Versatile Solutions to Current Demands for Automated Sample Cleanup, High Throughput, and High-Resolution Chromatography on a Single LC-MS Platform

Catherine Lafontaine, Keeley Murphy, Joseph DiBussolo
Thermo Fisher Scientific, San Jose, CA

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Overview

Purpose: Use two case studies to demonstrate the versatility of an LC-MS platform for research: high-throughput, online sample cleanup capabilities in a multi-channel UHPLC system coupled to a high-resolution, accurate-mass (HRAM) hybrid quadrupole-Orbitrap™ mass spectrometer (MS).

Methods:

Case Study #1:

An LC/MS accurate-mass screen of antiretrovirals (ARVs) in biological matrix for research was examined using online sample cleanup. In a high-throughput workflow configuration, standards in several biological matrices were injected across two LC channels (each with distinct sample extraction columns) in a cross-sequential manner to one at-source guard column.

Case Study #2:

One LC/MS research method for analysis of four major sulfatide molecular species in an organic standard was optimized for demonstration of two discrete high-throughput data acquisition styles. Four LC channels, each with distinct HPLC columns, were used and injections were made in both a cross-channel and a cross-sequential manner to the HRAM-MS.

Results:

Case Study #1:

A 100 s LC/MS method for ARVs in human biological matrices with online sample cleanup and chromatographic resolution were acquired within a data window of 30 s using a multi-channel LC system. Four standard curve matrices were investigated. All four curves gave linearity between R^2 = 0.96 and 0.99.

Case Study #2:

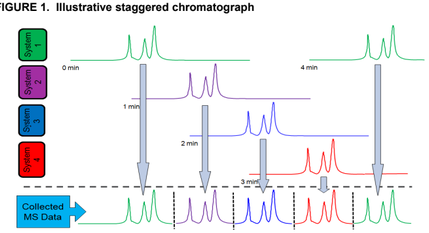
Through the two discrete high-throughput acquisition styles examined, the percent RSDs of each analyte across multiple injections of the organic standard were maintained at less than 5.8%.

Introduction

Investigative demands in both clinical research and pharmaceutical laboratories necessitate the use of simpler and faster LC/MS technologies. Market response to these demands has produced high-throughput technologies that can perform online sample cleanup followed by mass spectral analysis. These chromatographic capabilities, however, can be limiting and their results inconsistent.

Here we present two case studies highlighting the versatility of a single multichannel LC-MS platform as both a research and a pharmaceutical production tool. A multi-channel LC provides the ability to make staggered injections through multiple LC channels while utilizing a single MS, thus increasing sample throughput (Figure 1). This platform also has the capacity to perform online sample cleanup utilizing Thermo Scientific™ TurboFlow™ technology along with either baseline-resolved UHPLC chromatography or resolution based solely on accurate mass, all in a rugged high-throughput workflow.

FIGURE 1. Illustrative staggered chromatograph



Methods

Sample Preparation

Case Study #1: Four standard curve matrices of ARVs (saquinavir, nevirapine, efavirenz, and zidovudine) were prepared by a research [hospital] lab: acetonitrile-crashed plasma, water-diluted plasma, acetonitrile-crashed whole blood, and an acetonitrile-water neat mix. The dynamic range for each ARV was from 5 to 1000 ng/mL. Warfarin was added post-prep as internal standard for use with either the positive- or negative-ionizing ARVs.

Case Study #2: A 100 ng/mL sulfatide (from bovine) standard in neat solution, C8 UHPLC columns, and mobile phase reagents were supplied by a local pharmaceutical lab.

Liquid Chromatography

The multi-channel LC used in these experiments was a Thermo Scientific™ Transcend™ II LX-4 configured with four Thermo Scientific™ Dionex™ UltiMate™ 3000 Binary Rapid Separation HPG Pumps, a dual-valve VIM (valve interface module), and a CTC™ Dual-Arm DLW Autosampler (CTC Analytics AG, Zwingen, Switzerland). In both case studies, LC pump flow to the MS was diverted to waste, except during the data collection window of the method, to allow for faster LC flow rates and, therefore, faster column re-equilibration time.

Case Study #1: In a high-throughput workflow configuration, two LC channels (each with distinct TurboFlow sample extraction columns) were multiplexed to one at-source C18 guard column. The LC method details are outlined in Table 1. Use of the VIM's detector bypass position allowed for high flow rates (up to 5 mL/min) as necessary for TurboFlow technology.[1] Multiple injections of 30 µL were injected in staggered fashion across two channels of the LC system and were driven by Thermo Scientific™ Aria™ MX software version 2.1. Figure 2 illustrates a resultant chromatographic comparison of a neat standard with three in biological matrices.

Case Study #2: The sulfatide LC method details are outlined in Table 2. Multiple injections (10 µL) of the neat standard were injected onto a UHPLC column (C8, 2.1 x 50 mm, 1.7 µm) heated to 60 °C in both a cross-LC channel (four) and a cross-sequential manner and were driven by Aria OS software version 1.6.

TABLE 1. LC method details for Case Study #1

ARVs LC Method Details	
LC Method Length	100 s
Data Window	30 s
TurboFlow Columns	Thermo Scientific™ Cyclone™ column, 0.5 x 50 mm
Analytical Column	C18 guard cartridge, 10 x 4.6 mm
Loading Mobile Phase	10 mM ammonium formate + 0.05% formic acid (aq)
Eluting Mobile Phase	0.1% formic acid in acetonitrile
Extraction Column Wash	45:45:10 acetonitrile/isopropanol/acetone
Injection Volume	30 µL

TABLE 2. LC Method Details for Case Study #2

Sulfatide LC Method Details	
LC Method Length	96 s
Data Window	21 s (or 9 s)
Analytical Columns	C8, 2.1 x 50 mm, 1.7 µm heated to 60 °C
Loading Mobile Phase	5 mM ammonium formate + 0.2% formic acid (aq)
Eluting Mobile Phase	5 mM ammonium formate + 0.2% formic acid in 1:1 acetonitrile/methanol
Injection Volume	10 µL

Mass Spectrometry

All data were collected on the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap MS (HRAM, high-resolution accurate mass) with heated electrospray ionization source (HESI) and full scan exact mass extraction used to quantitate results.

Case Study #1: The HRAM MS was used in full scan positive (250-700 *m/z*) and negative (250-350 *m/z*) ionization modes at resolution 70,000. A 30 s MS data window, necessary for simultaneous positive/negative switching, was used.

Case Study #2: The Q Exactive HRAM MS was used in full scan negative ionization mode across a mass range of 804-895 *m/z* at resolution 36,000. Two acquisition styles of high-throughput data collection workflows were examined. The first style of acquisition (collection of 1000 individual data files with 21 s data windows) involved a high-throughput collection with complete baseline chromatographic separation of the analytes in order to avoid potential matrix interferences (data not shown). A two-tiered second acquisition style involved, first, 384 injections collected into a single data file using the 21 s data window (Figure 3). The second high-throughput tier demonstrated the use of the Q Exactive MS to theoretically separate compounds based solely on their accurate mass using a 9 s data window (Figure 4).

Data Analysis

Case Study #1: Post-acquisition data analysis was performed using Thermo Scientific™ Xcalibur™ Quan Browser software version 3.0.

Case Study #2: Post-acquisition data processing of single, individually collected data files was performed using Xcalibur Quan Browser software version 3.0. Post-acquisition data processing of the multiple data files collected into a single file was performed using Generic Chromatographic Viewer of Thermo Scientific™ QuickCalc™ software version 8.3.24.

FIGURE 2. Comparison of ARV standard (250 ng/mL) across four matrix preparations

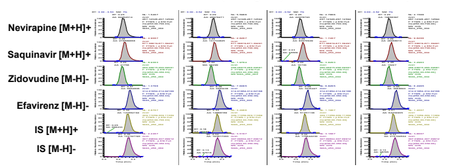


FIGURE 3. Upper trace: View of 384 sulfatide sample injections collected into a single data file, 21 s data window. Lower trace: Scaling to 1.75 min (four injections).

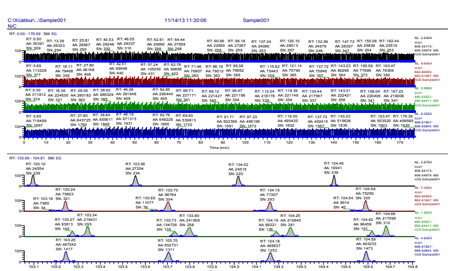
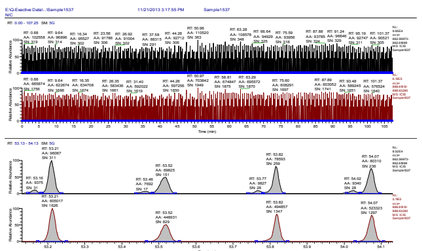


FIGURE 4. Upper trace: 384 sulfatide sample injections collected into a single data file, 9 s data window. Lower trace: Scaling to 1 min (four injections).



Results

Case Study #1:

Using a 100 s LC/MS research method with sample cleanup and chromatographic resolution, antiretrovirals were acquired within a data window of 30 s using a multiplexed (two-channel) LC system. Within the dynamic range investigated, all four standard curve matrices gave linearity between R^2 = 0.96 and 0.99. Internal standard %RSD across two channels was maintained at, or better than, 16% for each curve.

Case Study #2:

The first workflow (1000 separate data files using 21 s data windows) resulted in the acquisition of an average of 1.7 samples per minute with baseline chromatographic resolution of three analytes. The fourth analyte, co-eluting with another sulfatide, was earmarked as the internal standard.

The second workflow (three sets of 384 injections collected into 3 single data files using 21 s data windows) resulted in the acquisition of an average of 2.2 samples per minute with peaks chromatographically resolved.

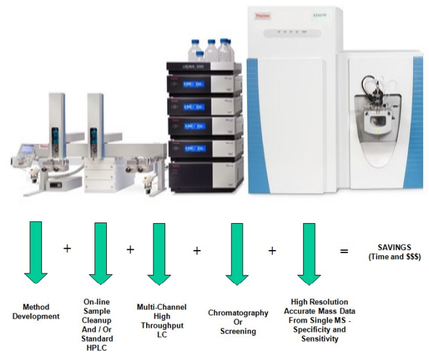
The final workflow (one set of 384 injections into a single file using 9 s data windows) resulted in the acquisition of 4.0 samples per minute with resolution by accurate mass in an ultra-high-throughput workflow. All workflows are summarized in Table 3.

Robustness of the assay was demonstrated with a combined %RSD of multiple injections (1152# multiplexed) for the 21 s data window workflow of ≤5.8% and with %RSDs of multiple injections (384# multiplexed) for the 9 s data window workflow of ≤2.8% .

TABLE 3. Overview of acquisition styles for data collection of 384-sample batches using the CTC Dual-Arm DLW AS with the four-channel Transcend II LX-4 system running cross-channel staggered injections to the Q Exactive HRAM MS.

Acquisition Style	Data Window Length (s)	LC Method Length (min)	Chromatography	Injections per minute
Standard LC-MS w/ 384 data files	N/A	1.6	Baseline separation of 3 analytes	0.63
Transcend II LX-4 system with 384 data files	21	1.6	Baseline separation of 3 analytes	1.7
Transcend II LX-4 system with 1 data file (DT_Submit)	21	1.6	Baseline separation of 3 analytes	2.2
Transcend II LX-4 system with 1 data file (DT_Submit)	9	1.0	Co-elution of 2 analytes	4.0

FIGURE 6. Versatility chart for Transcend II LX-4 system with Q Exactive hybrid quadrupole-Orbitrap MS platform.



Conclusion

- This work has effectively demonstrated the versatility of the Transcend II LX-4 with Q Exactive MS platform (Figure 6), which enables the user to perform fast online sample cleanup as needed or standard HPLC. Additionally, collection of multiple injections into a single data file can accomplish ultra-high sample throughput, whether for research screening applications or for true chromatography. The use of high-resolution, accurate-mass data collection adds in both the sensitivity and selectivity to the assay. The staggered injection capabilities of the multi-channel Transcend II LX-4 LC with the Q Exactive MS platform can be used to continuously acquire data during the window where the compounds are eluting. QuickCalc software offers accurate data parsing of those multiple injections into a single data file.
- In conclusion, it is important to note that any high-throughput workflow determinations must involve considerations to the requirements of the assay, such as chromatographic separation of isobaric compounds, removal of matrix interference, and carryover associated with either the LC column or the autosampler. Each of these considerations may pose restrictions to highest throughput. Overall, the greatest strength of this platform lies in the versatility that it can bring to either clinical research or drug discovery laboratories.

References

1. Chassaing, C.; Robinson, S.; *Chromatography Today,* **2009**, September, 20-24.

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