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Multi-Omics for Plasma: A Three-in-One End-to-End Automated Sample Preparation and LC/MS Metabolomics, Lipidomics, and Proteomics Workflow

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Introduction

Targeted LC/TQ Workflow for Multi-Omics

Comprehensive targeted proteomics, metabolomics, and lipidomics methods have been developed for multi-omic pathway discovery and quantification of biomarkers. Multi-omic studies enable analysis of metabolic pathway perturbations, which can provide insight into:

1. Therapeutic mode(s) of action
2. Off-target effects
3. Precision medicine development, efficacy correlation and cohort subgroups toxicity

Pseudo-Discovery with LC/TQ

These methods aim to measure a significant proportion of pathway relevant analytes with an LC/TQ. This takes advantage of the TQ performance in finding biomarkers which a HRAM untargeted platform may miss.

- Sensitivity at attomole level on column
- Precision – 0.5-fold change may be used with increased precision
- Linearity across 5-6 orders of magnitude
- Throughput with automation
- Annotation completed with TQ database

Agilent is combining hardware, consumables, and support to transfer polar metabolite, lipid, and peptide methods. Figure 1 describes the hardware and methods used for this workflow.

Experimental

Multi-Use Hardware for a Multi-Omic Dataset

Automated preparation of cell and plasma samples can be performed using the Bravo Metabolomics Sample Prep Platform.[1, 2] Manual extraction protocols are available, although automation improves reproducibility, reducing variation by ~50%.[2] Using a reproducible HILIC method and database, over 500 polar metabolites can be profiled with the Infinity II Bio LC and the new 6495D LC/TQ.[3] The speed of the 6495D allows for both positive and negative mode analysis in the same injection with reproducible peak areas even for low dwell times (Figure 7). This is impactful when trying to measure hundreds of biomarkers in the same injection. The same LC/TQ can measure over 700 lipids across 44 different classes with a reproducible C18 method annotated for isomers, with retention time alignment using NIST SRM-1950 for standardized retention time alignment. For protein digestion, an AssayMap Bravo may be used prior to peptide quant analysis with the same LC/TQ hardware and the MRM Proteomics products.[4] If a project is sample limited (<1 µg protein) and low flow is needed, then an Evosep One system with an Agilent nanoESI source may be used. This setup has proven robustness and reproducibility for low flow peptide analysis.[5] For all three methods, chromatographic specificity provides the ability to reproducibly monitor hundreds of analytes per injection.[6]

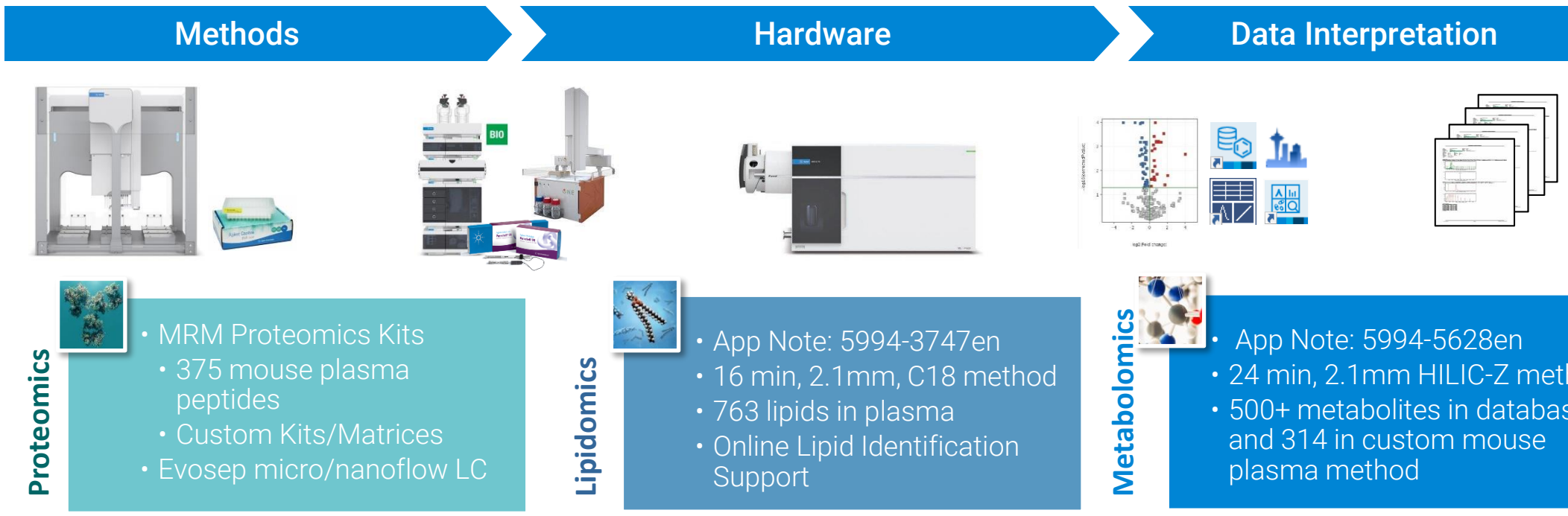

Figure 1. The multi-omic LC/TQ workflow begins with the Bravo Metabolomics Sample Prep Platform and AssayMAP Bravo for automated preparation of cell and plasma samples. Analyte separation is achieved using the Agilent Infinity II Bio LC for standard flow or the Evosep for low flow methods. The 6495D LC/TQ provides the sensitivity, robustness and precision for these methods while operating with dwell times as low as 0.5 ms per transition. Data analysis is completed with either MassHunter Quant or Skyline then Mass Profiler Professional with Pathway Architect used for statistics and pathway interpretation. Peptide, lipid, and metabolite methods are summarized here with references.

High Reproducibility of 3-in-1 Automated Bravo Sample Prep gives Greater Power to Studies

Automated sample preparation with the Bravo Metabolomics Sample Prep Platform with Captiva EMR-Lipid plates provides improved reproducibility and excellent metabolite recovery across representative chemical classes of compounds compared to manually pipetted extractions, (Figure 2).[7] For all metabolites, Bravo %RSDs were significantly lower than the combined %RSDs for manually prepared samples across 3 users. Reducing variation in this manner is crucial for large biological studies to increase the resulting statistical power.

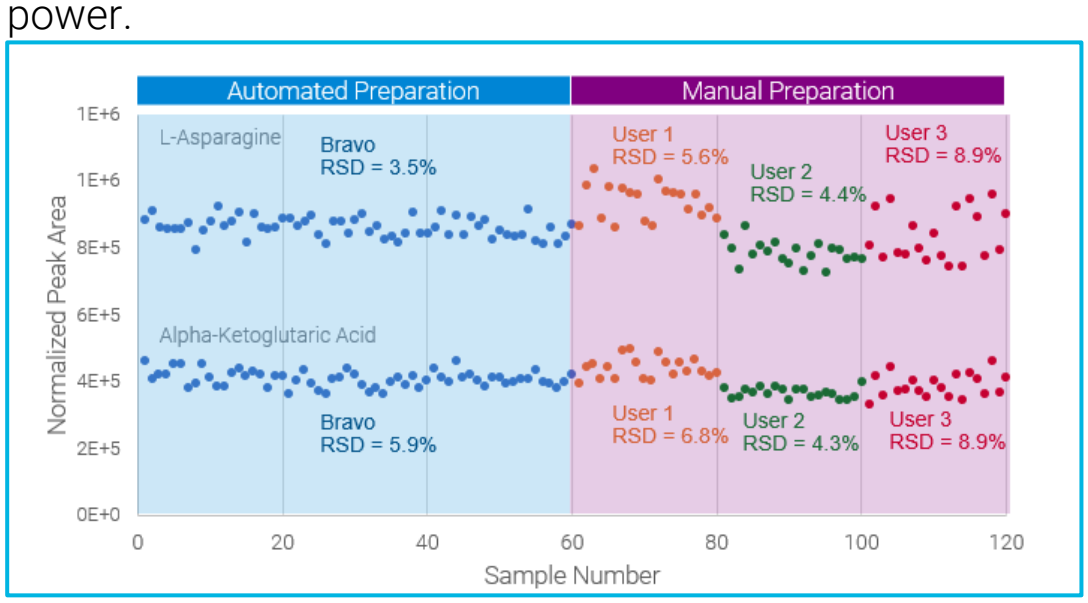

Figure 2. Reproducibility of results of metabolite extraction for asparagine and α-ketoglutaric acid with Captiva EMR Lipid plates for automated Bravo extraction (left) and manual extraction (right). Actual injection order was randomized.

Reproducible and Transferable Metabolite, Lipid and Peptide Methods

The peptide quant methods are enabled using MRM proteomics products. These cover 375 mouse plasma peptides and other products for other matrices are available. Data shown here shows amol sensitivity for mouse plasma peptides on this solution (Figure 3).

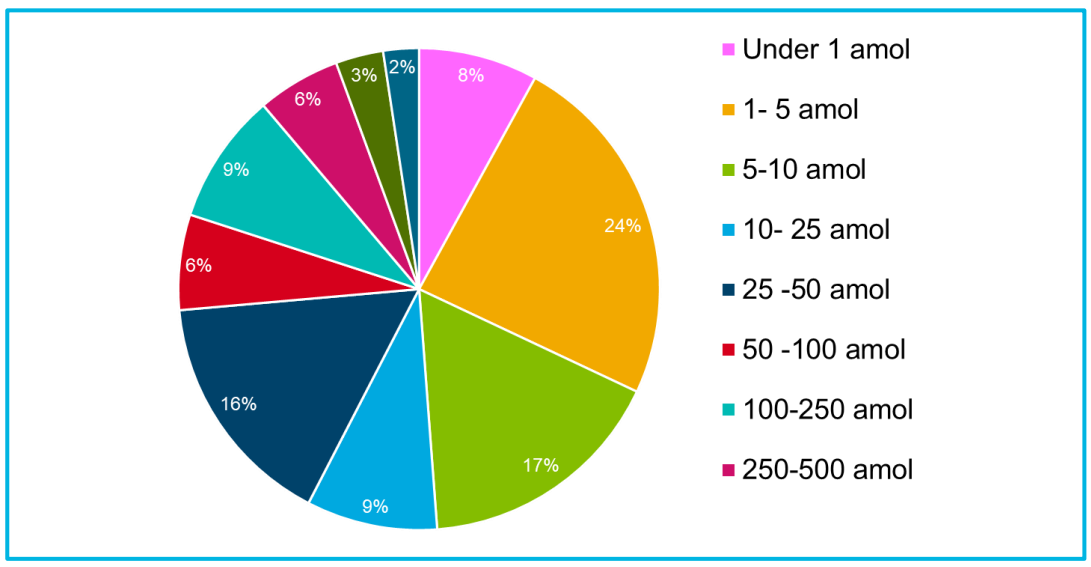

Figure 3. Sensitivity of 125 peptides in mouse plasma matrix. 74% LLOQ under 50 amol (on column) and 49% LLOQ under 10 amol (on column).

For an MRM method that has absolute quant, 375 mouse plasma peptides require 2250 transitions for 3 transition per peptide and internal standard. This methodology pushed the dwell time to <1 ms per transitions at times but the reproducibility is still very high. RSDs <30% for 95% of the peptides.

The lipid method is semi-quantitative and proven to be reproducible over large cohorts of plasma samples (3000+ samples).[6] It is very transferable with similar performance across different labs (Figure 5).

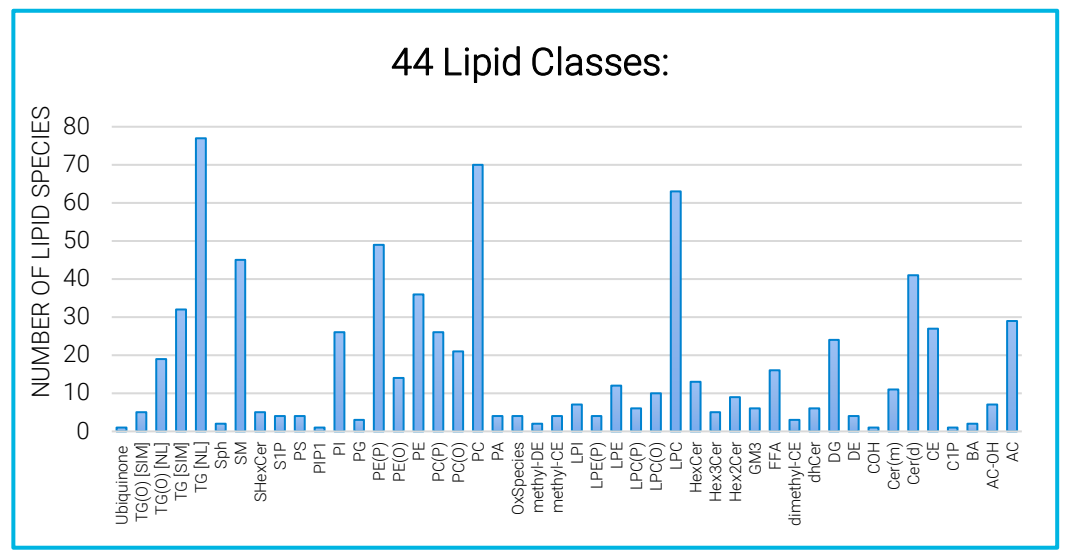

Figure 4. The lipid method covers 763 lipids across 44 different lipid classes. More details on which lipids are covered by the method in the corresponding App Note.[6]

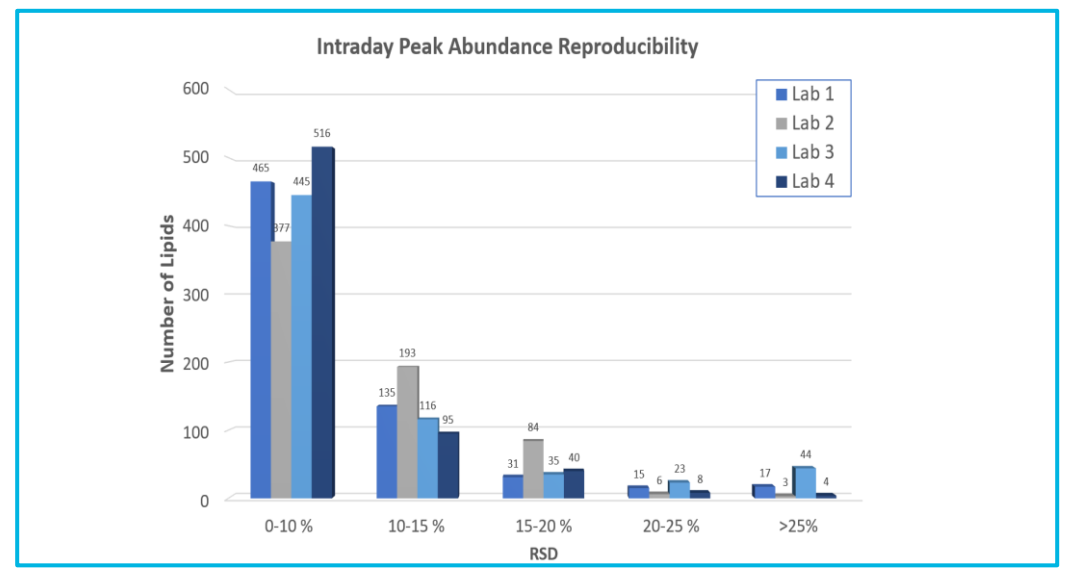

Figure 5. An inter-lab study using the NIST 1950 plasma standard was conducted. 4 different labs in 3 different states collected data with the same NIST extract. RSDs of the different lipids are reported here. The majority of lipids had <20% RSDs. And the 4 labs reported similar performance proving transferability.

New 6495D LC/TQ is Sensitive, Fast, and Precise

The metabolomics method and database can be used for a range of projects from discovery workflows of all 500 metabolites, to custom profiling of 200-300 analytes from plasma or cell samples, to quantitative analysis of a few key analytes of interest with fmol levels of detection in matrix.[3]

Results and Discussion

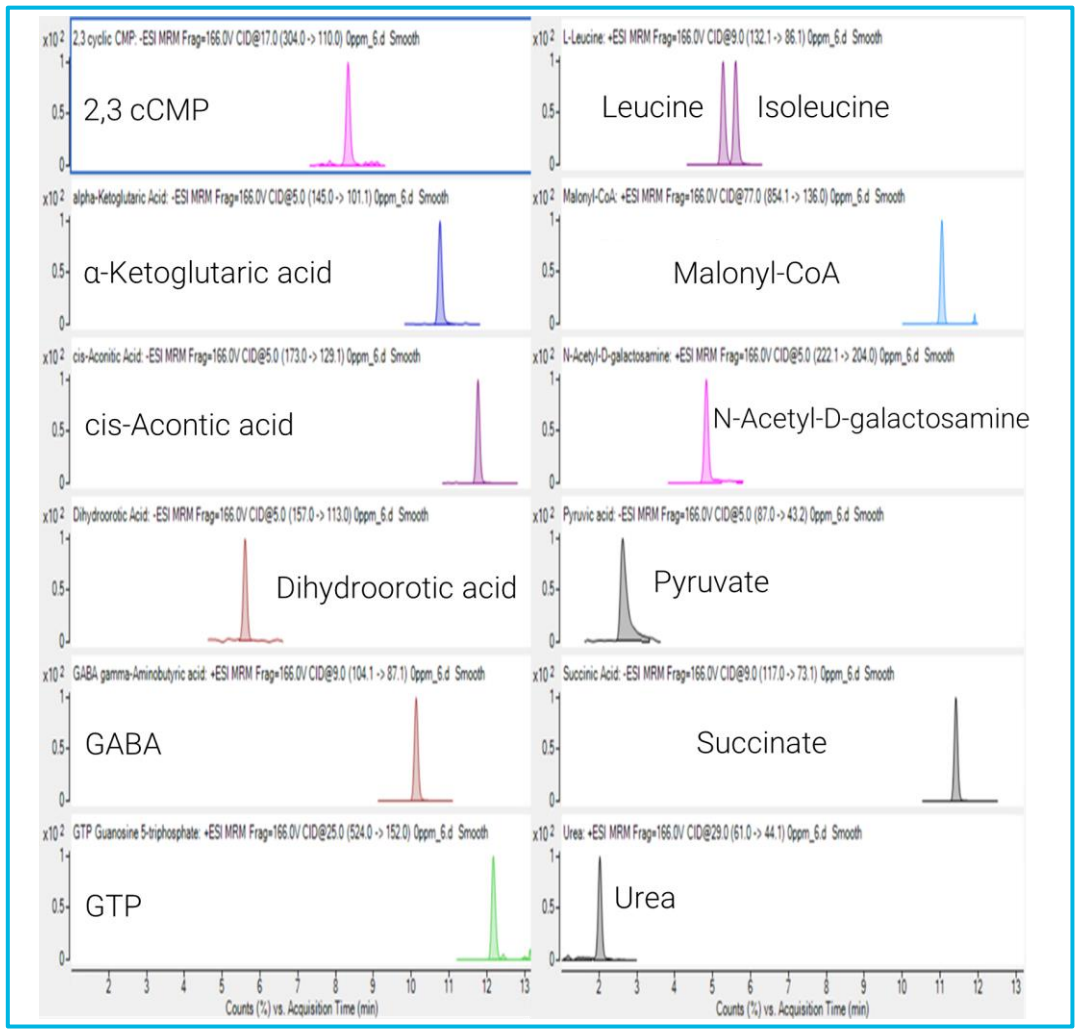

Figure 6. Analyte diversity in the HILIC LC/TQ database for metabolomics. 500+ metabolites in positive and/or negative mode with annotated retention times.

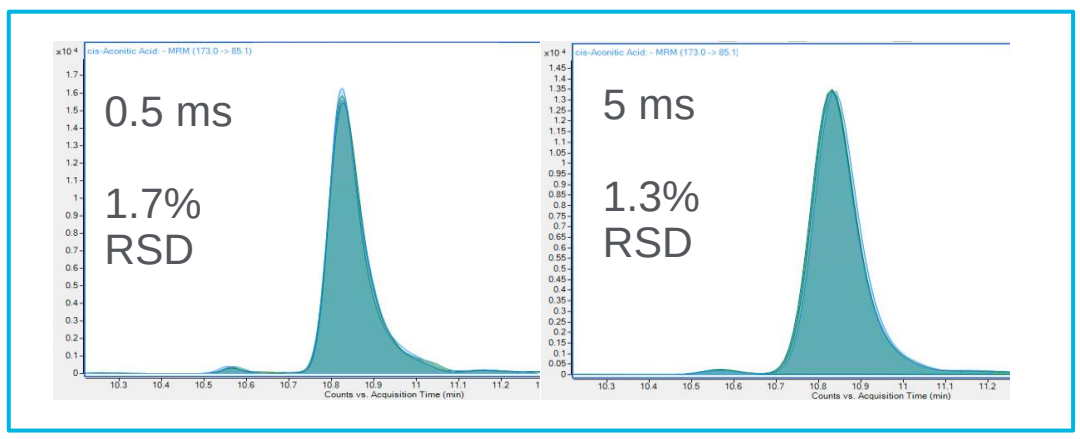

Figure 7. The 6495D LC/TQ can measure transitions as fast as 0.5 ms with excellent precision even in complex matrix. Here, 0.5 ms and 5 ms dwell times are tested with plasma matrix (n=6). At the 0.5 ms dwell time for *cis*-aconitic acid the RSD is 1.7% (left). When dwell time is increased to 5 ms, the RSD improves slightly to 1.3% (right). This hardware facilitates the measurement of more analytes per injection.

New Method Optimization for Ion Funnel Parameters Allow for Easy Analyte Customization

The new 6495D LC/TQ allows users to simplify and customize ion funnel parameters on an MRM-by-MRM basis. Settings include Fragile, Standard, and Large Molecule. Fragile and Standard iFunnel modes were evaluated using a cell matrix metabolite extract (n=6). The data shows improved sensitivity for some analytes with the Fragile setting (Figure 8A), while other analytes are less specific (Figure 8B).

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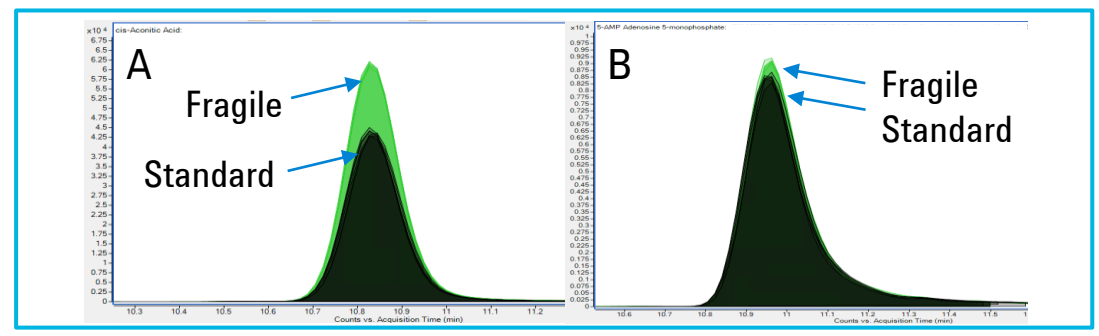

Figure 8: *cis*-Aconitic acid (A) and AMP (B) with Fragile and Standard iFunnel conditions in cell matrix (n=6) where Fragile had an 28% improvement over Standard conditions for *cis*-aconitic acid, while either condition could be used successfully for AMP.

Conclusions

Discover Impactful Pathway Information with Multi-Omic Studies Using easily Transferable Methods

- Agilent 1290 Infinity II Bio LC is configured to collect data on each omic method.
- The 6495D LC/TQ is robust, highly sensitive, very fast and easy to maintain enabling a pathway screening approach for labs looking for biological insights.
- Agilent and our partners at MRM Proteomics can provide transferable methods for 523 metabolites 763 lipids, and 375 mouse plasma peptides.
- LC/MS analyses are enabled by automated and manual sample preparation methods.

References

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