**Supplementary data:**

Table 1: Distribution of the subjects in the four study site cohorts by specimen type and study group

Tables 2- 9: LC/MS analyses of lipids and polar metabolites

**Table 1: Distribution of the subjects in the four study site cohorts by specimen type and study group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **LCC Study Cohort** | ***LRRK2-*/UC**  | ***LRRK2-*/PD**  | ***LRRK2+*/UC** | ***LRRK2+*/PD** | **Total subjects in each cohort** |
| **23andMe Blood Collection Study** |  |  |  |  |  |
|  Plasma | 0 | 0 | 60 | 27 | 87 |
|  CSF | 0 | 0 | 0 | 0 | 0 |
| **LRRK2 Longitudinal Study** |  |  |  |  |  |
|  Plasma | 19 | 27 | 14 | 27 | 87 |
|  CSF | 0 | 0 | 0 | 0 | 0 |
| **LRRK2 Cross-sectional Study**  **Europe, Asia, North**  **Africa**  |  |  |  |  |  |
|  Plasma | 25 | 23 | 30 | 48 | 126 |
|  CSF | 10 | 16 | 16 | 17 | 59 |
|  **North America**  |  |  |  |  |  |
|  Plasma | 21 | 20 | 11 | 16 | 68 |
|  CSF | 4 | 2 | 2 | 1 | 9 |

**Table 2: LC/MS analysis of GlcCer and GalCer species.** Glucosylceramide (GlcCer), galactosylceramide (GalCer), glucosylsphigosine (Glc sphingosine) and galactosylsphingosine (Gal sphingosine) analyses were performed by liquid chromatography (ACQUITY I-Class Plus UPLC FTN, Waters Corporation, Milford, MA USA) coupled to electrospray mass spectrometry (XEVO TQ-S Micro, Waters).

For each analysis, 2 µL of sample was injected on a HALO HILIC 2.0 µm, 3.0 × 150 mm column (Advanced Materials Technology, Wilmington, DE, USA) using a flow rate of 0.45 mL/min at 45°C. Mobile phase A consisted of 92.5/5/2.5 acetonitrile/ isopropyl alcohol/water with 5 mM ammonium formate and 0.5% formic acid. Mobile phase B consisted of 92.5/5/2.5 water/ isopropyl alcohol/acetonitrile with 5 mM ammonium formate and 0.5% formic acid. The gradient was programmed as follows: 0.0–2.0 min at 100% B, 2.1 min at 95% B, 2.11 min at 0% B, hold to 3.5 min at 0% B, ramp back to 100% at 3.60 min and hold to 4.5 min. Electrospray ionization was performed in the positive-ion mode applying the following settings: capillary voltage at 1.5 kV; source temperature at 150°C; desolvation temperature at 400°C; desolvation gas flow at 1000 L/hr; cone gas flow at 25 L/hr; cone voltage at 40 V; nebulizer gas at 7 bar. Data acquisition was performed in multiple reaction monitoring mode (MRM) with the precursor ion mass-to-charge ratio (Q1 m/z); fragment ion mass-to-charge ratio (Q3 m/z); and collision energy (CE) reported in Table 2. Peak areas were integrated using TargetLynx V4.2 (Waters).

Table 2. Acquisition parameters for the LC/MS analysis of GlcCer and GalCer species.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Internal Standard** | **Q1 m/z** | **Q3 m/z** | **CE (V)** |
| GlcCer(d18:1/16:0) | GlcCer (d18:1(d5)/18:0) | 700.6 | 264.3 | 32 |
| GlcCer(d18:1/18:0) | GlcCer (d18:1(d5)/18:0) | 728.6 | 264.3 | 32 |
| GlcCer(d18:2/18:0) | GlcCer (d18:1(d5)/18:0) | 726.6 | 262.3 | 32 |
| GlcCer(d18:1/20:0) | GlcCer (d18:1(d5)/18:0) | 756.6 | 264.3 | 35 |
| GlcCer (d18:2/20:0) | GlcCer (d18:1(d5)/18:0) | 754.6 | 262.3 | 35 |
| GlcCer (d18:1/22:0) | GlcCer (d18:1(d5)/18:0) | 784.7 | 264.3 | 35 |
| GlcCer (d18:1/22:1) | GlcCer (d18:1(d5)/18:0) | 782.7 | 264.3 | 35 |
| GlcCer (d18:2/22:0) | GlcCer (d18:1(d5)/18:0) | 782.7 | 262.3 | 35 |
| GlcCer (d18:1/24:1) | GlcCer (d18:1(d5)/18:0) | 810.7 | 264.3 | 35 |
| GlcCer (d18:1/24:0) | GlcCer (d18:1(d5)/18:0) | 812.7 | 264.3 | 35 |
| Glc Sphingosine | Glc Sphingosine (d5) | 462.2 | 264.3 | 11 |
| GlcCer (d18:1(d5)/18:0) | N/A | 733.6 | 269.3 | 32 |
| Glc Sphingosine (d5) | N/A | 467.2 | 269.3 | 11 |
| GalCer (d18:1/16:0) | GlcCer (d18:1(d5)/18:0) | 624.4 | 282.3 | 32 |
| GalCer (d18:1/18:0) | GlcCer (d18:1(d5)/18:0) | 700.6 | 264.3 | 32 |
| GalCer (d18:2/18:0) | GlcCer (d18:1(d5)/18:0) | 728.6 | 264.3 | 32 |
| GalCer (d18:1/20:0) | GlcCer (d18:1(d5)/18:0) | 726.6 | 262.3 | 35 |
| GalCer (d18:2/20:0) | GlcCer (d18:1(d5)/18:0) | 756.6 | 264.3 | 35 |
| GalCer (d18:1/22:0) | GlcCer (d18:1(d5)/18:0) | 754.6 | 262.3 | 35 |
| GalCer (d18:1/22:1) | GlcCer (d18:1(d5)/18:0) | 784.6 | 264.3 | 35 |
| GalCer (d18:2/22:0) | GlcCer (d18:1(d5)/18:0) | 782.6 | 264.3 | 35 |
| GalCer (d18:1/24:1) | GlcCer (d18:1(d5)/18:0) | 782.6 | 262.3 | 35 |
| GalCer (d18:1/24:0) | GlcCer (d18:1(d5)/18:0) | 810.7 | 264.3 | 35 |
| Gal sphingosine | Glc Sphingosine (d5) | 812.7 | 264.3 | 11 |

**Table 3: LC/MS analysis of Free fatty acid (FFA):** FFA analyses were performed by liquid chromatography (ACQUITY I-Class Plus UPLC FTN, Waters Corporation, Milford, MA USA) coupled to electrospray mass spectrometry (XEVO TQ-S Micro, Waters).

For each analysis, 2 µL of sample was injected on a ACQUITY UPLC BEH C18 1.7 µm, 2.1 × 50 mm column (Waters Corporation, Milford, MA USA) using a flow rate of 0.33 mL/min at 40°C. Mobile phase A consisted of methanol containing 0.25% acetic acid and 5 mM ammonium acetate. Mobile phase B consisted of water containing 0.25% acetic acid and 5 mM ammonium acetate. The gradient was programmed as follows: 0.1 min at 10% B, 0.1-3.5 min to 0% B,  ramp back to 10% B at 3.51 min and hold to 4.5 min. Electrospray ionization was performed in the negative-ion mode applying the following settings: For the XEVO TQ-S Micro we applied the following settings: capillary voltage at 2.0 kV; source temperature at 150°C; desolvation temperature at 500°C; desolvation gas flow at 1000 L/hr; cone gas flow at 25 L/hr; cone voltage at 56 V; nebulizer gas at 7 bar. Data acquisition was performed in multiple reaction monitoring mode (MRM) with the precursor ion mass-to-charge ratio (Q1 m/z); fragment ion mass-to-charge ratio (Q3 m/z); and collision energy (CE) reported in Table 3.  Peak areas were integrated using TargetLynx V4.2 (Waters).

Table 3. Acquisition parameters for the LC/MS analysis of FFA species.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Internal Standard** | **Q1 m/z** | **Q3 m/z** | **CE (V)** |
| FFA (16:0) | LPE 18:1 (d7) | 255.1 | 255.1 | 7 |
| FFA (16:1) | LPE 18:1 (d7) | 253.1 | 253.1 | 7 |
| FFA (18:0) | LPE 18:1 (d7) | 283.2 | 283.2 | 7 |
| FFA (18:1) | LPE 18:1 (d7) | 281.2 | 281.2 | 7 |
| FFA (18:2) | LPE 18:1 (d7) | 279.2 | 279.2 | 7 |
| FFA (18:3) | LPE 18:1 (d7) | 277.2 | 277.2 | 7 |
| FFA (20:1) | LPE 18:1 (d7) | 309.3 | 309.3 | 7 |
| FFA (20:3) | LPE 18:1 (d7) | 305.3 | 305.3 | 7 |
| FFA (20:4) | LPE 18:1 (d7) | 303.3 | 303.3 | 7 |
| FFA (20:5) | LPE 18:1 (d7) | 301.2 | 301.2 | 7 |
| FFA (22:5) | LPE 18:1 (d7) | 329.2 | 329.2 | 7 |
| FFA (22:6) | LPE 18:1 (d7) | 327.2 | 327.2 | 7 |
| FFA (24:0) | LPE 18:1 (d7) | 367.3 | 367.3 | 7 |
| FFA (24:1) | LPE 18:1 (d7) | 365.3 | 365.3 | 7 |
| FFA (24:6) | LPE 18:1 (d7) | 355.4 | 355.4 | 7 |
| LPE 18:1 (d7) | N/A | 485.3 | 288.3 | 35 |

**Table 4: LC/MS analysis of lipids.** Lipid analyses were performed by liquid chromatography (Nexera X2, Shimadzu Scientific Instrument, Columbia, MD, USA) coupled to electrospray mass spectrometry (QTRAP 6500+, Sciex, Framingham, MA, USA).

For each analysis, 5 µL of sample was injected on an ACQUITY UPLC BEH C18 1.7 µm, 2.1×100 mm column (Waters) using a flow rate of 0.25 mL/min at 55°C. For positive ionization mode, mobile phase A consisted of 60:40 acetonitrile/water (v/v) with 10 mM ammonium formate + 0.1% formic acid; mobile phase B consisted of 90:10 isopropyl alcohol/acetonitrile (v/v) with 10 mM ammonium formate + 0.1% formic acid. For negative ionization mode, mobile phase A consisted of 60:40 acetonitrile/water (v/v) with 10 mM ammonium acetate + 0.1% acetic acid; mobile phase B consisted of 90:10 isopropyl alcohol/acetonitrile (v/v) with 10 mM ammonium acetate + 0.1% acetic acid. The gradient was programmed as follows: 0.0-8.0 min from 45% B to 99% B, 8.0-9.0 min at 99% B, 9.0-9.1 min to 45% B, and 9.1-10.0 min at 45% B. Electrospray ionization was performed in either positive or negative ion mode. The following settings were applied: curtain gas at 30 V; collision gas was set at medium; ion spray voltage at 5500 V (positive mode) or -4500V (negative mode); temperature at 250°C (positive mode) or 600°C (negative mode); ion source Gas 1 at 50 psi; ion source Gas 2 at 60 psi; declustering potential at 80 V; entrance potential at 10 V; and collision cell exit potential at 12.5 V. Data acquisition was performed in multiple reaction monitoring mode (MRM) with the precursor ion mass-to-charge ratio (Q1 m/z); fragment ion mass-to-charge ratio (Q3 m/z); and collision energy (CE) reported in Tables 4 and 5.  Peak areas were integrated using MultiQuant 3.02 (Sciex).

Table 4. Acquisition parameters for the LC/MS analysis of lipid species in positive ionization mode.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Internal Standard** | **Q1 m/z** | **Q3 m/z** | **CE (V)**  |
| Sphingosine d17:1 | N/A | 286.2 | 268.3 | 20 |
| Sphingosine | Sphingosine d17:1 | 300.2 | 282.2 | 20 |
| Sphinganine | Sphingosine d17:1 | 302.2 | 284.2 | 20 |
| Hexosyl sphingosine | Glc Sphingosine(d5) | 462.3 | 282.2 | 16 |
| Lactosyl sphingosine | Glc Sphingosine(d5) | 624.4 | 282.3 | 16 |
| Cer(d18:1/17:0) | N/A | 552.4 | 264.3 | 40 |
| Cer(d18:1/16:0) | Cer(d18:1/17:0) | 538.5 | 264.4 | 40 |
| Cer(d18:1/18:0) | Cer(d18:1/17:0) | 566.6 | 264.4 | 40 |
| Cer(d18:1/24:0) | Cer(d18:1/17:0) | 650.6 | 264.4 | 40 |
| Cer(d18:1/24:1) | Cer(d18:1/17:0) | 648.6 | 264.4 | 40 |
| SM(d18:1(d9)/18:1) | N/A | 738.7 | 184.1 | 40 |
| SM(d18:1/16:0) | SM(d18:1(d9)/18:1) | 703.6 | 184.1 | 40 |
| SM(d18:1/18:0) | SM(d18:1(d9)/18:1) | 731.6 | 184.1 | 40 |
| SM(d18:1/24:0) | SM(d18:1(d9)/18:1) | 815.7 | 184.1 | 40 |
| SM(d18:1/24:1) | SM(d18:1(d9)/18:1) | 813.7 | 184.1 | 40 |
| GlcCer (d18:1(d5)/18:0) | N/A | 733.6 | 269.3 | 40 |
| HexCer(d18:1/16:0) | GlcCer (d18:1(d5)/18:0) | 700.6 | 264.6 | 40 |
| HexCer(d18:1/18:0) | GlcCer (d18:1(d5)/18:0) | 728.6 | 264.4 | 40 |
| HexCer(d18:1/24:0) | GlcCer (d18:1(d5)/18:0) | 812.7 | 264.4 | 40 |
| HexCer(d18:1/24:1) | GlcCer (d18:1(d5)/18:0) | 810.7 | 264.4 | 40 |
| LacCer(d18:1/16:0) | GlcCer (d18:1(d5)/18:0) | 862.6 | 264.6 | 40 |
| LacCer(d18:1/18:0) | GlcCer (d18:1(d5)/18:0) | 890.7 | 264.4 | 40 |
| LacCer(d18:1/24:0) | GlcCer (d18:1(d5)/18:0) | 974.8 | 264.4 | 40 |
| LacCer(d18:1/24:1) | GlcCer (d18:1(d5)/18:0) | 972.7 | 264.4 | 40 |
| LPC(18:1(d7)) | N/A | 529.3 | 184.1 | 40 |
| LPC(16:0) | LPC(18:1(d7)) | 496.3 | 184.1 | 40 |
| LPC(18:0) | LPC(18:1(d7)) | 524.3 | 184.1 | 40 |
| LPC(18:1) | LPC(18:1(d7)) | 522.3 | 184.1 | 40 |
| LPC(20:4) | LPC(18:1(d7)) | 544.3 | 184.1 | 40 |
| LPC(22:6) | LPC(18:1(d7)) | 568.3 | 184.1 | 40 |
| LSM | LPC(18:1(d7)) | 465.5 | 184.1 | 40 |
| PC(15:0/18:1(d7)) | N/A | 754.6 | 184.1 | 40 |
| PC(36:1) | PC(15:0/18:1(d7)) | 788.6 | 184.1 | 40 |
| PC(36:2) | PC(15:0/18:1(d7)) | 786.6 | 184.1 | 40 |
| PC(36:4) | PC(15:0/18:1(d7)) | 782.6 | 184.1 | 40 |
| PC(38:4) | PC(15:0/18:1(d7)) | 810.6 | 184.1 | 40 |
| PC(38:6) | PC(15:0/18:1(d7)) | 806.6 | 184.1 | 40 |
| PC(40:6) | PC(15:0/18:1(d7)) | 834.6 | 184.1 | 40 |
| PC(O-18:0/2:0) | LPC(18:1(d7)) | 524.3 | 184.1 | 40 |
| PE(15:0/18:1(d7)) | N/A | 711.6 | 570.5 | 40 |
| PE(36:1) | PE(15:0/18:1(d7)) | 746.6 | 605.5 | 40 |
| PE(36:2) | PE(15:0/18:1(d7)) | 744.6 | 603.5 | 40 |
| PE(36:4) | PE(15:0/18:1(d7)) | 740.6 | 599.5 | 40 |
| PE(38:4) | PE(15:0/18:1(d7)) | 768.6 | 627.5 | 40 |
| PE(38:6) | PE(15:0/18:1(d7)) | 764.6 | 623.5 | 40 |
| PE(40:6) | PE(15:0/18:1(d7)) | 792.6 | 651.5 | 40 |
| Cholesterol(d7) | N/A | 376.2 | 376.2 | 10 |
| Cholesterol | Cholesterol(d7) | 369.3 | 369.3 | 10 |
| CE(18:1(d7)) | N/A | 675.2 | 369.4 | 26 |
| CE(16:1) | CE(18:1(d7)) | 640.6 | 369.3 | 26 |
| CE(18:1) | CE(18:1(d7)) | 668.6 | 369.3 | 26 |
| CE(18:2) | CE(18:1(d7)) | 666.6 | 369.3 | 26 |
| CE(20:4) | CE(18:1(d7)) | 690.6 | 369.3 | 26 |
| CE(20:5) | CE(18:1(d7)) | 688.6 | 369.3 | 26 |
| CE(22:6) | CE(18:1(d7)) | 714.6 | 369.3 | 26 |
| TG(15:0/18:1(d7)/15:0) | N/A | 829.4 | 523.5 | 40 |
| TG 52:4/18:1 | TG(15:0/18:1(d7)/15:0) | 872.7 | 573.4 | 40 |
| TG 52:3/18:1 | TG(15:0/18:1(d7)/15:0) | 874.7 | 575.4 | 40 |
| TG 54:2/18:0 | TG(15:0/18:1(d7)/15:0) | 904.7 | 603.4 | 40 |
| TG 52:5/20:4 | TG(15:0/18:1(d7)/15:0) | 870.6 | 549.3 | 40 |
| TG 54:6/20:4 | TG(15:0/18:1(d7)/15:0) | 896.6 | 575.3 | 40 |
| TG 54:7/20:4 | TG(15:0/18:1(d7)/15:0) | 894.6 | 573.3 | 40 |
| TG 56:4/20:4 | TG(15:0/18:1(d7)/15:0) | 928.8 | 607.5 | 40 |
| TG 56:6/20:4 | TG(15:0/18:1(d7)/15:0) | 924.7 | 603.4 | 40 |
| TG 56:7/20:4 | TG(15:0/18:1(d7)/15:0) | 922.7 | 601.4 | 40 |
| TG 58:5/20:4 | TG(15:0/18:1(d7)/15:0) | 954.7 | 633.4 | 40 |
| TG 58:7/20:4 | TG(15:0/18:1(d7)/15:0) | 950.7 | 629.4 | 40 |
| TG 58:8/22:6 | TG(15:0/18:1(d7)/15:0) | 948.7 | 603.4 | 40 |
| TG 60:7/22:6 | TG(15:0/18:1(d7)/15:0) | 978.7 | 633.4 | 40 |
| TG 60:8/22:6 | TG(15:0/18:1(d7)/15:0) | 976.7 | 631.4 | 40 |
| Sphingosine-1-phosphate d17:1 | N/A | 366.3 | 250.3 | 25 |
| Sphingosine-1-phosphate | Sphingosine d17:1 | 380.3 | 264.3 | 25 |
| Sphinganine-1-phosphate | Sphingosine d17:1 | 382.3 | 266.3 | 18 |
| GB3(d18:1/16:0) | GlcCer (d18:1(d5)/18:0) | 1025 | 520.5 | 40 |
| GB3(d18:1/18:0) | GlcCer (d18:1(d5)/18:0) | 1053 | 548.6 | 40 |
| GB3(d18:1/24:0) | GlcCer (d18:1(d5)/18:0) | 1137 | 632.6 | 40 |
| GB3(d18:1/24:1) | GlcCer (d18:1(d5)/18:0) | 1135 | 630.6 | 40 |
| Cer(d18:1/16:0(d7)) | N/A | 545.5 | 271.4 | 40 |
| LPC(26:0) | LPC(18:1(d7)) | 636.5 | 104.1 | 40 |
| LPC(24:0) | LPC(18:1(d7)) | 608.5 | 184.1 | 40 |
| LPC(26:1) | LPC(18:1(d7)) | 634.5 | 104.1 | 40 |
| LPC(24:1) | LPC(18:1(d7)) | 606.5 | 184.1 | 40 |
| LPC(16:1) | LPC(18:1(d7)) | 494.5 | 184.1 | 40 |
| Cer(d18:0/16:0) | Cer(d18:1/17:0) | 540.6 | 522.3 | 40 |
| Cer(d18:0/18:0) | Cer(d18:1/17:0) | 568.7 | 550.4 | 40 |
| Cer(d18:0/24:0) | Cer(d18:1/17:0) | 652.9 | 634.4 | 40 |
| Cer(d18:0/24:1) | Cer(d18:1/17:0) | 650.9 | 632.4 | 40 |
| Glc-Cholesterol | Cholesterol(d7) | 566.6 | 369.3 | 17 |
| Glc-Sitosterol | Cholesterol(d7) | 594.6 | 397.4 | 17 |
| hydroxy-cholesterol | Cholesterol(d7) | 385.3 | 367.3 | 30 |
| DG(18:0/18:1) | DG(15:0/18:1(d7)) | 640.4 | 341.3 | 30 |
| DG(18:1/18:1) | DG(15:0/18:1(d7)) | 638.4 | 339.3 | 30 |
| DG(16:0/20:4) | DG(15:0/18:1(d7)) | 634.5 | 313.3 | 30 |
| DG(18:0/20:4) | DG(15:0/18:1(d7)) | 662.5 | 341.3 | 30 |
| DG(18:0/22:6) | DG(15:0/18:1(d7)) | 686.6 | 341.3 | 30 |
| DG(18:1/20:4) | DG(15:0/18:1(d7)) | 660.5 | 339.3 | 30 |
| DG(15:0/18:1(d7)) | N/A | 605.6 | 346.5 | 30 |
| MG(18:1(d7)) | N/A | 381.3 | 272.5 | 22 |
| MG(20:4) | MG(18:1(d7)) | 396.3 | 287.3 | 22 |
| MG(18:0) | MG(18:1(d7)) | 376.3 | 267.3 | 22 |
| MG(18:1) | MG(18:1(d7)) | 374.3 | 265.3 | 22 |
| MG(16:1) | MG(18:1(d7)) | 346.3 | 237.3 | 22 |
| MG (16:0) | MG(18:1(d7)) | 348.3 | 239.3 | 22 |
| 7-keto-cholesterol | Cholesterol(d7) | 401.3 | 383.3 | 15 |
| 4-beta-hydroxycholesterol | Cholesterol(d7) | 420.3 | 385.3 | 15 |
| CE oxoODE | CE(18:1(d7)) | 680.6 | 369.2 | 25 |
| CE HODE | CE(18:1(d7)) | 682.6 | 369.2 | 25 |
| CE HpODE | CE(18:1(d7)) | 698.6 | 369.2 | 25 |
| Glc Sphingosine(d5) | N/A | 467.2 | 269.3 | 16 |

**Table 5:** Acquisition parameters for LC/MS analysis of lipid species in negative ionization mode.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Internal Std** | **Q1 m/z** | **Q3 m/z** | **CE (V)** |
| PA(15:0/18:1(d7)) | N/A | 666.52 | 241.3 | -50 |
| PA(16:0\_18:1) | PA(15:0/18:1(d7)) | 673.5 | 255.3 | -50 |
| PA(18:0\_18:1) | PA(15:0/18:1(d7)) | 701.5 | 283.3 | -50 |
| PA(18:1\_18:1) | PA(15:0/18:1(d7)) | 699.5 | 281.3 | -50 |
| PA(18:0\_20:4) | PA(15:0/18:1(d7)) | 723.5 | 283.3 | -50 |
| PA(18:1\_22:6) | PA(15:0/18:1(d7)) | 745.5 | 281.3 | -50 |
| PA(18:0\_22:6) | PA(15:0/18:1(d7)) | 747.5 | 283.3 | -50 |
| PE(15:0/18:1(d7)) | N/A | 709.56 | 241.3 | -50 |
| PE(P-18:0/18:1) | PE(15:0/18:1(d7)) | 728.6 | 283.3 | -50 |
| PE(P-18:0/18:2) | PE(15:0/18:1(d7)) | 726.6 | 281.3 | -50 |
| PE(P-16:0/20:4) | PE(15:0/18:1(d7)) | 722.6 | 303.3 | -50 |
| PE(P-18:0/20:4) | PE(15:0/18:1(d7)) | 750.6 | 303.3 | -50 |
| PE(P-16:0/22:6) | PE(15:0/18:1(d7)) | 746.6 | 327.3 | -50 |
| PE(P-18:0/22:6) | PE(15:0/18:1(d7)) | 774.6 | 327.3 | -50 |
| Sulfatide C12:0 | N/A | 722.5 | 97 | -150 |
| Sulfatide C16:0 | Sulfatide C12:0 | 778.5 | 97 | -150 |
| Sulfatide C18:0 | Sulfatide C12:0 | 806.6 | 97 | -150 |
| Sulfatide C18:0(OH) | Sulfatide C12:0 | 822.6 | 97 | -150 |
| Sulfatide C24:0 | Sulfatide C12:0 | 890.7 | 97 | -150 |
| Sulfatide C24:0(OH) | Sulfatide C12:0 | 906.7 | 97 | -150 |
| Sulfatide C24:1 | Sulfatide C12:0 | 888.7 | 97 | -150 |
| Sulfatide C24:1(OH) | Sulfatide C12:0 | 904.7 | 97 | -150 |
| GM3 (d36:1 (d5)) | N/A | 1184.8 | 290.1 | -65 |
| GM3(d34:1) | GM3 (d36:1 (d5)) | 1151.7 | 290.1 | -65 |
| GM3(d36:1) | GM3 (d36:1 (d5)) | 1179.8 | 290.1 | -65 |
| GM3(d38:1) | GM3 (d36:1 (d5)) | 1207.8 | 290.1 | -65 |
| GM3(d40:1) | GM3 (d36:1 (d5)) | 1235.8 | 290.1 | -65 |
| CL(14:0/14:0/14:0/14:0) | N/A | 619.5 | 227.2 | -50 |
| CL(72:8) | CL(14:0/14:0/14:0/14:0) | 723.7 | 279.3 | -50 |
| Cholesterol Sulfate (d7) | N/A | 472.3 | 96.7 | -80 |
| Cholesterol Sulfate | Sulfatide C12:0 | 465.3 | 96.7 | -80 |
| PG(15:0/18:1(d7)) | N/A | 740.55 | 241.3 | -50 |
| PG(16:0\_18:1) | PG(15:0/18:1(d7)) | 747.5 | 255.3 | -50 |
| PG(18:0\_18:1) | PG(15:0/18:1(d7)) | 775.5 | 283.3 | -50 |
| PG(18:1/18:1) | PG(15:0/18:1(d7)) | 773.5 | 281.3 | -50 |
| PG(18:0\_20:4) | PG(15:0/18:1(d7)) | 797.6 | 283.3 | -50 |
| PI(15:0/18:1(d7)) | N/A | 828.6 | 241.3 | -50 |
| PI(18:0\_18:1) | PI(15:0/18:1(d7)) | 863.6 | 283.3 | -50 |
| PI(18:1/18:1) | PI(15:0/18:1(d7)) | 861.6 | 281.3 | -50 |
| PI(16:0\_20:4) | PI(15:0/18:1(d7)) | 857.6 | 255.3 | -50 |
| PI(18:0\_20:4) | PI(15:0/18:1(d7)) | 885.6 | 283.3 | -50 |
| PI(16:0\_22:6) | PI(15:0/18:1(d7)) | 881.6 | 255.3 | -50 |
| PI(18:0\_22:6) | PI(15:0/18:1(d7)) | 909.6 | 283.3 | -50 |
| PI(20:4/20:4) | PI(15:0/18:1(d7)) | 905.6 | 303.3 | -50 |
| PS(15:0/18:1(d7)) | N/A | 753.55 | 241.3 | -50 |
| PS(18:0\_18:1) | PS(15:0/18:1(d7)) | 788.6 | 283.3 | -50 |
| PS(18:0\_20:4) | PS(15:0/18:1(d7)) | 810.6 | 283.3 | -50 |
| PS(16:0\_22:6) | PS(15:0/18:1(d7)) | 806.6 | 255.3 | -50 |
| PS(18:1\_22:6) | PS(15:0/18:1(d7)) | 832.6 | 281.3 | -50 |
| PS(18:0\_22:6) | PS(15:0/18:1(d7)) | 834.6 | 283.3 | -50 |

**Table 6: LC/MS analysis of BMP species.** BMP species were analyzed by liquid chromatography (Nexera X2, Shimadzu Scientific Instrument) coupled to electrospray mass spectrometry (Sciex 6500+ QTRAP, Sciex).

For each analysis, 5 µL of sample was injected on an ACQUITY UPLC BEH C18 1.7 µm, 2.1×100 mm column (Waters) using a flow rate of 0.35 mL/min at 62°C. Mobile phase A consisted of 60:40 acetonitrile/water with 10 mM ammonium acetate + 0.1% acetic acid. Mobile phase B consisted of 90:10 isopropyl alcohol/acetonitrile with 10 mM ammonium acetate +0.1% acetic acid. The gradient was programmed as follows: 0.0–0.01 min from 45% B to 99% B, 0.1–3.0 min at 99% B, 3.0–3.01 min to 45% B, and 3.01–3.50 min at 45% B. Electrospray ionization was performed in the negative-ion mode applying the following settings: curtain gas at 30 psi; collision gas was set at medium; ion spray voltage at -4500V; temperature at 600°C; ion source Gas 1 at 50 psi; Gas 2 at 60 psi; CE at -50V, collision cell exit potential at -15V; declustering potential at -80V; entrance potential at -10V. Data acquisition was performed using Analyst 1.6 (Sciex) in multiple reaction monitoring mode (MRM) with the precursor ion mass-to-charge ratio (Q1 m/z) and fragment ion mass-to-charge ratio (Q3 m/z) reported in Table 6. Peak areas were integrated using MultiQuant 3.02 (Sciex).

Table 6. Acquisition parameters for LC/MS analysis of BMP species.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Internal Standard** | **Q1 m/z** | **Q3 m/z** |
| BMP di14:0 (Std) | N/A | 665.3 | 227.2 |
| BMP  di20:4 | BMP di14:0\_(Std) | 817.5 | 303.3 |
| BMP di22:6 | BMP di14:0\_(Std) | 865.5 | 327.3 |
| BMP di18:1 | BMP di14:0\_(Std) | 773.5 | 281.3 |

**Table 7: LC/MS analysis of polar metabolites.** Metabolites were analyzed by liquid chromatography (Nexera X2, Shimadzu Scientific Instrument) coupled to electrospray mass spectrometry (Sciex 6500+ QTRAP, Sciex).

For each analysis, 5 µL of sample was injected on an ACQUITY UPLC BEH amide 1.7 µm, 2.1×150 mm column (Waters Corporation, Milford, Massachusetts, USA) using a flow rate of 0.40 mL/min at 40°C. Mobile phase A consisted of  water with 10 mM ammonium formate + 0.1% formic acid. Mobile phase B consisted of acetonitrile with 0.1% formic acid. The gradient was programmed as follows: 0.0–1.0 min at 95% B; 1.0–7.0 min to 50% B; 7.0–7.1 min to 95% B; and 7.1–10.0 min at 95% B. Electrospray ionization was performed in positive ion mode or applying the following settings: curtain gas at 30 psi; collision gas was set at medium; ion spray voltage at 5500V; temperature at 550°C; ion source Gas 1 at 55 psi; ion source Gas 2 at 60 psi. Data acquisition was performed using multiple reaction monitoring mode (MRM) with the following parameters: entrance potential (EP) at 10V; collision cell exit potential (CXP) at 12.5V; the precursor ion mass-to-charge ratio (Q1 m/z); fragment ion mass-to-charge ratio (Q3 m/z); collision energy (CE) and declustering potential (DP) for each species reported in Table 7. Peak areas were integrated using MultiQuant 3.02 (Sciex).

Table 7. Acquisition parameters for LC/MS analysis of polar metabolites.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Internal Std** | **Q1 m/z** | **Q3 m/z** | **DP (V)** | **CE (V)** |
| 1-Methylhistamine | D4C13-Arginine | 126 | 109 | 30 | 20 |
| 1-Methylhistidine | D4C13-Arginine | 170 | 124 | 30 | 20 |
| 1-Methylinosine | 15N4-Inosine | 283.1 | 151.1 | 30 | 20 |
| 1-Methylnicotinamide | 6C13-Phenylalanine | 137 | 94 | 30 | 20 |
| 1-Methylxanthine | 6C13-Phenylalanine | 167 | 110 | 30 | 30 |
| 3-Hydroxy-N6,N6,N6-Trimethyllysine | D4C13-Arginine | 205.16 | 128.07 | 40 | 25 |
| 3-Hydroxyanthranilic acid | 6C13-Phenylalanine | 154 | 136 | 30 | 18 |
| 3-Hydroxybutyric acid | 6C13-Phenylalanine | 105 | 87 | 30 | 20 |
| 3-Methoxytyramine | 6C13-Phenylalanine | 168.1 | 151 | 30 | 15 |
| 3-Methoxytyrosine | D3-Methionine | 212.1 | 166.1 | 30 | 20 |
| 4-Hydroxy-L-proline | D4-Alanine | 132 | 68.1 | 30 | 20 |
| 4-trimethylammoniobutanal | D4C13-Arginine | 130.12 | 71.05 | 40 | 20 |
| 5-Hydroxyindoleacetic acid | 6C13-Phenylalanine | 192.07 | 146.06 | 30 | 20 |
| 5-Methylthioadenosine | 13C10,15N5-Adenosine | 298 | 136 | 30 | 20 |
| 7-HOCA | 6C13-Phenylalanine | 431.3 | 395.3 | 80 | 15 |
| 7-Methylguanine | 15N4-Inosine | 166.11 | 124.05 | 50 | 25 |
| 7-Methylxanthine | 13C5-Hypoxanthine | 167 | 69 | 30 | 30 |
| 8-Hydroxy-2’-deoxyGuanosine | 15N4-Inosine | 284.1 | 140 | 30 | 20 |
| Adenine | 6C13-Phenylalanine | 136.1 | 119 | 40 | 35 |
| Adenosine | 13C10,15N5-Adenosine | 268.15 | 136.1 | 50 | 27 |
| Allantoin | 15N4-Inosine | 159.1 | 116 | 50 | 12 |
| Asymmetric dimethylarginine | N15C13-Glycine | 203.15 | 70.3 | 30 | 40 |
| Betaine | D9-Carnitine | 118.1 | 59.1 | 30 | 20 |
| Bilirubin | 6C13-Phenylalanine | 585.3 | 299.1 | 30 | 20 |
| Butyrobetaine | D9-Carnitine | 146.11 | 87.04 | 30 | 20 |
| Butyrylcarnitine | D9-Carnitine | 232.2 | 85 | 30 | 30 |
| Caffeine | D3-Palmitoylcarnitine | 195.1 | 138 | 30 | 20 |
| Choline | D3-Acetylcarnitine | 104 | 60 | 30 | 21 |
| Citrulline | D2-Citrulline | 176.1 | 113.1 | 20 | 12 |
| Cotinine | 6C13-Phenylalanine | 177.1 | 80 | 50 | 32 |
| Creatine | D4-Alanine | 132 | 90 | 30 | 18 |
| Creatinine | 15N4-Inosine | 114 | 44.1 | 30 | 20 |
| Cytidine | 6C13-Phenylalanine | 244.1 | 112 | 30 | 14 |
| Cytosine | 6C13-Phenylalanine | 112.09 | 94.9 | 30 | 25 |
| D-Glucose | 6C13-Phenylalanine | 202.8 | 202.8 | 30 | 10 |
| D-Mannose | 6C13-Phenylalanine | 202.81 | 202.81 | 50 | 15 |
| Decanoylcarnitine | D3-Octanoylcarnitine | 316.2 | 85 | 30 | 35 |
| Deoxyadenosine | 6C13-Phenylalanine | 252.1 | 136 | 30 | 20 |
| Deoxyguanosine | 6C13-Phenylalanine | 268.1 | 152 | 30 | 20 |
| Deoxyinosine | 6C13-Phenylalanine | 253 | 137 | 30 | 20 |
| Dimethylethanolamine | 15N4-Inosine | 90 | 72 | 30 | 15 |
| Dopamine | 6C13-Phenylalanine | 154 | 137 | 70 | 13 |
| EPA Carnitine | 6C13-Phenylalanine | 446.3 | 85 | 40 | 45 |
| Epinephrine | 6C13-Phenylalanine | 166 | 107 | 30 | 20 |
| Ergothioneine | D3-Methionine | 230.1 | 127 | 50 | 22 |
| Ethanolamine | D3-Methionine | 62 | 44.2 | 30 | 12 |
| gamma-Aminobutyric acid | 6C13-Phenylalanine | 104.1 | 87 | 55 | 13 |
| Glutathione | N15C13-Glycine | 308.1 | 179.05 | 66 | 17 |
| Glutathione Oxidized | 6C13-Phenylalanine | 613.2 | 355.1 | 110 | 31 |
| Glycine | 6C13-Phenylalanine | 76.04 | 30 | 10 | 25 |
| Guanine | 6C13-Phenylalanine | 152.2 | 110 | 30 | 20 |
| Guanosine | 6C13-Phenylalanine | 284.1 | 152 | 30 | 20 |
| Hexanoylcarnitine | D3-Octanoylcarnitine | 260.2 | 85 | 30 | 35 |
| Histamine | N15C13-Glycine | 112.09 | 95 | 20 | 20 |
| Homocysteine | D3-Methionine | 136.12 | 90.1 | 30 | 17 |
| Hypoxanthine | 13C5-Hypoxanthine | 137.2 | 118.8 | 30 | 20 |
| Imidazoleacetic acid | D4-Alanine | 127 | 81 | 30 | 20 |
| Indole | 6C13-Phenylalanine | 118 | 91 | 30 | 20 |
| Inosine | 15N4-Inosine | 269.1 | 137.01 | 30 | 20 |
| Isovalerylcarnitine | D3-Octanoylcarnitine | 246.2 | 85 | 30 | 33 |
| Kynurenic acid | 15N4-Inosine | 190.05 | 116 | 30 | 36 |
| L-3-Hydroxykynurenine | 6C13-Phenylalanine | 225.1 | 179.1 | 40 | 15 |
| L-Acetylcarnitine | D3-Acetylcarnitine | 204.1 | 85 | 30 | 25 |
| L-Alanine | D4-Alanine | 90.1 | 44.2 | 10 | 20 |
| L-Arginine | D4C13-Arginine | 175.12 | 70 | 40 | 27 |
| L-Asparagine | N15C13-Glycine | 133.06 | 74.02 | 20 | 15 |
| L-Aspartic acid | 6C13-Phenylalanine | 134.04 | 74.02 | 20 | 18 |
| L-Carnitine | D9-Carnitine | 162.1 | 103.05 | 30 | 20 |
| L-Cysteine | D4-Alanine | 122.03 | 76.02 | 20 | 15 |
| L-Cystine | D4C13-Arginine | 241 | 74 | 30 | 25 |
| L-Dopa | D4-Alanine | 198 | 152 | 30 | 20 |
| L-Glutamic acid | D3-Glutamate | 148.06 | 84.04 | 20 | 20 |
| L-Glutamine | D3-Glutamate | 147.08 | 84 | 20 | 15 |
| L-Histidine | D4C13-Arginine | 156.08 | 110.07 | 16 | 16 |
| L-Homoserine | N15C13-Glycine | 120.15 | 56.2 | 30 | 24 |
| L-Kynurenine | 6C13-Phenylalanine | 209 | 146 | 30 | 25 |
| L-Leucine | D3-Leucine | 132.1 | 86.01 | 20 | 15 |
| L-Lysine | D4C13-Arginine | 147.11 | 84 | 20 | 20 |
| L-Methionine | D3-Methionine | 150.06 | 104.05 | 15 | 10 |
| L-Palmitoylcarnitine | D3-Palmitoylcarnitine | 400 | 85 | 30 | 40 |
| L-Phenylalanine | 6C13-Phenylalanine | 166.09 | 120.08 | 20 | 18 |
| L-Proline | D4C13-Arginine | 116 | 70.1 | 20 | 20 |
| L-Serine | N15C13-Glycine | 106 | 60 | 10 | 16 |
| L-Threonine | N15C13-Glycine | 120 | 74 | 30 | 13 |
| L-Tryptophan | 6C13-Phenylalanine | 205.01 | 146.06 | 20 | 20 |
| L-Tyrosine | 6C13-Tyrosine | 182.1 | 136 | 20 | 15 |
| L-Valine | D8-Valine | 118.09 | 55 | 11 | 20 |
| Linoleyl carnitine | D3-Palmitoylcarnitine | 424.3 | 85 | 40 | 45 |
| Malondialdehyde | 6C13-Phenylalanine | 73 | 55 | 30 | 25 |
| Mannitol | 6C13-Phenylalanine | 183 | 69 | 30 | 20 |
| Valerobetaine | D3-Propionylcartinine | 160 | 55.3 | 30 | 21 |
| Myristoylcarnitine | D3-Palmitoylcarnitine | 372 | 85 | 30 | 40 |
| N-Acetylglutamic acid | 6C13-Phenylalanine | 189 | 130 | 30 | 20 |
| N-Acetylputrescine | 6C13-Phenylalanine | 131.1 | 114 | 30 | 12 |
| N-Acetylserine | 6C13-Phenylalanine | 148 | 106 | 30 | 14 |
| N-Acetylserotonin | 6C13-Phenylalanine | 219.11 | 160.07 | 30 | 15 |
| N-Alpha-acetyllysine | D4-Alanine | 189 | 84 | 30 | 18 |
| N1-Acetylspermidine | N15C13-Glycine | 188.1 | 171 | 100 | 19 |
| N1-Acetylspermine | 6C13-Phenylalanine | 245.2 | 100.1 | 30 | 22 |
| N6,N6,N6-Trimethyllysine | D4C13-Arginine | 189.16 | 84.08 | 40 | 20 |
| NAD | 6C13-Phenylalanine | 664.1 | 428.2 | 90 | 24 |
| Niacinamide | D3-Octanoylcarnitine | 123.06 | 80 | 30 | 24 |
| Nicotinamide riboside | D8-Valine | 255 | 123 | 90 | 30 |
| Nicotinic acid | 6C13-Phenylalanine | 124.1 | 80 | 90 | 30 |
| Nitrotyrosine | 6C13-Phenylalanine | 227.1 | 210 | 30 | 12 |
| Norepinephrine | D3-Methionine | 152 | 107 | 30 | 15 |
| Octanoylcarnitine | D3-Octanoylcarnitine | 288.2 | 85 | 30 | 33 |
| Ornithine | D2-Ornithine | 133 | 70 | 20 | 15 |
| Paraxanthine | D3-Palmitoylcarnitine | 181.1 | 124 | 30 | 24 |
| Phosphorylcholine | 6C13-Phenylalanine | 184 | 125 | 30 | 20 |
| Propionylcarnitine | D3-Propionylcartinine | 218.1 | 85 | 30 | 20 |
| Putrescine | N15C13-Glycine | 89 | 72 | 30 | 20 |
| Pyridoxamine | 6C13-Phenylalanine | 169 | 152 | 30 | 20 |
| Pyroglutamic acid | D3-Octanoylcarnitine | 130 | 84 | 20 | 15 |
| S-Adenosylhomocysteine | N15C13-Glycine | 385.1 | 136 | 30 | 21 |
| S-Adenosylmethionine | D4C13-Arginine | 399 | 250 | 30 | 20 |
| Sarcosine | 6C13-Phenylalanine | 90.04 | 44.1 | 30 | 20 |
| Serotonin | 6C13-Phenylalanine | 177 | 160 | 30 | 15 |
| Spermidine | 6C13-Phenylalanine | 146.16 | 72 | 42 | 17 |
| Spermine | 6C13-Phenylalanine | 203.1 | 129.1 | 60 | 15 |
| Sucrose | N15C13-Glycine | 364.8 | 202.8 | 30 | 35 |
| Taurine | D3-Methionine | 126.02 | 80 | 30 | 25 |
| Theophylline | D3-Palmitoylcarnitine | 181.13 | 124 | 30 | 20 |
| Thiamine | D3-Methionine | 265 | 122 | 30 | 25 |
| Thymine | 6C13-Phenylalanine | 127.05 | 110 | 30 | 20 |
| Trigonelline | D3-Methionine | 138.1 | 94.1 | 50 | 20 |
| Trimethylamine-N-oxide | D3-Propionylcartinine | 76.08 | 58.07 | 60 | 20 |
| Uracil | D3-Palmitoylcarnitine | 113 | 70 | 30 | 23 |
| Ureidopropionic acid | D3-Palmitoylcarnitine | 133 | 115 | 30 | 20 |
| Uridine | 13C5-Hypoxanthine | 245 | 113 | 30 | 18 |
| Xanthine | 15N4-Inosine | 153 | 110 | 30 | 20 |
| Xanthosine | 13C5-Xanthosine | 285 | 153 | 30 | 20 |
| alpha-Tocopherol | 6C13-Phenylalanine | 431.4 | 165.1 | 50 | 20 |
| 6C13-Tyrosine |  N/A | 188.1 | 142.1 | 20 | 24 |
| 6C13-Phenylalanine |  N/A | 172.1 | 126.1 | 20 | 18 |
| 13C10,15N5-Adenosine |  N/A | 283 | 146.1 | 50 | 27 |
| 13C3\_Thiamine | N/A | 268 | 122.07 | 30 | 25 |
| 13C5-Hypoxanthine |  N/A | 142.2 | 124 | 30 | 20 |
| 15N4-Inosine |  N/A | 273.1 | 141.01 | 30 | 20 |
| 13C5-Xanthosine |  N/A | 290 | 153 | 30 | 20 |
| D2-Citrulline |  N/A | 178.11 | 115.1 | 20 | 12 |
| D2-Ornithine |  N/A | 135.1 | 117.2 | 10 | 15 |
| D3-Acetylcarnitine |  N/A | 207.14 | 85 | 30 | 25 |
| D3-Creatinine |  N/A | 117 | 47.1 | 50 | 21 |
| D3-Glutamate |  N/A | 151 | 133.1 | 20 | 20 |
| D3-Leucine |  N/A | 135.1 | 89.1 | 20 | 15 |
| D3-Methionine |  N/A | 153.07 | 107 | 20 | 20 |
| D3-Octanoylcarnitine |  N/A | 291.2 | 85 | 30 | 33 |
| D3-Palmitoylcarnitine |  N/A | 403.35 | 85 | 30 | 40 |
| D3-Propionylcartinine |  N/A | 221.15 | 85 | 30 | 20 |
| D4-Alanine |  N/A | 94.08 | 48.1 | 20 | 25 |
| D4C13-Arginine |  N/A | 180.1 | 75 | 40 | 27 |
| D8-Valine |  N/A | 126 | 80.1 | 30 | 20 |
| D9-Carnitine |  N/A | 171.16 | 85 | 30 | 20 |
| N15C13-Glycine |  N/A | 78.04 | 32 | 10 | 25 |
| U-13C6 Glucose |  N/A | 208.8 | 208.8 | 30 | 10 |
| U-13C6 Sucrose |  N/A | 370.8 | 208.8 | 30 | 35 |
| (4-Hydroxy-3-methoxyphenyl)ethanol | 6C13-Phenylalanine | 151 | 90.8 | 110 | 25 |
| 3-Methylglutaconic acid | 6C13-Phenylalanine | 145 | 99 | 50 | 20 |
| 3,4-Dihydroxybenzeneacetic acid | 6C13-Phenylalanine | 169.2 | 169.2 | 30 | 30 |
| 3,4-Dihydroxyphenylacetaldehyde | 6C13-Phenylalanine | 153 | 135 | 50 | 18 |
| 4-hydroxynonenal | 6C13-Phenylalanine | 157.1 | 139.1 | 50 | 25 |
| 6-Aminouracil | 6C13-Phenylalanine | 128 | 85 | 100 | 19 |
| 7-Methylguanosine | D8-Valine | 298 | 166 | 30 | 20 |
| AICA-riboside | 15N4-Inosine | 259 | 110 | 30 | 20 |
| Dimethylglycine | D4-Alanine | 104.02 | 58 | 30 | 21 |
| Erythronic acid | N15C13-Glycine | 137 | 119 | 50 | 15 |
| Glycerophosphorylcholine | 6C13-Phenylalanine | 258.1 | 104 | 30 | 16 |
| L-Gulonic acid | 6C13-Phenylalanine | 197 | 61 | 50 | 20 |
| L-Methionine S-oxide | N15C13-Glycine | 166.05 | 74.02 | 30 | 20 |
| N-Acetyl Threonine | D3-Methionine | 162 | 144 | 50 | 25 |
| N-Acetylneuraminic acid | D4C13-Arginine | 310.1 | 274 | 50 | 15 |
| N-acetylvaline | 6C13-Phenylalanine | 160.1 | 72.1 | 50 | 18 |
| N-succinyladenosine | 6C13-Phenylalanine | 384.1 | 251 | 50 | 24 |
| NADH | 6C13-Phenylalanine | 666.1 | 649.1 | 50 | 22 |
| Ribonic acid | 6C13-Phenylalanine | 167 | 91 | 50 | 22 |
| Betaine aldehyde | 6C13-Phenylalanine | 102 | 58 | 30 | 20 |
| Isobutyrylglycine | 6C13-Phenylalanine | 146 | 100.1 | 30 | 15 |
| Methylsuccinic acid | D8-Valine | 132 | 86.9 | 30 | 20 |
| N-formylanthranilic acid | 6C13-Phenylalanine | 166.05 | 120 | 62 | 16 |
| Anthranilate | D3-Methionine | 138.1 | 92 | 30 | 20 |
| dopamine 3-o-sulfate | 13C5-Hypoxanthine | 234 | 217 | 30 | 15 |
| dopamine 4-o-sulfate | 13C5-Hypoxanthine | 234 | 217 | 30 | 15 |
| 3-methoxytyramine-sulfate | 6C13-Phenylalanine | 238 | 150 | 30 | 15 |
| N-acetyl-aspartyl glutamate | D3-Methionine | 305.1 | 148 | 30 | 15 |
| prolyhydroxyproline | N15C13-Glycine | 229.1 | 70.1 | 30 | 22 |
| Ascorbate | 6C13-Phenylalanine | 177 | 95 | 30 | 20 |
| Arabitol | 6C13-Phenylalanine | 153.1 | 69 | 30 | 20 |
| Arabonate | 6C13-Phenylalanine | 157.1 | 131 | 30 | 30 |
| N-acetyl glutamate | 6C13-Phenylalanine | 197.1 | 84 | 30 | 24 |
| N-(3-acetamidopropyl)pyrrolidin-2-one | D4C13-Arginine | 185.1 | 141.1 | 30 | 20 |
| Dihydrothymine | D8-Valine | 129.1 | 68.9 | 30 | 20 |
| Ureidoisobutyric acid | 6C13-Phenylalanine | 147.1 | 86 | 30 | 20 |
| Piperine | 6C13-Phenylalanine | 286.1 | 201.1 | 30 | 20 |
| N-acetylaspartate | 6C13-Phenylalanine | 176.06 | 176.06 | 30 | 10 |
| L-Arabinitol | D4C13-Arginine | 175.1 | 175.1 | 118 | 5 |
| Vanillic Acid | 6C13-Phenylalanine | 169.1 | 92.9 | 88 | 18 |
| Homovanillic Acid | 6C13-Phenylalanine | 200.1 | 137 | 65 | 19 |
| 3,4-Dihydroxyphenylacetic acid | 6C13-Phenylalanine | 186.1 | 123.1 | 47 | 23 |
| 3-Hydroxytyrosol | 13C5-Hypoxanthine | 137.2 | 118.8 | 30 | 20 |
| mz285\_RT4.5 | 13C5-Xanthosine | 285 | 153 | 30 | 20 |
| L-isoleucine | D3-Leucine | 132.1 | 86 | 20 | 15 |

**Table 8: LC/MS analysis of Cardiolipins (CL)**

CL species were analyzed by liquid chromatography (Nexera X2, Shimadzu Scientific Instrument) coupled to electrospray mass spectrometry (Sciex 6500+ QTRAP, Sciex).

For each analysis, 5µL of sample was injected on an ACQUITY UPLC BEH C18 1.7 µm, 2.1×100 mm column (Waters) using a flow rate of 0.3 mL/min at 55°C. Mobile phase A consisted of 60:40 acetonitrile/water with 10 mM ammonium acetate.  Mobile phase B consisted of 90:10 isopropyl alcohol/acetonitrile with 10 mM ammonium acetate. The gradient was programmed as follows: 0.0–3.5 min from 60% B to 99% B, 3.5-4.5 min keep at 99% B, 4.5–4.6 min to 60% B, and stop at 5 min. Electrospray ionization was performed in the negative-ion mode applying the following settings: curtain gas at 30 psi; collision gas was set at medium; ion spray voltage at -4500V; temperature at 600°C; ion source Gas 1 at 50 psi; Gas 2 at 60 psi; CE at -50V, collision cell exit potential at -15V; declustering potential at -80V; entrance potential at -10V. Data acquisition was performed using Analyst 1.6 (Sciex) in multiple reaction monitoring mode (MRM) with the precursor ion mass-to-charge ratio (Q1 m/z) and fragment ion mass-to-charge ratio (Q3 m/z) reported in Table 8. Peak areas were integrated using MultiQuant 3.02 (Sciex).

Table 8. Acquisition parameters for LC/MS analysis of cardiolipin species.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Internal Standard** | **Q1 m/z** | **Q3 m/z** |
| CL (58:0/14:0)\_IS |  N/A | 619.5 | 227.2 |
| CL (72:8/18:2) | CL 58:0/14:0\_IS | 723.7 | 279.2 |
| CL (72:9/18:2) | CL 58:0/14:0\_IS | 722.7 | 279.2 |
| CL (72:7/18:2) | CL 58:0/14:0\_IS | 724.7 | 279.2 |
| CL (74:10/18:2) | CL 58:0/14:0\_IS | 735.7 | 279.2 |
| CL (72:8/18:2 ox) | CL 58:0/14:0\_IS | 755.7 | 279.2 |

**Table 9: LC/MS analysis of uric acid.**

Uric acid was analyzed by liquid chromatography (Nexera X2, Shimadzu Scientific Instrument) coupled to electrospray mass spectrometry (Sciex 6500+ QTRAP, Sciex).

For each analysis, 5 µL of sample was injected on an ACQUITY UPLC BEH amide 1.7 µm, 2.1×150 mm column (Waters Corporation, Milford, Massachusetts, USA) using a flow rate of 0.50 mL/min at 55°C. Mobile phase A consisted of  water with 10 mM ammonium formate + 0.1% formic acid. Mobile phase B consisted of acetonitrile with 0.1% formic acid. The gradient was programmed as follows: 0.0–0.3 min at 85% B; 0.3–2 min to 65% B; 2.0–2.5 min back to 85% B; 2.5–2.55 to 10% B; 2.55–2.7min hold at 10% B; 2.7–2.75 min back to 85% B; hold to 3.2 min at 85% B. Electrospray ionization was performed in negative ion mode or applying the following settings: curtain gas at 30 psi; collision gas was set at medium; ion spray voltage at -4500V; temperature at 550°C; ion source Gas 1 at 55 psi; ion source Gas 2 at 60 psi. Data acquisition was performed using multiple reaction monitoring mode (MRM) with the following parameters: entrance potential (EP) at 10V; collision cell exit potential (CXP) at 12.5V; collision energy (CE), declustering potential (DP), precursor ion mass-to-charge ratio (Q1 m/z) and fragment ion mass-to-charge ratio (Q3 m/z) for each species reported in Table 9.

Table 9. Acquisition parameters for LC/MS analysis of Uric Acid under negative mode.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Internal Standard** | **Q1 m/z** | **Q3 m/z** | **DP** | **CE** |
| Uric acid-1,3-15N |  N/A | 169.1 | 125 | -50 | -17 |
| Uric acid | Uric acid-1,3-15N | 167.000 | 124 | -50 | -17 |