**CHEAR Urine Reference Material**

**Biocrates Metabolomics Methods**

**Sample Preparation Prior to Biocrates p180 Kit Plate Analysis:**

Urine: Parent and Sub-Aliquots

Removed bulk urine from -80°C storage and thawed urine by storing it at 4°C overnight. The following day, the thawed urine was inspected to ensure there was no ice remaining and the bulk urine was then mixed in the 4°C room by repeatedly inverting the container.

Parent Aliquots were quickly prepared in the 4°C room using the Drummond pipet aid and serological pipet. 42 mL urine were transferred to 50 mL tubes in 4°C room and capped immediately and placed on ice. If needed, the bulk urine was mixed in between aliquots. The parent aliquots were labeled appropriately.

Sub-Aliquotswere prepared on ice at the bench. The parent aliquots were mixed by inverting the tube thoroughly before and in between aliquots as needed. 2.5 mL urine was transferred to 5 mL cryovials and capped immediately and stored on ice until sample splitting was competed. The sub aliquots were labeled appropriately.

Sub-aliquots were stored at -80°C.

Urine Aliquots for LCMS platforms:

Used sub-aliquot “UP\_A\_03” to prepare aliquots for various LCMS platforms. Allowed sub-aliquot to thaw on ice for 30 – 60 min. Vortexed aliquot briefly on vortexer, centrifuged at 4 °C for 2 minutes at 16,000 rcf. Aliquoted out 6 tubes for Biocrates (30 µL each) from “UP\_A\_03”. Aliquots were stored at -80 °C until analysis.

Sample preparation for Biocrates Plate:

CHEAR urine samples were thawed on ice for 30–60 min. All samples were vortexed on a multi-tube vortexer for 4 mins at 5,000 rpm and centrifuged at 4 °C for 5 minutes at 16,000 rcf before loading on the p180 plate. The samples were then placed on ice in the analysis order for sample loading on the p180 Biocrates plate.

**Biocrates Plate Preparation:**

A Biocrates p180 kit was prepared following the Absolute*IDQ*™ p180 Kit metabolomics procedure. Briefly, an internal standard mix was added to 95 of the 96 wells. Next, zero samples, QC standards and calibration standards were added to their corresponding wells. The samples (20 µL) were then added to the appropriate wells and dried for 30 minutes under nitrogen flow. The plate was derivatized using a 5% phenylisothiocyanate (PITC) solution in (1:1:1) ethanol:pyridine:water and, then, incubated for 20 minutes at room temperature followed by a drying step under nitrogen flow. An extraction solvent (5 mM ammonium acetate in methanol) was added to all wells. The plate was then shaken and centrifuged. After centrifugation, 150 µL was removed and transferred to a second 96-well plate (LCMS plate). This second plate was diluted with 150 µL of HPLC grade water for a subsequent LCMS (MRM analysis) for measuring amino acids and biogenic amines. All wells in the original plate were diluted with 400 µL of flow injection analysis (FIA) Running Solvent for a FIA-MS (MRM analysis) for measuring lipids, acylcarnitines, and hexose.

**MS Data Collection**

**LC-MS Method:**

LC-MS spectra were collected for all samples. LC was performed on an Agilent 1100 HPLC with an Agilent XDB-C18 column (2.1x 100mm x 3.5 µm) at 50 °C using a reversed-phase gradient separation. Water with 0.2% formic acid (mobile phase A) and acetonitrile with 0.2% formic acid (mobile phase B) were used as mobile phases and the metabolites were chromatographically separated using a gradient separation: (see below for the gradient information). Mass spectroscopy analysis was performed on an Applied BioSystem API 4000 using a 10 µL injection volume and scheduled MRM data collection in positive ion mode.

**LCMS Methods Parameters**

|  |  |
| --- | --- |
| **Mass Spectrometer** | API 4000 Triple Quadrupole with Turbo Ion Spray source |
| **HPLC** | Agilent 1100 |

|  |  |
| --- | --- |
| **Column** | Agilent XDB-C18, 3.5 µm (2.1 x 100mm); Temperature = 50 °C |
| **Injection Volume** | 10 µl |
| **Mobile Phases** | **MPA:** Water with 0.2% Formic Acid  **MPB**: Acetonitrile with 0.2% Formic Acid |
| **Flow Rate** | 0.5 mL/min |
| **Internal Standard** | Multiple labeled internal standards are used and listed in table below |

**LCMS Parameters**

Time(min) Flow Rate (mL/min) %A %B Curve

Initial 0.500 100.0 0.0 6

0.50 0.500 100.0 0.0 6

5.50 0.500 5.0 95.0 6

6.50 0.500 5.0 95.0 6

7.00 0.500 100.0 0.0 6

9.50 0.500 100.0 0.0 6

**MS Parameters**

Scan Type: MRM (MRM)

Scheduled MRM: Yes

Polarity: Positive

Ion Source: Turbo Spray

MRM detection window: 30 sec

Target Scan Time: 0.5 sec

CUR: 20.00

TEM: 500.00

GS1: 40.00

GS2: 50.00

ihe: ON

IS: 5500.00

CAD: 6.00

**List of LCMS MRMs**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Q1 Mass (Da)** | **Q3 Mass (Da)** | **Time (min)** | **ID** | **DP** | **EP** | **CE** | **CXP** |
| 114.1 | 44.1 | 0.9 | Creatinine | 60 | 10 | 17 | 15 |
| 117.1 | 47.1 | 0.9 | d3-Creatinine | 60 | 10 | 17 | 15 |
| 211.2 | 75.9 | 5.2 | Gly | 66 | 10 | 14 | 15 |
| 214.2 | 78.9 | 5.2 | 13C2-15N-Gly | 66 | 10 | 14 | 15 |
| 225.2 | 44.2 | 5.5 | Ala | 61 | 10 | 35 | 15 |
| 229.2 | 48.2 | 5.5 | d4-Ala | 61 | 10 | 35 | 15 |
| 241.2 | 60 | 5.1 | Ser | 41 | 10 | 31 | 15 |
| 244.2 | 63 | 5.1 | d3-Ser | 41 | 10 | 31 | 15 |
| 247 | 154 | 4.5 | Histamine | 56 | 10 | 19 | 15 |
| 251.2 | 70.3 | 5.5 | Pro | 51 | 10 | 43 | 15 |
| 253.2 | 72.2 | 6.1 | Val | 81 | 10 | 31 | 15 |
| 255.2 | 74.1 | 5.3 | Thr | 66 | 10 | 29 | 15 |
| 257.2 | 105.2 | 6.9 | PEA | 71 | 10 | 31 | 15 |
| 258.2 | 77.3 | 5.5 | d7-Pro | 51 | 10 | 43 | 15 |
| 259.2 | 77.1 | 5.3 | 13C4-15N-Thr | 66 | 10 | 29 | 15 |
| 261 | 126.1 | 4.6 | Taurine | 71 | 10 | 21 | 15 |
| 261.2 | 80.2 | 6.1 | d8-Val | 81 | 10 | 31 | 15 |
| 263 | 128 | 4.6 | 13C2-Taurine | 61 | 10 | 21 | 15 |
| 266.1 | 113.9 | 6.4 | Putrescine | 100 | 10 | 25 | 15 |
| 267.1 | 68 | 5.1 | c4-OH-Pro | 56 | 10 | 61 | 15 |
| 267.15 | 68 | 4.9 | t4-OH-Pro | 56 | 10 | 61 | 15 |
| 267.3 | 43 | 6.3 | Leu | 55 | 10 | 66 | 15 |
| 267.3 | 69 | 6.4 | Ile | 55 | 10 | 45 | 15 |
| 268.2 | 87 | 5.0 | Asn | 41 | 10 | 25 | 15 |
| 269.2 | 116.2 | 5.2 | Asp | 76 | 10 | 21 | 15 |
| 270.2 | 89.2 | 5.0 | 15N2-Asn | 66 | 10 | 27 | 15 |
| 272.2 | 119.2 | 5.2 | d3-Asp | 76 | 10 | 21 | 15 |
| 273.2 | 91.2 | 6.4 | 13C6-Ile | 66 | 10 | 27 | 15 |
| 282.2 | 130 | 5.0 | Gln | 71 | 10 | 25 | 15 |
| 283.2 | 130.2 | 5.2 | Glu | 76 | 10 | 23 | 15 |
| 285.1 | 104.2 | 6.0 | Met | 71 | 10 | 25 | 15 |
| 286.2 | 133.2 | 5.2 | d3-Glu | 76 | 10 | 23 | 15 |
| 287.2 | 135 | 5.0 | d5-Gln | 71 | 10 | 25 | 15 |
| 288.1 | 107.2 | 6.0 | d3-Met | 71 | 10 | 25 | 15 |
| 289.2 | 137.2 | 5.4 | Dopamine | 56 | 10 | 29 | 15 |
| 291.1 | 110.2 | 4.5 | His | 56 | 10 | 33 | 15 |
| 293.1 | 141.1 | 5.8 | d4-Dopamine | 56 | 10 | 29 | 15 |
| 297.1 | 115.2 | 4.5 | 13C6-His | 56 | 10 | 33 | 15 |
| 297.1 | 144.2 | 5.3 | alpha-AAA | 46 | 10 | 23 | 15 |
| 301.2 | 120.2 | 6.4 | Phe | 71 | 10 | 31 | 15 |
| 301.2 | 88.1 | 5.1 | Met-SO | 61 | 10 | 41 | 15 |
| 306.2 | 125.2 | 6.4 | d5-Phe | 71 | 10 | 31 | 15 |
| 310 | 217 | 4.6 | Arg | 76 | 10 | 23 | 15 |
| 310.2 | 217.3 | 5.2 | Ac-Orn | 46 | 10 | 15 | 15 |
| 311.2 | 113.1 | 5.0 | Cit | 56 | 10 | 29 | 15 |
| 312 | 219 | 4.6 | 15N2-Arg | 76 | 10 | 23 | 15 |
| 312.3 | 160.2 | 5.9 | Serotonin | 66 | 10 | 27 | 15 |
| 316.1 | 118.2 | 5.3 | 13C-d4-Cit | 56 | 10 | 29 | 15 |
| 316.3 | 164.2 | 5.9 | d4-Serotonin | 66 | 10 | 27 | 15 |
| 317.2 | 136.1 | 5.7 | Tyr | 76 | 10 | 31 | 15 |
| 321.2 | 140.1 | 5.7 | d4-Tyr | 76 | 10 | 31 | 15 |
| 333.1 | 198.1 | 5.5 | DOPA | 61 | 10 | 19 | 15 |
| 336.1 | 201.1 | 5.5 | d3-DOPA | 56 | 10 | 19 | 15 |
| 338.2 | 46 | 4.8 | ADMA | 71 | 10 | 61 | 15 |
| 338.2 | 70.1 | 4.8 | total DMA | 71 | 10 | 61 | 15 |
| 338.2 | 307 | 4.8 | SDMA | 81 | 10 | 17 | 15 |
| 340.2 | 188.2 | 6.2 | Trp | 96 | 10 | 25 | 15 |
| 342.2 | 189.2 | 6.2 | 15N2-Trp | 96 | 10 | 25 | 15 |
| 344.2 | 146.2 | 6.2 | Kynurenine | 46 | 10 | 33 | 15 |
| 345.2 | 77.2 | 4.8 | d7-ADMA | 71 | 10 | 61 | 15 |
| 362.2 | 110.1 | 4.5 | Carnosine | 46 | 10 | 47 | 15 |
| 362.2 | 136.1 | 6.2 | Nitro-Tyr | 65 | 10 | 41 | 15 |
| 363.2 | 270.1 | 6.4 | d4-Putrescine | 51 | 10 | 19 | 15 |
| 403.2 | 310.2 | 6.1 | Orn | 76 | 10 | 19 | 15 |
| 409.2 | 316.2 | 6.1 | d6-Orn | 76 | 10 | 19 | 15 |
| 417.2 | 324.2 | 6.2 | Lys | 81 | 10 | 19 | 15 |
| 551.2 | 193.2 | 6.9 | Spermidine | 71 | 10 | 41 | 15 |
| 743.3 | 193.2 | 7.2 | Spermine | 56 | 10 | 61 | 15 |
| 559.2 | 193.2 | 6.9 | d8-Spermidine | 71 | 10 | 41 | 15 |
| 751.3 | 193.2 | 7.2 | d8-Spermine | 56 | 10 | 61 | 15 |

Expected Calibrator Concentrations

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **20000611 Cal1** | **20000612 Cal2** | **20000613 Cal3** | **20000614 Cal4** | **20000615 Cal5** | **20000616 Cal6** | **20000617 Cal7** |
| **Ala** | 20 | 40 | 200 | 400 | 800 | 1200 | 1600 |
| **Arg** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Asn** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Asp** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Cit** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Gln** | 20 | 40 | 200 | 400 | 800 | 1200 | 1600 |
| **Glu** | 10 | 20 | 100 | 200 | 400 | 600 | 800 |
| **Gly** | 25 | 50 | 250 | 500 | 1000 | 1500 | 2000 |
| **His** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Ile** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Leu** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Lys** | 10 | 20 | 100 | 200 | 400 | 600 | 800 |
| **Met** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Orn** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Phe** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Pro** | 10 | 20 | 100 | 200 | 400 | 600 | 800 |
| **Ser** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Thr** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Trp** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Tyr** | 5 | 10 | 50 | 100 | 200 | 300 | 400 |
| **Val** | 10 | 20 | 100 | 200 | 400 | 600 | 800 |
| **Ac-Orn** | 0.5 | 1 | 5 | 10 | 20 | 30 | 40 |
| **ADMA** | 0.25 | 0.5 | 2.5 | 5 | 10 | 15 | 20 |
| **SDMA** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **total DMA** | 1.25 | 2.5 | 12.5 | 25 | 50 | 75 | 100 |
| **alpha-AAA** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **Carnosine** | 0.5 | 1 | 5 | 10 | 20 | 30 | 40 |
| **Creatinine** | 10 | 20 | 100 | 200 | 400 | 600 | 800 |
| **DOPA** | 0.5 | 1 | 5 | 10 | 20 | 30 | 40 |
| **Dopamine** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **Histamine** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **Kynurenine** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **Met-SO** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **Nitro-Tyr** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **c4-OH-Pro** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **t4-OH-Pro** | 1 | 2 | 10 | 20 | 40 | 60 | 80 |
| **PEA** | 0.1 | 0.2 | 1 | 2 | 4 | 6 | 8 |
| **Putrescine** | 0.1 | 0.2 | 1 | 2 | 4 | 6 | 8 |
| **Serotonin** | 0.1 | 0.2 | 1 | 2 | 4 | 6 | 8 |
| **Spermidine** | 0.25 | 0.5 | 2.5 | 5 | 10 | 15 | 20 |
| **Spermine** | 0.25 | 0.5 | 2.5 | 5 | 10 | 15 | 20 |
| **Taurine** | 2.5 | 5 | 25 | 50 | 100 | 150 | 200 |

**FIA Method:**

FIA-MS spectra were collected for all samples. FIA was performed using an Agilent 1100 HPLC without a column. Methanol with Biocrates FIA solvent I (mobile phase B) was used to transport sample from the injector to the source (see below for the flow rate information). Mass spectroscopy analysis was performed on an Applied BioSystem API 4000 using a 20 µL injection volume and MRM data collection in positive and negative ion modes.

**FIA-MS Methods Parameters**

|  |  |
| --- | --- |
| **Mass Spectrometer** | API 4000 Triple Quadrupole with Turbo Ion Spray source |
| **HPLC** | Agilent 1100 |

|  |  |
| --- | --- |
| **Column** | none |
| **Injection Volume** | 20 µl |
| **Mobile Phases** | **MPA:**  **MPB**: Methanol + Biocrates FIA Solvent I |
| **Flow Rate** | See below |
| **Internal Standard** | Class specific labeled internal standards are used |

**LCMS Parameters (Positive and Negative Ion FIA-MS)**

Time(min) Flow Rate(mL) %A %B Curve

0.00 0.020 0.0 100.0 6

0.50 0.020 0.0 100.0 6

1.00 0.030 0.0 100.0 6

2.40 0.200 0.0 100.0 6

2.80 0.200 0.0 100.0 6

3.00 0.030 0.0 100.0 6

**MS Parameters (Positive)**

Scan Type: MRM (MRM)

Scheduled MRM: no

Polarity: Positive

Ion Source: Turbo Spray

MRM cycle time: 3.1050 sec

CUR: 20.00

TEM: 190.00

GS1: 25.00

GS2: 40.00

ihe: ON

IS: 5500.00

CAD: 6.00

EP: 10.0

CXP: 15.0

**List of Positive Ion FIA-MS MRMs**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Q1 Mass (Da)** | **Q3 Mass (Da)** | **Time (msec)** | **ID** | **DP** | **EP** | **CE** | **CXP** |
| 398.2 | 184 | 10 |  | 76 | 10 | 30 | 15 |
| 468.3 | 184 | 10 |  | 79 | 10 | 30 | 15 |
| 494.3 | 184 | 10 |  | 79 | 10 | 30 | 15 |
| 496.3 | 184 | 10 |  | 81 | 10 | 31 | 15 |
| 510.3 | 184 | 10 |  | 82 | 10 | 31 | 15 |
| 520.3 | 184 | 10 |  | 82 | 10 | 31 | 15 |
| 522.3 | 184 | 10 |  | 82 | 10 | 31 | 15 |
| 524.3 | 184 | 10 |  | 85 | 10 | 31 | 15 |
| 544.3 | 184 | 10 |  | 85 | 10 | 31 | 15 |
| 546.3 | 184 | 10 |  | 87 | 10 | 31 | 15 |
| 563.4 | 184 | 10 |  | 94 | 10 | 32 | 15 |
| 608.4 | 184 | 10 |  | 96 | 10 | 33 | 15 |
| 622.4 | 184 | 10 |  | 98 | 10 | 33 | 15 |
| 634.4 | 184 | 10 |  | 98 | 10 | 33 | 15 |
| 636.5 | 184 | 10 |  | 101 | 10 | 34 | 15 |
| 650.5 | 184 | 10 |  | 103 | 10 | 35 | 15 |
| 662.5 | 184 | 10 |  | 103 | 10 | 35 | 15 |
| 664.5 | 184 | 10 |  | 105 | 10 | 35 | 15 |
| 676.5 | 184 | 10 |  | 106 | 10 | 35 | 15 |
| 678.5 | 184 | 10 |  | 108 | 10 | 36 | 15 |
| 688.5 | 184 | 10 |  | 108 | 10 | 36 | 15 |
| 689.6 | 184 | 10 |  | 108 | 10 | 36 | 15 |
| 690.5 | 184 | 10 |  | 109 | 10 | 36 | 15 |
| 692.6 | 184 | 10 |  | 111 | 10 | 37 | 15 |
| 701.6 | 184 | 10 |  | 111 | 10 | 37 | 15 |
| 702.5 | 184 | 10 |  | 111 | 10 | 37 | 15 |
| 703.6 | 184 | 10 |  | 112 | 10 | 37 | 15 |
| 706.5 | 184 | 10 |  | 114 | 10 | 37 | 15 |
| 716.6 | 184 | 10 |  | 114 | 10 | 37 | 15 |
| 717.6 | 184 | 10 |  | 114 | 10 | 38 | 15 |
| 718.6 | 184 | 10 |  | 115 | 10 | 38 | 15 |
| 728.5 | 184 | 10 |  | 117 | 10 | 38 | 15 |
| 729.6 | 184 | 10 |  | 117 | 10 | 38 | 15 |
| 730.5 | 184 | 10 |  | 117 | 10 | 38 | 15 |
| 731.6 | 184 | 10 |  | 117 | 10 | 38 | 15 |
| 732.6 | 184 | 10 |  | 118 | 10 | 39 | 15 |
| 734.6 | 184 | 10 |  | 119 | 10 | 39 | 15 |
| 742.6 | 184 | 10 |  | 120 | 10 | 39 | 15 |
| 744.6 | 184 | 10 |  | 120 | 10 | 39 | 15 |
| 746.6 | 184 | 10 |  | 121 | 10 | 39 | 15 |
| 748.6 | 184 | 10 |  | 122 | 10 | 40 | 15 |
| 754.5 | 184 | 10 |  | 122 | 10 | 40 | 15 |
| 755.6 | 184 | 10 |  | 123 | 10 | 40 | 15 |
| 756.6 | 184 | 10 |  | 123 | 10 | 40 | 15 |
| 758.6 | 184 | 10 |  | 123 | 10 | 40 | 15 |
| 760.6 | 184 | 10 |  | 125 | 10 | 41 | 15 |
| 766.6 | 184 | 10 |  | 125 | 10 | 41 | 15 |
| 768.6 | 184 | 10 |  | 126 | 10 | 41 | 15 |
| 770.6 | 184 | 10 |  | 126 | 10 | 41 | 15 |
| 772.6 | 184 | 10 |  | 127 | 10 | 41 | 15 |
| 774.6 | 184 | 10 |  | 127 | 10 | 41 | 15 |
| 776.7 | 184 | 10 |  | 128 | 10 | 42 | 15 |
| 778.5 | 184 | 10 |  | 128 | 10 | 42 | 15 |
| 780.6 | 184 | 10 |  | 128 | 10 | 42 | 15 |
| 781.6 | 184 | 10 |  | 129 | 10 | 42 | 15 |
| 782.6 | 184 | 10 |  | 129 | 10 | 42 | 15 |
| 784.6 | 184 | 10 |  | 130 | 10 | 42 | 15 |
| 786.6 | 184 | 10 |  | 130 | 10 | 42 | 15 |
| 788.6 | 184 | 10 |  | 131 | 10 | 43 | 15 |
| 790.6 | 184 | 10 |  | 131 | 10 | 43 | 15 |
| 792.6 | 184 | 10 |  | 132 | 10 | 43 | 15 |
| 794.6 | 184 | 10 |  | 132 | 10 | 43 | 15 |
| 796.6 | 184 | 10 |  | 133 | 10 | 43 | 15 |
| 798.6 | 184 | 10 |  | 133 | 10 | 43 | 15 |
| 799.7 | 184 | 10 |  | 133 | 10 | 43 | 15 |
| 800.7 | 184 | 10 |  | 133 | 10 | 43 | 15 |
| 801.7 | 184 | 10 |  | 134 | 10 | 43 | 15 |
| 802.7 | 184 | 10 |  | 134 | 10 | 44 | 15 |
| 804.7 | 184 | 10 |  | 135 | 10 | 44 | 15 |
| 806.6 | 184 | 10 |  | 135 | 10 | 44 | 15 |
| 808.6 | 184 | 10 |  | 136 | 10 | 44 | 15 |
| 810.6 | 184 | 10 |  | 136 | 10 | 44 | 15 |
| 812.6 | 184 | 10 |  | 136 | 10 | 44 | 15 |
| 813.7 | 184 | 10 |  | 137 | 10 | 45 | 15 |
| 815.7 | 184 | 10 |  | 137 | 10 | 45 | 15 |
| 816.7 | 184 | 10 |  | 138 | 10 | 45 | 15 |
| 818.7 | 184 | 10 |  | 138 | 10 | 45 | 15 |
| 820.6 | 184 | 10 |  | 139 | 10 | 45 | 15 |
| 822.6 | 184 | 10 |  | 139 | 10 | 45 | 15 |
| 824.7 | 184 | 10 |  | 140 | 10 | 45 | 15 |
| 826.7 | 184 | 10 |  | 140 | 10 | 46 | 15 |
| 828.7 | 184 | 10 |  | 141 | 10 | 46 | 15 |
| 829.7 | 184 | 10 |  | 141 | 10 | 46 | 15 |
| 830.7 | 184 | 10 |  | 141 | 10 | 46 | 15 |
| 834.6 | 184 | 10 |  | 143 | 10 | 46 | 15 |
| 836.6 | 184 | 10 |  | 143 | 10 | 47 | 15 |
| 838.6 | 184 | 10 |  | 144 | 10 | 47 | 15 |
| 840.7 | 184 | 10 |  | 144 | 10 | 47 | 15 |
| 841.7 | 184 | 10 |  | 144 | 10 | 47 | 15 |
| 842.7 | 184 | 10 |  | 144 | 10 | 47 | 15 |
| 843.7 | 184 | 10 |  | 145 | 10 | 47 | 15 |
| 844.7 | 184 | 10 |  | 145 | 10 | 47 | 15 |
| 846.7 | 184 | 10 |  | 146 | 10 | 48 | 15 |
| 850.7 | 184 | 10 |  | 147 | 10 | 48 | 15 |
| 852.7 | 184 | 10 |  | 148 | 10 | 48 | 15 |
| 854.7 | 184 | 10 |  | 148 | 10 | 48 | 15 |
| 856.7 | 184 | 10 |  | 149 | 10 | 48 | 15 |
| 858.7 | 184 | 10 |  | 141 | 10 | 46 | 15 |
| 860.8 | 184 | 10 |  | 150 | 10 | 49 | 15 |
| 862.6 | 184 | 10 |  | 150 | 10 | 49 | 15 |
| 864.7 | 184 | 10 |  | 151 | 10 | 49 | 15 |
| 866.7 | 184 | 10 |  | 152 | 10 | 50 | 15 |
| 870.7 | 184 | 10 |  | 153 | 10 | 50 | 15 |
| 872.7 | 184 | 10 |  | 153 | 10 | 50 | 15 |
| 874.7 | 184 | 10 |  | 154 | 10 | 50 | 15 |
| 876.7 | 184 | 10 |  | 154 | 10 | 50 | 15 |
| 878.7 | 184 | 10 |  | 155 | 10 | 51 | 15 |
| 880.7 | 184 | 10 |  | 156 | 10 | 51 | 15 |
| 882.7 | 184 | 10 |  | 156 | 10 | 51 | 15 |
| 162.1 | 85.1 | 25 |  | 61 | 10 | 27 | 15 |
| 165.1 | 85.1 | 25 |  | 61 | 10 | 27 | 15 |
| 204.1 | 85.1 | 25 |  | 41 | 10 | 27 | 15 |
| 207.1 | 85.1 | 25 |  | 41 | 10 | 27 | 15 |
| 216.1 | 85.1 | 25 |  | 49 | 10 | 27 | 15 |
| 218.1 | 85.1 | 25 |  | 46 | 10 | 29 | 15 |
| 230.1 | 85.1 | 25 |  | 52 | 10 | 29 | 15 |
| 232.2 | 85.1 | 25 |  | 46 | 10 | 29 | 15 |
| 235.2 | 85.1 | 25 |  | 46 | 10 | 29 | 15 |
| 234.1 | 85.1 | 25 |  | 53 | 10 | 30 | 15 |
| 244.2 | 85.1 | 25 |  | 55 | 10 | 31 | 15 |
| 246.2 | 85.1 | 25 |  | 46 | 10 | 29 | 15 |
| 255.2 | 85.1 | 25 |  | 46 | 10 | 29 | 15 |
| 248.1 | 85.1 | 25 |  | 55 | 10 | 32 | 15 |
| 258.2 | 85.1 | 25 |  | 57 | 10 | 33 | 15 |
| 260.2 | 85.1 | 25 |  | 56 | 10 | 27 | 15 |
| 263.2 | 85.1 | 25 |  | 56 | 10 | 27 | 15 |
| 262.2 | 85.1 | 25 |  | 58 | 10 | 33 | 15 |
| 274.1 | 85.1 | 25 |  | 60 | 10 | 35 | 15 |
| 276.2 | 85.1 | 25 |  | 61 | 10 | 35 | 15 |
| 288.2 | 85.1 | 25 |  | 66 | 10 | 33 | 15 |
| 291.2 | 85.1 | 25 |  | 66 | 10 | 33 | 15 |
| 302.2 | 85.1 | 25 |  | 66 | 10 | 39 | 15 |
| 304.2 | 85.1 | 25 |  | 66 | 10 | 39 | 15 |
| 312.2 | 85.1 | 25 |  | 67 | 10 | 40 | 15 |
| 314.2 | 85.1 | 25 |  | 68 | 10 | 40 | 15 |
| 316.2 | 85.1 | 25 |  | 56 | 10 | 37 | 15 |
| 319.3 | 85.1 | 25 |  | 56 | 10 | 37 | 15 |
| 342.3 | 85.1 | 25 |  | 73 | 10 | 44 | 15 |
| 344.3 | 85.1 | 25 |  | 73 | 10 | 44 | 15 |
| 368.3 | 85.1 | 25 |  | 78 | 10 | 47 | 15 |
| 370.3 | 85.1 | 25 |  | 78 | 10 | 47 | 15 |
| 372.3 | 85.1 | 25 |  | 86 | 10 | 45 | 15 |
| 374.3 | 85.1 | 25 |  | 86 | 10 | 45 | 15 |
| 384.3 | 85.1 | 25 |  | 81 | 10 | 49 | 15 |
| 386.3 | 85.1 | 25 |  | 81 | 10 | 50 | 15 |
| 396.3 | 85.1 | 25 |  | 83 | 10 | 51 | 15 |
| 398.3 | 85.1 | 25 |  | 84 | 10 | 51 | 15 |
| 400.3 | 85.1 | 25 |  | 84 | 10 | 51 | 15 |
| 403.3 | 85.1 | 25 |  | 84 | 10 | 51 | 15 |
| 412.3 | 85.1 | 25 |  | 86 | 10 | 53 | 15 |
| 414.3 | 85.1 | 25 |  | 87 | 10 | 53 | 15 |
| 416.3 | 85.1 | 25 |  | 87 | 10 | 53 | 15 |
| 424.3 | 85.1 | 25 |  | 89 | 10 | 54 | 15 |
| 426.4 | 85.1 | 25 |  | 89 | 10 | 55 | 15 |
| 428.4 | 85.1 | 25 |  | 96 | 10 | 63 | 15 |
| 431.4 | 85.1 | 25 |  | 96 | 10 | 63 | 15 |
| 442.4 | 85.1 | 25 |  | 92 | 10 | 57 | 15 |
| 290.2 | 85.1 | 25 |  | 63 | 10 | 37 | 15 |

**MS Parameters (Negative)**

Scan Type: MRM (MRM)

Scheduled MRM: no

Polarity: Negative

Ion Source: Turbo Spray

MRM cycle time: 0.4100 sec

CUR: 20.00

TEM: 190.00

GS1: 25.00

GS2: 40.00

ihe: ON

IS: -4500.00

CAD: 6.00

DP: -55.0

EP: -10.0

CE: -12

CXP: -15.0

**List of Negative Ion FIA-MS MRMs**

|  |  |  |
| --- | --- | --- |
| **Q1 Mass (Da)** | **Q3 Mass (Da)** | **Time (msec)** |
| 179 | 89 | 200 |
| 185 | 92 | 200 |