Evaluation of reproducibility and sensitivity of a new hardware workflow for LC/MS analysis of plasma samples.

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Introduction

LC/MS for Targeted Metabolomics

Targeted metabolomics studies using liquid chromatography mass spectrometry (LC/MS) are a well tested approach for interrogating known pathways. As these are becoming more common place in research laboratories, the boundaries are being pushed into making methods that are the most routine, reproducible, and analytically sensitive. This poster describes a workflow that can achieve reproducible results through automation of sample preparation coupled with an analytically sensitive analysis using a LC designed for metal sensitive analytes and an ion funnel triple quadrupole mass spectrometer (TQ).

Experimental

Automated Sample Preparation

A Metabolomics Bravo liquid handler platform (Figure 1) was used to process 10 µL aliquots of pooled bovine plasma. This Bravo-automated workflow has been described previously and provides more reproducible results than three individual technicians manually preparing samples.[1] Briefly, plasma aliquots in a 96-well plate have their proteins precipitated and the sample is transferred to the Captiva EMR-Lipid extraction plate. Here, the protein precipitate is captured by a filter, the lipids are captured by the EMR-Lipid resin, and the polar metabolites pass through the plate. Wash steps are then completed to boost metabolite recovery. The Metabolomics Bravo platform is equipped with a shaking station and vacuum filtration which facilitate sample mixing and collection of the metabolite-containing eluent. Lipids remain on the EMR-Lipid resin and can be extracted using a second method if desired. The metabolite-containing sample is dried down offline and reconstituted in the appropriate LC/MS analysis solvent. Data in this poster was obtained from 10 µL aliquots, but the protocols can be adjusted up to 100 µL sample.

Figure 1: Bravo chassie contains 9 positions for solvents, tips, plate shaker and vacuum filtration station.

Experimental

Infinity II Bio LC for Metal Sensitive Analytes

After the sample was prepared, 3 µL of the reconstituted extract was injected onto an Agilent HILIC-Z column (2.1 x 150 mm) and analyzed with an ammonium acetate buffer, pH 9. Standard LC systems need to be passivated to remove excess metal ions which can cause peak tailing and reduce overall analytical sensitivity. The new Infinity II Bio LC has MP35N metal alloy which is much lower in free metal ions. This system show significant improvement and was used for this method without passivation.

Figure 2: Infinity II Bio LC stack included a multisampler, column compartment with bio compatible switching valve, and high speed 1300 bar pump all made from bioinert material for improved detection limits for metal sensitive analytes. The 6495C LC/TQ was used in this method because the ion funnel improves detection limits.

6495C Ion Funnel TQ for Reproducible and Low Detection Levels

An Agilent Jet Stream (AJS) source was used to ionize analytes. The source conditions and ion funnel were optimized for the lowest responders, but the ion funnel on the 6495C (Figure 2) had a broad range of optimal conditions for various classes of analytes. A dynamic MRM method (dMRM) was used to collect one ion transition per metabolite. Ion transitions were in both positive and negative mode. Multiplexing was prioritized to target as many analytes as possible. All retention times were confirmed with pure standards. Dwell times were variable but as low as 6 ms. Adding more metabolites is possible with min dwell time of 0.5 ms.

Results and Discussion

BioLC is Easy to Use and Provides Low LODs and RT Reproducibly for Metabolites

The BioLC was working without any specialized cleaning or additives for metal sensitive analytes like adenosine diphosphate (ADP). 50 ng/mL standard had immediate signal after installation with the BioLC while a stainless-steel LC had no signal (Figure 3). After passivation of the stainless-steel LC the signal was regained. This shows increase ease of use with the BioLC for metabolomics applications.

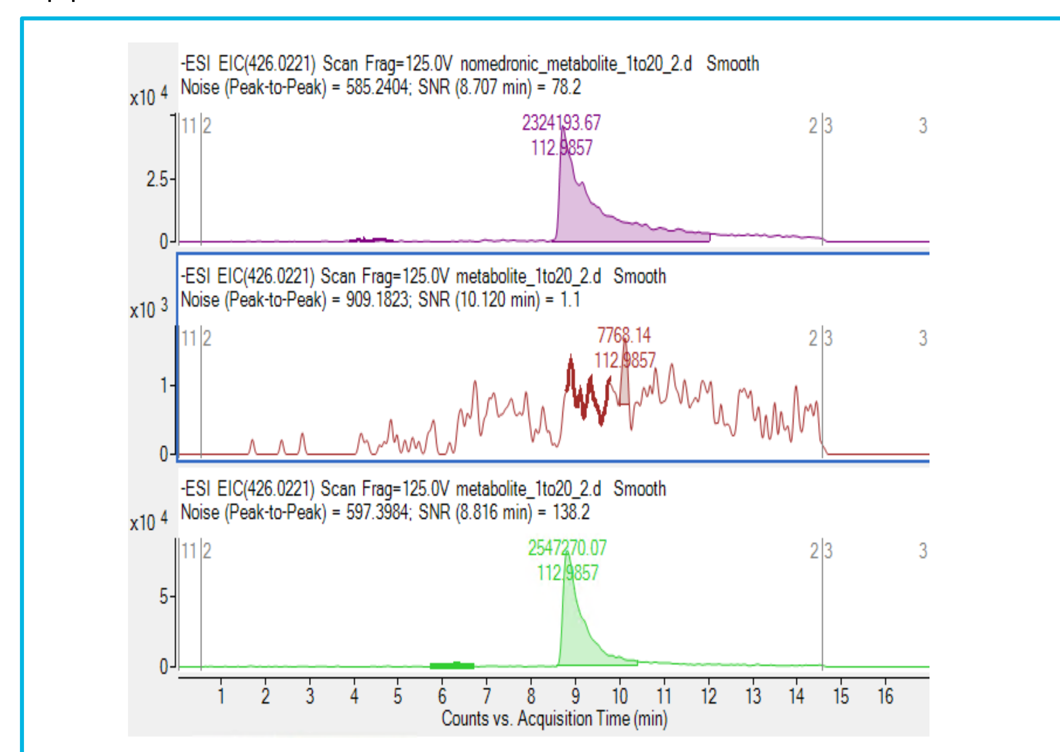

Figure 3: Extracted ion chromatogram for ADP on a new bio LC (top), new standard stainless-steel LC (middle) and passivated stainless-steel standard LC.

Reproducibility of the retention times (RT) over multiple HILIC columns was evaluated to ensure stability and that a narrow RT window can be used. Low variation was observed even when buffers and columns were changed frequently for the purpose of forcing variability in the experiment (Figure 4).

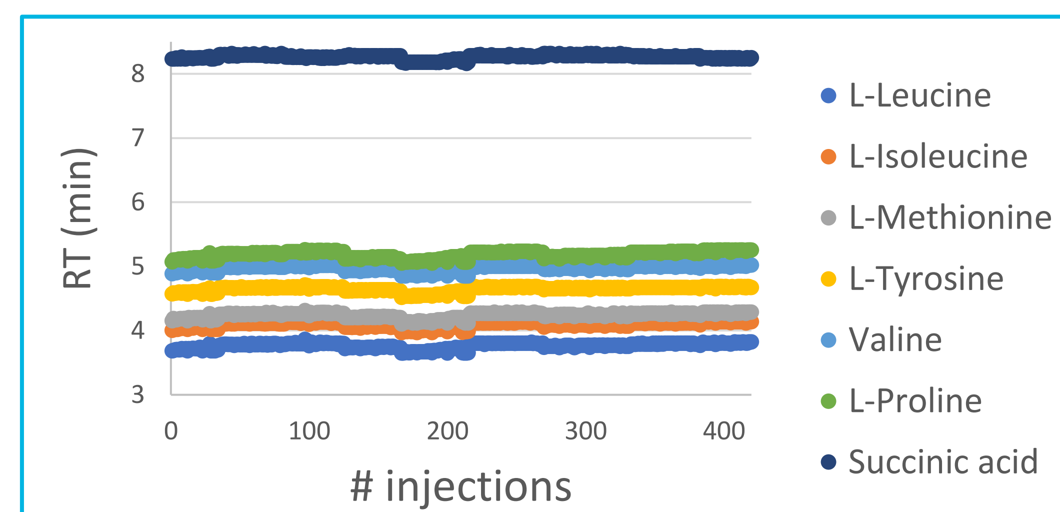

Figure 4: RT stability of various metabolites in the plasma extract over 400 injections and three different columns.

Results and Discussion

6495C had Reproducible Signal for the Metabolite Extract from 10 µL Bovine Plasma

Bovine extract injections led to sensitive and reproducible metabolite detection. 3 µL injection of the plasma sample extract had a relative standard deviation (RSD) of 1-14% for the analytes measured. Examples of the analytes detected, and their RSDs are in Figure 5.

The HILIC Z chromatography is stable (Figure 4) and also gives unique chromatographic separation of isobaric analytes like iso/leucine (Figure, a).

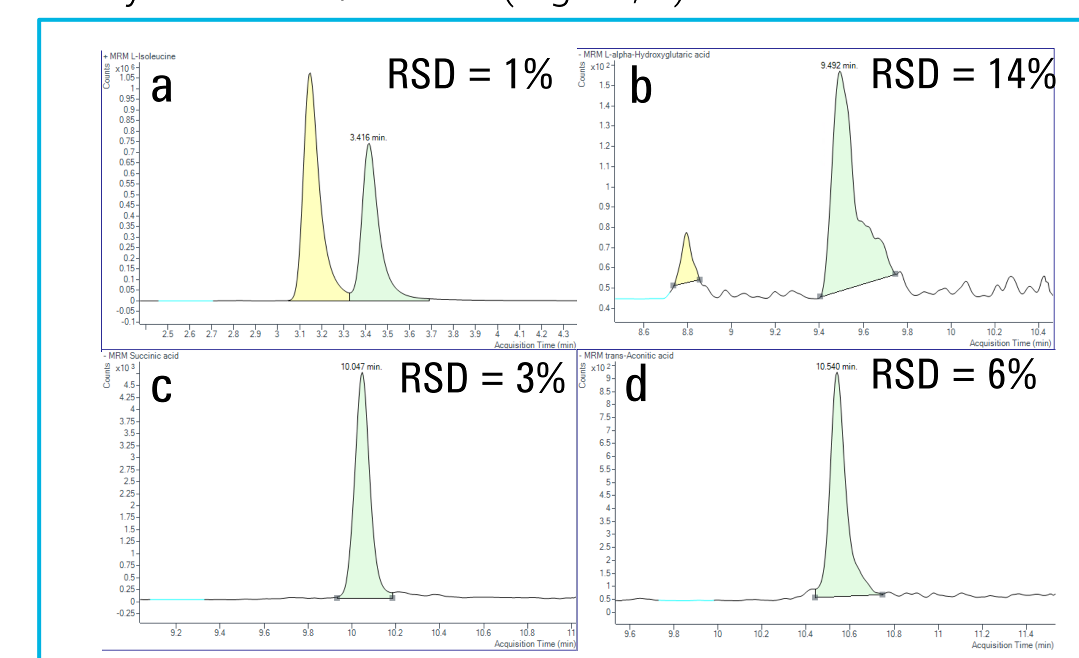

Figure 5: Leucine and Isoleucine (a), alpha-hydroxyglutaric acid (b), succinate (c), and trans-aconitic acid (d) and the RSD of 6 injections of bovine plasma extract.

Conclusions

The Combination of these Instruments Produce a Sensitive and Reproducible Platform for Targeted Metabolomics Studies

- Bravo Metabolomics Workbench was able to extract analytes from as little as 10 µL plasma and the extract was well separated by the HILIC Z column
- HILIC Z and the Infinity II Bio LC gave sensitive and reproducible analysis even over multiple columns.
- The 6495C Ion Funnel TQ gave sensitive and reproducible analysis when testing a complex matrix

References

[1] Sartain, M., Gomez, M., Van de Bittner, G., and Shu, H. Enabling Automated, Low-Volume Plasma Metabolite Extraction with the Agilent Bravo Platform. 5994-2156EN. 2020.

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