



Cambridge Isotope Laboratories, Inc.  
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MS/MS STANDARDS

# MS/MS Screening Mixtures and Standards



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## Amino Acid Reference Standard Mixtures (NSK-A and NSK-A1)

These sets contain 10 vials of a dry mixture of 12 stable isotope-labeled amino acids. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of 1:1 purified water:methanol.
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approaches for Working Stock

To prepare working stock solutions:

- Dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.
- If NSK-B (Carnitine/Acylcarnitine Standard Mix Set B) was purchased, mix 1 mL (or an aliquot of the reconstituted vial contents) of concentrated standards from Set A with 1 mL (or an aliquot) of the concentrated standards from Set B.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (µM)
L-Alanine (Ala)	2,3,3,3-D <sub>4</sub> , 98%	93.12	500
L-Arginine-HCl (Arg)	5- <sup>13</sup> C, 99%; 4,4,5,5-D <sub>4</sub> , 95%	215.68	500
L-Aspartic acid (Asp)	2,3,3-D <sub>3</sub> , 98%	136.12	500
L-Citrulline (Cit)	5,5-D <sub>2</sub> , 98%	177.20	500
DL-Glutamic acid (Glu)	2,4,4-D <sub>3</sub> , 98%	150.15	500
Glycine (Gly)	2- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%	77.05	2500
L-Leucine (Leu)	5,5,5-D <sub>3</sub> , 99%	134.19	500
L-Methionine (Met)	methyl-D <sub>3</sub> , 98%	152.23	500
L-Ornithine-HCl (Orn)*	5,5-D <sub>2</sub> , 98%	170.63	500
L-Phenylalanine (Phe)	ring- <sup>13</sup> C <sub>6</sub> , 99%	171.15	500
L-Tyrosine (Tyr)	ring- <sup>13</sup> C <sub>6</sub> , 99%	187.14	500
L-Valine (Val)	D <sub>8</sub> , 98%	125.20	500

\*NSK-A1 contains Orn 3,3,4,4,5,5,-D<sub>6</sub>, 98% (MW 174.66 Da). The remaining components are equivalent to NSK-A.

**Note:** A complementary set of these unlabeled amino acid reference standards (NSK-A-US-1) and/or a combined set with NSK-B (i.e., NSK-AB) is also available.

### Usage Specifications

Criteria	Recommendation
Use	960 samples/vial
<b>Before reconstitution:</b>	
Storage	≤25°C; protect from light
Recommended retest	4 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 5±3°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## 3-Plex Amino Acid Reference Standard Mixtures (NSK-AA3)

This dried-down mix comprises three stable isotope-labeled amino acids. Accurate and complete reconstitution of the vial's contents in 1 mL of highly pure water will produce the concentrations listed in the table below.

### Example Reconstitution Approach for Concentrated Stock

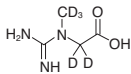
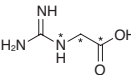
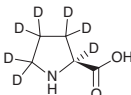
- Solubilize the dried-down mix in 1 mL of solvent (e.g., 1:1 purified water:acetonitrile)
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) of the concentrated amino acid standard with pure solvent.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)	Structure
Creatine (Cre)	N-Methyl-D <sub>3</sub> ; glycine-2,2-D <sub>2</sub> , 99%	154.18	500	
Guanidinoacetic acid (GAA)	1,2- <sup>13</sup> C <sub>2</sub> , 97-99%; 3- <sup>15</sup> N, 97-99% (CP 97%)	120.09	50	
L-Proline (Pro)	D <sub>7</sub> , 97-98%	122.17	500	

**Note:** The concentrations are also available at 10X (NSK-AA3-10X).

### Usage Specifications

Criteria	Recommendation
<b>Before reconstitution:</b>	
Storage	-5 to 5°C; protect from light
Recommended retest	2 years from date of manufacture
<b>After reconstitution:*</b>	
Storage	-20°C
Recommended retest	3 months

\*Represents minimum stability period when AA3 mix is reconstituted with 1:1 purified water:acetonitrile.

## Branched-Chain Amino Acid Reference Standard Mixtures (NSK-BCAA)

This dried-down mix comprises four stable isotope-labeled amino acids. Accurate and complete reconstitution of the vial's contents in 1 mL of highly pure water will produce the concentrations listed in the table below.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of solvent (e.g., 0.1 M HCl)
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) of the concentrated amino acid standard with pure solvent.

### Composition

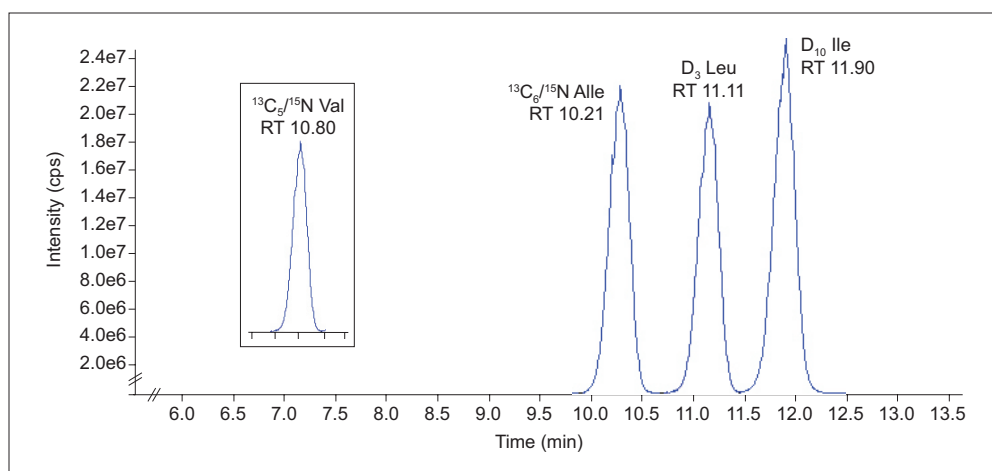
Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)
L-Allo-isoleucine (Alle)	$^{13}\text{C}_6$ , 97-99%; $^{15}\text{N}$ , 97-99%	138.2	400
L-Isoleucine (Ile)	$\text{D}_{10}$ , 98%	141.2	400
L-Leucine (Leu)	5,5,5- $\text{D}_3$ , 99%	134.2	400
L-Valine (Val)	$^{13}\text{C}_5$ , 99%; $^{15}\text{N}$ , 99%	123.1	400

**Note:** A mix of unlabeled BCAA standards (NSK-BCAA-US) is also available.

### Usage Specifications

Criteria	Recommendation
<b>Before reconstitution:</b>	
Storage	≤25°C; protect from light and moisture
Recommended retest	5 years from date of manufacture
<b>After reconstitution:*</b>	
Storage	4°C
Recommended retest	5 weeks

\*Represents minimum stability period when the BCAA mix is reconstituted with 100% water.



**Figure.** Representative XICs of the BCAA mix metabolites measured by RPLC-MRM/MS (ESI+, 5500+ QqQ). Separation of the isobaric metabolites is shown in the main plot, with Val in the inset. Displayed are the  $[\text{M}+\text{H}]^+$  ions (see Table on page 16 for precursor and product ion values).

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## Carnitine and Acylcarnitine Reference Standard Mixtures (NSK-B)

This set contains 10 vials of a dry mixture of eight stable isotope-labeled free carnitine and acylcarnitines. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of highly pure methanol.
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approaches for Working Stock

To prepare working stock solutions:

- Dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.
- If NSK-A (Amino Acid Standard Mix Set A) was purchased, mix 1 mL (or an aliquot) of the reconstituted vial contents of concentrated standards from Set B with 1 mL (or an aliquot) of the concentrated standards from Set A.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (µM)
L-Carnitine (C0)	trimethyl-D <sub>9</sub> , 98%	170.25	152
O-Acetyl-L-carnitine-HCl (C2)	N-methyl-D <sub>3</sub> , 98%	242.72	38
O-Propionyl-L-carnitine-HCl (C3)	N-methyl-D <sub>3</sub> , 98%	256.74	7.6
O-Butyryl-L-carnitine-HCl (C4)	N-methyl-D <sub>3</sub> , 98%	270.77	7.6
O-Isovaleryl-L-carnitine-HCl (C5)	N,N,N-trimethyl-D <sub>9</sub> , 98%	290.83	7.6
O-Octanoyl-L-carnitine-HCl (C8)	N-methyl-D <sub>3</sub> , 98%	326.87	7.6
O-Myristoyl-L-carnitine-HCl (C14)	N,N,N-trimethyl-D <sub>9</sub> , 98%	417.07	7.6
O-Palmitoyl-L-carnitine-HCl (C16)	N-methyl-D <sub>3</sub> , 98%	439.09	15.2

**Note:** A complementary set of these unlabeled carnitine/acylcarnitine standards (NSK-B-US-1) and a combined set with NSK-A (i.e., NSK-AB) is also available.

### Usage Specifications

Criteria	Recommendation
Use	960 samples/vial
<b>Before reconstitution:</b>	
Storage	≤8°C; protect from light
Recommended retest	1 year from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 5±3°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

### Example References

Lu, Y.; Zheng, Y.; Zhao, X.; et al. **2023**. The relationship between islet β-cell function and metabolomics in overweight patients with Type 2 diabetes. *Biosci Rep*, 43(2), BSR20221430-BSR20221444.

Schupper, A.; Almashanu, S.; Coster, D.; et al. **2021**. Metabolic biomarkers of small and large for gestational age newborns. *Early Hum Dev*, 160, 105422-105429.

Staretz-Chacham, O.; Daas, S.; Ulanovsky, I.; et al. **2021**. The role of orotic acid measurement in routine newborn screening for urea cycle disorders. *J Inher Metab Dis*, 44(3), 606-617.

Brennenstuhl, H.; Kohlmüller, D.; Gramer, G.; et al. **2020**. High throughput newborn screening for aromatic L-amino-acid decarboxylase deficiency by analysis of concentrations of 3-O-methyldopa from dried blood spots. *J Inher Metab Dis*, 43(3), 602-610.

Bai, Q.; Peng, B.; Wu, X.; et al. **2018**. Metabolomic study for essential hypertension patients based on dried blood spot mass spectrometry approach. *IUBMB Life*, 70(8), 777-785.

Céspedes, N.; Valencia, A.; Echeverry, C.A.; et al. **2017**. Reference values of amino acids, acylcarnitines and succinylacetone by tandem mass spectrometry for use in newborn screening in southwest Colombia. *Colomb Med (Cali)*, 48(3), 113-119.

Haynes, C.A.; De Jesús, V.R. **2016**. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA-ESI-MS/MS analysis of dried blood spot extracts for newborn screening. *Clin Biochem*, 49(1-2), 161-165.

Wang, Q.; Sun, T.; Cao, Y.; et al. **2016**. A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection. *Oncotargets Ther*, 9, 1389-1398.

Huang, T.; Cao, Y.; Zeng, J.; et al. **2016**. Tandem mass spectrometry-based newborn screening strategy could be used to facilitate rapid and sensitive lung cancer diagnosis. *Oncotargets Ther*, 9, 2479-2487.

George, R.S.; Moat, S.J. **2016**. Effect of dried blood spot quality on newborn screening analyte concentrations and recommendations for minimum acceptance criteria for sample analysis. *Clin Chem*, 62(3), 466-475.

**Note:** These references utilize NSK-A and NSK-B.

### Technical Note

Xie, X.; Kozak, M. **2020**. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. (Thermo Scientific technical note #73398).

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## Supplemental Acylcarnitine Reference Standard Mixtures (NSK-B-G1)

This set contains 10 vials of a dry mixture of five stable isotope-labeled acylcarnitines. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of highly pure methanol.
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approaches for Working Stock

To prepare working stock solutions:

- Dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.
- Mix 1 mL (or an aliquot) of the reconstituted vial contents of concentrated standards from NSK-A with 1 mL (or an aliquot) of the concentrated standards from NSK-B and 1 mL (or an aliquot) of the concentrated standards from NSK-B-G1.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (µM)
O-Glutaryl-L-carnitine-ClO <sub>4</sub> (C5-DC)	N-methyl-D <sub>3</sub> , 98% (CP 97%)	378.78	15.2
3-Hydroxyisovaleryl-L-carnitine-ClO <sub>4</sub> (C5-OH)	N-methyl-D <sub>3</sub> , 98%	364.79	7.6
O-Dodecanoyl-L-carnitine-HCl (C12)	N,N,N-trimethyl-D <sub>9</sub> , 98%	389.02	7.6
O-3-DL-Hydroxypalmitoyl-L-carnitine-ClO <sub>4</sub> (C16-OH)	N-methyl-D <sub>3</sub> , 98%	519.08	15.2
O-Octadecanoyl-L-carnitine-HCl (C18)	N-methyl-D <sub>3</sub> , 98%	467.15	15.2

### Usage Specifications

Criteria	Recommendation
Use	960 samples/vial
<b>Before reconstitution:</b>	
Storage	-5 to 5°C; protect from light
Recommended retest	2 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 5±3°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

**Note:** A complementary set of these unlabeled acylcarnitine standards (NSK-B-G1-US) is also available.

### Example References

- Schupper, A.; Almashanu, S.; Coster, D.; et al. **2021**. Metabolic biomarkers of small and large for gestational age newborns. *Early Hum Dev*, 160, 105422.
- Staretz-Chacham, O.; Daas, S.; Ulanovsky, I.; et al. **2021**. The role of orotic acid measurement in routine newborn screening for urea cycle disorders. *J Inherit Metab Dis*, 44(3), 606-617.
- Céspedes, N.; Valencia, A.; Echeverry, C.A.; et al. **2017**. Reference values of amino acids, acylcarnitines and succinylacetone by tandem mass spectrometry for use in newborn screening in southwest Colombia. *Colomb Med (Cali)*, 48(3), 113-119.
- Simcox, J.; Geoghegan, G.; Maschek, J.A.; et al. **2017**. Global analysis of plasma lipids identifies liver-derived acylcarnitines as a fuel source for brown fat thermogenesis. *Cell Metab*, 26(3), 509-522.
- Haynes, C.A.; De Jesús, V.R. **2016**. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA-ESI-MS/MS analysis of dried blood spot extracts for newborn screening. *Clin Biochem*, 49(1-2), 161-165.
- George, R.S.; Moat, S.J. **2016**. Effect of dried blood spot quality on newborn screening analyte concentrations and recommendations for minimum acceptance criteria for sample analysis. *Clin Chem*, 62(3), 466-475.

### Technical Note

Xie, X.; Kozak, M. **2020**. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. (Thermo Scientific technical note #73398).

## Lysophosphatidylcholine Reference Standard Mixtures (NSK-LPC)

This dried-down mix comprises four lysophosphatidylcholine (LPC or Lyso-PC) standards. Accurate and complete reconstitution of the vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of 95%:5% (v/v) methanol:water.
- Sonicate the reconstituted vial for 3 minutes then auto-vortex for 10 seconds or until complete dissolution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)	Structure
Lysophosphatidylcholine 20:0 (LysoPC C20:0)	eicosanoyl-12,12,13,13-D <sub>4</sub> , 98%	555.77	5.5	
Lysophosphatidylcholine 22:0 (LysoPC C22:0)	docosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	585.75	5.5	
Lysophosphatidylcholine 24:0 (LysoPC C24:0)	tetracosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	613.81	5.5	
Lysophosphatidylcholine 26:0 (LysoPC C26:0)	hexacosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	641.85	5.5	

**Note:** A mix of unlabeled LPC standards (NSK-LPC-US) is also available.

### Usage Specifications

Criteria	Recommendation
Use	~765 samples/vial
<b>Before reconstitution:</b>	
Storage	-20°C; protect from light
Recommended retest	2 years from date of manufacture
<b>After reconstitution:</b>	
Storage	5±3°C or -20±5°C
Recommended retest	6 weeks

## Steroid Reference Standard Mixtures (NSK-S and NSK-S-EXP)

These sets contain 10 vials of dried-down stable isotope-labeled steroids. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Vials of the individual mixes and compounds are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of highly pure methanol.
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted according to the following instructions, or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.

### Steroid Mix Set S (NSK-S)

#### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (nM)
4-Androstene-3,17-dione (A4)	2,2,4,6,6,16,16-D <sub>7</sub> , 97%	293.45	20
Cortisol (F)	9,11,12,12-D <sub>4</sub> , 98%	366.49	100
11-Deoxycortisol (11-S)	2,2,4,6,6-D <sub>5</sub> , 98%	351.49	20
21-Deoxycortisol (21-S)	2,2,4,6,6,21,21,21-D <sub>8</sub> , 97%	354.51	20
17 $\alpha$ -Hydroxyprogesterone (17-OHP)	2,2,4,6,6,21,21,21-D <sub>8</sub> , 98%	338.51	20

**Note:** The concentrations are also available at 40X (NSK-S-40X).

#### Example References

Lai, F.; Srinivasan, S.; Wiley, V. **2020**. Evaluation of a two-tier screening pathway for congenital adrenal hyperplasia in the New South Wales Newborn Screening Programme. *Int J Neonatal Screen*, 6(3), 63-74.

Gervasoni, J.; Schiattarella, A.; Primiano, A.; et al. **2016**. Simultaneous quantification of 17-hydroxyprogesterone, androstenedione, testosterone and cortisol in human serum by LC-MS/MS using TurboFlow online sample extraction. *Clin Biochem*, 49(13-14), 998-1003.

Hicks, R.A.; Yee, J.K.; Mao, C.S.; et al. **2014**. Precursor-to-product ratios reflect biochemical phenotype in congenital adrenal hyperplasia. *Metabolomics*, 10(1), 123-131.

Dhillon, K.; Ho, T.; Rich, P.; et al. **2011**. An automated method on analysis of blood steroids using liquid chromatography tandem mass spectrometry: application to population screening for congenital adrenal hyperplasia in newborns. *Clin Chem Acta*, 412(23-24), 2076-2084.

### Expanded Steroid Mix Set S (NSK-S-EXP)

#### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. ( $\mu$ M)
Aldosterone (A)	D <sub>7</sub> , 98%	367.49	0.52
4-Androstene-3,17-dione (A4)	2,2,4,6,6-D <sub>5</sub> , 98%	291.44	0.12
Corticosterone (B)	2,2,4,6,6,17 $\alpha$ ,21,21-D <sub>8</sub> , 97-98%	354.51	1.58
Cortisol (F)	9,12,12-D <sub>3</sub> , 98%	365.48	2.57
Dehydroepiandrosterone sulfate:sodium salt:2H <sub>2</sub> O (DHEAS)	2,2,3,4,4,6-D <sub>6</sub> , 95%	432.54	21.69
11-Deoxycortisol (11-S)	2,2,4,6,6-D <sub>5</sub> , 98% (CP 97%)	351.49	0.54
17 $\alpha$ -Hydroxyprogesterone (17-OHP)	2,2,4,6,6,21,21,21-D <sub>8</sub> , 98%	338.51	0.27
Progesterone (P)	2,2,4,6,6,17 $\alpha$ ,21,21,21-D <sub>9</sub> , 98%	323.52	0.14
Testosterone (T)	2,2,4,6,6-D <sub>5</sub> , 98%	293.46	0.12

Chemical purity (CP) is 98% or greater, unless otherwise specified.

#### Usage Specifications

Criteria	Recommendation
Use	48 samples/vial
<b>Before reconstitution:</b>	
Storage	-5 to 5°C; protect from light
Recommended retest	5 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 5 $\pm$ 3°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

#### Usage Specifications

Criteria	Recommendation
<b>Before reconstitution:</b>	
Storage	-5 to 5°C; protect from light
Recommended retest	1 year from date of manufacture



## Additional Standard Mixtures for Screening

The additional products outlined below are available as individual dried-down mixtures of their substrate and internal standard.

### $\alpha$ -Galactosidase Substrate and Internal Standard Mix (NSK-FA-1)

Each vial contains the following compounds at a molar ratio of 500:1.

Substrate	Internal Standard
(6-Benzoylamino-hexyl)-{2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-phenylcarbamoyl]-ethyl}-carbamic acid <i>tert</i> -butyl ester	(6-D <sub>5</sub> -Benzoylamino-hexyl)-[2-(4-hydroxy-phenyl-carbamoyl)-ethyl]-carbamic acid <i>tert</i> -butyl ester
C <sub>33</sub> H <sub>47</sub> N <sub>3</sub> O <sub>10</sub> MW: 645.7 Da	C <sub>27</sub> H <sub>32</sub> N <sub>3</sub> O <sub>5</sub> D <sub>5</sub> MW: 488.5 Da

### Glucocerebrosidase Substrate and Internal Standard Mix (NSK-GA-1)

Each vial contains the following compounds at a molar ratio of 50:1.

Substrate	Internal Standard
D-Glucosyl- $\beta$ 1-1'- <i>N</i> -dodecanoyl-D- <i>erythro</i> -sphingosine [C12-glucocerebroside]	<i>N</i> -Myristoyl-D- <i>erythro</i> -sphingosine [C14-ceramide]
C <sub>36</sub> H <sub>69</sub> NO <sub>8</sub> MW: 643.9 Da	C <sub>32</sub> H <sub>63</sub> NO <sub>3</sub> MW: 509.8 Da

### Galactocerebrosidase Substrate and Internal Standard Mix (NSK-KR-1)

Each vial contains the following compounds at a molar ratio of 150:1.

Substrate	Internal Standard
D-Galactosyl- $\beta$ 1-1'- <i>N</i> -octanoyl-D- <i>erythro</i> -sphingosine [C8-galactosylceramide]	<i>N</i> -Decanoyl-D- <i>erythro</i> -sphingosine [C10-ceramide]
C <sub>32</sub> H <sub>61</sub> NO <sub>8</sub> MW: 587.8 Da	C <sub>28</sub> H <sub>55</sub> NO <sub>3</sub> MW: 453.7 Da

### $\alpha$ -L-Duronidase Substrate and Internal Standard Mix (NSK-MP-1)

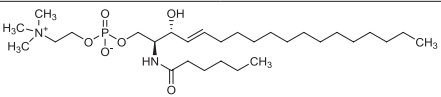
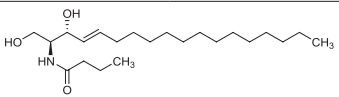
Each vial contains the following compounds at a molar ratio of 150:1.

Substrate	Internal Standard
(7-(1-Iduronic acid)-oxycoumarin-4-methylamine-(5'- <i>N</i> - <i>boc</i> -aminopentanoyl)-amide)	(7-Hydroxycoumarin-4-methylamine-(4'- <i>N</i> - <i>boc</i> -aminobutanoyl)-amide)
C <sub>26</sub> H <sub>34</sub> N <sub>2</sub> O <sub>12</sub> MW: 566.6 Da	C <sub>19</sub> H <sub>24</sub> N <sub>2</sub> O <sub>6</sub> MW: 376.4 Da

## Additional Standard Mixtures for Screening *(continued)*

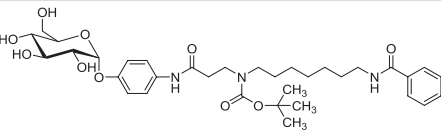
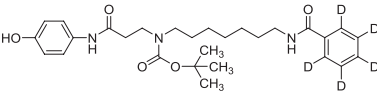
### Acid Sphingomyelinase Substrate and Internal Standard Mix (NSK-NI-1)

Each vial contains the following compounds at a molar ratio of 50:1.

Substrate	Internal Standard
<i>N</i> -Hexanoyl-D-erythro-sphingosylphosphorylcholine [C6-sphingomyelin]	<i>N</i> -Butyroyl-D-erythro-sphingosine [C4-ceramide]
C <sub>29</sub> H <sub>59</sub> N <sub>2</sub> O <sub>6</sub> P MW: 562.8 Da	C <sub>22</sub> H <sub>43</sub> NO <sub>3</sub> MW: 369.6 Da
	

### Acid α-Glucosidase Substrate and Internal Standard Mix (NSK-PO-1)

Each vial contains the following compounds at a molar ratio of 100:1.

Substrate	Internal Standard
(7-Benzoylamino-heptyl)-[2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenylcarbamoyl]-ethyl]-carbamic acid <i>tert</i> -butyl ester	(7-D <sub>5</sub> -Benzoylamino-heptyl)-[2-(4-hydroxy-phenyl-carbamoyl)-ethyl]-carbamic acid <i>tert</i> -butyl ester
C <sub>34</sub> H <sub>49</sub> N <sub>3</sub> O <sub>10</sub> MW: 659.8 Da	C <sub>28</sub> H <sub>34</sub> N <sub>3</sub> O <sub>5</sub> D <sub>5</sub> MW: 502.7 Da
	

### Usage Specifications

Criteria	Recommendation
Use	~600 samples/vial

<i>Before reconstitution:</i>		<i>After reconstitution:</i>	
Storage	-20°; protect from light	Storage	5±3°C or -20±5°C
Recommended retest	2 years from date of manufacture	Recommended retest	4 weeks

### Example References

Ribas, G.; De Mari, J.F.; Civallero, G.; et al. **2017**. Validation of a multiplex tandem mass spectrometry method for the detection of selected lysosomal storage diseases in dried blood spots. *JIMES*, 5, 1-7.

Tortorelli, S.; Turgeon, C.T.; Gavrilo, D.K.; et al. **2016**. Simultaneous testing for 6 lysosomal storage disorders and x-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. *Clin Chem*, 62(9), 1248-1254.

Cho, S.E.; Kwak, J.R.; Lee, H.; et al. **2016**. Triplex tandem mass spectrometry assays for the screening of 3 lysosomal storage disorders in a Korean population. *Clin Chim Acta*, 454, 20-27.

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## Succinylacetone Reference Standard (NSK-T)

This set contains 10 vials of dried-down stable isotope-labeled succinylacetone. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of highly pure methanol.
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (µM)
Succinylacetone (SUAC)	3,4,5,6,7- <sup>13</sup> C <sub>5</sub> , 99%	163.12	1000

**Note:** One vial of the unlabeled succinylacetone standard (NSK-T-US) is also available, having an unlabeled SUAC concentration of 0.1 mg/mL.

### Usage Specifications

Criteria	Recommendation
Use	9,600 samples/vial
<b>Before reconstitution:</b>	
Storage	≤25°C; protect from light and moisture
Recommended retest	5 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 5±3°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

### Example References

Fuenzalida, K.; Leal-Witt, M.J.; Guerrero, P.; et al. **2021**. NTBC treatment monitoring in Chilean patients with tyrosinemia type 1 and its association with biochemical parameters and liver biomarkers. *J Clin Med*, 10(24), 5832-5845.

Schupper, A.; Almashanu, S.; Coster, D.; et al. **2021**. Metabolic biomarkers of small and large for gestational age newborns. *Early Hum Dev*, 160, 105422-105429.

Staretz-Chacham, O.; Daas, S.; Ulanovsky, I.; et al. **2021**. The role of orotic acid measurement in routine newborn screening for urea cycle disorders. *J Inherit Metab Dis*, 44(3), 606-617.

Brennenstuhl, H.; Kohlmüller, D.; Gramer, G.; et al. **2020**. High throughput newborn screening for aromatic L-amino-acid decarboxylase deficiency by analysis of concentrations of 3-O-methyldopa from dried blood spots. *J Inherit Metab Dis*, 43(3), 602-610.

Jack, R.M.; Scott, C.R. **2019**. Validation of a therapeutic range for nitisinone in patients treated for tyrosinemia type 1 based on reduction of succinylacetone excretion. *JIMD Reports*, 46(1), 75-78.

Céspedes, N.; Valencia, A.; Echeverry, C.A.; et al. **2017**. Reference values of amino acids, acylcarnitines and succinylacetone by tandem mass spectrometry for use in newborn screening in southwest Colombia. *Colomb Med (Cali)*, 48(3), 113-119.

de Sain-van der Velden, M.G.M.; van der Ham, M.; Gerrits, J.; et al. **2017**. Quantification of metabolites in dried blood spots by direct infusion high resolution mass spectrometry. *Anal Chim Acta*, 979, 45-50.

Haynes, C.A.; De Jesús, V.R. **2016**. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA-ESI-MS/MS analysis of dried blood spot extracts for newborn screening. *Clin Biochem*, 49(1-2), 161-165.

Pankowicz, F.P.; Barzi, M.; Legras, X.; et al. **2016**. Reprogramming metabolic pathways *in vivo* with CRISPR/Cas9 genome editing to treat hereditary tyrosinaemia. *Nat Commun*, 7, 12642-12647.

### Technical Note

Xie, X.; Kozak, M. **2020**. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. (Thermo Scientific technical note #73398).

## Additional Example Standards for Screening

Catalog No.	Standard	Abbreviation	Label and Enrichment	Unit Size
CLM-3777	<i>N</i> -Acetylglycine	AG	2- <sup>13</sup> C, 99%	Please inquire
CLM-3678	Adenosine	Ado	ribose- <sup>13</sup> C <sub>5</sub> , 98% (CP 97%)	0.05 g, 0.1 g
CLM-8906	<i>S</i> -Adenosyl-L-homocysteine	SAH	adenosine- <sup>13</sup> C <sub>10</sub> , 98% (CP 95%)	0.1 mg
CNLM-3946	β-Alanine	BALA	<sup>13</sup> C <sub>3</sub> , 98%; <sup>15</sup> N, 96-99%	0.25 g
DLM-10008	5-Androstan-3α-ol-17-one (etiocholanolone)	etio	2,2,3,4,4-D <sub>5</sub> , 98%	1 mg
CNLM-9007	L-Argininosuccinic acid, barium salt·2H <sub>2</sub> O	ASA	arginine- <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N <sub>4</sub> , 99% (CP 90%)	0.1 mg, 0.5 mg
DLM-9276	<i>O</i> -Hexanoyl-L-carnitine-HCl	C6	<i>N</i> -methyl-D <sub>3</sub> , 98%	0.1 mg
DLM-9067	<i>O</i> -Decanoyl-L-carnitine-HCl	C10	<i>N</i> -methyl-D <sub>3</sub> , 98%	0.1 mg
DLM-6718	<i>O</i> -Hexacosanoyl-L-carnitine-HCl	C26	<i>N</i> -methyl-D <sub>3</sub> , 98% (CP 95%)	Please inquire
DLM-7347	Corticosterone	B	2,2,4,6,6,17α,21,21-D <sub>8</sub> , 97-98%	0.01 g
DLM-9976	Cortisone	E	2,2,4,6,6,9,12,12-D <sub>8</sub> , 98%	1 mg, 5 mg
CLM-7933	Creatine	Cre	guanidino- <sup>13</sup> C, 99%	0.1 g
DLM-1302	Creatine	Cre	methyl-D <sub>3</sub> , 98% (CP 97%)	0.25 g
DLM-3653	Creatinine	Crn	<i>N</i> -methyl-D <sub>3</sub> , 98%	0.1 g
CLM-10549	Dehydroepiandrosterone	DHEA	2,3,4- <sup>13</sup> C <sub>3</sub> , 99%	1 mg
DLM-8049	Dehydroepiandrosterone	DHEA	2,2,3,4,4,6-D <sub>6</sub> , 98% (CP 97%)	5 mg
CLM-10784	Dehydroepiandrosterone sulfate-sodium salt	DHEAS	2,3,4- <sup>13</sup> C <sub>3</sub> , 98%	1 mg
DLM-8337	Dehydroepiandrosterone sulfate sodium salt·2H <sub>2</sub> O	DHEAS	2,2,3,4,4,6-D <sub>6</sub> , 95%	5 mg
CLM-4579	2'-Deoxyadenosine·H <sub>2</sub> O	dAdo	ribose- <sup>13</sup> C <sub>5</sub> , 99%	Please inquire
DLM-7687	2'-Deoxyguanosine·H <sub>2</sub> O	2dG	ribose-5,5-D <sub>2</sub> , 98%	0.05 g, 0.1 g
DLM-3023	Dihydrotestosterone	DHT	16,16,17-D <sub>3</sub> , 98%	Please inquire
CLM-7824	L-Dihydroxyphenylalanine	L-Dopa	1- <sup>13</sup> C, ring- <sup>13</sup> C <sub>6</sub> , 99%	0.05 g
DLM-2084	L-Dihydroxyphenylalanine	L-Dopa	ring-D <sub>3</sub> , 98%	1 g
CLM-803	Estradiol	E2	3,4- <sup>13</sup> C <sub>2</sub> , 99%	Please inquire
DLM-2487	Estradiol	E2	2,4,16,16-D <sub>4</sub> , 95-97%	5 mg
CLM-9148	Estrone	E1	2,3,4- <sup>13</sup> C <sub>3</sub> , 99%	1 mg, 5 mg
DLM-3976	Estrone	E1	2,4,16,16-D <sub>4</sub> , 97%	5 mg
DLM-6013	Ethylmalonic acid	EMA	methyl-D <sub>3</sub> , 98%	0.1 g
CLM-1570	D-Galactose	Gal	U- <sup>13</sup> C <sub>6</sub> , 99%	0.1 g
CLM-9874	D-Galactose-1-phosphate, dipotassium salt	Gal-1P	galactose- <sup>13</sup> C <sub>6</sub> , 99%	Please inquire
CLM-1822-H	L-Glutamine	Gln	<sup>13</sup> C <sub>5</sub> , 99%	0.1 g, 0.25 g, 0.5 g
DLM-1826	L-Glutamine	Gln	2,3,3,4,4-D <sub>5</sub> , 97%	0.1 g
CLM-1017	Glycine	Gly	<sup>13</sup> C <sub>2</sub> , 97-99%	0.5 g, 1 g, 5 g
DLM-280	Glycine	Gly	D <sub>5</sub> , 98%	5 g
CNLM-8111	<i>N</i> -(3-Methylcrotonyl)glycine	3-MCG	glycine- <sup>13</sup> C <sub>2</sub> , 98%; <sup>15</sup> N, 98%	Please inquire
DLM-9715	<i>N</i> -(3-Phenylpropionyl)glycine	3-PPG	2,2,-D <sub>2</sub> , 98%	Please inquire
DLM-9998	Guanidinoacetic acid	GAA	2,2-D <sub>2</sub> , 97%	Please inquire
DLM-7689	Guanosine·H <sub>2</sub> O	Guo	ribose-5,5-D <sub>2</sub> , 98%	Please inquire
CNLM-8448	<i>N</i> -Hexanoylglycine	HG	<sup>13</sup> C <sub>2</sub> , 97-99%; <sup>15</sup> N, 97-99% (CP 95%)	Please inquire
CLM-373	Homovanillic acid	HVA	1,2- <sup>13</sup> C <sub>2</sub> , 98-99%	0.1 g
DLM-2738	Homovanillic acid	HVA	phenyl-D <sub>3</sub> , 2,2-D <sub>2</sub> , 96-98%	0.1 g
DLM-8118	3-Hydroxyglutaric acid	3-OHGA	D <sub>5</sub> , 98%	Please inquire
CLM-9936	5-Hydroxyindole-3-acetic acid	5-HIAA	(3α,4,5,6,7,7α- <sup>13</sup> C <sub>6</sub> , 98%)	1.2 mL
DLM-7206	17α-Hydroxypregnenolone	17-OHP5	21,21,21-D <sub>3</sub> , 97%	Please inquire
DLM-3619	DL-Homocystine	HCY	3,3,3',3',4,4,4',4'-D <sub>8</sub> , 98%	0.5 g, 1 g
NLM-4264	Inosine	Ino	<sup>15</sup> N <sub>4</sub> , 95%	0.01 g, 0.05 g
CLM-8742	L-allo-Isoleucine	alle	<sup>13</sup> C <sub>6</sub> , 97-99%	Please inquire
DLM-1505	L-allo-Isoleucine	alle	D <sub>10</sub> , 98%	0.1 g
CNLM-9291	<i>N</i> -Isovalerylglycine	IVG	glycine- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 99%	Please inquire
CLM-2247-H	L-Lysine·2HCl	Lys	<sup>13</sup> C <sub>6</sub> , 99%	0.05 g, 0.1 g, 0.25 g, 0.5 g, 1 g

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## Additional Example Standards for Screening *(continued)*

Catalog No.	Standard	Abbreviation	Label and Enrichment	Unit Size
DLM-10520	Lysophosphatidylcholine 20:0	LysoPC C20:0	eicosanoyl-12,12,13,13-D <sub>4</sub> , 98%	1 mg, 5 mg
CLM-10499	Lysophosphatidylcholine 22:0	LysoPC C22:0	docosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	1 mg, 5 mg
DLM-10500	Lysophosphatidylcholine 22:0	LysoPC C22:0	docosanoyl-12,12,13,13-D <sub>4</sub> , 98%	1 mg, 5 mg
CLM-10496	Lysophosphatidylcholine 24:0	LysoPC C24:0	tetracosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	1 mg, 5 mg
DLM-10497	Lysophosphatidylcholine 24:0	LysoPC C24:0	tetracosanoyl-12,12,13,13-D <sub>4</sub> , 98%	1 mg, 5 mg
CLM-9792	Lysophosphatidylcholine 26:0	LysoPC C26:0	hexacosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	1 mg, 5 mg
DLM-10501	Lysophosphatidylcholine 26:0	LysoPC C26:0	hexacosanoyl-12,12,13,13-D <sub>4</sub> , 98%	1 mg, 5 mg
DLM-205	Malonic acid	MA	D <sub>4</sub> , 98%	50 g
DLM-11341	L-3-O-Methyl-dopa·H <sub>2</sub> O	3-OMD	methoxy-D <sub>3</sub> , 98%	Please inquire
CLM-10350	2-Methylbutyric acid	2-MBA	methyl- <sup>13</sup> C, 99%	Please inquire
DLM-2312	DL-2-Methylcitric acid	MCA	methyl-D <sub>3</sub> , 98% (CP 90%)	Please inquire
CLM-9426	Methylmalonic acid	MMA	<sup>13</sup> C <sub>4</sub> , 99%	0.1 g
DLM-387	Methylmalonic acid	MMA	methyl-D <sub>3</sub> , 98%	0.25 g
CLM-4724	L-Ornithine·HCl	Orn	<sup>13</sup> C <sub>5</sub> , 99%	0.1 g
DLM-2969	L-Ornithine·HCl	Orn	3,3,4,4,5,5-D <sub>6</sub> , 98%	0.1 g, 0.25 g
NLM-1048	Orotic acid·H <sub>2</sub> O	Oro	1,3- <sup>15</sup> N <sub>2</sub> , 98%	0.25 g
DLM-7953	Progesterone	P4	2,2,4,6,6,17α,21,21,21-D <sub>9</sub> , 98%	0.01 g
DLM-6896	Pregnenolone	P5	17,21,21,21-D <sub>4</sub> , 98%	0.01 g
CLM-2260-H	L-Proline	Pro	<sup>13</sup> C <sub>5</sub> , 99%	0.1 g, 0.25 g, 0.5 g
DLM-487	L-Proline	Pro	D <sub>7</sub> , 97-98%	0.1 g, 0.25 g
CLM-7944	3-(3-Methyl-1H-pyrazol-5-yl)propanoic acid	MPP	methyl- <sup>13</sup> C, pyrazolyl- <sup>13</sup> C <sub>3</sub> , 3- <sup>13</sup> C, 99%	Please inquire
CLM-647	Propionic acid	PPA	<sup>13</sup> C <sub>3</sub> , 99%	1 g
DLM-1919	Propionic acid	PPA	D <sub>5</sub> , 98%	5 g
CNLM-9292	<i>N</i> -Propionylglycine	PG	glycine- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 99%	Please inquire
DLM-6874	Sarcosine·HCl ( <i>N</i> -methylglycine·HCl)	Sar	methyl-D <sub>3</sub> , 98%	0.1 g, 0.25 g
CNLM-8183	Suberylglycine	SG	glycine- <sup>13</sup> C <sub>2</sub> , 98%; <sup>15</sup> N, 98% (CP 95%)	Please inquire
CLM-9164	Testosterone	T	2,3,4- <sup>13</sup> C <sub>3</sub> , 99%	5 mg, 10 mg
DLM-8085	Testosterone	T	2,2,4,6,6-D <sub>5</sub> , 98%	Please inquire
CLM-6725	L-Thyroxine	T4	tyrosine-ring- <sup>13</sup> C <sub>6</sub> , 99% (CP 90%)	0.1 mg
CLM-8931	L-Thyroxine	T4	ring- <sup>13</sup> C <sub>12</sub> , 99% (CP 97%)	0.1 mg
DLM-10758	Trisodium 2-methylcitrate, racemic mixture of diastereomers	MCA	methyl-D <sub>3</sub> , 98% (CP 90%)	5 mg, 10 mg
CLM-4290-H	L-Tryptophan	Trp	<sup>13</sup> C <sub>11</sub> , 99%	0.1 g
DLM-6903	L-Tryptophan	Trp	D <sub>8</sub> , 97-98%	0.25 g
ULM-11135	Uridine diphosphate-α- <i>N</i> -acetyl-D-glucosamine, disodium salt	UDP-GlcNAc	unlabeled	Please inquire

**Note:** Unlabeled standards are also available. Please inquire for size and pricing or visit [isotope.com](http://isotope.com).

### Example References

Daas, S.; Salah, N.A.; Anikster, Y.; et al. **2023**. Addition of galactose-1-phosphate measurement enhances newborn screening for classical galactosemia. *J Inherit Metab Dis*, 46(2), 232-242.

Young, A.; Hendricks, J.; Foreman, D.; et al. **2020**. Development of dried blood spot quality control materials for adenosine deaminase severe combined immunodeficiency and an LC-MS/MS method for their characterization. *Clin Mass Spec*, 17, 4-11.

Coene, K.L.M.; Kluijtmans, L.A.J.; van der Heeft, E.; et al. **2018**. Next-generation metabolic screening: targeted and untargeted metabolomics for the diagnosis of inborn errors of metabolism in individual patients. *J Inherit Metab Dis*, 41(3), 337-353.

Monostori, P.; Klinke, G.; Richter, S.; et al. **2017**. Simultaneous determination of 3-hydroxypropionic acid, methylmalonic acid and methylcitric acid in dried blood spots: Second-tier LC-MS/MS assay for newborn screening of propionic acidemia, methylmalonic acidemias and combined remethylation disorders. *PLoS One*, 12(9), e0184897.

Nakano, M.; Uemura, O.; Honda, M.; et al. **2017**. Development of tandem mass spectrometry-based creatinine measurement using dried blood spot for newborn mass screening. *Pediatr Res*, 82(2), 237-243.

Prinsen, H.C.M.T.; Schiebergen-Bronkhorst, B.G.M.; Roeleveld, M.W.; et al. **2016**. Rapid quantification of underivatized amino acids in plasma by hydrophilic interaction liquid chromatography (HILIC) coupled with tandem mass-spectrometry. *J Inherit Metab Dis*, 39(5), 651-660.

Held, P.K.; Haynes, C.A.; De Jesús, V.R.; et al. **2014**. Development of an assay to simultaneously measure orotic acid, amino acids, and acylcarnitines in dried blood spots. *Clin Chem Acta*, 436, 149-154.

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## MS/MS Parameter Examples

### Neutral Loss (NL) Scan (for NSK-A and NSK-A1 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	NL $m/z$	Precursor Ion $m/z$	NL $m/z$
Alanine (D <sub>4</sub> )	Ala	94	46	150	102
Arginine ( <sup>13</sup> C/D <sub>4</sub> )	Arg	180	105	236	161
Aspartate (D <sub>3</sub> )	Asp	137	46	249	102
Citrulline (D <sub>2</sub> )	Cit	178	63	234	119
Glutamate (D <sub>3</sub> )	Glu	151	46	263	102
Glycine ( <sup>13</sup> C/ <sup>15</sup> N)	Gly	78	46	134	102
Leucine (D <sub>3</sub> )	Leu	135	46	191	102
Methionine (D <sub>3</sub> )	Met	153	46	209	102
Ornithine (D <sub>2</sub> )	Orn	135	63	191	119
Ornithine (D <sub>6</sub> )	Orn	139	63	195	119
Phenylalanine ( <sup>13</sup> C <sub>6</sub> )	Phe	172	46	228	102
Tyrosine ( <sup>13</sup> C <sub>6</sub> )	Tyr	188	46	244	102
Valine (D <sub>8</sub> )	Val	126	46	182	102

**Note:** The MS/MS fragmentation mechanism of amino acids during NL scan is well established (e.g., PMID: 14578311). For example, the losses for the underivatized amino acids reflect HCOOH ( $m/z$  46), HCOOH and NH<sub>3</sub> ( $m/z$  63), and HCOOH and H<sub>2</sub>NCNHNH<sub>2</sub> ( $m/z$  105).

### Precursor (Pre) Ion Scan (for NSK-B and NSK-B-G1 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	Product Ion $m/z$	Precursor Ion $m/z$	Product Ion $m/z$
Carnitine (D <sub>9</sub> )	C0	171	85	227	85
Acetylcarnitine (D <sub>3</sub> )	C2	207	85	263	85
Propionylcarnitine (D <sub>3</sub> )	C3	221	85	277	85
Butyrylcarnitine (D <sub>3</sub> )	C4	235	85	291	85
Isovalerylcarnitine (D <sub>9</sub> )	C5	255	85	311	85
Glutarylcarnitine (D <sub>3</sub> )	C5-DC	279	85	391	85
Hydroxyisovalerylcarnitine (D <sub>3</sub> )	C5-OH	265	85	321	85
Octanoylcarnitine (D <sub>3</sub> )	C8	291	85	347	85
Dodecanoylcarnitine (D <sub>9</sub> )	C12	353	85	409	85
Myristoylcarnitine (D <sub>9</sub> )	C14	381	85	437	85
Palmitoylcarnitine (D <sub>3</sub> )	C16	403	85	459	85
Hydroxypalmitoylcarnitine (D <sub>3</sub> )	C16-OH	419	85	475	85
Octadecanoylcarnitine (D <sub>3</sub> )	C18	431	85	487	85

**Note:** The common fragment ion of  $m/z$  85 corresponds to <sup>+</sup>CH<sub>2</sub>-CH=CH-COOH and is consistent between nonderivatized and derivatized acylcarnitines (e.g., PMID: 9365395 for background and fragmentation mechanism).

## MS/MS Parameter Examples *(continued)*

### MRM Acquisition Mode (for NSK-A, NSK-A1, and NSK-T Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	Product Ion $m/z$	Precursor Ion $m/z$	Product Ion $m/z$
Alanine ( $D_4$ )	Ala	94	48	150	48
Arginine ( $^{13}C/D_4$ )	Arg	180	75	236	75
Aspartate ( $D_3$ )	Asp	137	119	249	147
Citrulline ( $D_2$ )	Cit	178	115	234	115
Glutamate ( $D_3$ )	Glu	151	133	263	161
Glycine ( $^{13}C/^{15}N$ )	Gly	78	32	134	78
Leucine ( $D_3$ )	Leu	135	89	191	89
Methionine ( $D_3$ )	Met	153	136	209	107
Ornithine ( $D_2$ )	Orn	135	72	191	72
Ornithine ( $D_6$ )	Orn	131	68	195	68
Phenylalanine ( $^{13}C_6$ )	Phe	172	126	228	126
Tyrosine ( $^{13}C_6$ )	Tyr	188	142	244	142
Valine ( $D_8$ )	Val	126	80	182	80
Succinylacetone ( $^{13}C_2$ )	SUAC	160	114	216	142

### MRM Acquisition Mode (for NSK-B and NSK-B-G1 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	Product Ion $m/z$	Precursor Ion $m/z$	Product Ion $m/z$
Carnitine ( $D_9$ )	C0	171	103	227	103
Acetylcarnitine ( $D_3$ )	C2	207	85	263	85
Propionylcarnitine ( $D_3$ )	C3	221	85	277	85
Butyrylcarnitine ( $D_3$ )	C4	235	85	291	85
Isovalerylcarnitine ( $D_9$ )	C5	255	85	311	85
Glutaryl carnitine ( $D_3$ )	C5-DC	279	85	335	85
Hydroxyisovalerylcarnitine ( $D_3$ )	C5-OH	265	85	321	85
Octanoylcarnitine ( $D_3$ )	C8	291	85	347	85
Dodecanoylcarnitine ( $D_9$ )	C12	353	85	409	85
Myristoylcarnitine ( $D_9$ )	C14	381	85	437	85
Palmitoylcarnitine ( $D_3$ )	C16	403	85	459	85
Hydroxypalmitoylcarnitine ( $D_3$ )	C16-OH	419	85	475	85
Octadecanoylcarnitine ( $D_3$ )	C18	431	85	487	85

#### Technical Note

Xie, X.; Kozak, M. 2020. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. Thermo Fisher Scientific, San Jose, CA.

## MS/MS Parameter Examples *(continued)*

### MRM Acquisition Mode (for NSK-AA3 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
Creatine (D <sub>3</sub> )	Cre	137	95
Guanidinoacetic acid ( <sup>13</sup> C <sub>2</sub> / <sup>15</sup> N)	GAA	121	79
L-Proline (D <sub>7</sub> )	Pro	123	77

### MRM Acquisition Mode (for NSK-BCAA Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
L-Allo-isoleucine ( <sup>13</sup> C <sub>6</sub> / <sup>15</sup> N)	Alle	139	92
L-Isoleucine (D <sub>10</sub> )	Ile	142	96
L-Leucine (D <sub>3</sub> )	Leu	135	89
L-Valine ( <sup>13</sup> C <sub>6</sub> / <sup>15</sup> N)	Val	124	77

### MRM Acquisition Mode (for NSK-S and NSK-S-EXP Standards)

(all  $m/z$  as  $[M+H]^+$  unless otherwise specified)

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
Aldosterone (D <sub>7</sub> )	A	350 for $[M+H^+-H_2O]^+$	173
4-Androstene-3,17-dione (D <sub>5</sub> )	A4	292	100
Corticosterone (D <sub>8</sub> )	B	355	125
Cortisol (D <sub>3</sub> )	F	366	121
11-Deoxycortisol (D <sub>9</sub> )	11-S	352	100
21-Deoxycortisol (D <sub>8</sub> )	21-S	355	319
17 $\alpha$ -Hydroxyprogesterone (D <sub>8</sub> )	17-OHP	339	100
Progesterone (D <sub>9</sub> )	P	324	100
Testosterone (D <sub>5</sub> )	T	294	100

### MRM Acquisition Mode (for NSK-LPC Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
Lysophosphatidylcholine 20:0 (D <sub>4</sub> )	LPC 20:0	556	104
Lysophosphatidylcholine 22:0 ( <sup>13</sup> C <sub>6</sub> )	LPC 22:0	586	104
Lysophosphatidylcholine 24:0 ( <sup>13</sup> C <sub>6</sub> )	LPC 24:0	614	104
Lysophosphatidylcholine 26:0 ( <sup>13</sup> C <sub>6</sub> )	LPC 26:0	643	104

Chemical purity (CP) is 98% or greater, unless otherwise specified.



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## Setting the Standard...

Donald H. Chace, PhD MSFS FACB  
Medolac Laboratories

“In clinical chemistry-based applications of mass spectrometry, the first lesson the laboratory learns is the requisite nature of stable isotope-enriched standards for quantification of metabolites in biological fluids. In newborn screening of amino acids and acylcarnitines, Cambridge Isotope Laboratories, Inc., set the standard for quantification of these metabolites in dried blood spots. As research and development of the newborn screening analysis by mass spectrometry progressed, it was clear that a half dozen isotope-labeled internal standards would not be adequate for the analysis of an amino acid and acylcarnitine profile, together comprising a range of 500 separate mass units and more than 30 important metabolites, most of which require accurate quantification. When screening began to expand beyond research, it was clear that weighing out small quantities of individual standards would reduce accuracy and introduce unnecessary error. Therefore, together, we set out to develop sets of standards for amino acids and acylcarnitine analysis that would enable quantification. We started this development more than 20 years ago adding, changing and improving these standards. CIL, together with the early developers of tandem mass spectrometry-based newborn screening, set the standard by which all other laboratories follow. CIL's commitment to supporting the metabolic and newborn screening community is exceptional. It is our good fortune in the clinical chemistry and mass spectrometry community to have CIL as part of our laboratory solutions.”

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