Electronic Supplementary Material (ESI) for Analyst.


# Supplementary Information for:

## Information-Rich High-Throughput Cellular Assays using Acoustic Mist Ionisation Mass Spectrometry

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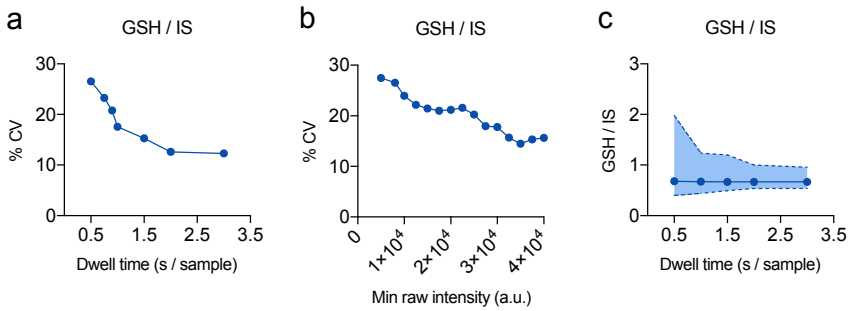


**Fig. S1**. Reproducibility of relative quantitation of glutathione (GSH) across a single 384-well plate (MCF7 cells) using GSH-$^{13}C_2$,$^{15}N$ as an internal standard (IS) spiked into the lysis buffer. (a) Relationship between the coefficient of variation (CV) and sample throughput. (b) Relationship between the coefficient of variation and a minimum raw intensity threshold for individual 100 µs MS scans (*x* axis). The threshold is calculated as the sum of GSH and IS signal intensities. (c) Relationship between the measurements of GSH (IS corrected) and sample throughput. Shown are average ± range of measurements across a single 384-well plate.

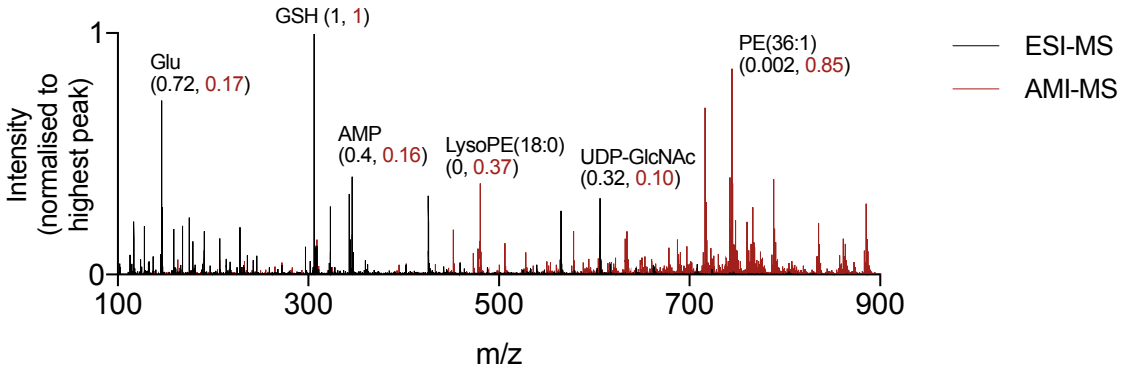


**Fig. S2**. Negative ion spectra of crude aqueous cell lysates (untreated caco-2 cells) acquired on AMI-MS and ESI-MS and normalized to the highest peak (glutathione at m/z 306.0765). AMI spectra are visibly richer in phospholipid species (650 – 900 Da), while ESI is more efficient for small polar species such as amino acids and nucleotides. Several species are highlighted, and their normalised intensities shown in brackets. Glu = glutamic acid, AMP = adenosine monophosphate, PE = phosphatydilethanolamine, UDP-GlcNAc = uridine diphosphate N-acetyl glucosamine.

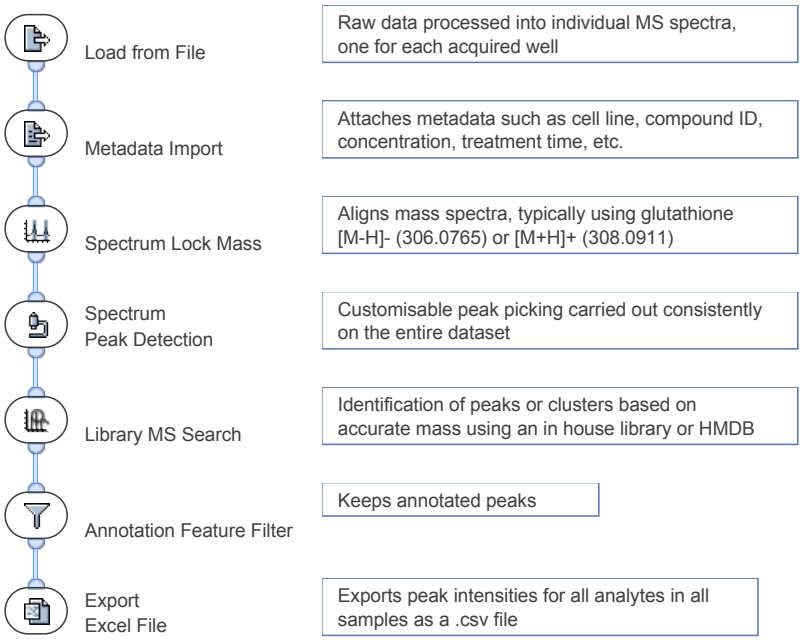


**Fig. S3**. Backbone of a typical data processing workflow in Genedata Expressionist. Many additional activities are available depending on the experimental design, biological questions or assay type.

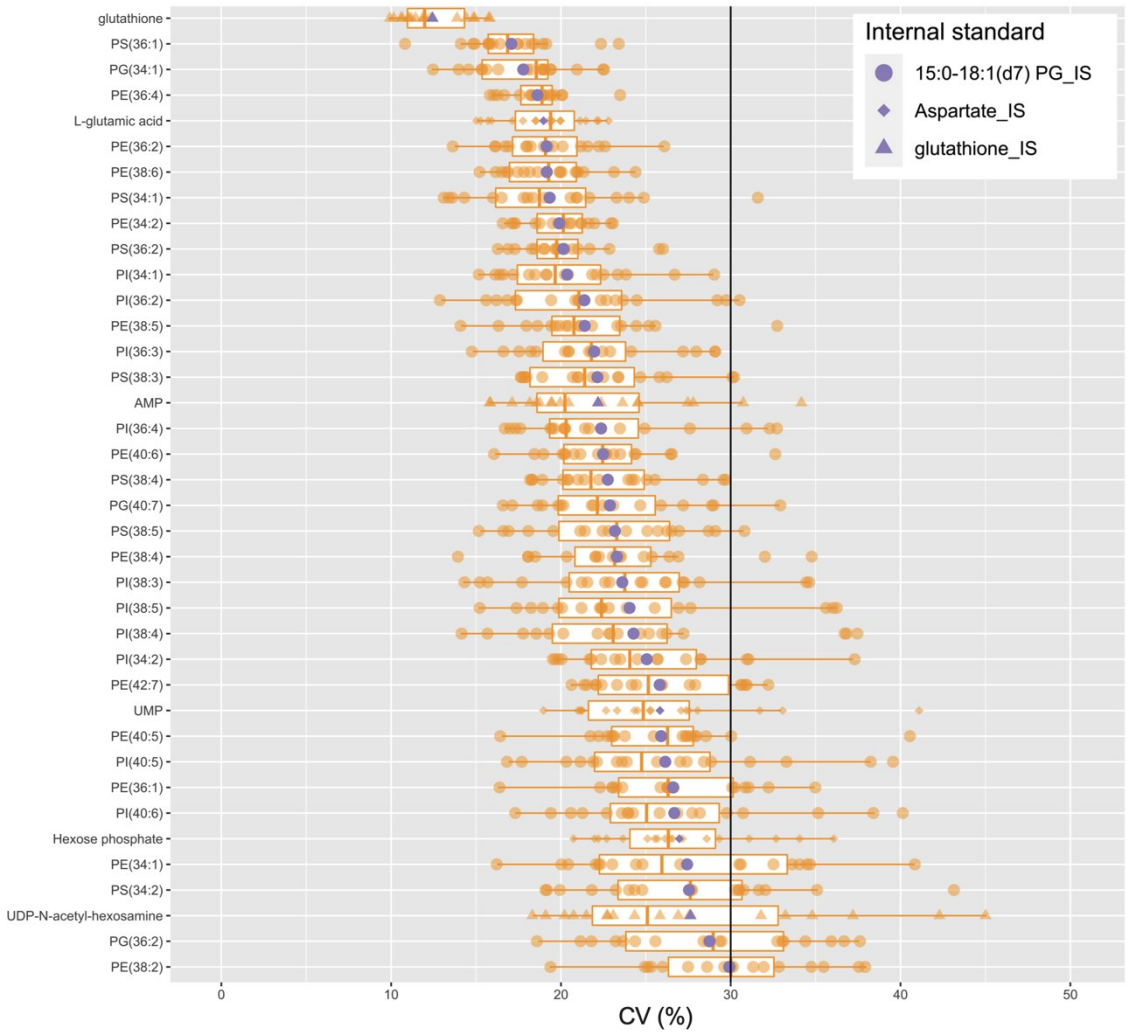


**Fig. S4**. Reproducibility of cellular assays with an AMI-MS end point. Untreated HepG2 cells were analysed across 7 independent experiments using a seeding density of 3500 cells per well and a 48 h incubation period. Shown are mean coefficient of variation (CV) (purple symbols, n = 18 plates) and CV from individual plates (orange symbols, n = 352 wells per plate). Underlaid box plots denote the median and interquartile range of individual plate CVs (n = 18). All analytes were normalised with an internal standard (see corresponding symbol shape on top right). AMP = adenosine monophosphate, UMP = uridine monophosphate, PE = phosphatidylethanolamine, PS = phosphatidylserine, PI = phosphatidylinositol, PG = phosphatidylglycerol.

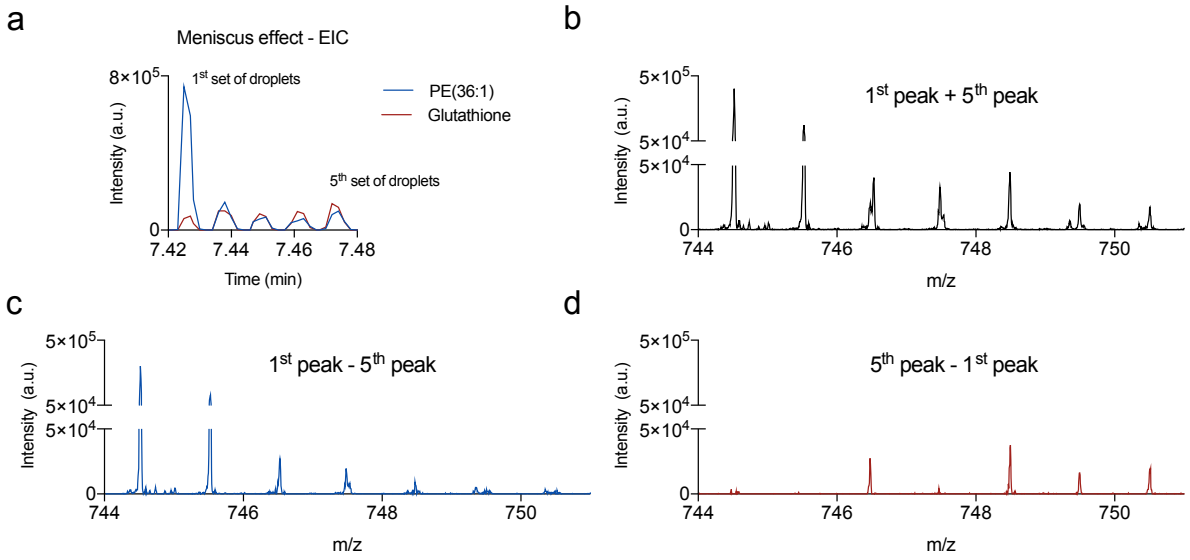


**Figure S5**. Natural enrichment of phospholipids at the surface of an aqueous lysate of human epithelial colorectal adenocarcinoma cells (10,000 Caco-2 cells per well). (a) Extracted ion chromatograms of an abundant phospholipid (PE(36:1)) and an abundant water-soluble metabolite (GSH) in 5 consecutive sets of misting events (20 nL each, total time 3.5 s). (b) Mass spectra combined from 1st and 5th peak. Natural isotopes of an abundant phospholipid PE(36:1) prevent relative quantitation of a lower abundant species (m/z 746.5). (c,d) Subtracted mass spectra (1st vs 5th peak) can reveal lower abundant species (e.g., 746.5 and 748.5 Da in (d)) and allow their relative quantitation.

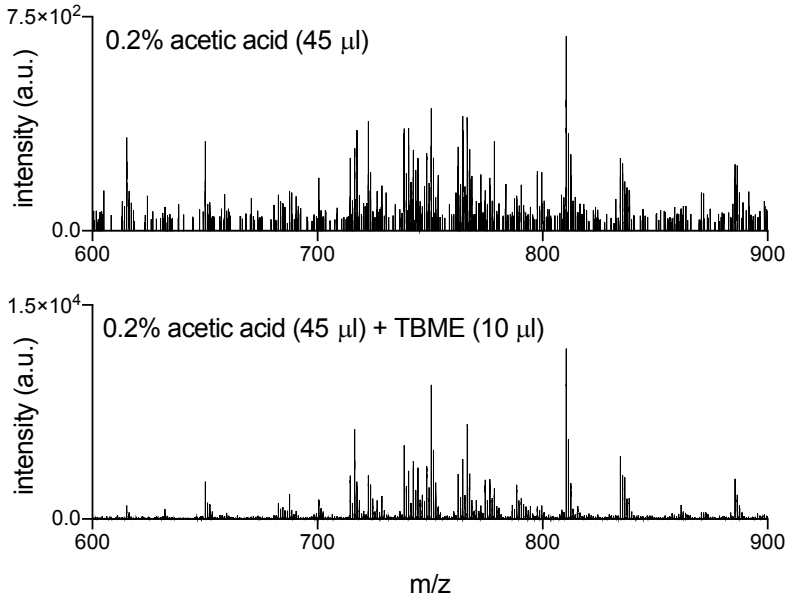


**Figure S6**. Addition of *tert*-butyl methyl ether (TBME) into an aqueous cell lysate further enriches phospholipids at the meniscus, resulting in an enhanced phospholipid region in AMI-MS spectra compared to aqueous lysate only. Spectra were acquired from an individual well containing 1000 hepatocytes (HepG2) before and after the addition of TBME.

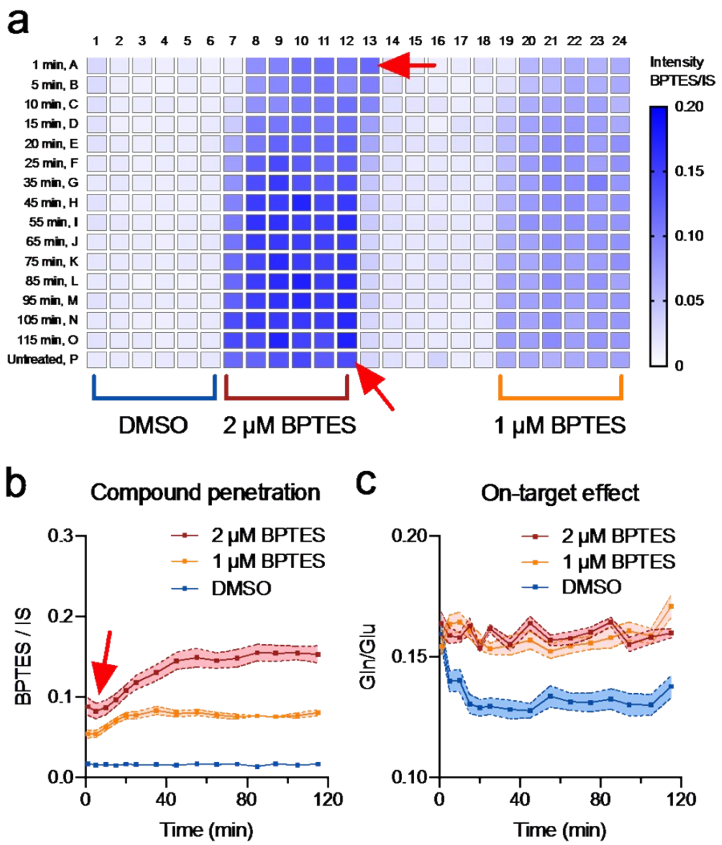


**Fig. S7**. Flow injection ESI-MS measurements of BPTES-treated caco-2 cells highlighting the carryover problems that can be encountered with chemically adsorbing compounds using contact-based in-solution MS methods. (a) Assay plate layout and a heatmap of BPTES intensity normalized to $^{13}C_2$,$^{15}N$-glutathione internal standard (IS) across one dosed cell plate. Samples were injected in a column-wise discontinuous sequence (i.e., A1, B1, … , P1, A2, B2, …, P24), with acidified 60% organic mobile phase, 3 s pre-injection needle wash step using acidified acetonitrile, and a sample-to-sample run time of 45 s. Red arrows indicate clear carryover in untreated samples in samples P7-12, column 13, and also in early time points (e.g., wells A7-12). (b) BPTES penetration measurements are skewed as a result of this carryover (red and orange curves not originating close to 0). (c) Glutamine and glutamate (not chemically adsorbing) eluted cleanly within the first 12 s of sample run time and validated the AMI-MS measurements in Fig. 3c. Shown are mean ± s.e.m. of 12 replicates for each condition and time point in (b) and (c).

**Table S1**. Selected commonly observed metabolites in AMI mass spectra of human cell lysates (here MCF7 cells) acquired in the negative ion mode. Shown are measured m/z values (deprotonated species only), signal-to-noise ratio (maximum intensity in a mass spectrum derived as a geometrical mean of all MS scans from 384 wells acquired in 3.4 min), and relative deviation from reference mass in parts per million (ppm). FA = fatty acid, PE = phosphatidylethanolamine, PS = phosphatidylserine, PI = phosphatidylinositol, PG = phosphatidylglycerol.

| m/z (M-H) | ID | signal:noise | Error (ppm) | m/z (M-H) | ID | signal:noise | Error (ppm) |
|---|---|---|---|---|---|---|---|
| 124.0069 | Taurine | 148.8 | -4.1 | 506.3284 | LysoPE(20:1) | 9.9 | 6.4 |
| 127.0507 | Pyroglutamine | 12.5 | -4.4 | 565.0521 | UDP hexose | 9.1 | 7.7 |
| 128.0350 | Pyroglutamic acid | 46.8 | -2.3 | 571.2929 | LysoPI(16:0) | 9.5 | 7.0 |
| 131.0462 | Asparagine | 5.1 | -0.1 | 579.0296 | UDP-hexuronic acid | 5.1 | 4.6 |
| 131.0822 | Ornithine | 5.4 | -2.8 | 599.3216 | LysoPI(18:0) | 15.6 | 2.4 |
| 132.0295 | Aspartic acid | 5.7 | -5.6 | 606.0784 | UDP-N-acetyl-hexosamine | 83.9 | 6.8 |
| 145.0614 | Glutamine | 206.9 | -3.5 | 662.4812 | PE(30:0) | 13.9 | 7.0 |
| 146.0453 | Glutamic acid | 134.9 | -4.3 | 688.4973 | PE(32:1) | 46.8 | 7.3 |
| 154.0614 | Histidine | 9.1 | -5.1 | 690.5106 | PE(32:0) | 127.2 | 3.9 |
| 173.1037 | Arginine | 24.7 | -3.8 | 714.5162 | PE(34:2) | 119 | 11.6 |
| 180.0655 | Tyrosine | 6.8 | -6.0 | 716.5311 | PE(34:1) | 454.2 | 10.5 |
| 214.0476 | Glycerophosphoethanolamine | 251.8 | -4.7 | 718.5409 | PE(34:0) | 130.6 | 2.3 |
| 218.1007 | Pantothenic acid | 5.8 | -12.4 | 742.5465 | PE(36:2) | 409.1 | 9.8 |
| 255.2315 | Palmitic acid | 10.3 | -5.8 | 744.5600 | PE(36:1) | 525.2 | 6.9 |
| 281.2480 | Oleic acid | 5.6 | -2.0 | 747.5247 | PG(34:1) | 10.8 | 8.7 |
| 283.2642 | Stearic acid | 93.7 | -0.2 | 760.5209 | PS(34:1) | 18.5 | 9.9 |
| 297.2432 | FA(oxo-18:0) | 22.6 | -1.0 | 762.5336 | PS(34:0) | 45.4 | 5.9 |
| 299.2597 | FA(hydroxy-18:0) | 27.2 | 1.6 | 764.5306 | PE(38:5) | 76.6 | 9.2 |
| 306.0765 | Glutathione, reduced | 915.8 | 0.0 | 766.5405 | PE(38:4) | 68.9 | 1.7 |
| 323.0287 | UMP | 30.4 | 0.4 | 772.5952 | PE(38:1) | 19.5 | 11.7 |
| 346.0567 | AMP | 116.8 | 2.7 | 773.5417 | PG(36:2) | 20.1 | 10.2 |
| 362.0511 | GMP | 6.1 | 1.0 | 776.5648 | PE(P-40:5) | 8.9 | 6.2 |
| 402.9971 | UDP | 15.1 | 5.5 | 786.5340 | PS(36:2) | 5.9 | 6.2 |
| 426.0241 | ADP | 59.6 | 4.6 | 788.5492 | PS(36:1) | 60.2 | 5.7 |
| 442.0199 | GDP | 6.2 | 6.4 | 807.5132 | PI(32:1) | 13.3 | 12.7 |
| 450.2651 | LysoPE(16:1) | 58.8 | 5.5 | 809.5273 | PI(32:0) | 80.3 | 10.7 |
| 452.2804 | LysoPE(16:0) | 250.2 | 4.6 | 819.5249 | PG(40:7) | 13.9 | 8.2 |
| 465.3070 | cholesterol sulfate | 210 | 5.5 | 833.5290 | PI(34:2) | 10.6 | 12.5 |
| 478.2964 | LysoPE(18:1) | 192 | 5.2 | 835.5435 | PI(34:1) | 119.3 | 11.1 |
| 480.3123 | LysoPE(18:0) | 280.6 | 5.6 | 837.5582 | PI(34:0) | 87.4 | 9.9 |
| 500.2824 | LysoPE(20:4) | 13.1 | 8.3 | 861.5588 | PI(36:2) | 32.8 | 10.3 |
| 505.9918 | ATP | 10.4 | 6.5 | 863.5737 | PI(36:1) | 127.2 | 9.5 |