

# TruQuant Workflow Calibration Experiment

## 01 TruQuant Workflow Calibration Experiment

If this is the first time you are using the TruQuant Workflow, your first experiment should be a “calibration” experiment, namely to test different amounts of your matrix (blood, cells, tissues, urine etc.) with the same amount of IROA-IS to figure out how much to balance with the IROA-IS.

The calibration of IS to your SOP simply requires the generation of a single large portion of prepared “prepped” sample (the matrix sample type you plan to use for your experiment, i.e. plasma, urine or tissue, etc.). Prepare approximately 30X the normal sample size that is routinely used for this experiment. This experiment is only needed once but should be performed for each different matrix sample type prior to performing the entire Workflow.

**VERY IMPORTANT FOR HILIC AND OTHER NORMAL PHASE SYSTEMS: THE MORE POLAR COMPOUNDS IN THE IS AND LTRS WILL NOT DISSOLVE IN RELATIVELY NON-POLAR SOLVENTS. THESE REAGENTS MAY NEED TO BE DISSOLVED IN DIFFERENT SOLVENTS FOR EACH CHROMATOGRAPHIC CONDITION.**

# TruQuant Workflow Calibration Experiment

## 02 Setting up the Calibration Experiment

1. Pool and filter prepped sample to create a single homogeneous sample.
2. Deliver varying aliquots of the pooled sample, ranging from half of the normal amount delivered to 3 times the normal amount to Eppendorf capped sample tubes. (For example: 0.5X, 0.75X, 1X, 1.5X, and 3X.) Make triplicate samples.
3. Dry aliquots under a gentle nitrogen stream.
4. Be sure to add a blank sample, i.e. an empty tube that has no experimental material but will receive an aliquot of Internal Standard. This will not only serve as a QA/QC sample but a sample which will provide important qualitative and quantitative information. This sample, referred to as the "IS-Only" sample, should be injected three or four times randomly within the collection of experimental samples.
5. Resolvate a single vial of IROA LTRS with 30  $\mu$ L of a solvent that will work for injections (based on the chromatographic condition) but is reasonably polar to maximize the number of compounds that will go into solution. (The compounds in the LTRS and IS are mostly polar.) Thoroughly mix to ensure complete solvation. Note: It should go into solution freely. Keep on ice until ready to use. Inject 3, 4 and 5  $\mu$ L of the LTRS to determine the best injection size. An injection size that gives you a total of several hundred peaks is the goal.
6. Add 900  $\mu$ L of the same solvent to a single vial of IS. Thoroughly mix to ensure a single homogeneous solution. Note: Again, it should go into solution freely. Keep on ice until ready to use.
7. Use a 30  $\mu$ L aliquot of the IS to resolvate each of the dried experimental samples. Upon addition of IS, mix thoroughly.
8. Analyze samples using the chromatographic method you would normally use using injection volumes determined in step 5. The analysis may be repeated in multiple modes, i.e. positive reverse phase, negative reverse phase, positive HILIC, or negative HILIC. The IROA LTRS should be injected before and after analytical samples and should also be injected approximately every 10th injection. (If your injection size will require more than the original 30  $\mu$ L volume then pool two LTRS prior to the initial injection.)
9. The higher concentrated samples may overload the column so we do not recommend running them in random order, but rather run them from most dilute to most concentrated to reduce any carry-over effects. The randomly run LTRS and IS-Only samples will provide evidence of any carry-over.
10. There is no need for msms in the Calibration (experimental) samples, only do msms on the LTRS. Since msms tends to retard the peaks, run msms on only one or two LTRS injections.

# TruQuant Workflow Calibration Experiment

11. Use the ClusterFinder software to find and identify all IROA peaks in the LTRS in an unbiased analysis.
12. Export the compounds found in the previous step to run a targeted analysis of the IS containing samples. Use the ClusterFinder software to export all of the quantitative data from the IS containing samples for statistical analysis. Export all of the compound identification and QA/QC data from the LTRS. 13.
13. Analyze the samples to identify the amount of the prepped samples that yield an overall mass spectral signal that is equal to the overall mass spectral signal of the IS. This is the amount of sample that will most accurately be measured using the IS in the future, i.e. well balanced by the standard 30  $\mu$ L of IS. This is illustrated in Figure 1 below.

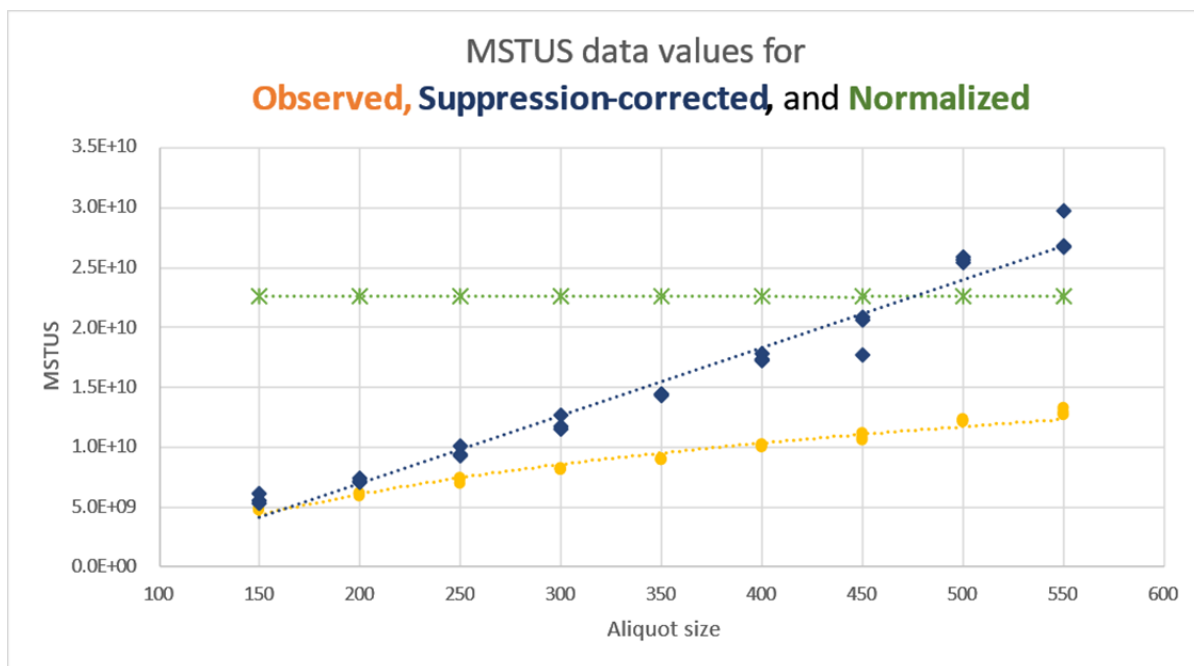


Figure 1. Suppression-correction and normalization for IS related peaks. The intersection of the suppression corrected MSTUS values (dark blue values), and the MSTUS C13 values for normalized data (green values) represents the aliquot size that will have optimal quantitation, i.e. any aliquot volume in this region will have the maximal number of 1:1 ratios between the natural abundance sample and the C13 IS when used in the Workflow protocol that is discussed in Section 5.03.