Integrated instrumental and statistical approach for improved diagnostics of inborn errors of metabolism using acylcarnitine profiling

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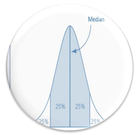
Introduction

Acylcarnitines (ACs) are important biomarkers for inborn errors of metabolism (IEM) and are increasingly implicated in diabetes and cardiovascular disease. Currently, flow injection analysis tandem mass spectrometry (FIA-MS/MS) serves as the gold standard for screening; however, it cannot distinguish between many AC isomers, leading to a higher rate of false positives - particularly in complex cases. Additionally, AC concentrations vary across populations, making it difficult to establish consistent and reliable cutoff values. Therefore, there is a growing need for advanced LC-MS/MS methods, supported by robust, age-specific reference ranges, to improve the accuracy and diagnostic power of IEM screening.

Methodology



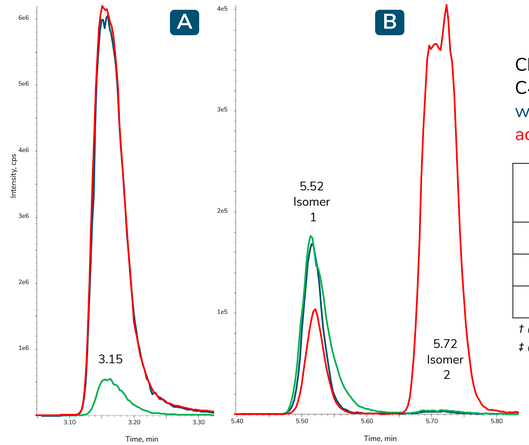



Sample type	Sample preparation	Instrumental analysis	Statistical analysis
>600 Dried blood spot (DBS) samples including 77 confirmed IEM cases 1 x ø 3 mm discs (~3.1 µl blood)	Standard FIA-MS/MS protocol - extraction wia mixture of ISTD in MeOH, followed by butylation	Reversed-phase chromatography; SCIEX 4000QTRAP; MRM mode	Percentile-based reference ranges (2.5–97.5), validated with ERNDIM QA.

Conclusion

- ✓ Reference ranges differ between children and adults, varying for particular AC isomers, likely due to distinct metabolic activities, nutritional statuses and differences in hematocrit levels (age and gender specific) between this groups.
- ✓ A higher outlier rate was observed in adult samples, likely due to other coexisting metabolic disturbances such as fasting, diet, diseases other than IEM, drug therapy etc. highlighting the complexity of AC profiling in the elderly.
- ✓ The developed method has demonstrated significant diagnostic potential, based on its ability to differentiate between several acidurias, including: PA versus MMA, 3MCCD versus BKTD and IVA versus SBCADD.
- ✓ Analysed data suggest that due to variability in ACs' levels in children and adult population, dedicated cut-off values can additionally improve IEM detection and diagnosis.

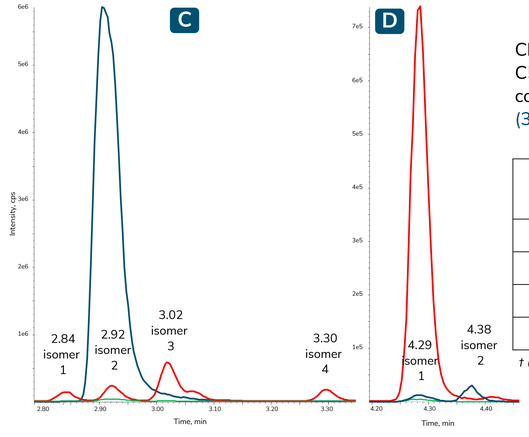
Results



Chromatogram of C3-carnitine (fig. A) and C4DC-carnitine (fig. B) for healthy individual and patient with aconfirmed propionic acidu rias (PA) or methylmalonic acidurias (MMA)

	Ref. range [µmol\l]	PA [µmol\l]	MMA [µmol\l]
C3	< 4.85	29.3	31.3
C4DC_1†	< 0.77	0.29	0.23
C4DC_2‡	< 0.06	0.01	1.54

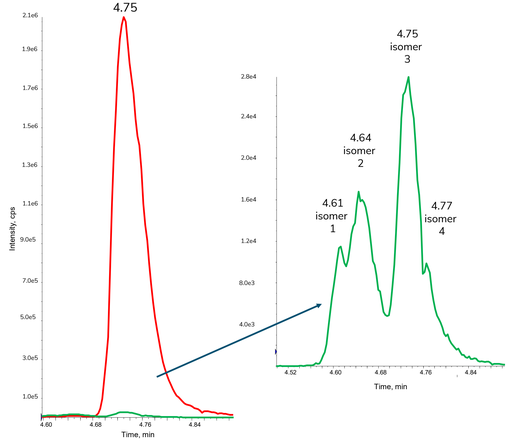
† *C4DC_1 - O-succinyl-L-carnitine*
‡ *C4DC_2 - methylmalonyl-L-carnitine*



Chromatogram of C5:1-carnitine (fig. C) and C5-OH-carnitine (fig. D) healthy individual and patient with confirmed 3-methylcrotonyl-CoA carboxylase deficiency (3MCC) or beta-ketothiolase deficiency (BKT)

	Ref. range [µmol\l]	3MCC [µmol\l]	BKT [µmol\l]
C5-OH†	< 0.17	0.17	1.24
C5-OH_2	<0.42	10.4	0.28
C5:1_1	<0.02	0.01	0.81
C5:1_2	<0.01	0.26	0.00

† *C5-OH defined as sum of isomers 1, 3 and 4*



Chromatogram of four C5-carnitine isomers for healthy individual and patient with confirmed propionic acidurias (IVA).

	Ref. range [µmol\l]	SBCADD	IVA [µmol\l]
C5_1†	<0.21†	Looking for samples to confirm	0.058
C5_2††			
C5_3‡	<0.22‡		12.72
C5_4‡‡			

† *pivaloyl-carnitine;* †† *2-methylbutyryl-carnitine;* ‡ *isovaleryl-carnitine,* ‡‡ *valeryl-carnitine*

Over 80 ACs' isomers were separated, including qualitative biomarkers, and gender-specific reference ranges were established for 32 ACs' isomers. Metabolic profiles from 300 pediatric and 300 adult DBS samples were analyzed to provide clinically relevant cut-off values (2.5th and 97.5th percentiles).

Age-dependent reference ranges for acylcarnitine isomers

Cut-off values (2.5th and 97.5th percentiles) and medians for children ( ) and adults ( )