

# Application News

## No. C94

### Liquid Chromatography Mass Spectrometry

## Simultaneous Analysis of Amino Acids and Acylcarnitines in DBS (Dried Blood Spot) with LCMS-8040

LC-MS/MS is a powerful tool in the analysis of amino acids (as indicators of amino acid metabolism) and acylcarnitines (considered to be useful indicators of fatty acid metabolism) as it delivers high sensitivity with minimal sample preparation. In this paper we describe the analytical protocol developed by Mass Spectrometry, Clinical Chemistry and Pharmacology Lab. of Meyer Children's Hospital (Florence, Italy) to measure amino acids and acylcarnitines from dried blood spot samples using the LCMS-8040 high-performance liquid chromatograph triple quadrupole mass spectrometer.

### ■ Sample Extraction from DBS and MS Analysis

The sample preparation method is shown in Fig. 1. Filter paper blotted with blood (DBS) was used to prepare the sample. After cutting 3.2-mm diameter disks from the DBS for the 96-well plate, sample preparation was performed in accordance with the protocol. After sample preparation, flow injection analysis (FIA) was performed with the extracted solution. Stable isotopes such as adenosine and deoxyadenosine mixed with stable isotopes of amino acids, carnitine, and acylcarnitine (manufactured by Cambridge Isotope Laboratories, Inc.) were used as internal standards for standard extraction.

Analysis was performed by neutral loss scanning of 46 Da for amino acids, precursor ion scanning of 85 m/z for acylcarnitines, and multiple reaction monitoring (MRM) for some amino acids, succinylacetone, adenosine, and deoxyadenosine. Information on the target compounds is shown in Table 1. Quantitation of target compounds was performed using internal standards. LC and MS conditions are shown in Table 2.

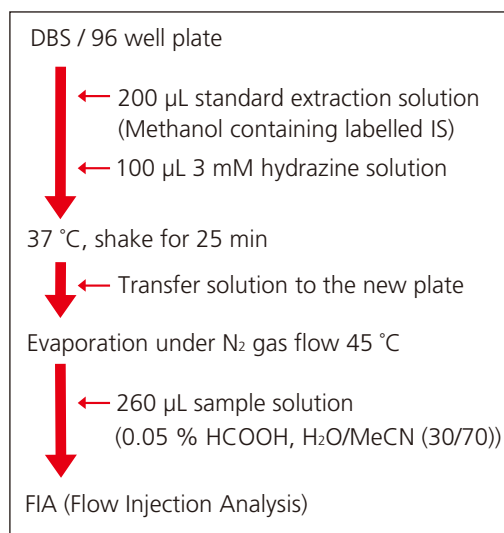


Fig. 1 Preparation Protocol

Table 1 Target Compounds

Compound	type	m/z	event	Compound	type	m/z	event	Compound	type	m/z	event
C0	Target	162.1	2-Precursor(+)	C3DC	Target	248.1	2-Precursor(+)	Ala	Target	90	3.NLS(+)
C0 IS	ISTD	171.2	2-Precursor(+)	C3DC IS	ISTD	221.1	2-Precursor(+)	Ala IS	ISTD	94	3.NLS(+)
C5	Target	246.2	2-Precursor(+)	C4OH	Target	248.1	2-Precursor(+)	Val	Target	118.1	3.NLS(+)
C5 IS	ISTD	255.2	2-Precursor(+)	C4OH IS	ISTD	235.2	2-Precursor(+)	Val IS	ISTD	126.1	3.NLS(+)
C6	Target	260.2	2-Precursor(+)	C5:1	Target	244.2	2-Precursor(+)	Xleu	Target	132.1	3.NLS(+)
C8	Target	288.2	2-Precursor(+)	C5:1 IS	ISTD	255.2	2-Precursor(+)	Xleu IS	ISTD	135.1	3.NLS(+)
C8 IS	ISTD	291.2	2-Precursor(+)	C5DC	Target	276.1	2-Precursor(+)	Met	Target	150.1	3.NLS(+)
C8:1	Target	286.2	2-Precursor(+)	C5DC IS	ISTD	291.2	2-Precursor(+)	Met IS	ISTD	153.1	3.NLS(+)
C8:1 IS	ISTD	291.2	2-Precursor(+)	C5OH	Target	262.2	2-Precursor(+)	Tyr	Target	182.1	3.NLS(+)
C6 IS	ISTD	255.2	2-Precursor(+)	C5OH IS	ISTD	255.2	2-Precursor(+)	Tyr IS	ISTD	188.1	3.NLS(+)
C2	Target	204.1	2-Precursor(+)	C12OH	Target	360.3	2-Precursor(+)	Asp	Target	134.1	3.NLS(+)
C2 IS	ISTD	207.1	2-Precursor(+)	C12OH IS	ISTD	381.3	2-Precursor(+)	Asp IS	ISTD	137.1	3.NLS(+)
C3	Target	218.1	2-Precursor(+)	C14OH	Target	388.3	2-Precursor(+)	Glu	Target	148.1	3.NLS(+)
C3 IS	ISTD	221.1	2-Precursor(+)	C14OH IS	ISTD	381.3	2-Precursor(+)	Glu IS	ISTD	151.3	3.NLS(+)
C4	Target	232.2	2-Precursor(+)	C16OH	Target	416.3	2-Precursor(+)	Phe	Target	166.1	3.NLS(+)
C4 IS	ISTD	235.2	2-Precursor(+)	C16OH IS	ISTD	403.3	2-Precursor(+)	Phe IS	ISTD	172.1	3.NLS(+)
C10	Target	316.1	2-Precursor(+)	C18OH	Target	444.4	2-Precursor(+)	Gly	Target	76.00-30.10	1.MRM(+)
C10 IS	ISTD	291.2	2-Precursor(+)	C18OH IS	ISTD	403.3	2-Precursor(+)	Gly IS	ISTD	78.00-32.10	1.MRM(+)
C10:1	Target	314.1	2-Precursor(+)	C18:1OH	Target	442.4	2-Precursor(+)	Cit MRM	Target	176.10-113.10	1.MRM(+)
C10:1 IS	ISTD	291.2	2-Precursor(+)	C18:1OH IS	ISTD	403.4	2-Precursor(+)	Cit MRM IS	ISTD	178.10-115.10	1.MRM(+)
C12	Target	344.3	2-Precursor(+)	C14:2	Target	368.3	2-Precursor(+)	Arg MRM	Target	175.10-116.10	1.MRM(+)
C12 IS	ISTD	381.3	2-Precursor(+)	C14:2 IS	ISTD	381.3	2-Precursor(+)	Arg MRM IS	ISTD	180.10-121.10	1.MRM(+)
C12:1	Target	342.3	2-Precursor(+)	C16:1OH	Target	414.3	2-Precursor(+)	Arg Succ MRM	Target	291.10-176.20	1.MRM(+)
C12:1 IS	ISTD	381.3	2-Precursor(+)	C16:1OH IS	ISTD	403.3	2-Precursor(+)	Arg Succ MRM IS	ISTD	180.10-121.10	1.MRM(+)
C14	Target	372.3	2-Precursor(+)	C10:2	Target	312.2	2-Precursor(+)	Orn	Target	133.10-70.10	1.MRM(+)
C14 IS	ISTD	381.3	2-Precursor(+)	C10:2 IS	ISTD	291.2	2-Precursor(+)	Orn IS	ISTD	135.10-72.10	1.MRM(+)
C14:1	Target	370.3	2-Precursor(+)	C4DC	Target	262.1	2-Precursor(+)	Met MRM	Target	150.10-104.10	1.MRM(+)
C14:1 IS	ISTD	381.3	2-Precursor(+)	C4DC IS	ISTD	235.2	2-Precursor(+)	Met MRM IS	ISTD	153.10-107.10	1.MRM(+)
C16	Target	400.3	2-Precursor(+)	C6DC	Target	290.2	2-Precursor(+)	SuAC	Target	155.00-137.20	1.MRM(+)
C16 IS	ISTD	403.3	2-Precursor(+)	C6DC IS	ISTD	255.2	2-Precursor(+)	SuAC IS	ISTD	159.00-141.20	1.MRM(+)
C16:1	Target	398.3	2-Precursor(+)	C10 OH	Target	332.2	2-Precursor(+)	ADO	Target	268.20-136.10	1.MRM(+)
C16:1 IS	ISTD	403.3	2-Precursor(+)	C10 OH IS	ISTD	291.2	2-Precursor(+)	ADO IS	ISTD	269.20-136.10	1.MRM(+)
C18	Target	428.4	2-Precursor(+)	C8DC	Target	318.2	2-Precursor(+)	Deoxi ADO	Target	252.20-136.10	1.MRM(+)
C18 IS	ISTD	403.3	2-Precursor(+)	C8DC IS	ISTD	381.3	2-Precursor(+)	Deoxi ADO IS	ISTD	257.20-136.10	1.MRM(+)
C18:1	Target	426.4	2-Precursor(+)	C18:2 OH	Target	440.4	2-Precursor(+)	Guanosina	Target	284.10-152.00	4.MRM(+)
C18:1 IS	ISTD	403.3	2-Precursor(+)	C18:2 OH IS	ISTD	403.4	2-Precursor(+)	Guanosina IS	ISTD	289.10-156.90	4.MRM(+)
C18:2	Target	424.3	2-Precursor(+)					Deoxy Guanosina	Target	268.00-152.00	4.MRM(+)
C18:2 IS	ISTD	403.3	2-Precursor(+)					Deoxy Guanosina IS	ISTD	270.10-151.90	4.MRM(+)
								Inosina	Target	269.00-137.10	4.MRM(+)
								Inosina IS	ISTD	273.10-140.90	4.MRM(+)
								Deoxy Inosina	Target	253.10-137.10	4.MRM(+)
								Deoxy Inosina IS	ISTD	273.10-140.90	4.MRM(+)

Table 2 Analytical Conditions

Mobile Phase A	: 0.1 % HCOOH-H <sub>2</sub> O	Ionization Mode	: ESI (+)
Mobile Phase B	: 0.1 % HCOOH-CH <sub>3</sub> CN	Probe Voltage	: +4.5 kV
Ratio	: 70 % B	Nebulizing Gas Flow	: 3.0 L/min
Flowrate	: 0.07 mL/min	Drying gas Flow	: 20.0 L/min
Injection Volume	: 40 µL	DL Temperature	: 300 °C
Analysis Time	: 2.2 min	Block Heater Temperature	: 500 °C

■ **Example of Analysis Results**

Results from measuring a control sample are shown in Table 3. The Neonatal Solution amino acid/acylcarnitine analytical support software was used to calculate the concentration (µM) of target compounds automatically,

and to show whether the concentration was within the range of criteria that were set in advance. The results for the control sample shows that the concentrations were within the range of the criteria set in advance.

**Table 3 Analysis Results for Control Samples**

Compound	C0	C2	C3	C3DC	C4	C4OH	C4DC	C5
Criteria Upper Limit (Caution)	-	-	-	-	-	-	-	-
Criteria Upper Limit (Notice)	45.00	48.00	3.30	0.70	0.92	0.50	0.54	0.56
Criteria Lower Limit (Notice)	5.50	6.30	0.20	0.01	0.01	0.01	0.04	0.01
Criteria Lower Limit (Caution)	-	-	-	-	-	-	-	-
test_001	8.32	19.84	1.47	0.20	0.14	0.18	0.19	0.07
test_002	9.99	19.36	2.43	0.20	0.15	0.17	0.23	0.06
test_003	7.64	21.79	1.20	0.17	0.12	0.15	0.16	0.05
test_004	7.16	15.14	1.48	0.18	0.27	0.17	0.27	0.09
test_005	8.12	21.15	1.30	0.21	0.11	0.20	0.25	0.07
test_006	11.70	24.70	1.63	0.23	0.19	0.22	0.30	0.04
test_007	12.31	12.52	1.66	0.10	0.14	0.09	0.24	0.04
test_008	12.37	18.24	1.57	0.16	0.13	0.14	0.16	0.06
test_009	12.46	16.08	1.41	0.11	0.14	0.10	0.20	0.07
test_010	6.93	17.85	1.23	0.25	0.15	0.21	0.21	0.06
test_011	15.53	24.85	2.62	0.24	0.21	0.23	0.27	0.08
test_012	8.67	10.11	0.56	0.17	0.12	0.15	0.12	0.06
test_013	5.83	12.29	0.99	0.15	0.17	0.14	0.26	0.06
test_014	5.70	13.08	1.14	0.23	0.44	0.23	0.26	0.07
test_015	7.33	14.44	1.61	0.28	0.15	0.26	0.38	0.05
test_016	10.18	20.75	1.08	0.33	0.17	0.29	0.13	0.05
test_017	10.42	15.67	2.14	0.13	0.12	0.13	0.16	0.05
test_018	11.79	21.11	1.54	0.18	0.16	0.16	0.27	0.05

■ **Analysis Results for Prepared Samples**

Results from measuring 7 samples are shown in Table 4. Samples A through G were analyzed, and target compounds were detected that did not meet the set criteria (yellow cells in Table 4). The chromatogram

profiles of each target compound are compared with that of the control sample in Fig. 2. Target compounds that did not meet the criteria are shown to have a profile that differs from the control sample.

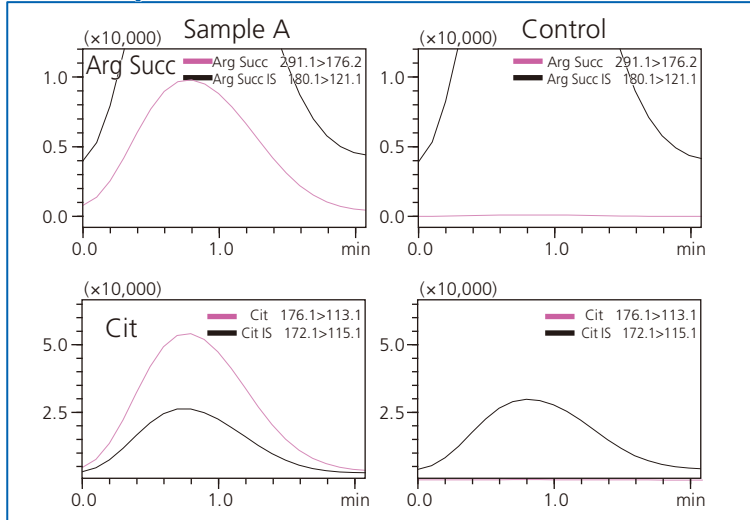
**Table 4 Analysis Results for 7 Samples**

Sample A: ArgSuc, Sample B: VLCAD, Sample C: MCAD, Sample D: Cit I, Sample E: GAL I, Sample F: Tyr I, Sample G: PA

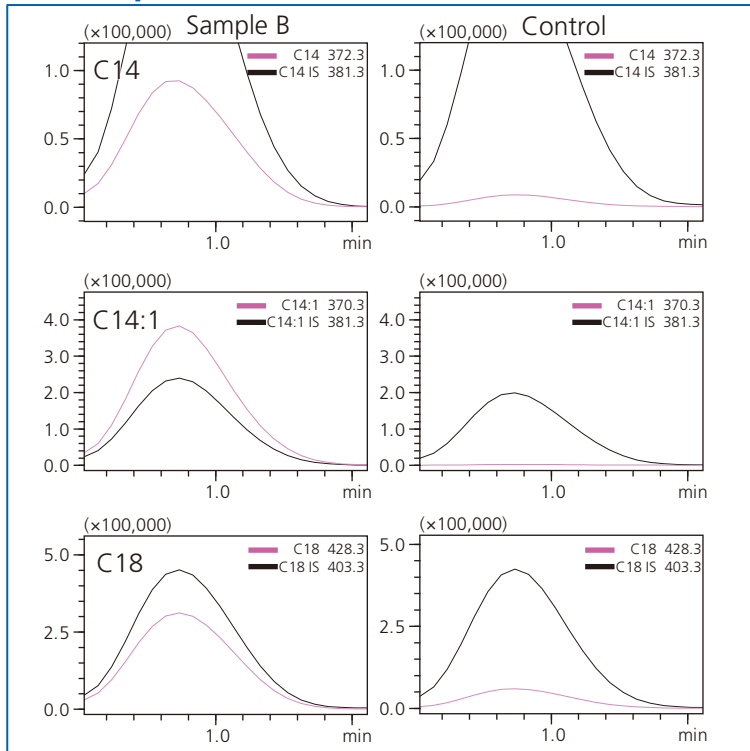
Compound	C3	C5DC	C6	C8	C14	C14:1	C18:1	Tyr	Cit	Arg Succ
Criteria Upper Limit (Caution)	-	-	-	-	-	-	-	-	-	-
Criteria Upper Limit (Notice)	3.30	0.15	0.25	0.40	0.57	0.44	2.43	200.00	30.00	1.00
Criteria Lower Limit (Notice)	0.20	0.01	0.01	0.01	0.01	0.01	0.39	0.01	3.00	0.01
Criteria Lower Limit (Caution)	-	-	-	-	-	-	-	-	-	-
Sample A_ArgSuc	0.33	0.01	0.02	0.09	0.11	0.03	0.37	50.93	71.01	57.85
Sample B_VLCAD	0.59	0.02	0.04	0.04	0.91	3.54	3.16	68.26	38.28	0.78
Sample C_MCAD	0.32	0.01	0.44	2.49	0.10	0.02	0.63	57.79	5.57	0.04
Sample D_Cit I	0.91	0.01	0.01	0.01	0.06	0.02	0.32	57.02	116.82	0.44
Sample E_GAL I	0.28	1.66	0.01	0.01	0.08	0.03	0.68	87.22	7.86	0.67
Sample F_Tyr I	0.23	0.00	0.01	0.05	0.06	0.08	1.09	344.21	31.90	0.74
Sample G_PA	8.58	0.00	0.02	0.02	0.03	0.03	0.54	23.28	13.74	0.29

Compound	SuAC	C14:1/C4	C3/C0	C3/C4	C3/C16	C5DC/C4	C5DC/C8	C5DC/C12	C8/C10
Criteria Upper Limit (Caution)	-	-	-	-	-	-	-	-	-
Criteria Upper Limit (Notice)	2.00	2.14	0.30	25.80	1.60	0.56	0.75	1.07	2.42
Criteria Lower Limit (Notice)	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01
Criteria Lower Limit (Caution)	-	-	-	-	-	-	-	-	-
Sample A_ArgSuc	1.71	0.21	0.01	2.56	0.31	0.10	0.15	0.32	1.01
Sample B_VLCAD	1.23	23.85	0.05	3.94	0.22	0.12	0.43	0.13	0.32
Sample C_MCAD	1.12	0.21	0.03	2.72	0.28	0.12	0.01	0.33	12.17
Sample D_Cit I	1.07	0.25	0.04	13.05	0.65	0.13	0.67	0.75	1.36
Sample E_GAL I	0.97	0.40	0.03	3.95	0.25	23.60	223.72	59.59	0.16
Sample F_Tyr I	10.43	1.19	0.01	3.44	0.28	0.04	0.05	0.05	0.67
Sample G_PA	0.96	0.57	0.33	154.82	12.51	0.07	0.21	0.30	0.73

### Sample A



### Sample B



### Sample C

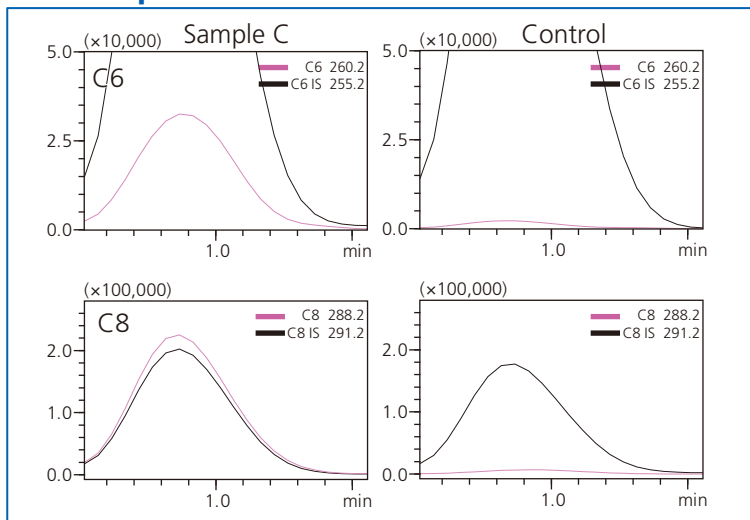
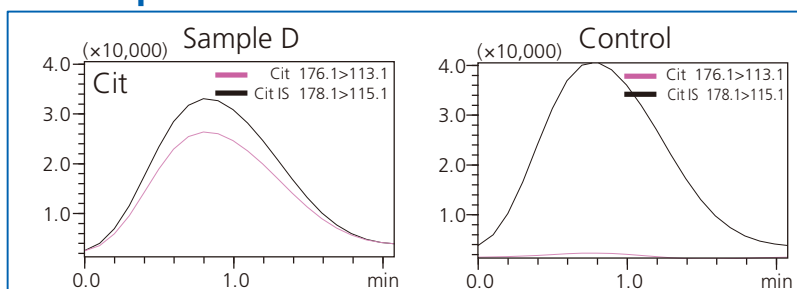
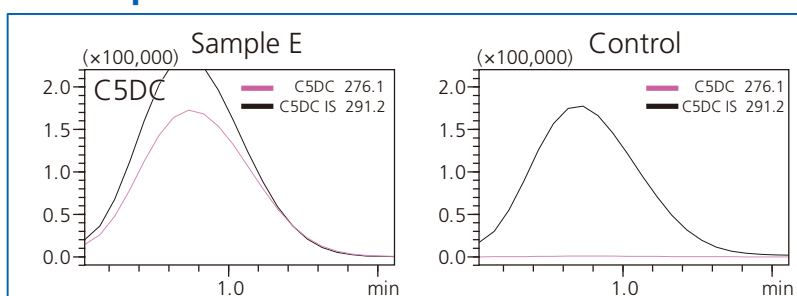


Fig. 2-1 Extracted-Ion Chromatograms of Target Compounds

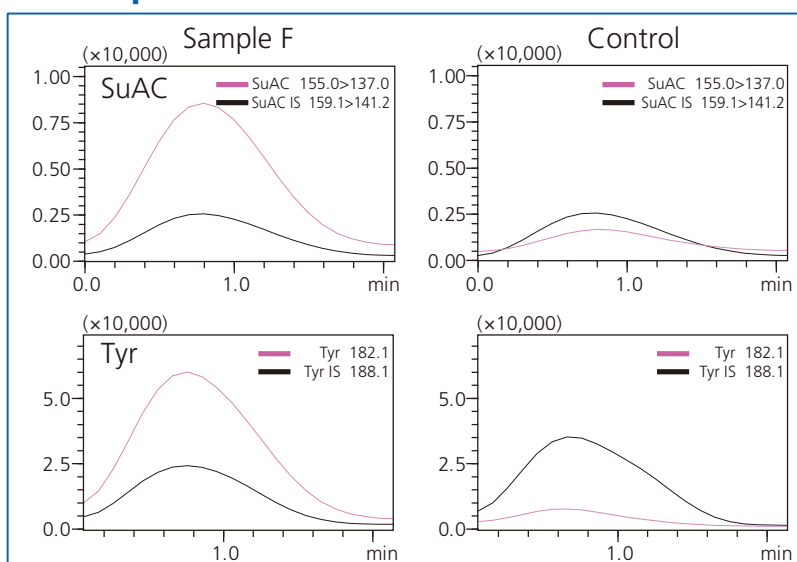
### Sample D



### Sample E



### Sample F



### Sample G

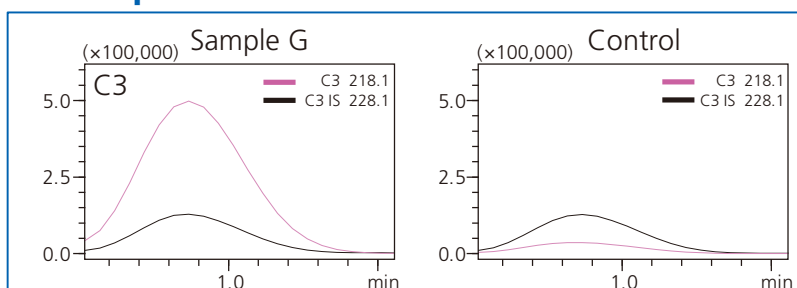


Fig. 2-2 Extracted-Ion Chromatograms of Target Compounds

Note: This analytical system may only be used for research applications, and may not be used for clinical diagnosis.