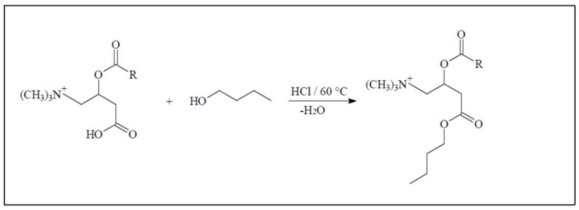
Introduction

Flow injection tandem mass spectrometry (FIA-MS/MS) has been frequently used for analysis of amino acids and acylcarnitines in dried blood spots from newborns in order to detect inborn errors of metabolism for research (1, 2). Derivatised sample preparation method was initially deployed due to its increased sensitivity. However, with the development of new MS instrument, non-derivatised method is attracting attention because of simplicity and cost-effectiveness.

FIGURE 1. Derivatisation of representative amino acid and acylcarnitine



Derivatisation of phenylalanine to phenylalanine butyric ester



Derivatisation of acylcarnitines to acylcarnitine butyric esters

The kit from Chromsystems® and internal standards from CIL are two commercially available products for mass spectrometric analysis of amino acids and acylcarnitines in dried blood spots. Each product can be used for either derivatised or non-derivatised method.

Method

Sample Preparation

DBS QC samples were kindly provided by CDC. Four levels of AA, AC, and SUAC were enriched on dried blood spot filter papers. A 1/8 inch disc was punched out into 96-well plate.

Chromsystems® product (derivatised and non-derivatised)

The protocols from instruction manuals were followed.

CIL product (derivatised and non-derivatised, ref. 3)

1.Add 100 µL of working internal standard solution to each well.

2.Shake the plate for 45 min at 45°C.

3.Transfer the eluates into another plate and evaporate at 50°C under nitrogen flow.

4.Pipet 50 µL of methanol into each sample well and evaporate 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 (for derivatised method only).

6.Reconstitute each sample well with 100 µL of 50:50:0.02 Acetonitrile/Water/Formic acid.

7.The plate is ready for mass spectrometric analysis.

LC method

The HPLC used was Thermo Scientific™ Dionex™ Ultimate™ 3000 and the autosampler was Thermo Scientific™ Dionex™ Ultimate™ 3000 RS autosampler. The mobile phase was 50:50:0.02 Acetonitrile/Water/Formic acid. No column was used.

FIGURE 2. HPLC gradient method

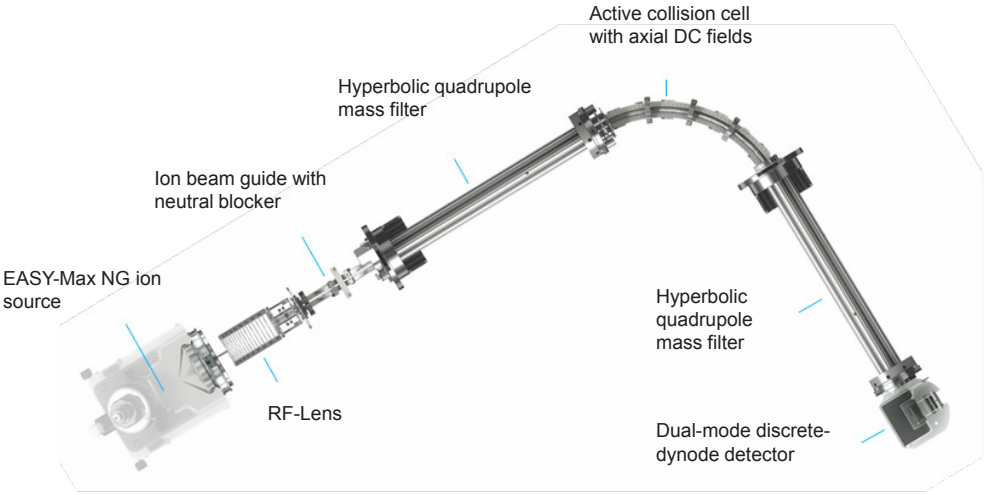
Time (min)	Flow rate (mL/min)	%A (mobile phase)
0.00	0.09	100
1.23	0.09	100
1.25	0.30	100
1.50	0.09	100

Mass Spectrometry

With best in class sensitivity, unprecedented usability, and exceptional robustness, the TSQ Endura triple quadrupole mass spectrometer delivers exceptional value.

- Ion optics—RF-Lens, ion beam guide with neutral blocker and quadrupole mass filter -- combine to reduce noise and increase sensitivity for enhanced quantitative performance
- Ultrafast selected-reaction monitoring (SRM) as high as 500 SRM/second, with up to 30,000 definable SRMs, enables quantification of more compounds in less time
- Five order of dynamic range increases quantitative confidence

FIGURE 3. Schematic diagram of the new TSQ Endura triple quadrupole mass spectrometer.



Data Analysis

LC-MS/MS data were processed using a new meta-calculation software, iRC PRO. The off-line automated data processing tool can process peak area, concentration and user-defined formulas.

The meta calculation 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 4. iRC PRO intuitive workflow – icon based user interface

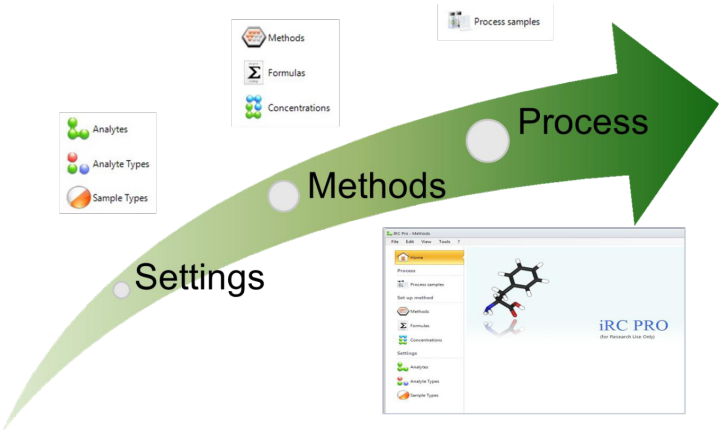


FIGURE 5. SRM transitions of targets and internal standards (CIL derivatised method).

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Alanine	146.20	44.20	17	62
Alanine-D4	150.20	48.18	17	62
Arginine	231.28	70.13	30	87
Arginine-D4^{13}C	236.28	75.13	30	87
Aspartic acid	246.18	144.13	15	103
Aspartic acid-D3	249.25	147.13	15	103
Citrulline	232.28	113.13	20	85
Citrulline-D2	234.28	115.18	20	85
Glutamic acid	260.28	157.93	16	94
Glutamic acid-D3	263.33	161.13	16	94
Glycine	131.80	76.05	8	55
Glycine-^{13}C^{15}N	134.20	78.10	8	55
Leucine	188.25	86.10	15	75
Leucine-D3	191.25	89.18	15	75
Methionine	206.23	104.13	16	81
Methionine-D3	209.20	107.23	16	81
Ornithine	189.25	70.10	24	79
Ornithine-D2	191.18	72.13	24	79
Phenylalanine	222.25	120.13	19	105
Phenylalanine-^{13}C6	228.33	126.18	19	105
Tyrosine	238.30	136.13	18	93
Tyrosine-^{13}C6	244.28	142.15	18	93
Valine	174.25	72.13	16	73
Valine-D8	182.23	80.18	16	73
SUAC	211.18	137.05	12	91
SUAC-^{13}C5	216.18	142.05	12	91

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Carnitine	218.28	85.05	28	104
Carnitine-D9	227.33	85.05	28	104
C2-Carnitine	260.30	85.05	25	113
C2-Carnitine-D3	263.30	85.05	25	113
C3-Carnitine	274.33	85.05	25	121
C3-Carnitine-D3	277.33	85.05	25	121
C3DC-Carnitine	360.33	85.05	25	121
C4-Carnitine	288.33	85.05	27	117
C4-Carnitine-D3	291.33	85.05	27	117
C4OH-Carnitine	304.33	85.05	27	117
C5-Carnitine	302.33	85.05	30	113
C5-Carnitine-D9	311.38	85.05	30	113
C5DC-Carnitine	388.35	85.05	31	138
C5DC-Carnitine-D3	391.35	85.05	31	138
C5OH-Carnitine	318.38	85.05	31	138
C5OH-Carnitine-D3	321.38	85.05	31	138
C6-Carnitine	316.35	85.05	30	135
C8-Carnitine	344.38	85.05	32	141
C8-Carnitine-D3	347.38	85.05	32	141
C10-Carnitine	372.40	85.05	31	158
C12-Carnitine	400.43	85.05	36	184
C12-Carnitine-D9	409.43	85.05	36	184
C14-Carnitine	428.48	85.05	35	193
C14-Carnitine-D9	437.48	85.05	35	193
C16-Carnitine	456.55	85.05	37	183
C16OH-Carnitine	472.55	85.05	37	183
C16-Carnitine-D3	459.55	85.05	37	183
C18-Carnitine	484.55	85.05	38	215
C18OH-Carnitine	500.55	85.05	38	215
C18-Carnitine-D3	487.55	85.05	38	215

FIGURE 6. SRM transitions of targets and internal standards (CIL non-derivatised method).

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Alanine	90.15	44.20	13	45
Alanine-D4	94.15	48.20	13	45
Arginine	175.23	70.15	24	92
Arginine-D4^{13}C	180.23	75.15	24	92
Aspartic acid	134.20	116.13	6	54
Aspartic acid-D3	137.20	119.13	6	54
Citrulline	176.20	113.13	18	59
Citrulline-D2	178.20	115.13	18	59
Glutamic acid	148.15	130.08	9	62
Glutamic acid-D3	151.15	133.08	9	62
Glycine	76.08	30.25	13	43
Glycine-^{15}N^{13}C	78.08	32.25	13	43
Leucine	132.25	86.13	11	56
Leucine-D3	135.25	89.13	11	56
Methionine	150.18	133.08	9	60
Methionine-D3	153.18	136.08	9	60
Ornithine	133.15	70.15	19	63
Ornithine-D2	135.15	72.15	19	63
Phenylalanine	166.20	120.15	16	69
Phenylalanine-^{13}C6	172.20	126.15	16	69
Tyrosine	182.15	136.18	15	71
Tyrosine-^{13}C6	188.15	142.18	15	71
Valine	118.23	72.15	13	53
Valine-D8	126.23	80.15	13	53
SUAC	155.18	109.12	22.1	63
SUAC-^{13}C5	160.18	114.12	22.1	63

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Carnitine	162.23	85.05	23	69
Carnitine-D9	171.23	85.05	23	69
C2-Carnitine	204.23	85.05	21	96
C2-Carnitine-D3	207.23	85.05	21	96
C3-Carnitine	218.23	85.05	23	91
C3-Carnitine-D3	221.23	85.05	23	91
C3DC-Carnitine	248.23	85.05	23	91
C4-Carnitine	232.18	85.05	21	78
C4-Carnitine-D3	235.18	85.05	21	78
C4OH-Carnitine	248.25	85.05	21	78
C5-Carnitine	246.30	85.05	25	96
C5-Carnitine-D9	255.30	85.05	25	96
C5DC-Carnitine	276.30	85.05	25	96
C5DC-Carnitine-D3	279.30	85.05	25	96
C5OH-Carnitine	262.30	85.05	25	96
C5OH-Carnitine-D3	265.30	85.05	25	96
C6-Carnitine	260.30	85.05	25	96
C8-Carnitine	288.33	85.05	26	108
C8-Carnitine-D3	291.33	85.05	26	108
C10-Carnitine	316.33	85.05	26	108
C12-Carnitine	344.45	85.05	39	152
C12-Carnitine-D9	353.45	85.05	39	152
C14-Carnitine	372.45	85.05	39	152
C14-Carnitine-D9	381.45	85.05	39	152
C16-Carnitine	400.45	85.05	36	185
C16OH-Carnitine	416.45	85.05	36	185
C16-Carnitine-D3	403.45	85.05	36	185
C18-Carnitine	428.45	85.05	36	185
C18OH-Carnitine	444.45	85.05	36	185
C18-Carnitine-D3	431.45	85.05	36	185

Results

FIGURE 7. Spectrum of derivatised acylcarnitines and their corresponding internal standards (Chromsystems®)

m/z	Target	m/z	IS
218.44	C0	227.56	C0-D9
260.33	C2	265.44	C2-D3
274.56	C3	277.44	C3-D3
288.44	C4	291.33	C4-D3
302.44	C5	311.56	C5-D9
388.33	C5DC	394.44	C5DC-D6
316.56	C6	319.56	C6-D3
344.33	C8	347.67	C8-D3
372.67	C10	375.56	C10-D3
400.67	C12	403.56	C12-D3
428.67	C14	431.67	C14-D3
456.78	C16	459.78	C16-D3
484.78	C18	487.67	C18-D3

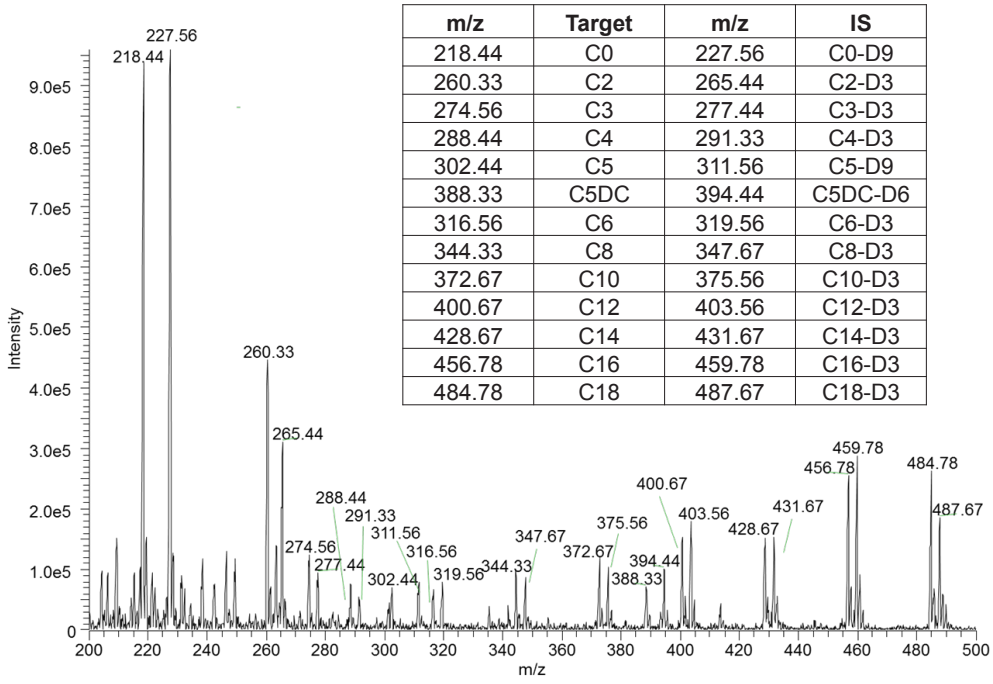
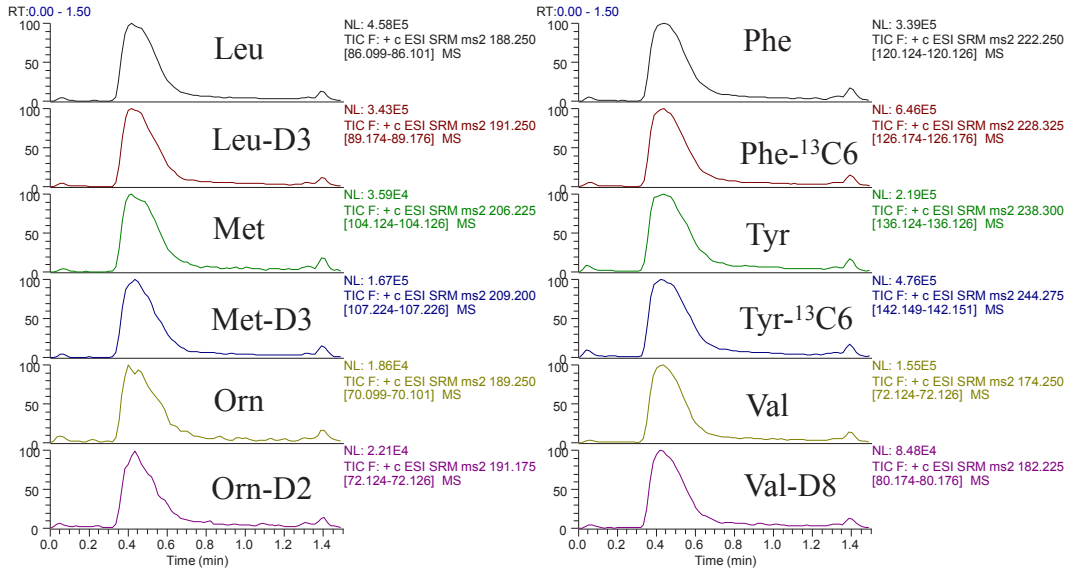


FIGURE 8. Flow injection analysis profiles of representative amino acids and acylcarnitines (CIL derivatised method)

- **Amino acids (half of monitored amino acids were shown due to space limit):**



- **Acylcarnitines (half of monitored acylcarnitines were shown due to space limit):**

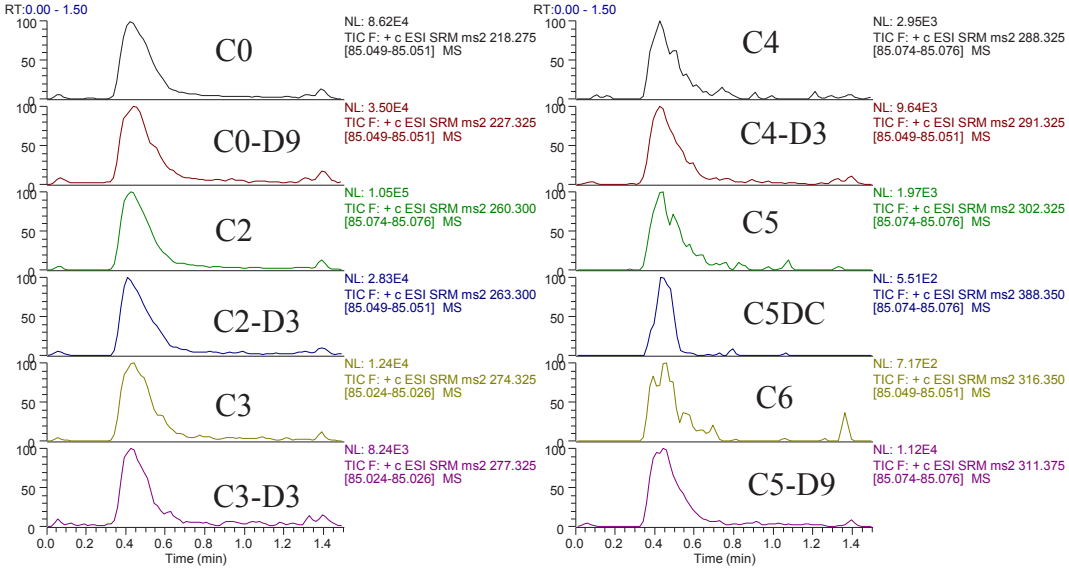
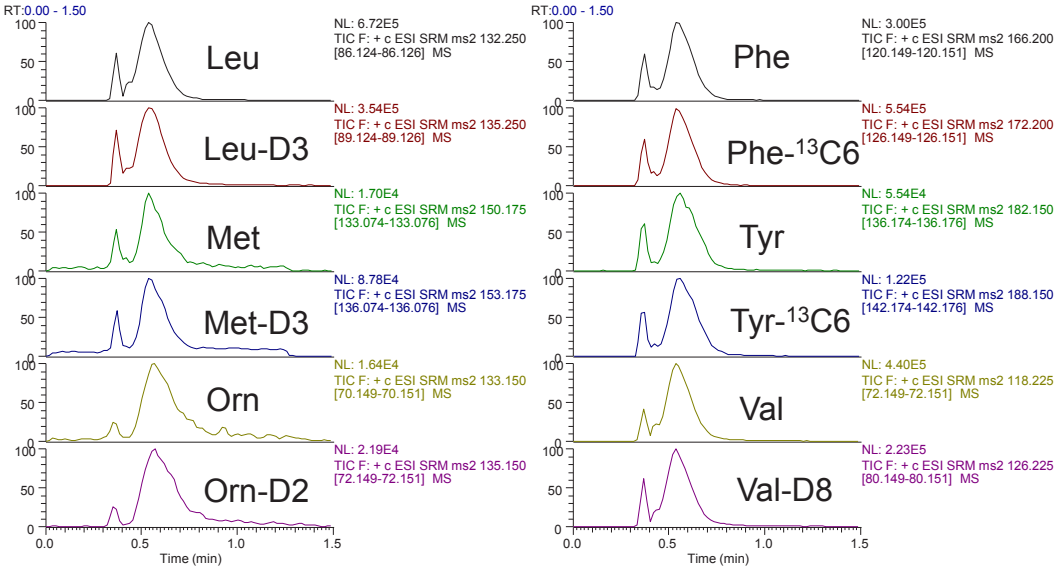


FIGURE 9. Flow injection analysis profiles of representative amino acids and acylcarnitines (CIL non-derivatised method)

- **Amino acids (half of monitored amino acids were shown due to space limit):**



- **Acylcarnitines (half of monitored acylcarnitines were shown due to space limit):**

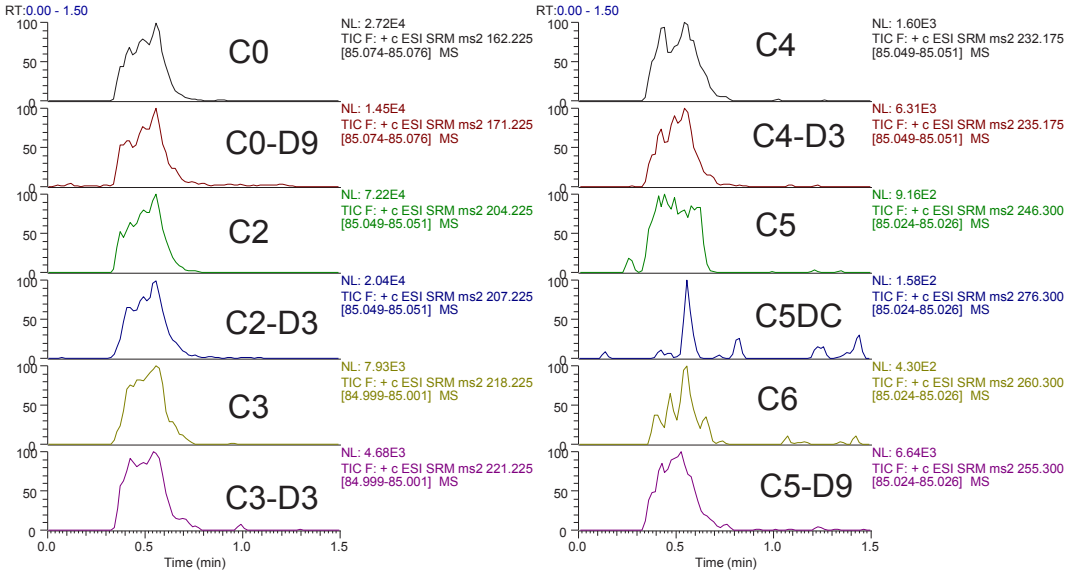


FIGURE 10. Analytical precision of four different methods (coefficient of variation of analytical methods, %)

Target	Chromsystems derivatised	Chromsystems non-derivatised	CIL derivatised	CIL non-derivatised
Alanine	4.5	3.4	7.1	7.1
Arginine	7.9	3.2	3.5	9.6
Aspartic acid	5.2	5.0	7.7	9.7
Citrulline	7.5	4.6	7.5	9.0
Glutamic acid	3.3	7.2	8.2	8.2
Glycine	3.3	7.9	8.9	7.3
Leucine	2.5	7.0	9.4	7.7
Methionine	3.3	7.9	5.5	9.8
Ornithine	8.6	7.2	8.7	6.9
Phenylalanine	2.6	5.7	4.7	6.3
Proline	8.2	6.7	N/A	N/A
Tyrosine	3.6	5.9	9.4	5.6
Valine	8.8	5.5	6.7	8.6
Carnitine	7.6	7.3	9.1	8.3
C2-Carnitine	3.8	7.3	8.3	5.9
C3-Carnitine	7.1	7.1	6.8	9.7
C4-Carnitine	3.2	7.3	9.9	3.9
C5-Carnitine	8.0	7.5	5.3	8.4
C5DC-Carnitine	8.0	7.4	14.2	8.0
C6-Carnitine	8.7	8.4	8.2	6.2
C8-Carnitine	9.1	6.4	6.3	6.3
C10-Carnitine	9.0	7.6	9.6	8.8
C12-Carnitine	6.5	6.0	9.6	6.0
C14-Carnitine	4.4	7.4	8.1	7.3
C16-Carnitine	5.9	7.5	7.1	4.8
C18-Carnitine	7.2	6.5	7.7	5.2
Average	**6.1**	**6.6**	**7.9**	**7.4**

Conclusion

- Derivatised and non-derivatised methods from two commercially available products for mass spectromtric research analysis of amino acids and acylcarnitines in dried blood spots were evaluated using a novel TSQ Endura triple quadrupole mass spectrometer
- The exceptional sensitivity and selectivity of TSQ Endura MS SRM mode can detect and quantify AA, AC, and SUAC simultaneously in 1.5 min on either derivatised or non-derivatised method from two products. LC-MS/MS data processing can be automated using a new meta-calculation software, iRC PRO.
- Using the new TSQ Endura triple quadrupole MS, the average analytical precision of 6-8% can be achieved on either derivatised or non-derivatised method from two products.

References

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