Quantitative Comparison of the Suppression Between HILIC and Reverse Phase Chromatography

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Overview

The degree of ion suppression observed for a variety of classes of metabolites over a range of aliquot concentrations was determined and supression-corrected values were calculated using the IROA TQ-IS and its associated LTRS.

- MS signal suppresion was investigated using HILIC and RPC chromatographic methods in both positive and negative ionization modes.
- Suppression-corrected values were calculated for both the C12 and C13-enriched peaks for every standard compound detected; generally 500 compounds were observed for HILIC and RPC.
- Almost all compounds exhibit some level of suppression and compounds are suppressed to different extents between HILIC and RPC-MS.

Introduction

Hydrophilic Interaction Liquid Chromatography (HILIC) and Reverse Phase Chromatography (RPC) represent the major chromatographic methods for metabolomic analysis today. Here we investigate and compare HILIC- to RPC-associated ion suppression using the IROA TruQuant Internal Standard (TQ-IS) and its associated Long-Term Reference Standard (LTRS), testing the hypothesis that TQ-IS and LTRS could be used to correct for ion suppression over a range of metabolite concentrations. We determined the degree of ion suppression for various classes of metabolites over a range of aliquot concentrations and calculated suppression-corrected values using the TQ-IS and LTRS for both the C12 and C13-enriched peaks for every standard compound detected; generally 500 compounds were observed for HILIC and RPC.

Because the internal standard was introduced at the same concentration for all samples, the Area Under the Curve (AUC) and peak height of each metabolite indicated that both the natural abundance isotopomer (Na) and the IROA isotopomeric collection (IROA-IS) for each compound was always subjected to the same level of suppression, and, therefore, TQ-IS and LTRS could be used to consistently correct for suppression across a range of sample volumes. Notably, the ratio of Na:IROA-IS was consistent with concentration and unaffected by suppression. On that basis, we were able to calculate corrected values for suppression losses and precisely calculate suppression for all compounds. This technique proved to be appropriate for both HILIC and RPC, and in theory should be applicable to all chromatographic systems and source types that do not show performance differences between isotopomeric species.

The LTRS was used to verify the identity of all compounds, and in each chromatographic system, to determine their RI. Their identity was verified by both the shape of their symmetrical IROA peak clusters, which assures formula, and fragmentation data to verify isobaric compounds. The LTRS from their respective analyses were used to map compounds between RPC to HILIC so that we could see the percent suppression for the same compounds in each system. In RPC, when performing standard metabolomic "non-targeted" analysis, the percentage of compounds that suffer from suppression is frequently assumed by most users to be low and is disregarded. Our initial analysis found that almost all compounds exhibit some level of suppression and that ion suppression is a seriously underestimated phenomenon in LC-MS. Similarly, we found that compounds are suppressed to different extents between the two chromatographic systems.

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Methods

1. **Sample Preparation.** Pooled plasma was extracted using -80°C methanol (methanol:plasma, 20:1). Six replicate aliquots of pooled extract at 150, 200, 250, 300, 350, 400, 450, 500, and 550 µL were delivered to vials, dried using a centrifugal vacuum concentrator, and stored at -80°C. For analysis, samples were reconstituted with 40 µL of the TQ-IS in distilled water. The LTRS was resolvated by addition of 40 µL distilled water. Samples were randomized and analyzed according to their respective chromatographic systems.
2. **LC-MS.** The first chromatographic system consisted of a Phenomenex Kinetex HILIC column (1.7 µm, 100 Å, 100 x 2.1 mm). The second chromatographic system consisted of a Phenomenex Kinetex C18 column (2.6 µm, 100 Å, 150 x 2.1 mm) C18 column. Mass spectrometry was performed on a Thermo Scientific Orbitrap Fusion Lumos Tribrid operated in FTMS full-scan mode with a scan range of 70-1,400 m/z and a resolution of 240,000.
3. **Data analysis.** Data were analyzed using ClusterFinder software (version 3.1).

Results

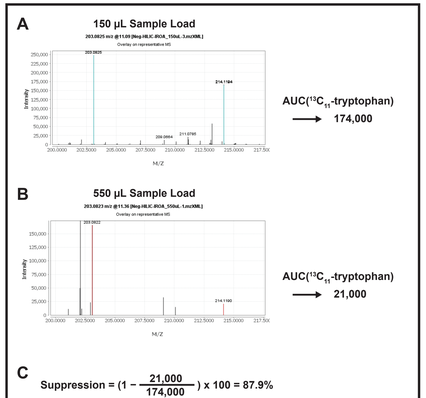


Figure 1. Suppression of tryptophan in HILIC negative ionization mode. Extracted ion chromatograms (XICs) from the analysis of (A) 150 µL and (B) 550 µL plasma extract. (C) Calculation of the degree of suppression at 550 µL relative to 150 µL sample load.

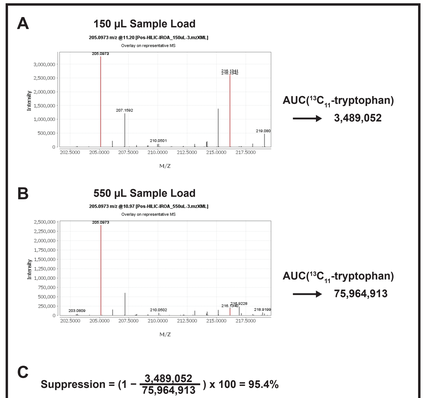


Figure 2. Suppression of tryptophan in HILIC positive ionization mode. Extracted ion chromatograms (XICs) from the analysis of (A) 150 µL and (B) 550 µL plasma extract. (C) Calculation of the degree of suppression at 550 µL relative to 150 µL sample load.

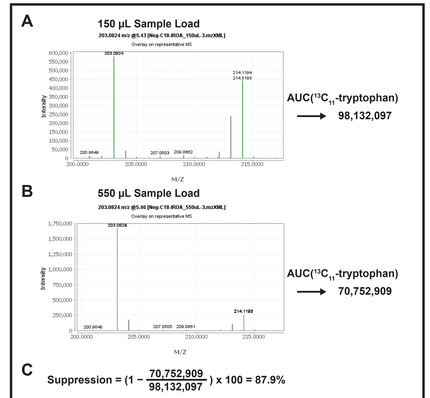


Figure 3. Suppression of tryptophan in RP negative ionization mode. Extracted ion chromatograms (XICs) from the analysis of (A) 150 µL and (B) 550 µL plasma extract. (C) Calculation of the degree of suppression at 550 µL relative to 150 µL sample load.

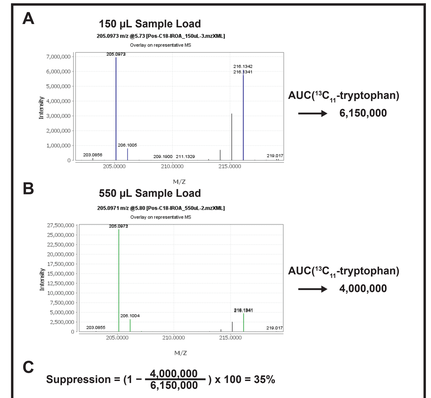


Figure 4. Suppression of tryptophan in RP positive ionization mode. Extracted ion chromatograms (XICs) from the analysis of (A) 150 µL and (B) 550 µL plasma extract. (C) Calculation of the degree of suppression at 550 µL relative to 150 µL sample load.

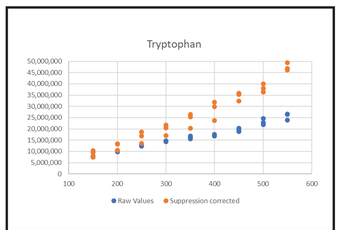


Figure 5. IROA-based correction of tryptophan ion suppression increases proportionality to sample load, resulting in more accurate quantitation. Raw AUC values are shown in blue, and corrected values (calculated by dividing raw ^{12}C AUC values by the corresponding ^{13}C AUC values) are shown in orange. Data are presented as a log-log plot to facilitate assessment of signal proportionality.

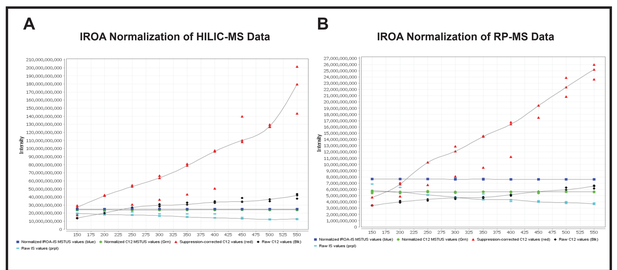


Figure 6. IROA successfully normalizes and corrects ion suppression in global metabolite profiles acquired by (A) HILIC-MS and (B) RP-MS. With both HILIC and RP approximately 500 metabolites were assessed. Corrected values were calculated by dividing raw ^{12}C AUC values by the corresponding ^{13}C AUC values.

Conclusions

- **Ion suppression appears to be greater in HILIC-MS than in RP-MS**
- **Nearly all compounds exhibit suppression**
- **A biochemically complex LTRS and IROA internal standards successfully correct for ion suppression across measured metabolites**

Future Directions

- **Assess additional chromatographic systems**
- **Develop protocols for the correction of lipid ion suppression**
- **Assess the degree of suppression observed in additional biological matrices**

Acknowledgements

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