Ion suppression correction and normalization for non-targeted metabolomics

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Abstract

Ion suppression is a major problem in mass spectrometry (MS)-based metabolomics; it can dramatically decrease measurement accuracy, precision, and signal-to-noise sensitivity. Here we report a new method, the IROA TruQuant Workflow, that uses a stable isotope-labeled internal standard (IROA-IS) library plus novel companion algorithms to 1) measure and correct for ion suppression, and 2) perform Dual MSTUS normalization of MS metabolomic data. We have evaluated the method across ion chromatography (IC), hydrophilic interaction liquid chromatography (HILIC), and reverse phase liquid chromatography (RPLC)-MS systems in both positive and negative ionization modes, with clean and unclean ion sources, and across different biological matrices. Across the broad range of conditions tested, all detected metabolites exhibited ion suppression ranging from 1% to 90+% and coefficient of variations ranging from 1% to 20%, but the Workflow and companion algorithms were highly effective at nulling out that suppression and error. Overall, the Workflow corrects ion suppression across diverse analytical conditions and produces robust normalization of non-targeted metabolomic data.

Introduction

Metabolite levels reflect metabolic function and the integrated output of genomics, epigenomics, transcriptomics, and proteomics, including inputs from lifestyle and environment[1–3]. Hence, metabolomics is an effective approach for elucidating candidate drug targets[4], candidate biomarkers of disease progression[5], candidate biomarkers of therapeutic response[6,7], mechanism(s) of drug sensitivity[8], mechanism(s) of drug resistance[9], and mechanisms of drug toxicity[10]. Unfortunately, rigorous, reproducible quantitation of metabolites is difficult. Standardization across laboratories, biological matrices, and analytical conditions is a major challenge for both research and clinical implementation of metabolomics.

Ion suppression is a type of matrix effect in mass spectrometry (MS) and a major contributor to those challenges (**Fig. 1**). The authors of a recent perspective article on best practices in metabolomics noted: “While there is no universal solution to the ion suppression problem, assessing the effects of ion suppression affords greater confidence in the accuracy of the results.”[11] Indeed, until now, no universal solution has existed to counteract the negative effects of ion suppression across all analytes in a non-targeted metabolite profiling study. We present such a solution.

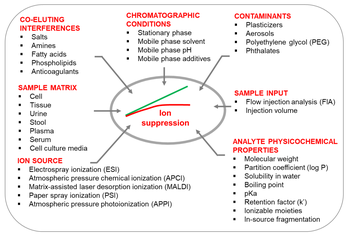

Fig. 1 | Pre-analytical and analytical variables that affect quantitative rigor and reproducibility in MS-based metabolomics. The red line represents observed, suppressed signal, which can be caused by the listed factors. The green line represents suppression-corrected signal, which can be realized by the techniques described in this paper. Representative examples of factors that affect the quantitative accuracy of MS-based measurements are listed and have been reviewed elsewhere[12,13].

The mechanisms of ion suppression (e.g., in plasma, urine, cell culture, or tumor) are reviewed in detail elsewhere[11–13]; the type of ionization source[5,14–17], mobile phase composition[18], gas temperature, and physicochemical properties (e.g., pKa, polarity/aromaticity, hydrophobicity/lipophilicity) of analytes and matrix components are examples of factors that can contribute to ion suppression[12,19,20].

Ion suppression for small numbers of analytes can be addressed to some degree by diluting samples, modifying analytical conditions to eliminate interferences, conducting a sample cleanup procedure such as solid phase extraction, and/or adding a chemically matched stable isotope-labeled internal standard[11,21]. However, because the source and magnitude of ion suppression can vary extensively across metabolites and samples[11,22], counteracting ion suppression across all analytes and all samples in a non-targeted profiling study remains an unsolved challenge[11,23].

Stable isotope-labeled internal standards can correct for variability in ionization efficiency and ion suppression. However, isobaric isotopologs (e.g., the M+0 isotopolog of lactate and the M+1 isotopolog of alanine) are difficult to distinguish. That has been a barrier to the effective use of stable isotope mixtures. Isotopic Ratio Outlier Analysis (IROA) protocols[24–27] solve that problem by generating clearly identifiable isotopolog patterns. IROA also facilitates removal of non-biological signals, which are common artifacts in MS data.

We introduce here a novel Workflow that effectively corrects ion suppression and uses a Dual-MSTUS normalization algorithm to improve the quantitative accuracy, precision, and signal-to-noise sensitivity of metabolomic data across diverse origins and analytical conditions.

Conceptual overview

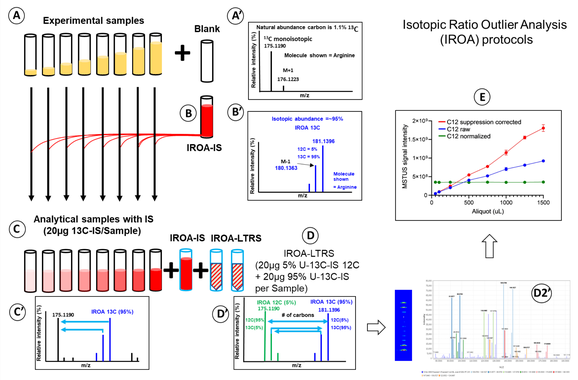

Fig. 2 | IROA TruQuant Workflow. In this protocol the experimental samples (**A**) are prepared and dried. They are then reconstituted with a solvent containing the IROA-IS (**B**) to yield the analytical samples (**C**). The analytical samples are randomized and injected within a sequence that starts and ends with injections of the IROA-LTRS (**D**), which is also injected approximately every 10 injections. Based on the presence of the IROA-IS, each sample can be suppression-corrected and normalized despite significant differences in sample input (original sample aliquot volume prior to dry down) (**E**).

Ion suppression correction in non-targeted metabolomics

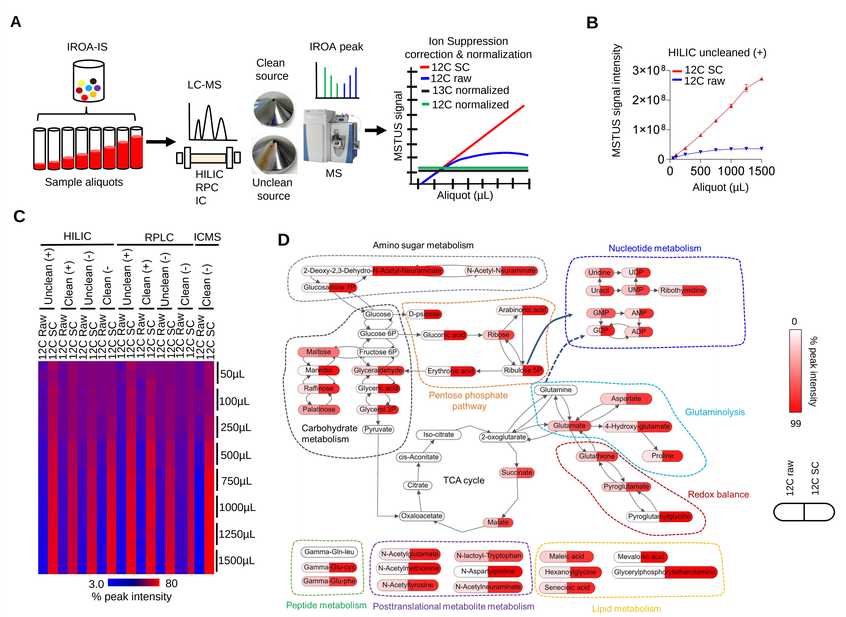

Fig. 3 | Ion suppression correction workflow. (B) An example of raw MSTUS-12C (blue lines) and suppression-corrected MSTUS-12C (red lines) values are shown for HILIC positive mode with uncleaned source. (**L**) Ratio of raw MSTUS-12C to suppression-corrected MSTUS-12C peak intensity across chromatographic methods and experimental conditions. (D) Global metabolic pathway analysis illustrating the effects of IROA ion suppression correction as an example determined by IC-MS before and after ion suppression correction. Data were drawn in SBGN (system biology graphical notation), Process Description (PD) and Activity Flow (AF) languages or Simple Interaction Format (SIF). Metabolites are color-coded based on the percent peak intensity. Raw = 12C raw; SC= 12C suppression corrected.

Development of Dual MSTUS for robust normalization of metabolomic data

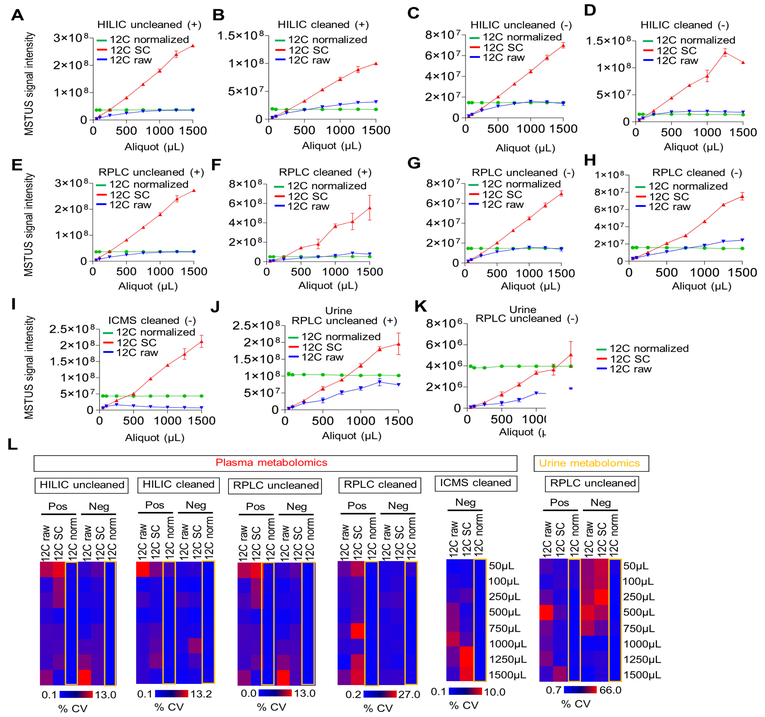

Fig. 4 | Dual MSTUS normalization of metabolomic data. (A-K) Raw MSTUS-12C values (blue lines), normalized MSTUS-13C values (green lines), and suppression-corrected MSTUS-12C values (red lines) for indicated chromatographic systems and conditions in plasma & urine. (**L**) Percent coefficient of variation (%CV) for raw, suppression-corrected, and normalized data from uncleaned and cleaned source conditions across different sample matrices and chromatographic systems including IC, HILIC, and RPLC.

Conclusions

1. We present an IROA-based Dual MSTUS Workflow that is applicable across chromatographic systems, ion sources, and biological matrices.
2. The Workflow, including suppression correction and Dual MSTUS normalization algorithms, facilitates accurate comparison of metabolomic profiling data across: i) unclean and clean ionization sources; ii) IC-, HILIC-, and RPLC-MS systems; and iii) plasma and urine matrices.
3. Overall, the Workflow provides an almost universal antidote to the negative effects of ion suppression across all analytes in a non-targeted metabolite profiling study. By doing so, it can increase the sensitivity, accuracy, precision, and biological and statistical significance of such measurements.

References

Mahmud et al. **An IROA Workflow for correction and normalization of ion suppression in mass spectrometry-based metabolomic profiling data.** Research Square, 2024 Feb 1:rs.3.rs-3914827. doi: 10.21203/r.3.rs-3914827/v1. **https://pubmed.ncbi.nlm.nih.gov/38352620/**

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