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 Workflow Measurement System for use in the analysis of any type of research or clinical sample.

3 STEP PROCESS: (see Figure)

(1) Generate compound library with analysis of MATRIX using ClusterFinder software.

MATRIX (paired 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.

(2) Analyze experimental samples spiked with IS. Inject MATRIX periodically i.e. 10 sample intervals, as QC Standard to account for fluctuations in mass and retention time drift.

(3) Use ClusterFinder to identify and quantitate compound using library

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. Compounds may have different RTs but will exhibit the same mass and physical characteristics.

Benefits of the IROA Workflow

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

- Used to build a reference **library of 100's of compounds** for each LC-MS 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)

- Used 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 WORKFLOW

MEASUREMENT SYSTEM FOR TARGETED OR UNTARGETED ANALYSES

Suggested Use

Research or Clinical Samples

QC Standard

Build a 400-600 compound library for every LC-MS Mode to analyze and quantitate metabolites

IROA-WORKFLOW 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 -80°C
FOR RESEARCH USE ONLY

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

IROA WORKFLOW

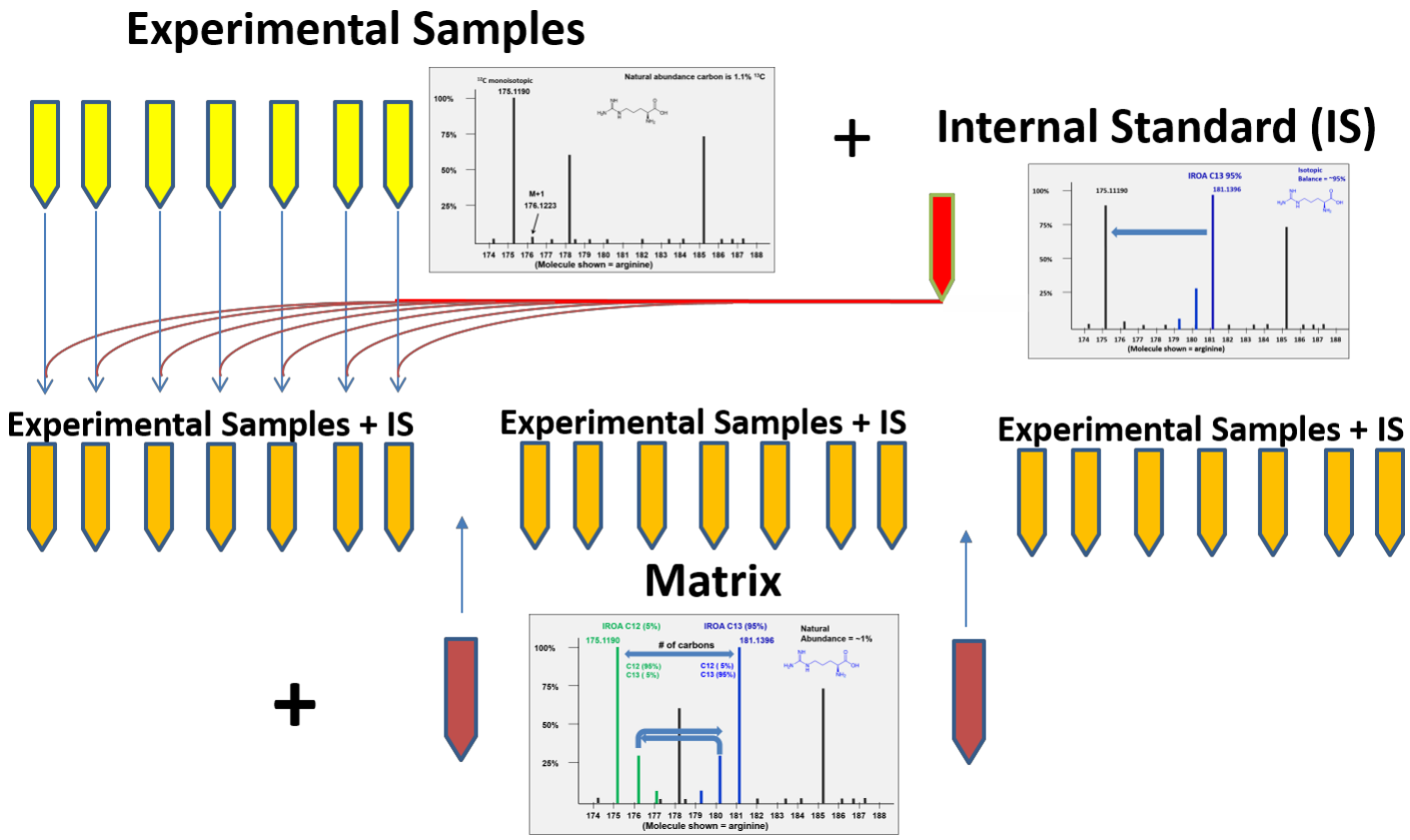

Figure. The IROA-based workflow adds a consistent biochemically complex Internal Standard into every experimental sample for enhanced quantitation, and two injections of a specially-developed, pure IROA MATRIX sample are analyzed every 10 samples to support identification, compound location and to create 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 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.