Quantification of amino acids and acylcarnitines in dried blood spots by FIA-MS/MS

Author: Mariana Barcenas,
Thermo Fisher Scientific, Les Ulis, France

Keywords: Amino acids, acylcarnitines, dried blood spots, non-derivatized, mass spectrometry, TSQ Fortis MS

Goal

Implementation of a flow injection analysis-tandem mass spectrometry (FIA-MS/MS) method for simultaneous quantification of 11 amino acids and 13 acylcarnitines in dried blood spots

Introduction

In this report, an MS-based analytical method for determination of amino acids and acylcarnitines in dried blood spots (DBS) for inborn errors of metabolism (IEM) research is reported. FIA was performed on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system. Detection was performed on a Thermo Scientific™ TSQ Fortis™ triple-stage quadrupole mass spectrometer with heated electrospray ionization (HESI) by selected reaction monitoring (SRM). Reagents and controls were obtained from the NeoBase™ MSMS Complete Kit for Amino Acids and Acylcarnitines in Dried Blood Spots (Ref 3040-0010) from PerkinElmer, Inc. (Waltham, USA). The NeoBase kit enables the determination of up to 11 amino acids, with corresponding controls for nine of them.

Experimental

Target analytes

Target analytes and their respective internal standards are summarized in Table 1.

Sample preparation

Reagents from PerkinElmer included an extraction reagent and two controls, as well as the internal standards.

Samples were prepared as described by PerkinElmer using the following procedure:

1. The internal standards were reconstituted using the extraction reagent.
2. 3.2 mm punches were placed in a well plate, and 100 µL of extraction reagent (containing the internal standards) were added to each well.
3. The plate was covered to minimize evaporation.
4. The plate was kept at 45 °C for 45 minutes while shaking at 700 rpm.
5. Extracted samples were transferred to a clean plate.

Table 1. List of analytes and internal standards

Amino acids	Amino acid internal standards	Acylcarnitines	Acylcarnitine internal standards
Alanine	d_4-Alanine	Carnitine (C0)	d_9-C0
Citrulline	d_2-Citrulline	Acetylcarnitine (C2)	d_3-C2
Glycine	$^{13}C_2$,^{15}N-Glycine	Propionylcarnitine (C3)	d_3-C3
Leucine/Isoleucine	d_3-Leucine	Butyrylcarnitine (C4)	d_3-C4
Methionine	d_3-Methionine	Isovalerylcarnitine(C5)	d_9-C5
Phenylalanine	$^{13}C_6$-Phenylalanine	Glutarylcarnitine (C5DC)	d_6-C5DC
Proline	$^{13}C_5$-Proline	Hexanoylcarnitine (C6)	d_3-C6
Tyrosine	$^{13}C_6$-Tyrosine	Octanoylcarnitine (C8)	d_3-C8
Valine	d_8-Valine	Decanoylcarnitine (C10)	d_3-C10
	d_4,^{13}C-Arginine	Dodecanoylcarnitine (C12)	d_3-C12
	d_6-Ornitine	Tetradecanoylcarnitine (C14)	d_3-C14
		Hexadecanoylcarnitine (C16)	d_3-C16
		Octadecanoylcarnitine (C18)	d_3-C18

Liquid chromatography

Flow injection analysis was performed on a Vanquish Flex Binary UHPLC system, using an injection volume of 10 µL of extracted sample. The mobile phase was provided by PerkinElmer. Details of the analytical method are reported in Table 2. Total runtime was 1.5 minutes.

Table 2. LC method description

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

Mass spectrometry

Analytes and internal standards were detected by SRM on a TSQ Fortis triple-stage quadrupole mass spectrometer with heated electrospray ionization operated in positive ionization mode. A summary of the MS source conditions is reported in Table 3. SRM transitions with optimized collision energy and tube lens values are summarized in Table 4.

Method evaluation

The method performance was evaluated in terms of intra- and inter-assay precision and accuracy for all analytes.

Table 3. MS settings

Source type	Heated electrospray inization (HESI)
Vaporizer temperature	100 °C
Capillary temperature	300 °C
Spray voltage (positive mode)	3500 V
Sheath gas	25 AU
Sweep gas	0 AU
Auxiliary gas	5 AU
Data acquisition mode	Selected-reaction monitoring (SRM)
Source fragmentation	5 V
Collision gas pressure	1.5 mTorr
Cycle time	0.800 s
Q1 mass resolution (FWMH)	0.7
Q3 mass resolution (FWMH)	0.7

Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the controls (batch # 670641) at two different concentration levels in replicates of five (n=5). Inter-assay precision was evaluated for both levels as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days, n=15). Analytical accuracy was evaluated in terms of percentage bias between nominal and average calculated concentrations using the quality control samples at both levels.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Quantification of the analytes is done by comparison with the corresponding isotopically labeled internal standard, using the formula Conc = A Area/IS Area × IS conc.

Table 4. SRM transitions, collision energies, and tube lens values

Analyte	Precursor ion	Product ion	Internal standard	Precursor ion	Product ion	Collision energy (V)	Tube lens (V)
Alanine	90.088	44.054	d_4-Alanine	94.088	48.054	12	100
Citrulline	176.125	113.125	d_2-Citrulline	178.125	115.125	16	60
Glycine	76.088	30.179	$^{13}C_2{}^{15}N$-Glycine	79.088	32.179	11	120
Leucine	132.125	86.125	d_3-Leucine	135.125	89.125	10	73
Methionine	150.088	133.054	d_3-Methionine	153.088	136.054	10	63
Phenylalanine	166.162	120.125	$^{13}C_6$-Phenylalanine	172.162	126.143	13	100
Proline	116.175	70.125	$^{13}C_5$-Proline	121.175	74.125	15	109
Tyrosine	182.138	136.125	$^{13}C_6$-Tyrosine	186.138	142.125	13	108
Valine	118.125	72.125	d_8-Valine	126.125	80.179	11	105
Arginine	175.125	70.125	$d_4{}^{13}C$-Arginine	180.125	75.125	19	131
Ornitine	133.125	70.125	d_6-Ornitine	139.125	76.125	18	114
C0	162.162	85.054	d_9-C0	171.162	85.054	21	103
C2	204.175	85.054	d_3-C2	207.175	85.054	19	108
C3	218.162	85.054	d_3-C3	221.162	85.054	19	120
C4	232.162	85.054	d_3-C4	235.212	85.054	20	106
C5	246. 212	85.125	d_9-C5	255.212	85.125	21	114
C5DC	276.175	85.054	d_6-C5DC	282.175	85.054	24	83
C6	260.150	85.054	d_3-C6	263.15	85.054	21	111
C8	288.175	85.054	d_3-C8	291.175	85.054	22	122
C10	316.300	85.125	d_3-C10	319.300	85.125	24	125
C12	344.300	85.054	d_3-C12	347.300	85.054	24	129
C14	372.350	85.054	d_3-C14	375.350	85.054	25	133
C16	400.350	85.054	d_3-C16	403.350	85.054	27	148
C18	428.400	85.125	d_3-C18	431.400	85.125	27	141

Results and discussion

Representative chromatograms for tyrosine, octanoylcarnitine, and the corresponding internal standards are reported in Figure 1.

The reported method showed good reproducibility, with the maximum intra- and inter-assay precision below 10.1% and 10.3%, respectively, for nine of the analytes. Arginine and ornitine are not present in the control samples, but since internal standards are included in the kit, their concentrations can be calculated in authentic samples. The kit manufacturer uses surrogate controls (citrulline) for these two amino acids. Results for intra- and inter-assay precision are reported in Tables 5 and Table 6, respectively.

Analytical accuracy for the evaluated analytes was always within the acceptance range provided by the supplier, with values between -8.6% and 8.8% (Table 7).

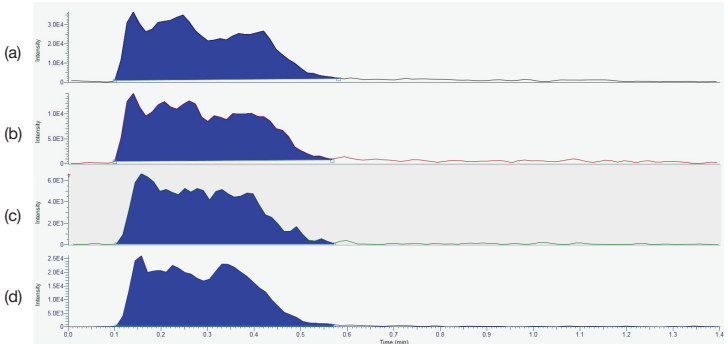

Figure 1. FIA-MS/MS profiles for (a) tyrosine, (b) $^{13}C_6$-tyrosine, (c) octanoylcarnitine (C8), and (d) d_3-octanoylcarnitine

Table 5. Analytical intra-assay precision for batch #670641

Analyte	Control I			Control II		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
	CV (%)	CV (%)	CV (%)	CV (%)	CV (%)	CV (%)
Alanine	5.5	6.7	6.3	5.3	6.3	8.1
Citrulline	4.8	4.1	5.1	3.7	4.7	6.5
Glycine	8.5	5.9	6.0	5.4	6.0	7.3
Leucine	3.7	2.7	3.2	2.4	3.6	4.7
Methionine	4.7	4.5	4.8	2.9	5.1	5.9
Phenylalanine	4.1	3.0	3.7	2.5	3.9	5.4
Proline	4.5	2.8	3.6	2.7	4.1	4.7
Tyrosine	4.2	3.0	4.1	3.1	5.0	6.3
Valine	4.5	3.3	3.8	2.7	4.2	5.7
C0	5.9	2.0	3.6	2.6	3.7	5.4
C2	4.6	2.1	3.7	2.4	4.3	5.7
C3	4.1	2.3	2.8	2.5	4.0	5.6
C4	4.6	2.3	4.4	3.5	3.4	5.3
C5	4.9	2.7	4.2	3.0	4.0	5.3
C5DC	10.1	6.6	8.2	9.8	10.0	8.3
C6	4.2	3.8	4.9	4.2	3.3	5.7
C8	5.4	3.0	4.5	2.6	4.7	6.1
C10	4.0	3.9	3.9	2.2	3.8	5.7
C12	4.2	3.5	3.3	2.9	3.0	5.3
C14	4.0	3.1	3.5	3.1	4.2	5.3
C16	3.2	3.5	3.4	2.8	3.6	6.5
C18	3.8	3.5	3.0	2.6	3.9	4.7

Table 6. Analytical inter-assay precision results for batch #670641

Analyte	Control I CV (%)	Control II CV (%)
Alanine	6.7	7.6
Citrulline	6.9	7.0
Glycine	8.1	7.4
Leucine	3.9	3.4
Methionine	4.8	4.3
Phenylalanine	4.1	3.6
Proline	4.3	3.9
Tyrosine	4.0	4.2
Valine	4.6	3.9
C0	4.7	6.6
C2	3.9	3.8
C3	4.0	3.9
C4	4.0	4.0
C5	4.4	4.2
C5DC	8.3	10.3
C6	4.8	4.8
C8	4.5	4.2
C10	4.0	3.8
C12	4.4	3.9
C14	4.2	4.2
C16	3.8	3.7
C18	4.3	3.7

Table 7. Accuracy for controls #670641

Analyte	Control I (µmol/L)	Calculated conc. (µmol/L)	Bias (%)	Control I range (µmol/L)	Outcome	Control II (µmol/L)	Calculated conc. (µmol/L)	Bias (%)	Control II range (µmol/L)	Outcome
Alanine	787.0	727.6	-7.6	686.0–888.0	Normal	1643	1569	-4.5	1433–1853	Normal
Citrulline	96.00	101.9	6.2	84.00–108.0	Normal	266.0	289.3	8.8	232.0–300.0	Normal
Glycine	1062	1050	-1.2	923.0–1201	Normal	2530	2490	-1.6	2199–2861	Normal
Leucine	391.0	380.6	-2.7	343.0–439.0	Normal	791.0	820.0	3.7	693.0–889.0	Normal
Methionine	112.0	106.1	-5.2	98.00–126.0	Normal	358.0	359.8	0.50	314.0–402.0	Normal
Phenylalanine	232.0	218.2	-6.0	203.0–261.0	Normal	615.0	632.0	2.8	538.0–692.0	Normal
Proline	551.0	541.8	-1.7	482.0–620.0	Normal	1459	1546	6.0	1277–1641	Normal
Tyrosine	360.0	330.6	-8.2	315.0–405.0	Normal	1066	1047	-1.8	933.0–1199	Normal
Valine	441.0	403.2	-8.6	385.0–497.0	Normal	955.0	941.4	-1.4	833.0–1077	Normal
C0	102.0	98.10	-3.8	89.00–115.0	Normal	233.0	235.6	1.1	203.0–263.0	Normal
C2	58.30	58.46	0.3	50.90–65.70	Normal	137.8	146.8	6.6	120.3–155.3	Normal
C3	10.90	10.03	-8.0	9.500–12.30	Normal	26.20	25.69	-2.0	22.80–29.60	Normal
C4	2.460	2.262	-8.0	2.150–2.770	Normal	6.080	5.880	-3.3	6.020–7.580	Normal
C5	1.060	0.9794	-7.6	0.9200–1.200	Normal	2.580	2.452	-5.0	2.230–2.930	Normal
C5DC	0.5100	0.5444	6.7	0.4300–0.5900	Normal	1.170	1.229	5.1	0.9900–1.350	Normal
C6	0.5100	0.4760	-6.7	0.4400–0.5800	Normal	1.260	1.215	-3.6	1.080–1.440	Normal
C8	0.6400	0.5960	-6.9	0.5400–0.7400	Normal	1.570	1.527	-2.7	1.330–1.690	Normal
C10	0.8900	0.8266	-7.1	0.7600–1.020	Normal	2.200	2.121	-3.6	1.890–2.510	Normal
C12	1.860	1.716	-7.8	1.620–2.100	Normal	4.780	4.628	-3.2	4.170–5.390	Normal
C14	1.940	1.792	-7.6	1.700–2.180	Normal	4.800	4.744	-1.2	4.200–5.400	Normal
C16	12.70	11.67	-8.1	11.10–14.30	Normal	30.50	30.72	0.70	26.60–34.40	Normal
C18	2.510	2.351	-6.4	2.190–2.830	Normal	4.930	4.742	-3.8	4.300–5.560	Normal

Conclusion

The NeoBase MSMS Complete Kit for Amino Acids and Acylcarnitines in DBS from Perkin Elmer was implemented on a Vanquish Flex Binary UHPLC system coupled to a TSQ Fortis triple-stage quadrupole mass spectrometer. SRM data acquisition was used to provide high selectivity and sensitivity. The method met sensitivity requirements, accuracy, and precision expectations typically demanded by clinical research laboratories.

Find out more at **thermofisher.com/clinicalresearch**