The IROA TruQuant IQQ Workflow

Measurement and Normali*zation System for targeted and untargeted MS analysis*

***Identification*, *quantitation* and QC of metabolites across time and instruments**

"IQQ"
Identification: Confidence in identifying common and new metabolites
Quantification: Comprehensive correction for all source ionization losses and biological variability
Quality Control: Convenient and automated tools for assuring reproducibility within/across studies

1. **Daily instrument QA/QC.**
2. **No false data. Reports only compounds of biological origin; excludes artefactual peaks.**
3. **Accurate compound formula ID for MS alone; complete ID with addition of SWATH, or IM.**
4. **All source ionization losses corrected for significantly better quantitation.**
5. **Reproducibility. Sample normalization to a universal standard for complete comparability.**
6. **ClusterFinder software solution** builds libraries, IDs/quantitates compounds, corrects for ion-suppression and normalizes data.
7. **Mass-spec vendor agnostic; specific extensions for inclusion of Sciex SWATH, and Agilent IM.**

Kit contents

Materials and tools for the analysis of 90 experimental samples

Unique fully-labeled *S. Cerevisiae* Yeast Extract*

- 3 vials of lyophilized IROA-IS; U-^{13}C, 95%
- 3 vials of lyophilized IROA-LTRS (Long-Term Reference Standard); paired U-^{13}C, 95% and 5%; mixed 1:1
- ClusterFinder™ software (including library of IROA peaks in the LTRS and their physical characteristics)
- User manual

Storage

-80ºC, protected from light

IROA Long-Term Reference Standard – IROA-LTRS (complex mixture of fully labeled 5% and 95% U-^{13}C metabolites, mixed 1:1) is used to build a daily, verified-identity "dictionary" of Retention Time (RT), *m/z* and physical characteristics for all IROA peaks. Identity of isobaric compounds is verified using fragmentation and/or Collisional Cross Section (CCS) data. As a Standard Reference Material (SRM) it also provides complete daily QA/QC on instrument performance. The IROA-LTRS data are collected randomly within every day's injection sequences.

IROA Internal Standard – IROA-IS (equivalent to the 95% U-^{13}C component of IROA-LTRS) is added to analytical samples to identify and quantitate metabolites using the co-incidentally-run LTRS "dictionary". The chemical makeup and chromatographic behavior of the IROA-LTRS is identical to that of the IROA-IS; so the IROA-LTRS "dictionary" is completely applicable to the IROA-IS containing analytical samples. Isobaric compounds distinguished in the IROA-LTRS based on fragmentation or CCS are distinguished in the IROA-IS by reference to the IROA-LTRS. Even if analyzed on different chromatographic systems, results can be equated using the dictionary because of comparability of the IROA-LTRS and IROA-IS, by querying the same mass and secondary physical characteristics across systems.

*Prepared from proprietary U-5%/U-95% ^{13}C IROA-labeled glucoses specially produced for IROA Technologies by Cambridge Isotope Laboratories, Inc.

3 STEP PROCESS:

1. LC-MS analysis of experimental samples resolvated with IROA-IS (experimental sample + IROA IS = analytical sample) are randomly analyzed. Within the random analytical injections the IROA-LTRS is injected approximately once for every 10 analytical samples, as a QA/QC Standard to account for fluctuations in mass and RT drift.
2. Generate "dictionary" of RT, m/z, formula and physical characteristics from the analysis of IROA-LTRS using ClusterFinder software to validly identify all compounds in the IROA-LTRS.
3. Use ClusterFinder and dictionary to identify and quantitate, suppression-correct and normalize all compounds in original experimental samples.

Summary of Benefits of the IROA TruQuant (IQQ) Measurement System

- **Cost effective** simultaneous metabolite identification, quantitation and platform QC
- Accurate compound formula ID for MS alone; complete ID with addition of SWATH, or IM, even at low concentrations
- **Unique IROA patterns discriminate peaks of biological origin from artefactual peaks** allowing the removal of false data
- All IROA-based **fragments** will have the IROA ratio pattern derived from their parent peaks and can be **identified** as such using the "peak correlation" ClusterFinder module
- **Alignment of all peaks** in the chromatogram using IROA-IS generated Retention Time (RT) ladder
- Retention Index within the sample **allows "unknowns" to be tracked** with accuracy and precision
- **All source ionization losses corrected for significantly better quantitation.**
- **Normalizes** (using suppression-corrected) experimental samples to overcome day-to-day, sample-to-sample, or instrument-to-instrument variances
- **ClusterFinder software solution** builds libraries, IDs/quantitates compounds and normalizes data
- Ensures **high level QC** for accurate and **reproducible** results