

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:

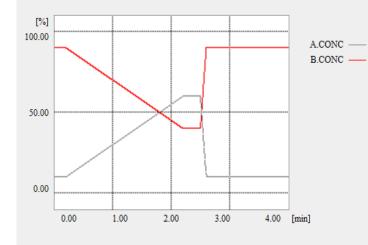
ALL LC/MS/MS analysis was performed on an ADDA (Apricot Designs Dual Arm) autosampler.

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:

Compound ID 🔹 1/1/2000 📅 To 5/22/2015 😈 Get Atlantis HILIC Silica Load Data Q3 Mass 1 CE 1 109.000 102.000 88.000 111.000 Glutamine Glutamine Glutamate Glutamate Aspartate Aspartate Negative Negative ChromaTune Experiment 70.900 · 43.100 · Atlantis HILIC Silica none Atlantis HILIC Silica Cogent Diamond Hydride 100A Halo C18

Kinetex C18

XBridge

00.000

Kinetex HILIC 100A

WJL CT Test 05-11-15

Sprite Echelon

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 nam

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ChromaTune ADDA		
Autosampler ADDA 💌		
Analyst Starter Method filepath C:\Analyst Data\Projects\Default\Acquisition Methods\ADME Hub Starter.dam	Top x Fragments 1 Injection Vol 20.00 🔄 ul Run time 4.000 🐑 min Dwell Time (msec) 1.00 🔄	
Column ID Atlantis : HILIC Silica : Sum : 2mm x 50.00mm	Plate Code 96 - mid Concentration Units µg/ml ▼	
Mobile Phase A Mobile Phase B Mobile Phase C Aqueous Water: H20 90 🚔 % Organic Acetonitrile: CH3CN 10 🚔 % Acid/Base - none - 0 🚔 % Buffer Ammonium Formate 10 🚔 mM Use pH? 0 🚔 pH	Plate Location Concentration Plate 1 Plate 1 Plate 1	
Column ID Column ID LC Notes Column ID Conore Attantis : HILIC Silice : Sum : 2mm x 500 - none - Attantis : HILIC Silice : Sum : 2mm x Attantis : HILIC Silice : Sum : 2mm x Cogent : Diamond Hydride 100A : 4 Halo : C18 : 3um : 20mm x 2.10mr Kinetex : C18 : 3um : 30mm x 2.10mr Kinetex : HILIC IOA : 3um : 50mm x Sprite : Echelon : 4um : 20mm x 2.10 XBridge : BEH Amide : 2um : 2mm x	50.00mm 50.00mm um : 30mm x 2.10mm n 2.10mm mm	





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

RESULTS:

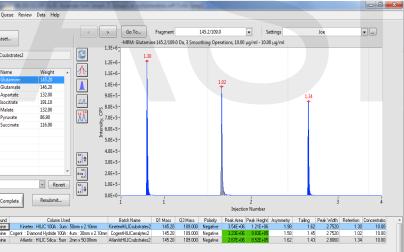
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chemistries tested.

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:

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Status	Compound	Plate Code	Vial Position	MW						
Unsampled	Glutamine	96 - mid	C1	146.140						
Unsampled	Glutamate	96 - mid	C2	147.130						
Unsampled	Aspartate	96 - mid	C3	133.100						
Unsampled	Isocitrate	96 - mid	C4	192.125						
Unsampled	Malate	96 - mid	C5	134.090						
Unsampled	Pyruvate	96 - mid	C6	88.060						
Unsampled	Succinate	96 - mid	C7	118.090						



e '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

RESULTS:

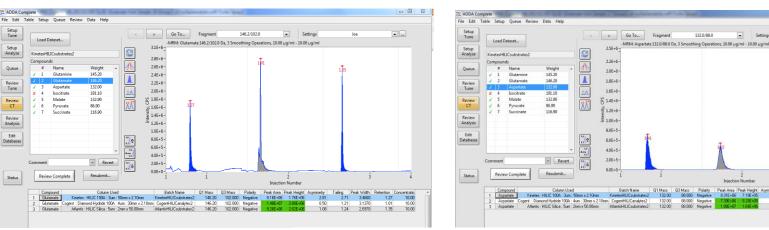


Fig. 4b. During data review (for Glutamate (left panel) and Aspartate (right panel)) it was determined that the Atlantis HILIC chemistry was optimal for the substrates tested. Note: all x-y chromatographic response data is stored in central database, this facilitates comparison across LC chemistries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

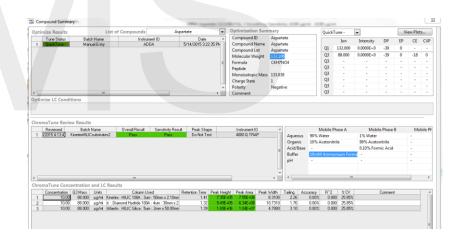


Fig. 5. The 'Compound Summary' provides a tabular view of all optimization results.

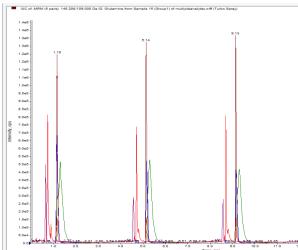


Fig. 6. The substrates were pooled (succinate, alphaketoglutarate, glutamine, glutamate, aspartate, at equivalent conc, 1µM) and tested using the Atlantis HILIC column

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.

