Comparison of Non-derivatization and Derivatization Tandem Mass Spectrometry Methods for Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots

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Introduction

Flow injection tandem mass spectrometry (FIA-MS/MS) has been frequently used to analyze amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC) in dried blood spots (DBS) for inborn errors of metabolism research. [1-3]. The original sample preparation techniques detect butyl esterification (i.e., derivatized) of AAs, ACs, and SUAC. However, with improved sensitivity of MS instruments, it is possible to detect AAs, ACs, and SUAC as their native free acids (i.e., non-derivatized). This simplifies analytical operation and minimizes the use of corrosive chemicals. Using quality control (QC) DBS samples enriched with different levels of analytes, we conducted a comprehensive study to evaluate and compare non-derivatization and derivatization methods on a triple quadrupole mass spectrometer.

Method

Sample Preparation

The following protocols were used to prepare the DBS samples:

Non-derivatization

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. Reconstitute each sample well with 100 µL of 50:50:0.02

Derivatization

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 50:50:0.02 acetonitrile/water/formic acid.

Liquid Chromatography and Mass Spectrometry

Flow injection MS/MS analysis was performed on a Thermo Scientific™ Dionex™ UltiMate™ 3000 RS LC pump without using an analytical column and a TSQ Endura triple quadrupole mass spectrometer. Selected Reaction Monitoring (SRM) parameters were optimized for each analyte (data not shown).

Data Processing

FIA-MS/MS data were processed using a streamlined 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 1).

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.

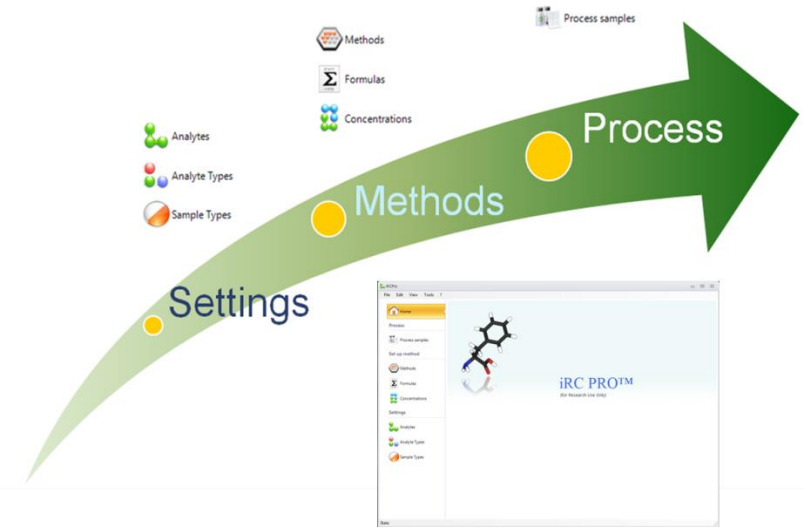


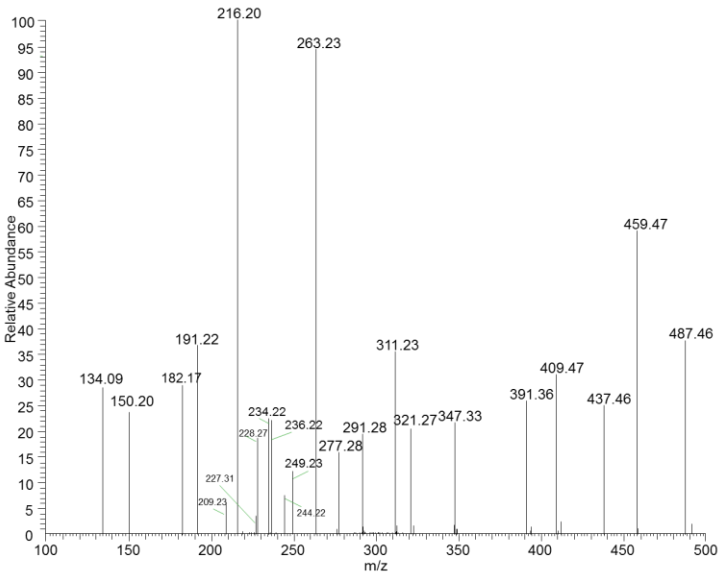
Figure 1. iRC PRO intuitive workflow – icon based user interface

Analytical Precision Evaluation

The within-run precision was determined at three concentrations by means of 10 successive, independent measurement of DBS samples (n=10). The run-to-run precision was determined at three concentrations by means of 10 independent measurement of DBS samples in 7 different test series (n=70).

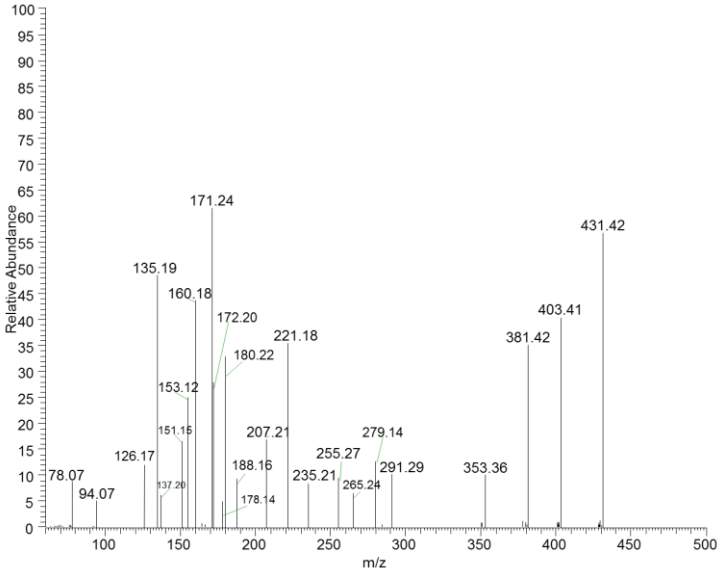
Results

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 non-derivatized internal standards respectively.



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 2. Full-scan spectra of derivatized internal standards.



Internal Standard	*m/z*	Internal Standard	*m/z*
Alanine-D4	94.07	C0-Carnitine-D9	171.24
Arginine-^{13}C-D4	180.22	C2-Carnitine-D3	207.21
Aspartic acid-D3	137.20	C3-Carnitine-D3	221.18
Citrulline-D2	178.14	C4-Carnitine-D3	235.21
Glutamic acid-D3	151.15	C5-Carnitine-D9	255.27
Glycine-^{13}C-^{15}N	78.07	C5DC-Carnitine-D3	279.14
Leucine-D3	135.19	C5OH-Carnitine-D3	265.24
Methionine-D3	153.12	C8-Carnitine-D3	291.29
Ornithine-D2	135.19	C12-Carnitine-D9	353.36
Phenylalanine-^{13}C6	172.20	C14-Carnitine-D9	381.42
Tyrosine-^{13}C6	188.16	C16-Carnitine-D3	403.41
Valine-D8	126.17	C18-Carnitine-D3	431.42
Succinylacetone-^{13}C5	160.18		

Figure 3. Full-scan spectra of non-derivatized 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 (Figure 4 and 5).

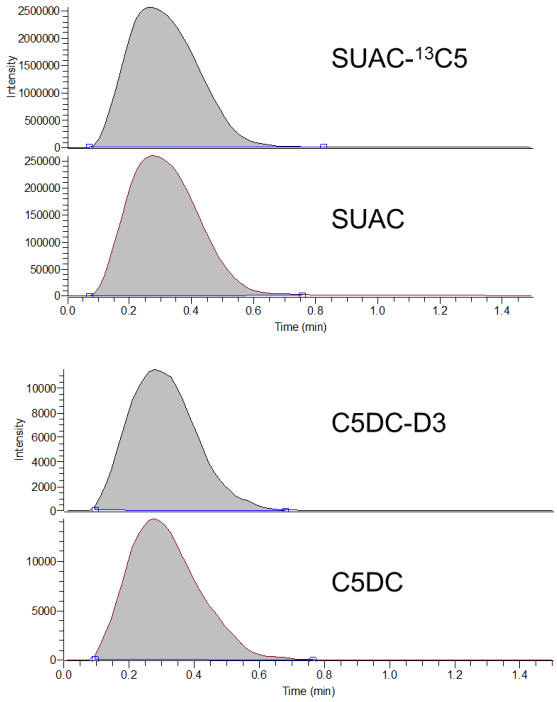


Figure 4. Flow injection analysis (FIA) profiles of SUAC-13C5, SUAC and C5DC-D3, C5DC using derivatization method.

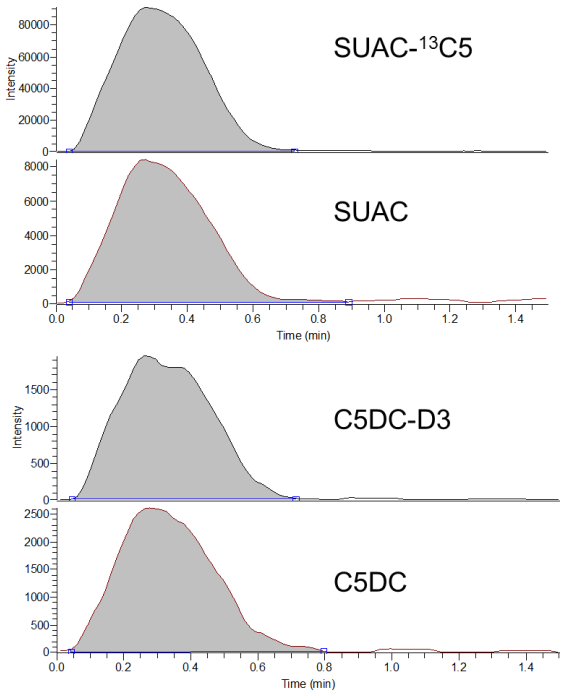


Figure 5. Flow injection analysis (FIA) profiles of SUAC-^{13}C5, SUAC and C5DC-D3, C5DC using non-derivatization method.

Within-run Precision

For the derivatization method, the within-run precisions (n=10) for 12 AAs and SUAC at three concentrations were less than 7.9% (low), 8.0% (intermediate), and 8.0% (high). The within-run precisions for 18 ACs were less than 8.9% (low), 8.3% (intermediate), and 9.0% (high).

For the non-derivatization method, the within-run precisions (n=10) for AAs and SUAC at three concentrations were less than 6.1% (low), 7.2% (intermediate), and 9.8% (high). The within-run precisions for ACs were less than 7.6% (low), 6.2% (intermediate), and 8.2% (high).

Run-to-run Precision

For the derivatization method, the run-to-run precisions (n=70) for 12 AAs and SUAC at three concentrations were less than 13.5% (low), 12.9% (intermediate), and 12.5% (high). The run-to-run precisions for 18 ACs were less than 15.0% (low), 15.6% (intermediate), and 16.1% (high).

For the non-derivatization method, the run-to-run precisions (n=70) for AAs and SUAC at three concentrations were less than 12.8% (low), 12.8% (intermediate), and 12.6% (high). The run-to-run precisions for ACs were less than 12.7% (low), 10.5% (intermediate), and 11.8% (high).

Method Comparison

The concentration of analytes obtained from non-derivatization and derivatization methods were compared. The method differences of 12 AAs and SUAC between quantitative values resulting from butyl esters and free acid techniques at three concentrations were less than 3.8% (low), 4.8% (intermediate), and 3.2% (high). The method differences of 18 ACs were less than 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 an empirical analysis [5].

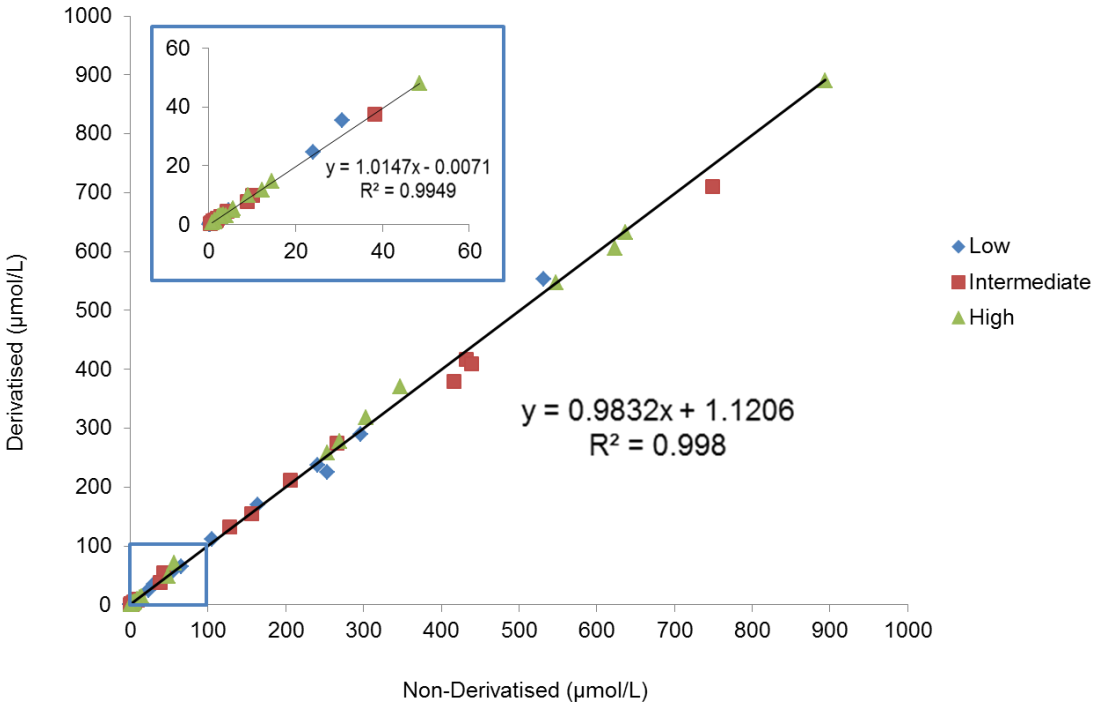


Figure 6. Comparisons between quantitative values of 12 AAs, 18 ACs, and SUAC resulting from non-derivatization and derivatization methods.

Conclusions

- Flow injection MS/MS methods were developed to simultaneously detect and quantify amino acids, acylcarnitines, and succinylacetone in a single extraction step in dried blood spots. Rapid data processing was performed using iRC PRO meta-calculation software.
- Both non-derivatization and derivatization methods were capable of accurately quantifying AAs, ACs, and SUAC on TSQ Endura triple quadrupole MS with a run time of 1.5 min. The quantitative values were well within confidence limits.
- The methods had excellent analytical precision performance. The within-run precision (n=10) at three enriched concentrations was less than 10% and the run-to-run precision (n=70) was less than 15%.
- The method difference between quantitative values resulting from non-derivatization and derivatization methods was minor (<15%) for the majority of analytes and both methods are highly correlated.

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), succinylacetone (SUAC)

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

References

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