Automated LC/MS/MS methods development for targeted bioanalysis of metabolic intermediates.

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OVERVIEW:

Metabolic intermediates (e.g. glutamine, glutamate, aspartate, isocitrate, malate, pyruvate and succinate) are small (MW<200amu) highly polar molecules, that are poorly retained using reverse phase chromatography.

Hydrophilic-interaction chromatography (HILIC) is commonly used for separations in this molecular class.

We used an automated LC/MS/MS methods development approach ('Chromatune' application software) to rapidly characterize and optimize LC conditions across metabolic intermediates and common biomarkers.

Optimized conditions can be easily retrieved via a database by individual users.

METHODS:

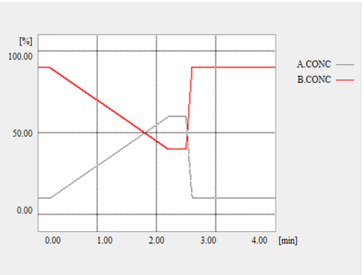
ALL LC/MS/MS analysis was performed on an ADDA (Apricot Designs Dual Arm) autosampler.

ADDA Complete (ADDA-C) software was used to set up LC methods development experiments

Two mobile phase compositions were tested (**MP1**: A:90% 10mM Am Formate/ 10% Acetonitrile, B: 90% Acetonitrile/ 10% AmFormate; **MP2:** A: 90% 0.1% formic acid/10% Methanol, B: 90% Methanol/ 10% 0.1% formic acid

The user recalls substrates to be tested from the DiscoveryQuant database (Fig. 1)

LC Gradient conditions (flow rate 0.5-1.0 ml/min)



METHODS:

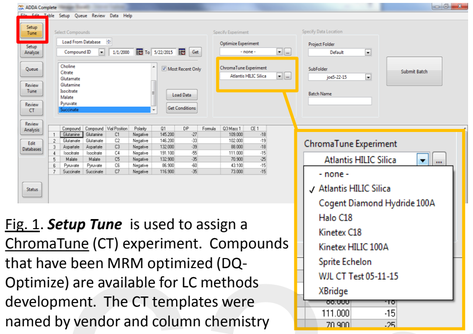


Fig. 1. ***Setup Tune*** is used to assign a ChromaTune (CT) experiment. Compounds that have been MRM optimized (DQ-Optimize) are available for LC methods development. The CT templates were named by vendor and column chemistry

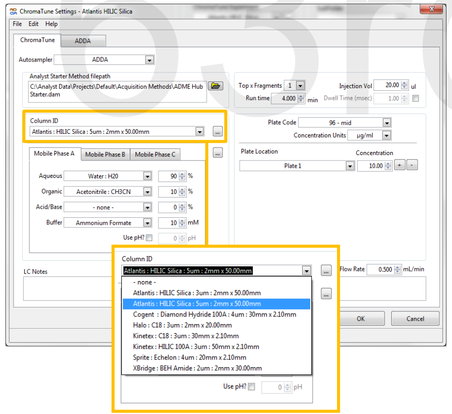


Fig. 2. The ***Chromatune template*** file, a drop-down menu displays column chemistries listed in the database. New LC chemistries can be added to the database on-the-fly via template dialog. It seems worthwhile to standardize naming where possible allowing more efficient querying across substrates and LC chemistries in the future.

METHODS:

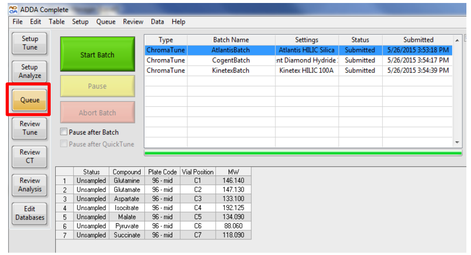


Fig. 3. The batch is submitted to the ADDA Queue and analysis begins

RESULTS:

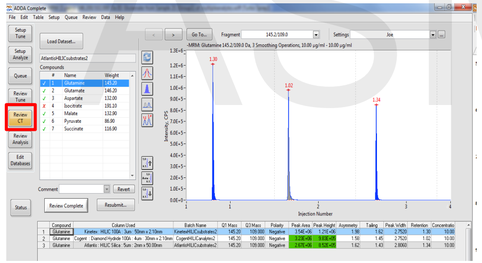
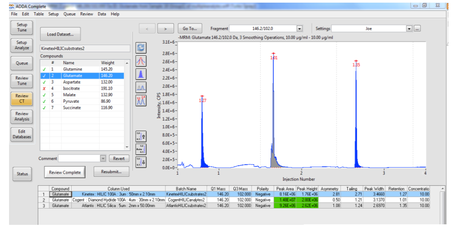


Fig. 4a. The 'Review CT' panel loads and reviews experimental results. In this example, the LC trace for Glutamine is compared across the three column chemistries tested. Performance was similar across HILIC chemistries tested.

RESULTS:



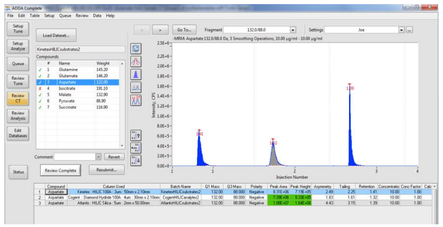


Fig. 4b. During data review (for Glutamate (left panel) and Aspartate (right panel)) it was determined that the Atlantis HILIC chemistry was optimal for the substrates tested. ***Note: all x-y chromatographic response data is stored in central database, this facilitates comparison across LC chemistries and columns .***

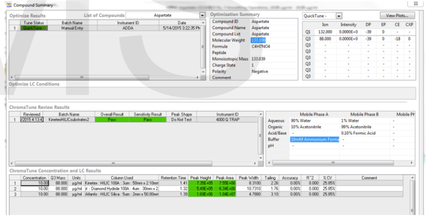


Fig. 5. The 'Compound Summary' provides a tabular view of all optimization results.

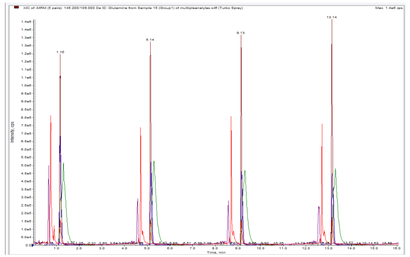


Fig. 6. The substrates were pooled (succinate, alpha-ketoglutarate, glutamine, glutamate, aspartate, at equivalent conc , 1µM) and tested using the Atlantis HILIC column.

CONCLUSIONS:

- Commercially available HILIC stationary phases offer quite distinct separation chemistries.
- ADDA-C/ChromaTune software should allow us to survey numerous LC chemistries in development of high-throughput LC/MS/MS methods for biomarker analysis.
- The ADDA autosampler coupled with ADDA-Complete/Chromatune software provide a means to survey multiple LC chemistries 'on-the-fly' , **and/or** using a more deliberate approach (as described in this poster) wherein substrates are methodically tested across LC chemistries and resultant knowledge is accumulated and stored in a central database that can be accessed across the bionalytical organization/infrastructure.