

# IROA TruQuant IQQ WORKFLOW

## TAKE THE IROA TruQuant-IQQ CHALLENGE: Generate a Calibration curve

We are all aware that metabolomics data suffers from severe ion suppression. The IROA workflow can be used to generate a calibration curve to test for suppression. This information can be used to help get better results with quantitative accuracy.

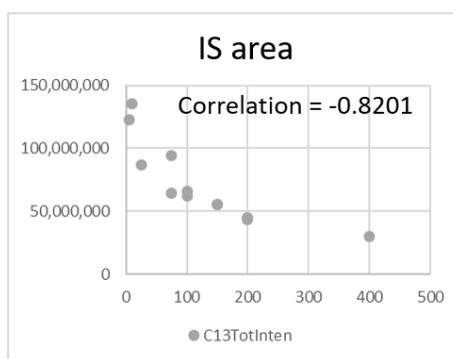
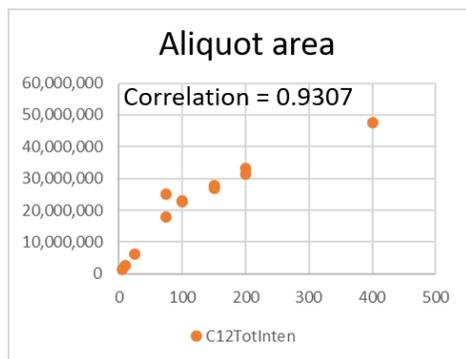
### Purpose of the Experiment

- 1) Allows the generation of a calibration curve specific to your sample prep methodology.
- 2) Allows familiarization with the IROA IQQ Workflow kit, fundamental IROA concepts and the ClusterFinder software.
- 3) Helps to understand the ideal amount/quantity of experimental sample required for analysis.
- 4) Results can be used to help you determine whether you need to normalize your samples.

### Experiment

- 1) Prepare an experimental sample for metabolomics analysis using your favorite sample type and standard prep method. The “prep” should represent approx. 30 times “normal” (sample volume usually injected) sample size and be homogeneous.
- 2) Make triplicate aliquot samples to generate a standard curve. We suggest using a range of 5% normal volume to 400% normal volume, i.e. Recommended: 5%, 10%, 25%, 50%, 100% (normal amount), 150%, 200%, and 400%.
- 3) Dry down the aliquots.
- 4) Resolubilize by adding 40 ul of Internal Standard (IS) from the IROA-IQQ kit.
- 5) Run standard LC-MS analytical method(s) on the samples.
- 6) Analyze the data using ClusterFinder software. (Follow the ClusterFinder User Manual and watch the [instructional ClusterFinder tutorials](#)).
- 7) Choose a compound of interest where good data has been collected. Plot the volume of prep (x-axis) vs. mass resolution (y-axis) for the C12 natural abundance signal (generated from the experimental compound) and generate a plot of the volume of prep (x-axis) vs. mass resolution for the compound isotopologue (generated from the C13 Internal standard). Look at the plots, it is usually obvious that the signal has been distorted by ion suppression, indicating that any quantitation based on these measurements will be inaccurate.

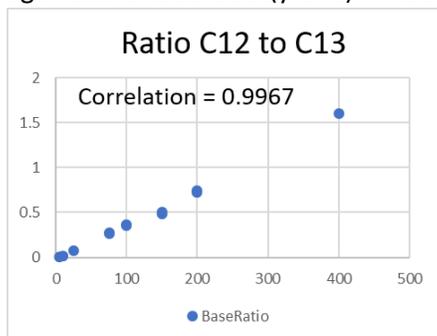
See example below:



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Compound = glutamic acid; human plasma was used as the “prep” sample and LC-MS analysis was conducted on an Orbitrap.

- 8) Calculate the “Base ratio” of C12 to C13 for each measurement. Plot the volume of prep (x-axis) against the base ratio (y-axis). This should be linear and the correlation coefficient close to 1.



- 9) Should you wish to normalize your data, we recommend a variant of the MS “total useful signal” (MSTUS)\* normalization procedure which uses the total intensity of components which are common to all samples, avoiding artifacts, to normalize data. Calculate the total area ion count for all C12 signals, make the same calculation for the C13 signals and calculate the C12/C13 ratio. Multiply the individual C12 compound areas with the normalization ratio to adjust for variability.
- 10) Please contact us ([chris@iroatech.com](mailto:chris@iroatech.com); [felice@iroatech.com](mailto:felice@iroatech.com) or [info@iroatech.com](mailto:info@iroatech.com)) and share your data.

For further background reading on the IROA-IQQ Workflow kit, please visit our [website](#) or read the WHITEPAPER “[Challenges in Metabolomics Analysis \(and a solution\)](#)” and contact us if you have any questions.

\*Warrack *et al.*, J. Chrom. B 877: 547-552 (2009).