**Supporting Information**

# Phenotypic mapping of metabolic profiles using self-organizing maps of high-dimensional mass spectrometry data

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**Abstract**

A metabolic system is comprised of inherently interconnected metabolic precursor, intermediates, and products. The analysis of untargeted metabolomics data has conventionally been performed through the use comparative statistics or multivariate statistical analysis-based approaches; however, each falls short in representing the related nature of metabolic perturbations. Herein, we describe a complementary method for the analysis of large metabolite inventories using a data-driven approach based upon a self-organizing map algorithm. This work flow allows for the unsupervised clustering, and subsequent prioritization, of correlated features through Gestalt comparisons of metabolic heat maps. We describe this methodology in detail, including a comparison to conventional metabolomics approaches, and demonstrate the application of this method to the analysis of the metabolic repercussions of prolonged cocaine exposure upon rat sera profiles.

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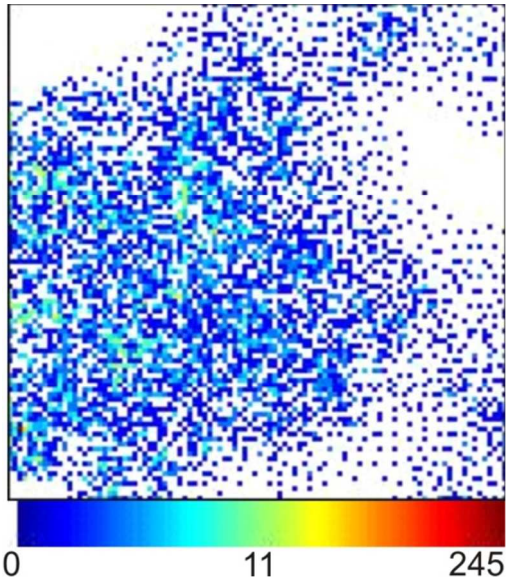


**Figure S1.** Density map for Figure 2(b). Color indicates the density of features per node. These features are then summed to determine the node intensity.

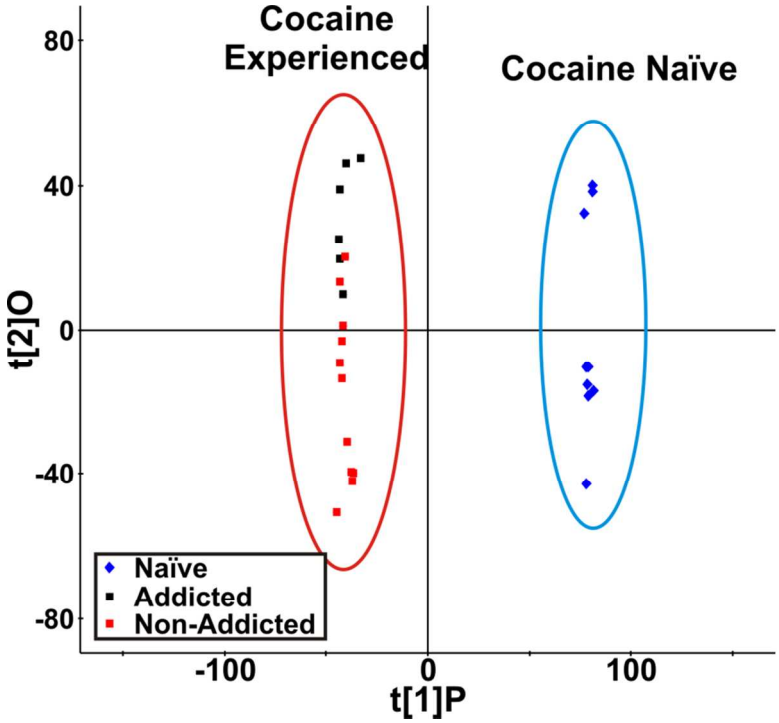


**Figure S2.** Orthogonal partial least square-discriminant analysis comparing cocaine-experienced (-1) to cocaine-naïve (+1) rat sera profiles.

**Table S1.** Region “a” statistical summary.

| Blue | Non-addicted averages | Addicted averages | Naïve averages | Experienced averages |
|---|---|---|---|---|
| | 6200.54 | 8959.59 | 3002.5 | 6200.54 |
| | 10163.6 | 7979.64 | 2892.6 | 10163.6 |
| | 8724.59 | 11042.4 | 2606.9 | 8724.59 |
| | 7838.07 | 8700.31 | 2218.3 | 7838.07 |
| | 9355.55 | 8140.3 | 2057.7 | 9355.55 |
| | 5919.37 | 8945.93 | 2298.2 | 5919.37 |
| | 8781.63 | | 2461 | 8781.63 |
| | 5323.97 | | 2551.5 | 5323.97 |
| | 6343.24 | | 2704.5 | 6343.24 |
| | 6202.28 | | | 6202.28 |
| | | | | 8959.59 |
| | | | | 7979.64 |
| | | | | 11042.4 |
| | | | | 8700.31 |
| | | | | 8140.3 |
| | | | | 8945.93 |

**Kruskal-Wallace One-way ANOVA**

**SUMMARY**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| **Column 1** | 10 | 74852.9 | 7485.286 | 2859614 |
| **Column 2** | 6 | 53768.1 | 8961.355 | 1207373 |
| **Column 3** | 9 | 22793.3 | 2532.586 | 96271.43 |
| **Column 4** | 16 | 128621 | 8038.812 | 2662921 |

**ANOVA**

| H=22.02 | df=3 | Non-addicted | Addicted | Naïve | Experienced |
|---|---|---|---|---|---|
| **p<0.0001** | Mean Ranks for Sample | 22.7 | 30.2 | 5 | 25.5 |

***Post hoc* test: Bonferroni-Holm**

| Group 1 | Group 2 | Critical | P | Significant? |
|---|---|---|---|---|
| Addicted average | Naïve average | 0.008333 | 3.25E-10 | **Yes** |
| Naïve average | Experienced average | 0.01 | 8.69E-10 | **Yes** |
| Non-addicted average | Naïve average | 0.0125 | 1.28E-07 | **Yes** |
| Non-addicted average | Addicted average | 0.016667 | 0.078596 | No |
| Addicted average | Experienced average | 0.025 | 0.218321 | No |
| Non-addicted average | Experienced average | 0.05 | 0.414697 | No |

**Test for Equal Variance**

| F | DFn | DFd | P | |
|---|---|---|---|---|
| 6.716522861 | 3 | 37 | 0.000987 | FAIL-equal variance cannot be assumed |

**Table S2.** Region “b” statistical summary.

| Gray | Non-addicted averages | Addicted averages | Naïve averages | Experienced averages |
|---|---|---|---|---|
| | 2170.11 | 1016.38 | 245.32 | 2170.11 |
| | 2101.32 | 692.3 | 327.03 | 2101.32 |
| | 1542.41 | 782.36 | 331.68 | 1542.41 |
| | 843.41 | 879.43 | 400.34 | 843.41 |
| | 748.87 | 879.85 | 338.84 | 748.87 |
| | 814.03 | 1082.14 | 439.14 | 814.03 |
| | 737.19 | | 401.79 | 737.19 |
| | 704.62 | | 476.22 | 704.62 |
| | 593.51 | | 515.46 | 593.51 |
| | 620.41 | | | 620.41 |
| | | | | 1016.38 |
| | | | | 692.3 |
| | | | | 782.36 |
| | | | | 879.43 |
| | | | | 879.85 |
| | | | | 1082.14 |

**ANOVA: Single Factor**

| **SUMMARY** | | | | | | |
|---|---|---|---|---|---|---|
| ***Groups*** | ***Count*** | ***Sum*** | ***Average*** | ***Variance*** | | |
| **Column 1** | 10 | 10875.9 | 1087.588 | 376374.2 | | |
| **Column 2** | 6 | 5332.46 | 888.7433 | 20753.32 | | |
| **Column 3** | 9 | 3475.82 | 386.2022 | 7077.63 | | |
| **Column 4** | 16 | 16208.3 | 1013.021 | 242627.1 | | |
| **ANOVA** | | | | | | |
| ***Source of Variation*** | ***SS*** | ***df*** | ***MS*** | ***F*** | ***P-value*** | ***F crit*** |
| **Between Groups** | 2908170 | 3 | 969389.9 | 4.990485 | 0.00525 | 4.3595 |
| **Within Groups** | 7187162 | 37 | 194247.6 | | | |
| **Total** | 10095332 | 40 | | | | |

***Post hoc* test: Bonferroni-Holm**

| Group 1 | Group 2 | Critical | P | Significant? |
|---|---|---|---|---|
| Addicted average | Naïve average | 0.008333 | 1.02E-06 | Yes |
| Naïve average | Experienced average | 0.01 | 0.001037 | Yes |
| Non-addicted average | Naïve average | 0.0125 | 0.003471 | Yes |
| Non-addicted average | Addicted average | 0.016667 | 0.453466 | No |
| Addicted average | Experienced average | 0.025 | 0.555188 | No |
| Non-addicted average | Experienced average | 0.05 | 0.735433 | No |

**Test for Equal Variance**

| F | DFn | DFd | P | |
|---|---|---|---|---|
| 2.31283416 | 3 | 37 | 0.091936 | PASS - equal variance may be assumed (p > 0.05). |

**Table S3.** Region “c” statistical summary.

| Green | Non-addicted averages | Addicted averages | Naïve averages | Experienced averages |
|---|---|---|---|---|
| | 1878.46 | 2836.05 | 504.97 | 1878.46 |
| | 1469.38 | 3042.55 | 448.33 | 1469.38 |
| | 1993.71 | 2200.57 | 345.58 | 1993.71 |
| | 2543.64 | 1483.77 | 251.39 | 2543.64 |
| | 1045.84 | 1382.98 | 293.38 | 1045.84 |
| | 8985.16 | 726.63 | 296.93 | 8985.16 |
| | 6632.13 | | 263.09 | 6632.13 |
| | 7836.09 | | 405.93 | 7836.09 |
| | 7118.23 | | 333.42 | 7118.23 |
| | 6014.89 | | | 6014.89 |
| | | | | 2836.05 |
| | | | | 3042.55 |
| | | | | 2200.57 |
| | | | | 1483.77 |
| | | | | 1382.98 |
| | | | | 726.63 |

**Kruskal-Wallace one-way ANOVA**

**SUMMARY**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| **Column 1** | 10 | 45517.5 | 4551.753 | 9223617 |
| **Column 2** | 6 | 11672.6 | 1945.425 | 815385.2 |
| **Column 3** | 9 | 3143.02 | 349.2244 | 7550.15 |
| **Column 4** | 16 | 57190.1 | 3574.38 | 7504202 |

**ANOVA**

| H=21.83 | df=3 | Non-addicted | Addicted | Naïve | Experienced |
|---|---|---|---|---|---|
| **p<0.0001** | Mean Ranks for Sample | 28.1 | 21.2 | 5 | 25.5 |

***Post hoc* test: Bonferroni-Holm**

| Group 1 | Group 2 | Critical | P | Significant? |
|---|---|---|---|---|
| Addicted average | Naïve average | 0.008333 | 0.000128 | Yes |
| Naïve average | Experienced average | 0.01 | 0.000688 | Yes |
| Non-addicted average | Naïve average | 0.0125 | 0.001938 | Yes |
| Non-addicted average | Addicted average | 0.016667 | 0.062533 | No |
| Addicted average | Experienced average | 0.025 | 0.174181 | No |
| Non-addicted average | Experienced average | 0.05 | 0.404085 | No |

**Test for Equal Variance**

| F | DFn | DFd | P | |
|---|---|---|---|---|
| 11.32572354 | 3 | 37 | 2.05E-05 | FAIL-equal variance cannot be assumed |

**Table S4.** Region “d” statistical summary.

| Purple | Non-addicted averages | Addicted averages | Naïve averages | Experienced averages |
|---|---|---|---|---|
| | 1961.77 | 1068.04 | 564.75 | 1961.77 |
| | 1714.38 | 1373.17 | 459.78 | 1714.38 |
| | 1701.73 | 1044.29 | 582.72 | 1701.73 |
| | 1803.42 | 1004.43 | 388.91 | 1803.42 |
| | 3796.06 | 1516.42 | 345.29 | 3796.06 |
| | 964.43 | 1350.24 | 536.04 | 964.43 |
| | 841.67 | | 772.68 | 841.67 |
| | 1642.09 | | 675.7 | 1642.09 |
| | 1295.13 | | 673.84 | 1295.13 |
| | 1657.07 | | | 1657.07 |
| | | | | 1068.04 |
| | | | | 1373.17 |
| | | | | 1044.29 |
| | | | | 1004.43 |
| | | | | 1516.42 |
| | | | | 1350.24 |

**ANOVA: Single Factor**

| **SUMMARY** | | | | | | |
|---|---|---|---|---|---|---|
| ***Groups*** | ***Count*** | ***Sum*** | ***Average*** | ***Variance*** | | |
| **Column 1** | 10 | 17377.8 | 1737.775 | 656170.8 | | |
| **Column 2** | 6 | 7356.59 | 1226.098 | 45700.29 | | |
| **Column 3** | 9 | 4999.71 | 555.5233 | 19740.94 | | |
| **Column 4** | 16 | 24734.3 | 1545.896 | 474389.1 | | |
| **ANOVA** | | | | | | |
| ***Source of Variation*** | ***SS*** | ***df*** | ***MS*** | ***F*** | ***P-value*** | ***F crit*** |
| **Between Groups** | 7871591 | 3 | 2623864 | 7.240781 | 0.00061 | 4.3595 |
| **Within Groups** | 13407803 | 37 | 362373.1 | | | |
| **Total** | 21279394 | 40 | | | | |

***Post hoc* test: Bonferroni-Holm**

| Group 1 | Group 2 | Critical | P | Significant? |
|---|---|---|---|---|
| Addicted average | Naïve average | 0.008333 | 5.35E-06 | **Yes** |
| Naïve average | Experienced average | 0.01 | 0.00032 | **Yes** |
| Non-addicted average | Naïve average | 0.0125 | 0.000476 | **Yes** |
| Non-addicted average | Addicted average | 0.016667 | 0.156613 | No |
| Addicted average | Experienced average | 0.025 | 0.283378 | No |
| Non-addicted average | Experienced average | 0.05 | 0.524272 | No |

**Test for Equal Variance**

| F | DFn | DFd | P | |
|---|---|---|---|---|
| 2.801616821 | 3 | 37 | 0.053266 | PASS - equal variance may be assumed (p > 0.05). |

**Table S5.** Region “e” statistical summary.

| Red | Non-addicted averages | Addicted averages | Naïve averages | Experienced averages |
|---|---|---|---|---|
| | 411.94 | 655.04 | 174.65 | 411.94 |
| | 386.85 | 238.99 | 176.65 | 386.85 |
| | 1046.58 | 487.88 | 209.55 | 1046.58 |
| | 889.8 | 897.8 | 155.11 | 889.8 |
| | 1555.52 | 652.19 | 151.64 | 1555.52 |
| | 494.63 | 1134.66 | 158.86 | 494.63 |
| | 581.41 | | 384.33 | 581.41 |
| | 878.57 | | 344.2 | 878.57 |
| | 417.64 | | 345.88 | 417.64 |
| | 723.63 | | | 723.63 |
| | | | | 655.04 |
| | | | | 238.99 |
| | | | | 487.88 |
| | | | | 897.8 |
| | | | | 652.19 |
| | | | | 1134.66 |

**ANOVA: Single Factor**

| **SUMMARY** | | | | | | |
|---|---|---|---|---|---|---|
| ***Groups*** | ***Count*** | ***Sum*** | ***Average*** | ***Variance*** | | |
| **Column 1** | 10 | 7386.57 | 738.657 | 135840.8 | | |
| **Column 2** | 6 | 4066.56 | 677.76 | 97383.75 | | |
| **Column 3** | 9 | 2100.87 | 233.43 | 9164.929 | | |
| **Column 4** | 16 | 11453.1 | 715.8206 | 114892.8 | | |

**ANOVA**

| H=18.9 | df=3 | Non-addicted | Addicted | Naïve | Experienced |
|---|---|---|---|---|---|
| **p<0.0001** | Mean Ranks for Sample | 25.5 | 25 | 5.7 | 25.3 |

***Post hoc* test: Bonferroni-Holm**

| Group 1 | Group 2 | Critical | P | Significant? |
|---|---|---|---|---|
| Addicted average | Naïve average | 0.008333 | 0.000395 | Yes |
| Naïve average | Experienced average | 0.01 | 0.000963 | Yes |
| Non-addicted average | Naïve average | 0.0125 | 0.001348 | Yes |
| Non-addicted average | Addicted average | 0.016667 | 0.740765 | No |
| Addicted average | Experienced average | 0.025 | 0.813415 | No |
| Non-addicted average | Experienced average | 0.05 | 0.872901 | No |

**Test for Equal Variance**

| F | DFn | DFd | P | |
|---|---|---|---|---|
| 5.963301904 | 3 | 37 | 0.002013 | FAIL - equal variance cannot be assumed. |

**Table S6.** Region “f” statistical summary.

| White | Non-addicted averages | Addicted averages | Naïve averages | Experienced averages |
|---|---|---|---|---|
| | 1950.42 | 1520.41 | 470.8 | 1950.42 |
| | 786.35 | 2827.55 | 511.69 | 786.35 |
| | 915.45 | 2014.75 | 446.62 | 915.45 |
| | 1068.64 | 2372.6 | 359.06 | 1068.64 |
| | 2538.34 | 3169.89 | 414.26 | 2538.34 |
| | 1199.5 | 2626.97 | 294.02 | 1199.5 |
| | 694.17 | | 408.2 | 694.17 |
| | 1263.33 | | 300.77 | 1263.33 |
| | 1087.39 | | 318.73 | 1087.39 |
| | 728.13 | | | 728.13 |
| | | | | 1520.41 |
| | | | | 2827.55 |
| | | | | 2014.75 |
| | | | | 2372.6 |
| | | | | 3169.89 |
| | | | | 2626.97 |

**Kruskal-Wallis One-way ANOVA**

**SUMMARY**

| ***Groups*** | ***Count*** | ***Sum*** | ***Average*** | ***Variance*** | | |
|---|---|---|---|---|---|---|
| **Column 1** | 10 | 12231.7 | 1223.172 | 345940.3 | | |
| **Column 2** | 6 | 14532.2 | 2422.028 | 349396.1 | | |
| **Column 3** | 9 | 3524.15 | 391.5722 | 6081.414 | | |
| **Column 4** | 16 | 26763.9 | 1672.743 | 683343.6 | | |

**ANOVA**

| H=25.6 | df=3 | Non-addicted | Addicted | Naïve | Experienced |
|---|---|---|---|---|---|
| **p<0.0001** | Mean Ranks for Sample | 20.3 | 34.2 | 5 | 25.5 |

***Post hoc* test: Bonferroni-Holm**

| Group 1 | Group 2 | Critical | P | Significant? |
|---|---|---|---|---|
| Addicted average | Naïve average | 0.008333 | 1.18E-07 | Yes |
| Naïve average | Experienced average | 0.01 | 0.000128 | Yes |
| Non-addicted average | Naïve average | 0.0125 | 0.000606 | Yes |
| Non-addicted average | Addicted average | 0.016667 | 0.00148 | Yes |
| Addicted average | Experienced average | 0.025 | 0.05688 | No |
| Non-addicted average | Experienced average | 0.05 | 0.148061 | No |

**Test for Equal Variance**

| F | DFn | DFd | P | |
|---|---|---|---|---|
| 10.23623293 | 3 | 37 | 4.79E-05 | FAIL - equal variance cannot be assumed. |

**Table S7.** Features within region extracted from the S-plot in Figure 4(b). Included are the retention time, mass, loadings and correlation coefficients, averages for each group, factor of change with uncertainty, in addition to the regional location in the MEDI heat map in Figure 4(c).

| Retention Time | Mass | p[1]P | p(corr)[1]P | Experienced Average | Naïve Average | Factor of Change | Uncertainty | Region |
|---|---|---|---|---|---|---|---|---|
| 11.76 | 130.066 | -0.077 | -0.730 | 60.693 | 4.294 | 14.1 | 5.121 | blue |
| 12.87 | 614.174 | -0.046 | -0.584 | 25.551 | 0.000 | -3.4E+07 | 10000 | green |
| 12.83 | 616.179 | -0.186 | -0.689 | 354.740 | 0.000 | 10000 | 10000 | blue |
| 13.22 | 158.154 | -0.056 | -0.576 | 51.840 | 13.964 | 3.7 | 0.709 | blue |
| 10.19 | 162.056 | -0.051 | -0.739 | 26.495 | 1.509 | 17.6 | 12.02 | purple |
| 9.92 | 182.192 | -0.198 | -0.811 | 475.820 | 139.393 | 3.4 | 0.274 | blue |
| 10.82 | 185.080 | -0.066 | -0.633 | 64.801 | 17.044 | 3.8 | 0.653 | blue |
| 10.46 | 186.223 | -0.031 | -0.577 | 16.783 | 5.390 | 3.1 | 0.819 | red |
| 11.78 | 190.048 | -0.059 | -0.720 | 35.808 | 1.642 | 21.8 | 14.47 | blue |
| 9.09 | 201.068 | -0.057 | -0.670 | 47.493 | 13.496 | 3.5 | 0.548 | grey |
| 1.2 | 203.054 | -0.049 | -0.636 | 31.450 | 4.884 | 6.4 | 1.842 | white |
| 9.98 | 211.143 | -0.056 | -0.583 | 39.752 | 2.159 | 18.4 | 8.956 | blue |
| 9.6 | 215.125 | -0.078 | -0.615 | 96.993 | 29.218 | 3.3 | 0.497 | blue |
| 11.94 | 223.066 | -0.042 | -0.628 | 25.111 | 5.240 | 4.8 | 1.275 | white |
| 12.62 | 225.088 | -0.065 | -0.591 | 54.029 | 3.078 | 17.6 | 7.917 | green |
| 9.85 | 229.091 | -0.084 | -0.549 | 139.021 | 49.404 | 2.8 | 0.44 | blue |
| 10.53 | 229.142 | -0.182 | -0.709 | 418.809 | 97.261 | 4.3 | 0.527 | blue |
| 10.77 | 243.095 | -0.065 | -0.643 | 46.404 | 0.571 | 81.3 | 89.034 | blue |
| 10.85 | 257.136 | -0.055 | -0.602 | 48.442 | 14.323 | 3.4 | 0.556 | blue |
| 12.63 | 261.109 | -0.100 | -0.575 | 165.129 | 44.117 | 3.7 | 0.613 | blue |
| 9.55 | 269.132 | -0.050 | -0.704 | 35.318 | 9.933 | 3.6 | 0.595 | purple |
| 10.34 | 273.175 | -0.069 | -0.577 | 97.670 | 39.432 | 2.5 | 0.327 | blue |
| 9.09 | 279.688 | -0.044 | -0.571 | 33.130 | 10.547 | 3.1 | 0.667 | blue |
| 9.22 | 305.159 | -0.032 | -0.555 | 16.740 | 3.643 | 4.6 | 1.467 | green |
| 10.19 | 308.091 | -0.049 | -0.659 | 27.579 | 2.347 | 11.8 | 5.586 | blue |
| 9.49 | 338.087 | -0.053 | -0.598 | 32.848 | 0.000 | -2.4E+07 | 10000 | blue |
| 9.74 | 340.104 | -0.049 | -0.630 | 29.279 | 2.385 | 12.3 | 6.83 | blue |
| 10.55 | 361.138 | -0.049 | -0.609 | 54.713 | 27.654 | 2 | 0.225 | blue |
| 9.48 | 371.227 | -0.052 | -0.606 | 41.785 | 10.601 | 3.9 | 0.875 | purple |
| 10.71 | 383.113 | -0.042 | -0.556 | 21.828 | 0.000 | 10000 | 10000 | white |
| 10.58 | 392.138 | -0.046 | -0.594 | 30.563 | 5.803 | 5.3 | 1.534 | green |
| 9.54 | 399.625 | -0.037 | -0.610 | 15.955 | 0.000 | 10000 | 10000 | red |
| 9.6 | 406.164 | -0.046 | -0.600 | 27.037 | 1.929 | 14 | 7.712 | green |
| 12.18 | 430.296 | -0.067 | -0.712 | 44.363 | 0.000 | -4.2E+07 | 10000 | green |
| 9.96 | 431.090 | -0.046 | -0.728 | 19.933 | 0.000 | -4E+07 | 10000 | green |
| 9.58 | 432.280 | -0.048 | -0.680 | 33.715 | 10.099 | 3.3 | 0.601 | gray |
| 12.18 | 448.306 | -0.050 | -0.626 | 29.513 | 1.137 | 26 | 20.871 | green |
| 12.19 | 466.318 | -0.067 | -0.556 | 58.968 | 2.487 | 23.7 | 11.808 | green |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 14.09 | 500.277 | -0.063 | -0.577 | 48.380 | 1.157 | 41.8 | 33.792 | blue |
| 9.72 | 520.336 | -0.069 | -0.854 | 51.818 | 12.795 | 4 | 0.548 | blue |
| 14.11 | 542.327 | -0.045 | -0.582 | 24.440 | 0.000 | -3.2E+07 | 10000 | white |
| 9.86 | 547.334 | -0.031 | -0.558 | 18.339 | 6.459 | 2.8 | 0.559 | purple |
| 9.93 | 608.387 | -0.034 | -0.680 | 18.797 | 7.236 | 2.6 | 0.481 | purple |

**Table S8.** *Putative metabolite identification.* Metabolites were given preliminary identifications based upon accurate mass, ion type, and database searching.

| Retention Time (min) | *m/z* | MEDI Region | Putative Identification | Mass Accuracy (ppm) | Database |
|---|---|---|---|---|---|
| 11.76 | 130.066 | a | 3-Methylene-indolenine | 6.9 | HMDB |
| 9.92 | 182.192 | a | No Database Match | | |
| 10.82 | 185.080 | a | (3-Methoxy-4-hydroxyphenyl) ethylene glycol | 4.3 | HMDB |
| 11.78 | 190.048 | a | Kynurenic acid | 10.0 | METLIN |
| 9.85 | 229.091 | a | Deoxyuridine | 28.8 | METLIN |
| 10.53 | 229.142 | a | No Database Match | N/A | |
| 10.77 | 243.102 | a | Thymidine | 14.4 | METLIN |
| 10.34 | 273.175 | a | Estradiol | 37.7 | LIPID MAPS |
| 10.19 | 308.091 | a | Glutathione | 0.3 | METLIN |
| 9.49 | 338.087 | a | 3-Indole carboxylic acid glucuronide | 0.9 | HMDB |
| 9.74 | 340.104 | a | 5-Hydroxy-6-methoxyindole glucuronide | 3.8 | HMDB |
| 10.55 | 361.138 | a | Dityrosine | 3.9 | HMDB |
| 14.09 | 500.277 | a | LPE(20:5) | 0.4 | LIPID MAPS |
| 9.72 | 520.336 | a | LPC(18:2) | 7.3 | LIPID MAPS |
| 9.09 | 201.068 | b | Bilirubin oxidation product | 21.3 | HMDB |
| 12.62 | 225.088 | c | 3-Hydroxykynurenine | 5.3 | HMDB |
| 9.22 | 305.159 | c | Sodiated steroid-like molecule | N/A | |
| 12.18 | 430.296 | c | Glycocholic acid $-2H_2O$ | 1.9 | METLIN |
| 12.18 | 448.306 | c | Glycocholic acid-$H_2O$ | 0.7 | METLIN |
| 12.19 | 466.318 | c | Glycocholic acid | 3.6 | METLIN |
| 10.19 | 162.056 | d | 4,6-Dihydroxyquinoline | 6.1 | HMDB |
| 9.55 | 269.132 | d | 3-Carboxy-4-methyl-5-pentyl-2-furanpropionic acid | 24.2 | HMDB |
| 9.48 | 371.227 | d | 6-Keto-prostaglandin F1a | 21.5 | LIPID MAPS |
| 9.54 | 399.625 | e | $[M+2H]^{2+}$ | N/A | |
| 1.20 | 203.054 | f | Succinylacetoacetate | 4.9 | HMDB |
| 11.94 | 223.066 | f | No Database Match | N/A | |
| 14.11 | 542.327 | f | LPC(20:5) | 5.3 | LIPID MAPS |

**Figure S3.** Retention time selected isotope envelope for 3-methylene-indolenine.

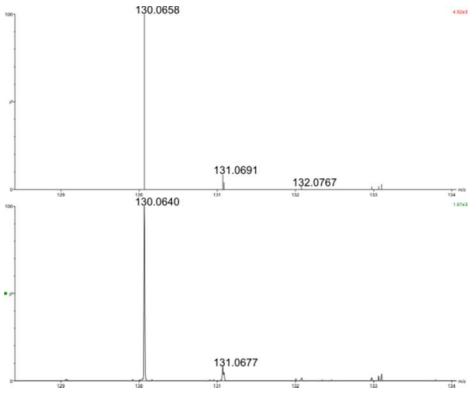


**Figure S4.** Retention time selected isotope envelope for (3-methoxy-4-hydroxyphenyl) ethylene glycol.

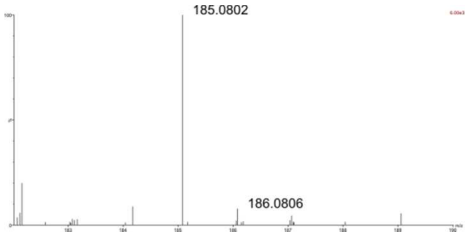


**Figure S5.** Retention time selected isotope envelope for kynurenic acid.

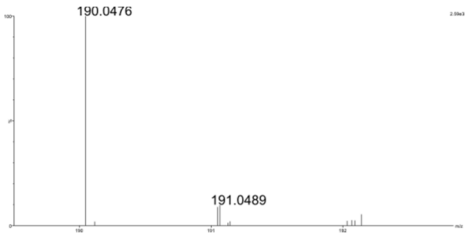


**Figure S6.** Retention time and drift time selected isotope envelope for deoxyuridine.

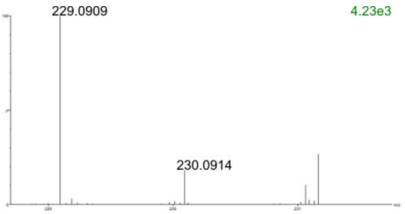


**Figure S7.** Intact spectrum of the isotopic envelope for thymidine.

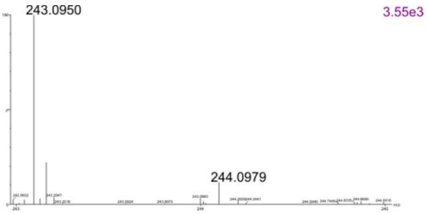


**Figure S8.** Retention time and drift time selected isotope envelope for estradiol.

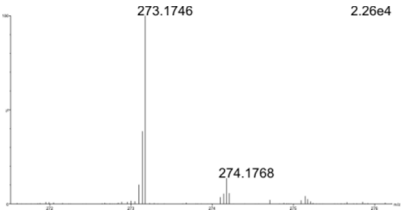


**Figure S9.** Retention time selected isotope envelope for glutathione.

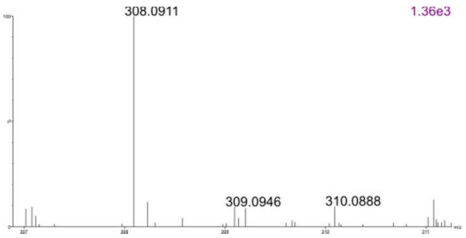


**Figure S10.** Retention time selected isotope envelope for 3-indole carboxylic acid glucuronide. We observe the loss of glucuronic acid (-176 Da) to generate a 162 *m/z* ion in the fragmentation spectrum.

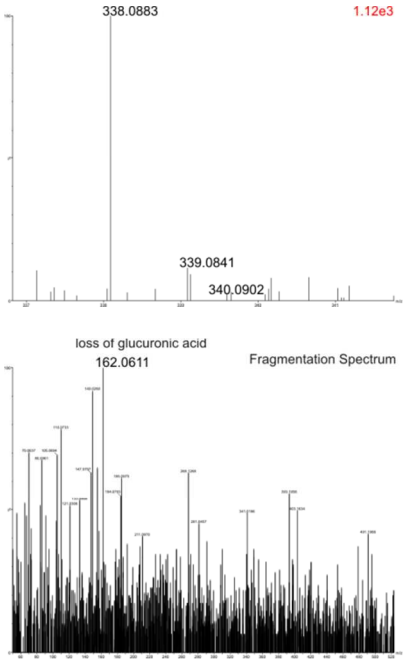


**Figure S11.** Retention time selected isotope envelope for 5-hydroxy-6-methoxyindole glucuronide. We observe the loss of glucuronic acid (-176 Da) to generate a 164 *m/z* ion in the fragmentation spectrum.

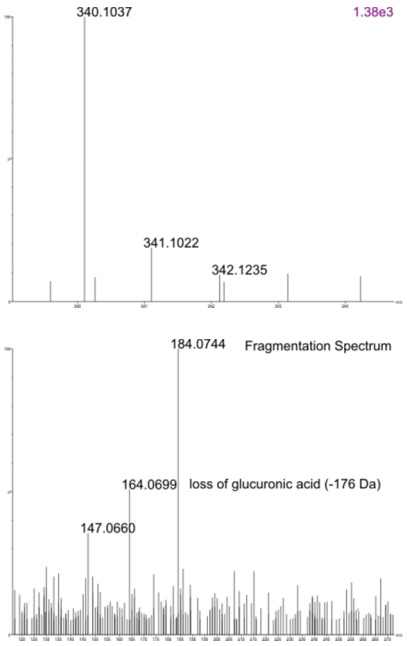


**Figure S12.** Retention time selected isotope envelope for dityrosine.

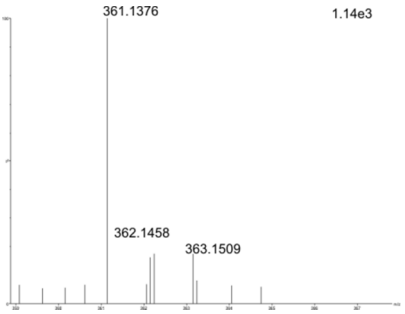


**Figure S13.** Retention time selected isotope envelope for LPE (20:5).

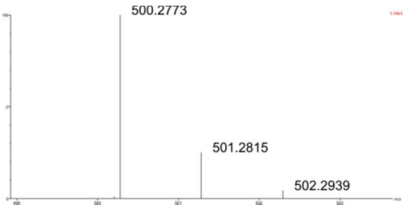


**Figure S14.** Retention time selected isotope envelope for LPC (18:2). A 184 head group fragment is also observed in the fragmentation spectrum below.

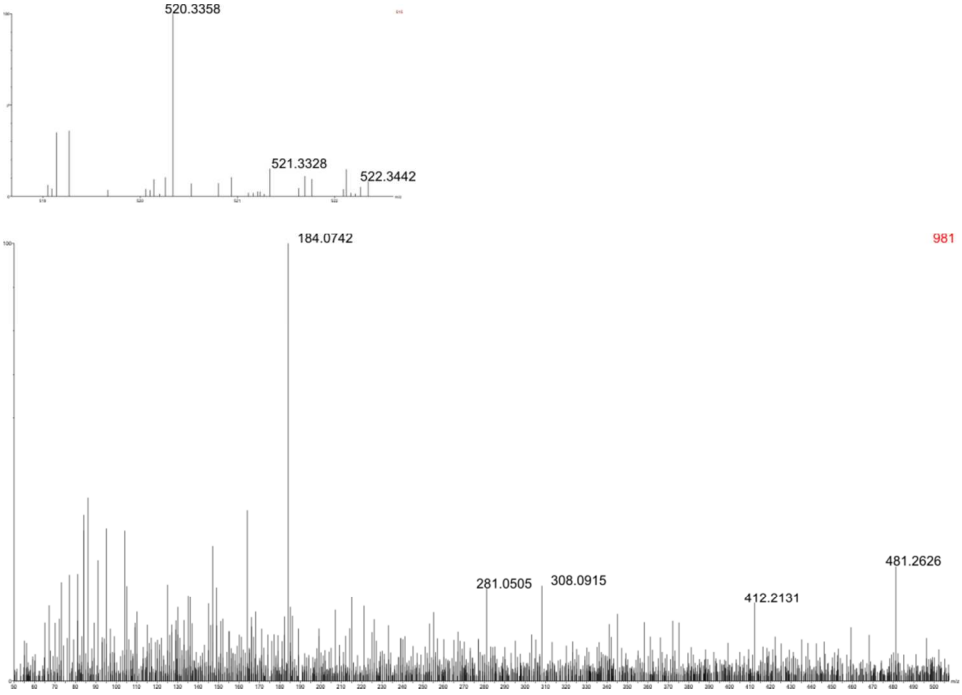


**Figure S15.** Representative structure for bilirubin oxidation product.

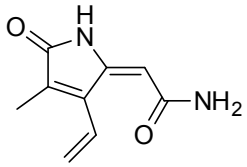


**Figure S16.** Retention time and drift time selected isotope envelope for 3-hydroxykynurenine.

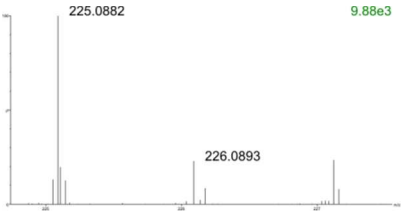


**Figure S17.** Retention time selected spectrum for glycocholic acid and in-source fragments.

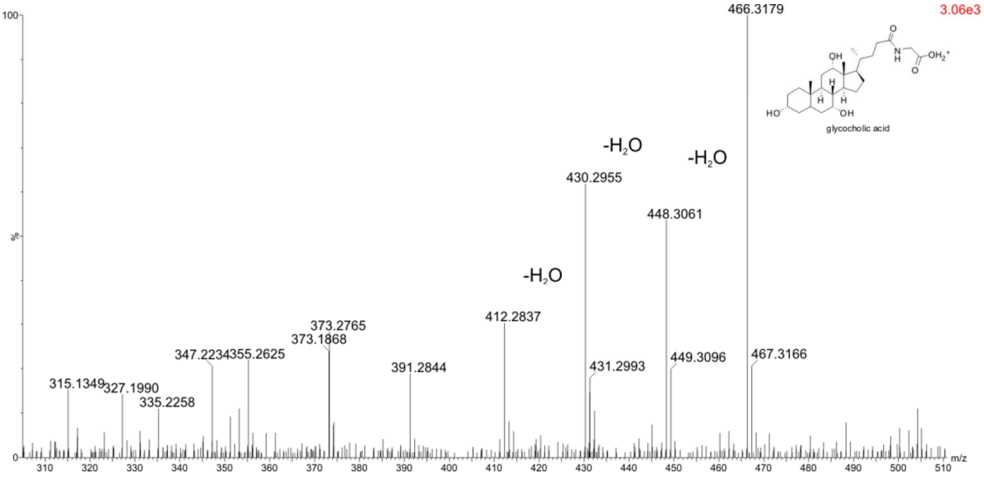


**Figure S18.** Retention time selected isotope envelope for 4,6-dihyroxyquinoline.

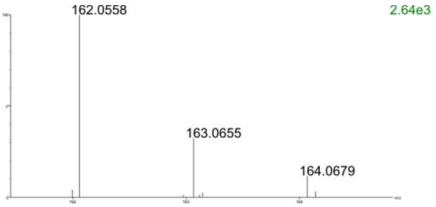


**Figure S19.** Retention and drift time selected isotope envelope for 3-carboxy-4-methyl-5-pentyl-2-furanpriopionic acid.

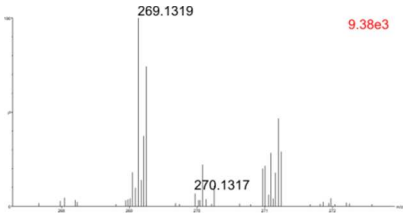


**Figure S20.** Retention and drift time selected isotope envelope for 6-keto-prostaglandin F1a.

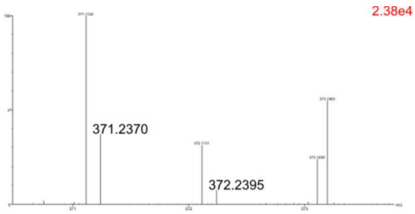


**Figure S21.** Retention time selected isotope envelope displaying doubly charged unknown analyte.

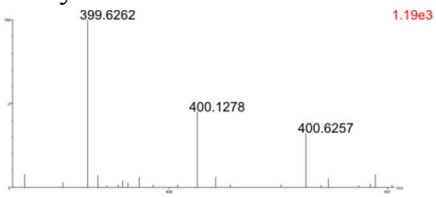


**Figure S22.** Retention time selected intact and fragmentation spectra for LPC(20:5). Desaturation locations are representative and not exact.