IDENTIFICATION AND COMPARABILITY OF 100s OF METABOLITES ACROSS TIME AND INSTRUMENTS

Background

LC-MS/MS metabolomics methods use isotopically labeled internal standards to ensure reproducibility and accuracy as they behave physically and chemically identical to the analytes under measurement. To ensure reproducibility and accuracy of measurements, IROA Technologies developed the IROA ID-QUANT-QC Workflow Measurement System for use in the analysis of any type of research or clinical sample.

3 STEP PROCESS: (see Figure)

(1) LC-MS analysis of experimental samples spiked with IS and MATRIX. MATRIX is injected periodically i.e. 10 sample intervals, as QC Standard to account for fluctuations in mass and retention time drift.

(2) Generate "dictionary" of RT, m/z, formula and physical characteristics from the analysis of MATRIX using ClusterFinder software.

(3) Use ClusterFinder and dictionary to identify and quantitate compounds in experimental samples.

MATRIX (paired 1:1 mixture of labeled U-5% and - 95% ^{13}C metabolites): used to build a triply redundant dictionary (library) of RT, *m/z* and physical characteristics including fragmentation data.

IROA INTERNAL STANDARD – IS (labeled 95% ^{13}C metabolites): spiked into experimental samples and used as a yardstick in which to identify and quantitate metabolites using library. Even if analyzed on a different chromatographic system, results can be equated using dictionary because of IS. Experimental compounds may have different RTs but will exhibit the same mass and physical characteristics.

Benefits of the IROA ID-QUANT-QC Workflow

MATRIX (paired 1:1 mixture of Internal Standard; U- 95% ^{13}C and its U-5% ^{13}C mirror image)

- Used to build a reference **dictionary of 100's of compounds** for each LC-MS and MSMS mode
- Analyzed every 10 samples during sample analysis to create a Retention Index specific to the chromatographic run each day
  - Enables comparison of data across time and instruments
  - MATRIX ensures **high level QC** for accurate and reproducible results

IROA Internal Standard - **IROA-IS** (U- 95% ^{13}C component of Matrix only)

- Used as a yard stick to spike into native experimental samples for the identification and measurement of 100's of analytes
- Native and labeled metabolites chromatographically separate together and ionize with the same intensity
- Native and labeled metabolites easily distinguished; co-location of native compounds in experimental samples, even at low concentrations
- Fragments and adducts have identical IROA labeling patterns of precursor ions and can be identified as such
- Artefactual or non-paired peaks can be eliminated
- IROA-IS generated Retention Time (RT) ladder allows alignment of all peaks
- Metabolite data may be normalized to the IROA-IS to overcome day-to-day, instrument-to-instrument variances

ClusterFinder Software

- Generates libraries for each LC-MS mode from Matrix analysis data
- Automatically finds, quantitates and identifies all natural abundance peaks in experimental samples corresponding to known IROA isotopomers in the IROA-IS, and remove artifacts
- Performs Peak Correlation Analysis - finds correlated adducts and fragments

IROA ID-QUANT-QC Workflow

MEASUREMENT SYSTEM FOR TARGETED OR UNTARGETED ANALYSES

Suggested Use

Research or Clinical Samples

Built-in QC Standard

Build a 400-600 reference dictionary for every LC-MS and MSMS Mode to analyze and quantitate experimental samples

Kit includes

materials and tools for the analysis of 90 experimental samples

Unique fully-labeled Yeast Extract

- 3 vials of lyophilized IROA-IS
- 3 vials of lyophilized IROA-Matrix
- **ClusterFinder™ software**
- User manual

Store at -20°C, long-term at -80°C
FOR RESEARCH USE ONLY

Proprietary IROA-labeled materials specially produced for IROA Technologies by Cambridge Isotope Laboratories (CIL).

IROA WORKFLOW

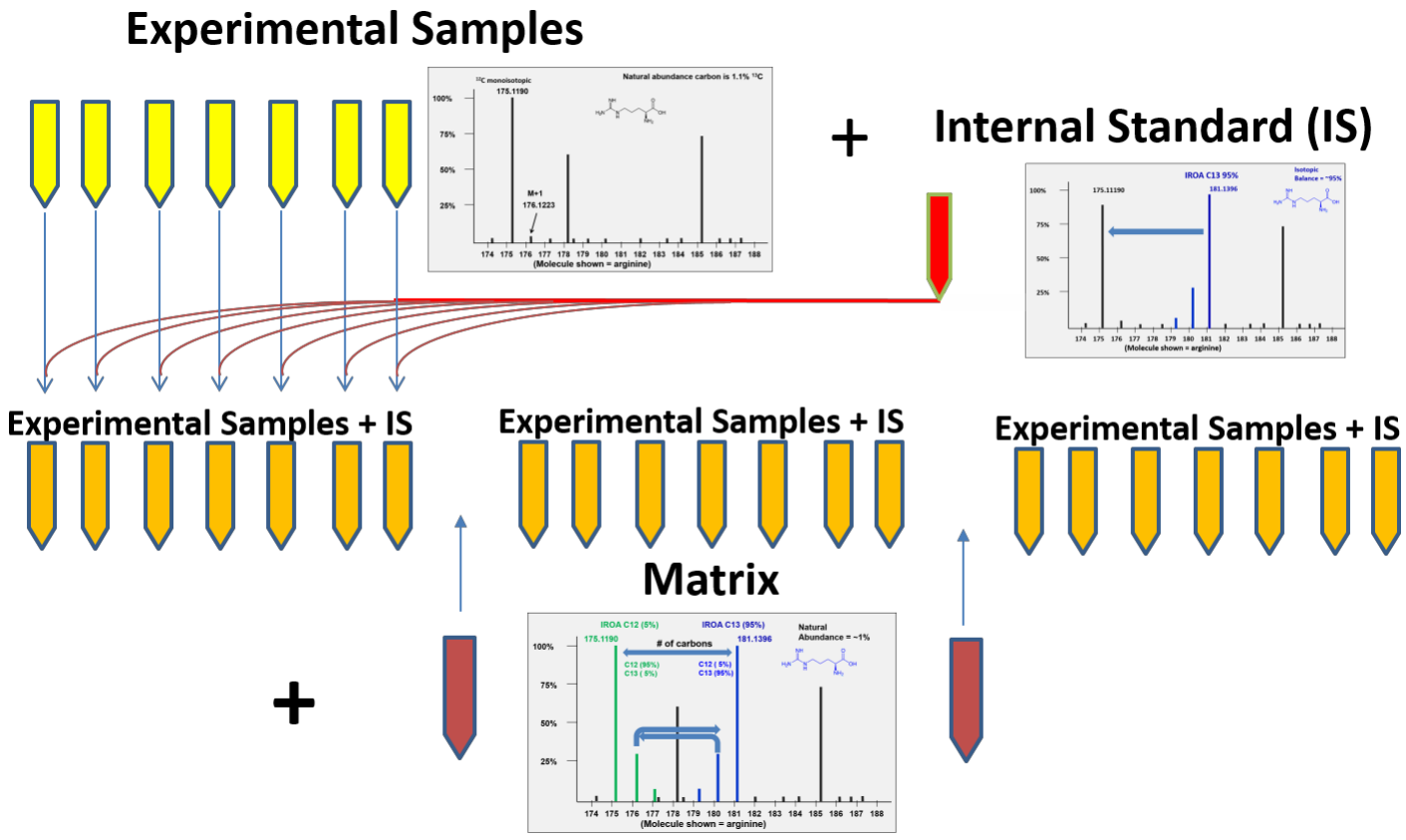

Figure. The IROA-based ID-QUANT-QC workflow adds a consistent biochemically complex Internal Standard into every experimental sample for enhanced quantitation, and injections of specially-developed, IROA MATRIX standards are analyzed every 10 samples to support identification, compound location and to create a Retention Index specific to the chromatographic run each day. Together these assure the comparability of accurate data across time and instruments.

The IROA-Matrix "U-shaped smile" pattern of peaks contains both the IROA-IS envelope (U-95% ^{13}C peaks; M-1 etc.) and its mirror-image envelope (U-5% ^{13}C peaks; M+1 etc.). The height of the M+1 and M-1 differ directly according to the number of carbons in a molecule; here 32% the height of their monoisotopic peaks, ^{12}C and ^{13}C, for a six-carbon molecule. This is true not only for the M+1 and M-1, but also the shape of the entire isotopic envelope is different for every number of carbons. The number of carbons in a biological molecule can be also determined by the distance between the two monoisotopic peaks. Since the relative height of the M+1, the relative height of M-1, and the distance between the monoisotopic peaks all provide confirmation of this fact, this results in a triply redundant quality control check point.

The MATRIX is initially analyzed separately using LC-MS and MSMS methods to build a "dictionary" of RT, m/z and physical characteristics stored in ClusterFinder software. The dictionary is subsequently used to ID metabolites in experimental samples spiked with the IS. The IS serves as a yardstick. Even if a different chromatographic system is used the software can rely on the physical characteristics stored in the dictionary to accurate ID compounds.