Open-Access LC/MS/MS Platform for Rapid Bioanalysis in Drug Discovery

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Overview

A software system for early-ADME studies is presented that provides a robust platform for developing and storing optimal LC conditions as Open Access Ready (OAR) methods. These methods are retrieved from a central database on an OAR LC/MS/MS workstation and applied to target compounds creating an streamlined solution for Drug Discovery bioanalysis.

Introduction

The workflow associated with in vitro ADME sample analysis is highly automated. The current process from receipt of samples, through assignment of MS/MS conditions, batch submission, LC/MS/MS analysis, data review and upload has been automated within the DiscoveryQuant™ software infrastructure. We have further expanded the automated workflow by inclusion of the latest version of DiscoveryQuant/ADDA Complete software. This version includes the ability to create and store LC conditions, acquire multiple injections per data file and retain sample information which can then be processed in an automated fashion. Here we describe the fully integrated system that facilitates an open-access approach allowing non-experts to perform complex LC/MS/MS bioanalysis.

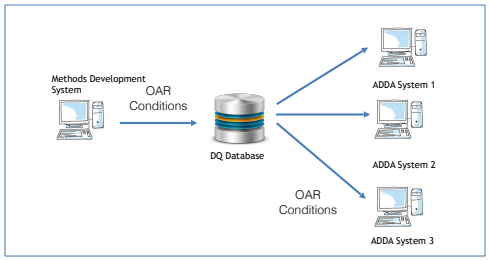

Figure 1: Methods are developed on a dedicated LC MS system. The resultant Method details are stored in central database. Each of the High Throughput Mass Spectrometer workstations can access these OAR Conditions when submitting assays.

Methods

A 96-well plate containing a set of eight distinct compounds was prepared. The compounds were injected into pre-defined chromatography setups. Criteria were set for peak sensitivity. Low concentrations of the compounds of interest were chosen to increase the likelihood of a sub-optimal chromatographic result. A side-by-side visualization of the resulting data from the multiple chromatography setups was then displayed for each compound, along with a color to mark the pass/fail criteria. All the parameters for the process were automatically stored within a database for later use.

Experimental Conditions:
Compounds in 75/25 Acetonitrile / Water
Glutamine (10 µg/ml), Glutamate (10 µg/ml), Aspartate (10 µg/ml), Isocitrate (10 µg/ml), Malate (10 µg/ml), Pyruvate (10 µg/ml), Succinate (10 µg/ml), alpha-ketoglutarate (10 µg/ml)

LC Analysis
Pumps: Shimadzu LC-20 gradient pump
Injection Volume: 20µl

Gradient Conditions (all ports):
Aqueous: 90% Water, 10% Acetonitrile, with 10mM Ammonium Formate buffer
Organic: 1% Water, 99% Acetonitrile 0.1% Formic acid

Chromatune 1: Chromatographic Conditions
Column: Atlantis HILIC Silica, 5µm, 50x2.1mm
Gradient Time: 240 seconds
Flow rate: 1.0ml/min

Chromatune 2: Chromatographic Conditions
Column: Kinetex HILIC 100A, 2.6µm, 50x2.1mm
Gradient Time: 240 seconds
Flow rate: 1.0ml/min

Chromatune 3: Chromatographic Conditions
Column: Cogent Diamond Hydride, 4µm, 30x2.1mm
Gradient Time: 240 seconds
Flow rate: 1.0ml/min

Results

ChromaTune™ Analysis
All compounds were sampled using the three Chromatographic setups on an Apricot Designs Dual Arm (ADDA) autosampler, using a Shimadzu LC-20 pumping system, and an ABSCIEX 4000 QTRAP. The ADDA Complete™ software was used to analyze the results.

The samples were reviewed using the ChromaTune™ review panel which performed an automated check for Peak Height and Peak Area. Each parameter check had tolerance limits set in the software (see Figure 2).

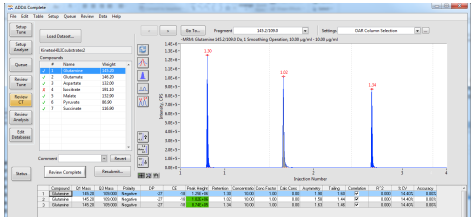

Figure 2: ChromaTune quality visualization panel of each compound set (with three ChromaTune setups selected). Results for each chromatographic setup are shown in the chart under the peaks. As the user scrolls down the compound list (upper left in the figure), each set of ChromaTune results is updated in the graph, and on the chart. In this way, results from multiple runs can be compared using the same review criteria.

Database Storage & Retrieval
The compound and ChromaTune optimization parameters were stored in the compound database for later retrieval. Retrieved compounds were evaluated using the Compound Summary Panel (Figure 3). Failed Tests were shown in red; successful results were shown in green. This panel allowed a user to decide on the best chromatographic conditions to select for the compound based on a set of conditions from different ChromaTune runs. The selected condition is tagged with a user specified flag.

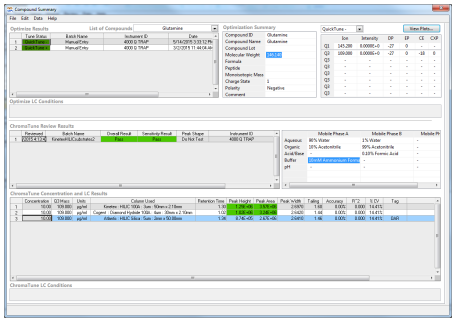

Figure 3: ChromaTune results are reviewed and the set of conditions with the best response is tagged as being the Open Access Ready set of conditions (see Tag entry in the far right of the bottom table).

Condition Selection
Each Open Access Ready ADDA Workstation (ADDA-OAR) running the ADDA Complete software has access to the shared database that stores the tuning and LC conditions. The Saved Search functionality in the ADDA Complete program was utilized to return Chromatographic conditions for the provided compounds based on Open Access Ready flag (Figure 4).

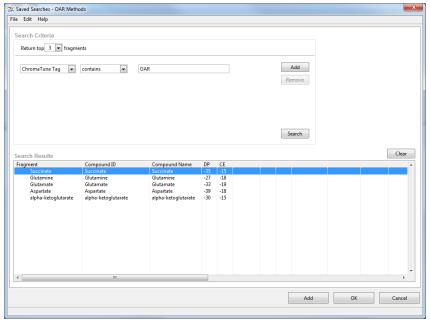

Figure 4: Conditions recalled from the database based on a search of the ChromaTune Tag.

Each ADDA-OAR workstation includes Template configurations for the target LC conditions that correspond to those in the database. These configurations define a LC/MS/MS setup on the ADDA autosampler including column and mobile phase selection, valve timings, pump settings, and gradient pump time schedule. Compounds IDs and locations were imported from a spreadsheet file. The batch was then added to the autosampler queue without the requirement of any additional configuration.

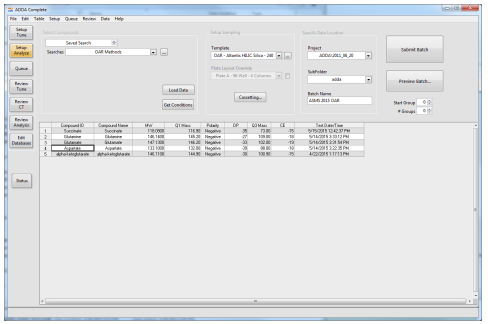

Figure 5: Conditions for the compounds are loaded based on the Compound ID and the ChromaTune Tag. A Template is selected that configures the system for the appropriate LC Conditions.

Conclusions

There are numerous types of in vitro ADME assays conducted in lead optimization phases of small molecule drug discovery. Nearly all of these assays use LC/MS/MS for bioanalytical quantification. The myriad of assays and formats can make communication around sample handoff between bioassay and bioanalytical scientist time consuming and prone to error. With the additional advancements in automation described here we have been able to improve productivity associated with the sample analysis. The open-access approach allows both bioanalytical and bioassay scientist to treat ADDA-OAR workstation as an appliance and/or plate-reader style measurement device and proficiently run routine samples. This approach has further shown resource efficiency as it allows bioanalytical personnel time to develop difficult or specialty LC/MS/MS methods, thereby accelerating project progression overall.

We expect that future work will center around further automation of the batch submission based on the compounds provided. A single batch can be divided up and assigned to the correct LC settings based on the results contained in the database.