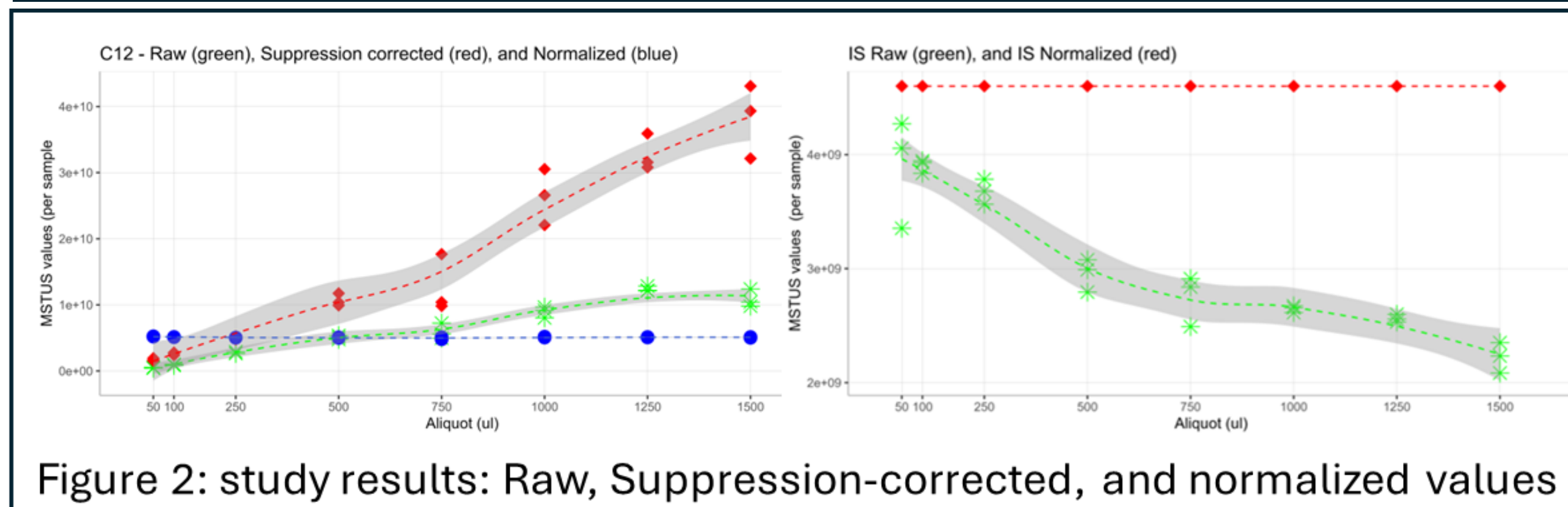
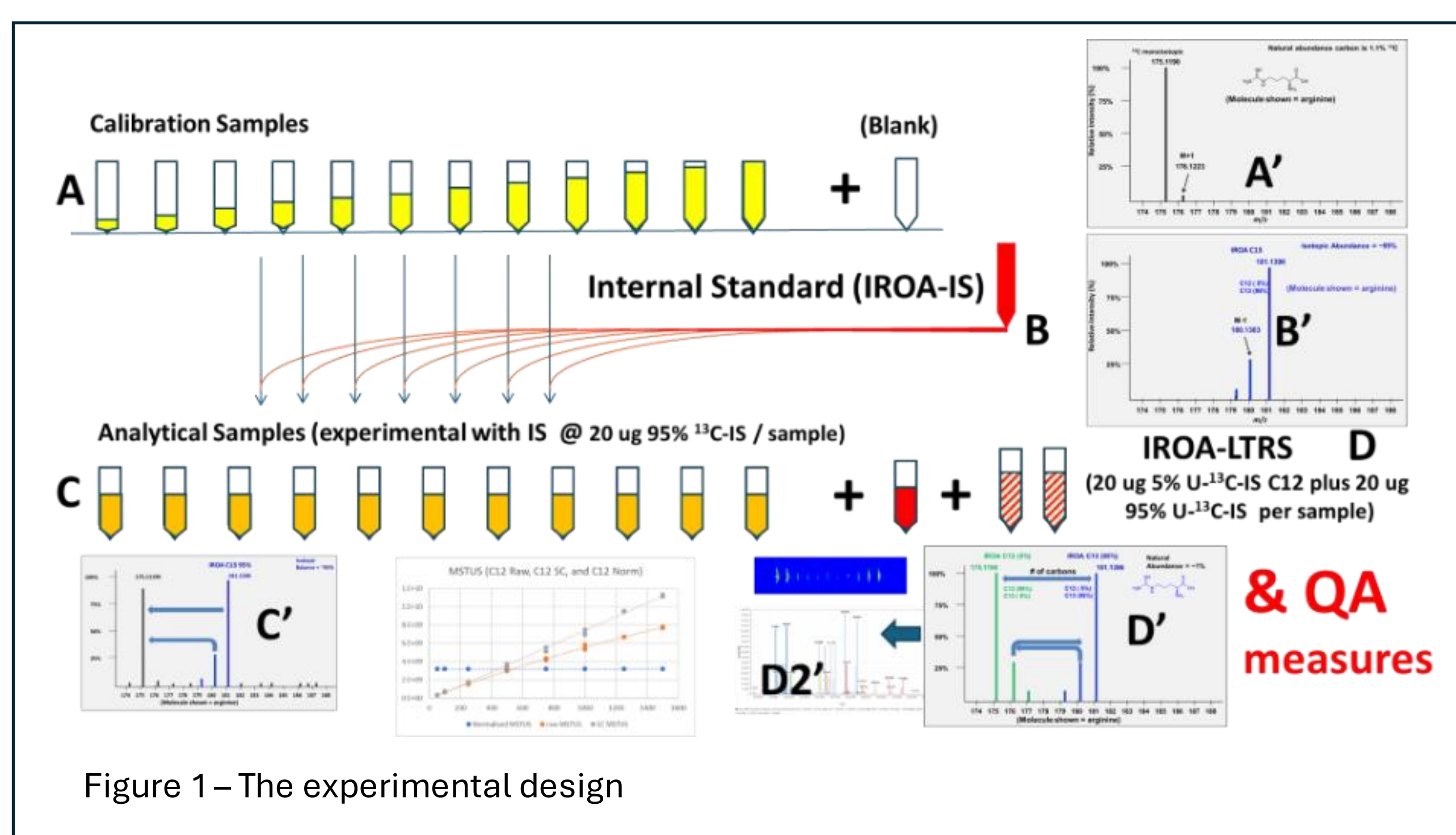


OVERVIEW

Ion-suppression in biological matrices is a complex phenomenon in which ionization inefficiencies cause the AUC of a peak to be less than proportional to analyte concentration. We have (i) developed a method (Figure 1) for quantitating suppression for large mixtures of molecules and (ii) used Self-Organizing Maps (SOM) to visualize patterns of suppression for 539 diverse analytes in plasma across serial dilutions using LC-MS/MS. At low concentration, analyte peaks may not show up; at higher concentration, peaks for easily-suppressed compounds may disappear entirely. A given chemical mixture shows different patterns of suppression depending on the analyte, the concentration of analyte, and the nature, volume, and concentration of matrix components in the LC injectate. Such complex effects are ubiquitous in MS of biological samples.



METHODS

To model ion suppression, we created a single methanol extract of plasma, divided it into aliquots ranging from 50 to 1,500 µL, dried the aliquots, and reconstituted them with a fixed volume and concentration of IROA-Internal Standard (IROA-IS). Immediately before analysis, samples were reconstituted in 40 µL of IROA-IS solution, prepared by dissolving the contents of an IROA-IS vial in 1.2 mL H₂O, vortexing, and briefly centrifuging. IROA-LTRS, an isotopically-labeled complex standard composed of extracts universally labeled at both 5% and 95% U-¹³C in a 1:1 ratio, was prepared by dissolving the contents of an IROA-LTRS vial in 40 µL H₂O. A 3-µL injection of IROA-LTRS and 5-µL injection of each sample were analyzed using LC-MS/MS.

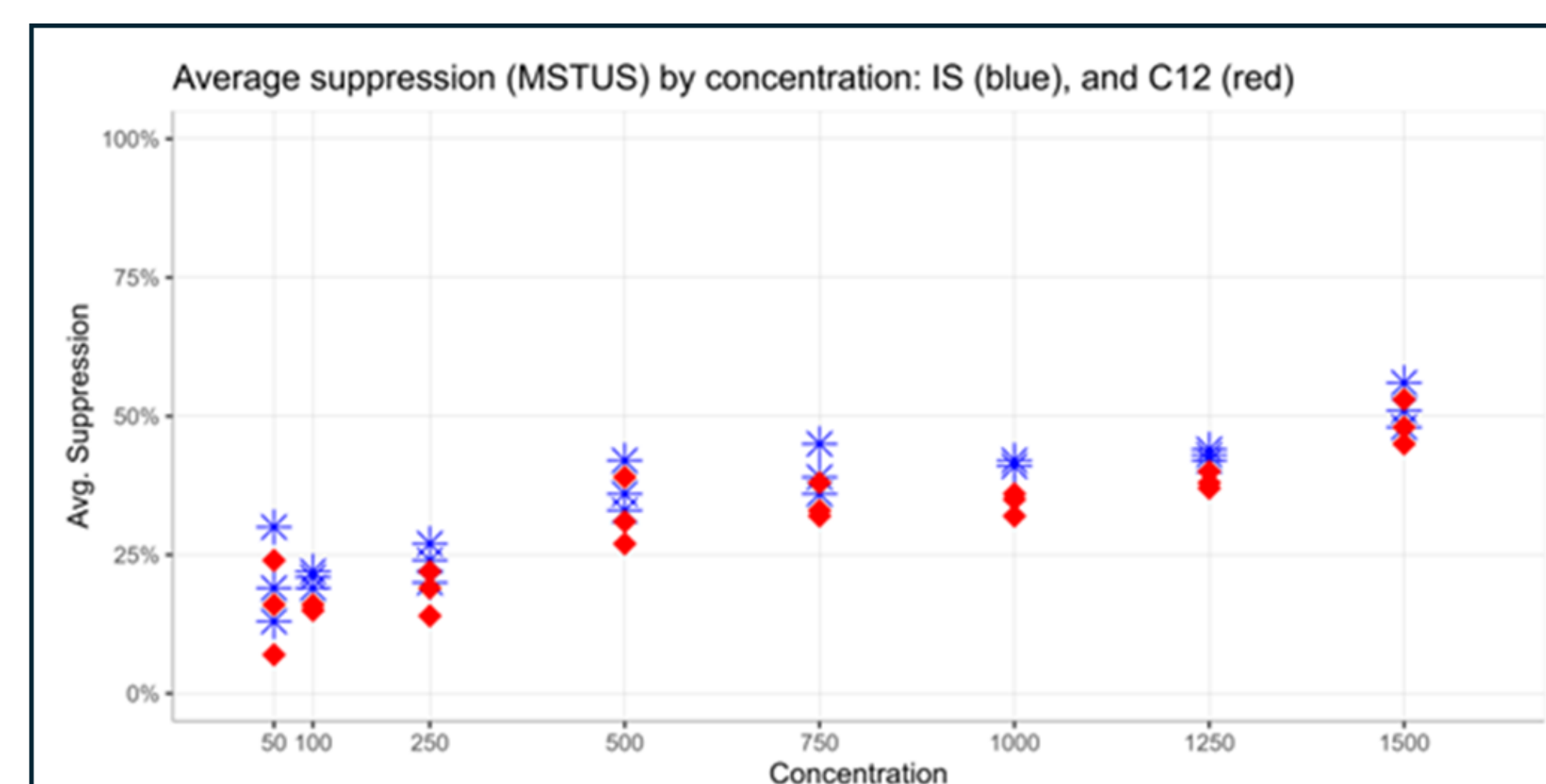


Figure 3: Average suppression by concentration makes it clear that both C12 and C13 track with one another

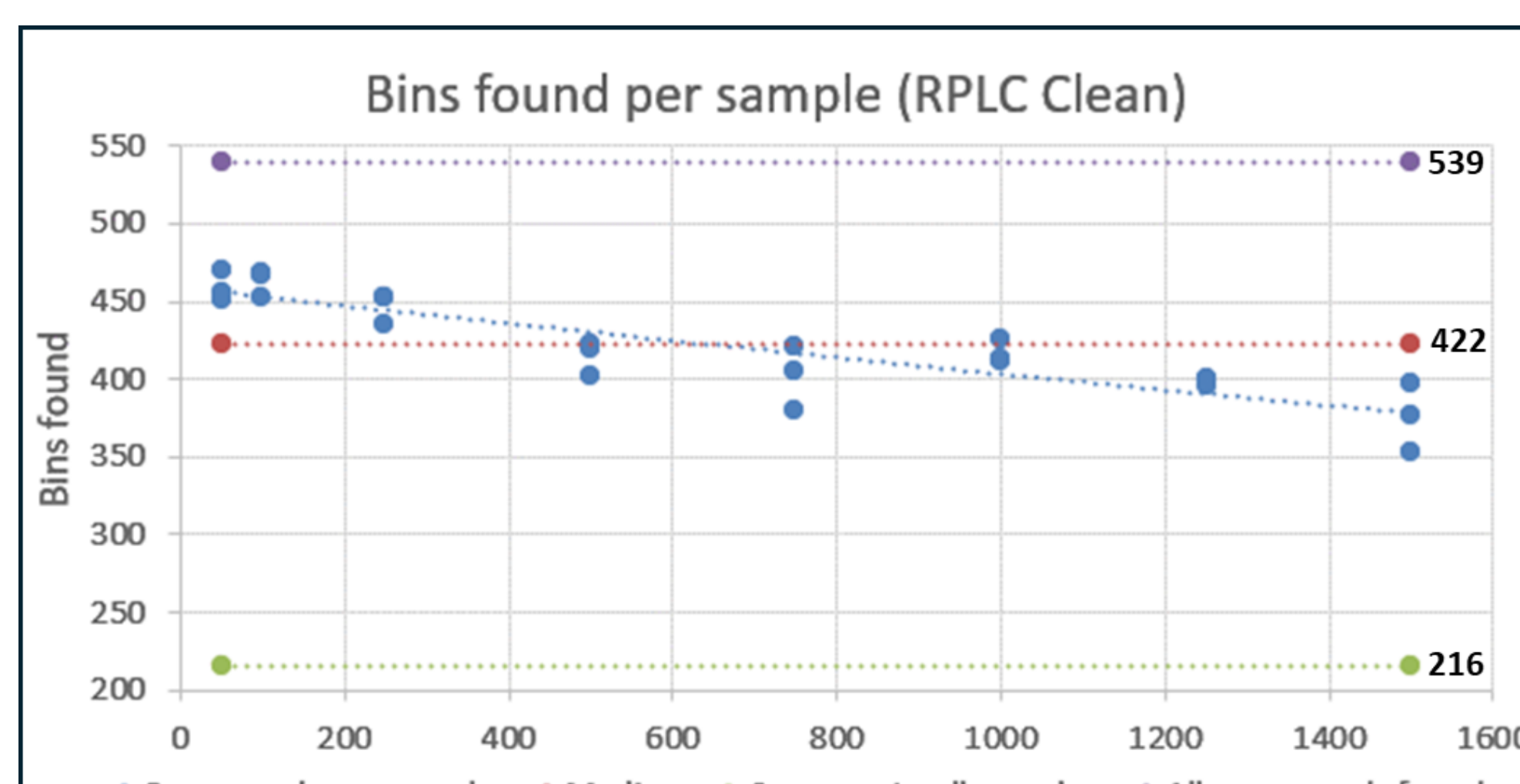


Figure 5 - Number of bins found at different concentrations

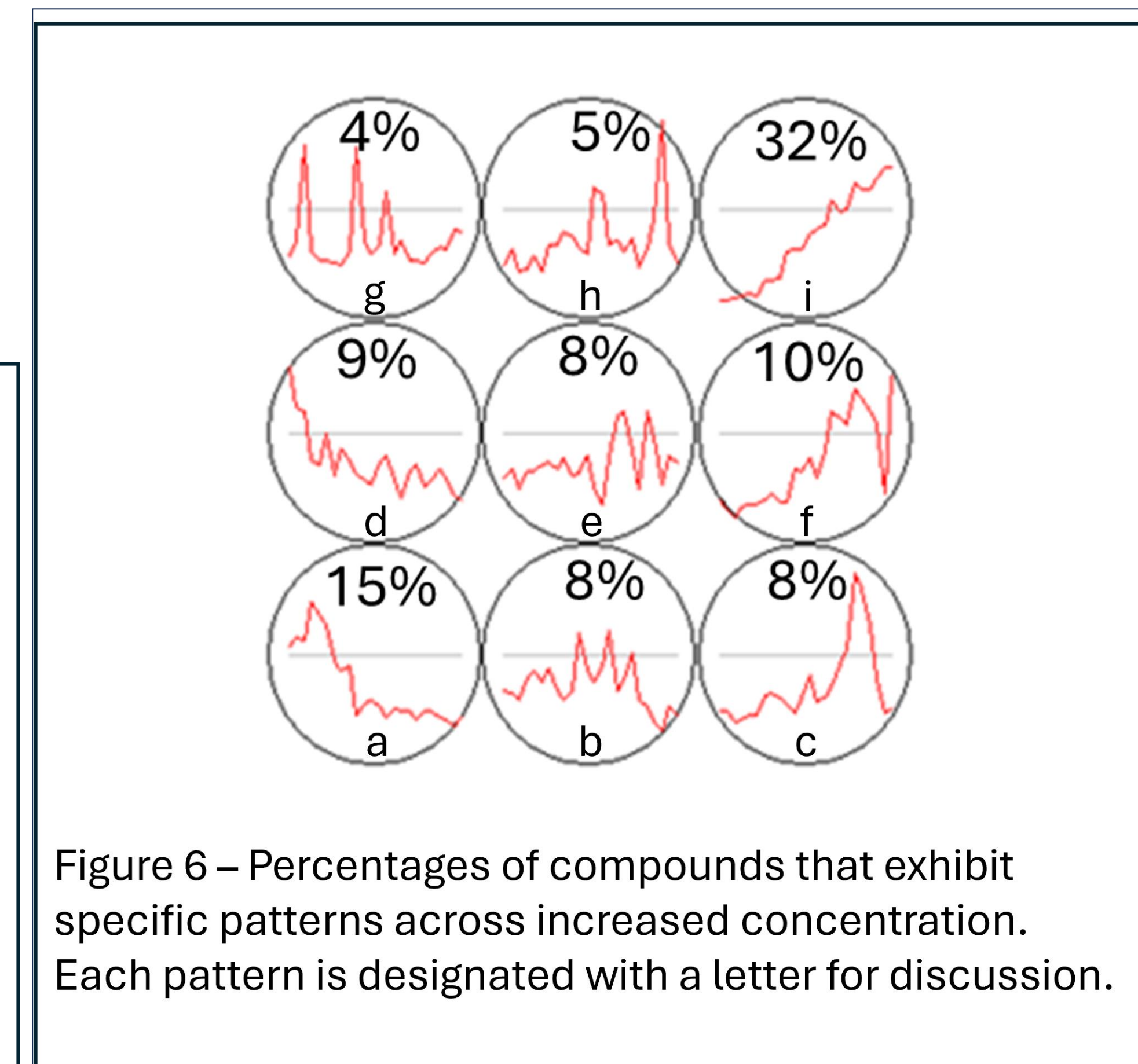
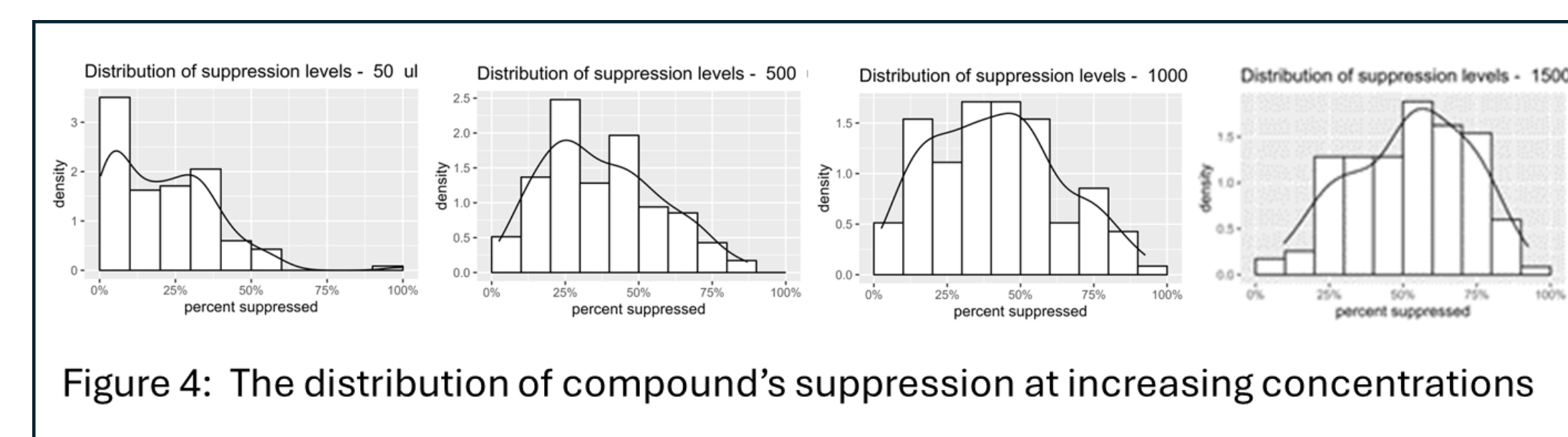
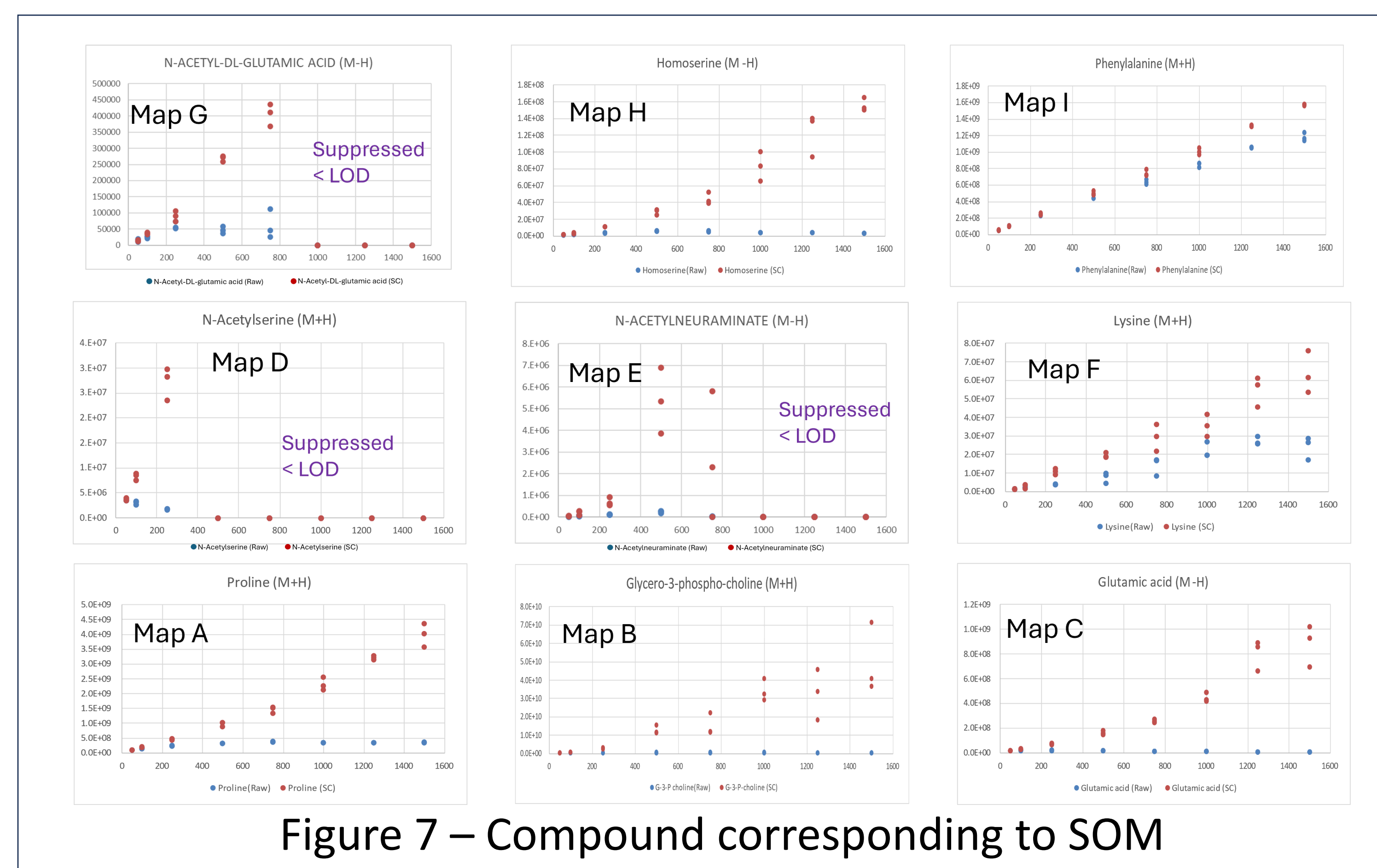


Figure 6 – Percentages of compounds that exhibit specific patterns across increased concentration. Each pattern is designated with a letter for discussion.



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Conflict of interest: Chris Beecher and Felice de Jong are co-founders of IROA Technologies

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RESULTS

The ability to see a compound in a mass spectrometer relies on 1) the efficiency of creating an ion, and 2) generating enough ions to be above the limit of detection (LOD). These two factors are closely interrelated.

The experiment detailed in this report provided a way to examine these effects in detail in real world samples. The results of this systematic approach have allowed us to quantitate and examine patterns of suppression seen for many compounds across a considerable concentration gradient. We are presenting our first observations but believe that there are many additional aspects that can be drawn from this dataset.

- The samples in the experimental design (see Figure 1) were analyzed.
- The overall results demonstrated that suppression did increase, as expected, as a function of sample concentration (see Figure 2).
- The rate of suppression seen in the natural abundance compounds was determined to be very comparable to the rate of suppression in the compounds in the Internal Standard (see Figure 3).
- The distribution of suppression across all of the compounds seen at any concentration (see Figure 4) indicates that in the high concentration samples the majority of peaks are intensely suppressed.
- In Figure 5, we analyze the responses of the 539 compounds we examined for this dataset.
 - The average number of compounds seen across all concentrations was 422 (~80%). However, only 216 (40% of the compounds) were seen in all samples.
 - The largest number of compounds seen were found in the lowest concentration samples. However, 22% of the compounds were present at concentrations below their LOD and were only seen at higher concentrations.
- In Figure 6, we see the results of a Self-Organizing Map (SOM) analysis of the appearance and disappearance of the compounds as a function of concentration across these samples.
 - Both patterns "a" and "d" represent compounds seen in low concentration samples that are heavily ion-suppressed ("a"), or slightly less heavily suppressed ("d").
 - Both patterns "i" and "f" represent compounds that are less effected by ion-suppression. Compounds represented by map "i" seem to be compounds present at higher concentrations and are less sensitive to ion suppression.
 - The remaining patterns "G", "D", "E", and "B", are indicative of compounds that appear episodically and disappear, i.e. drop below LOD, at different concentrations.
- In Figure 7 are compounds that are illustrative of the SOM maps. It should be noted that as long as the Internal standard peak can be found the peak can be corrected for the suppression it has endured, although in extreme conditions there appears to be more variance.

CONCLUSIONS

We used the IROA TruQuant Workflow plus novel companion algorithms to measure and correct for ion suppression, and then SOM to visualize the patterns of suppression of 539 peak pairs (IS and NA) seen across all concentrations.

Using a dilution series of plasma we were able to see distinct SOM patterns of suppression stressing the critical impact of ion suppression on the reproducibility of metabolomics analysis and the importance of study design for unbiased biomarker discovery.