Maximizing Triple Quadrupole Mass Spectrometry Productivity Through the Automated Use of an Expanded Dual-Channel HPLC System with Online Sample Cleanup

Kevin McCann, Sameer Nene, Doug McIntyre, Edmond Neo, Dennis Nagtalon; Agilent Technologies, Santa Clara, CA

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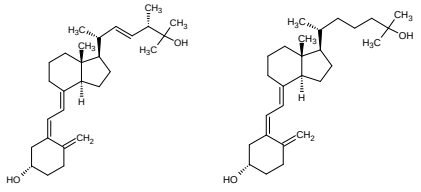
Introduction

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is ideally suited for the direct, rapid analysis of prepared biological samples. While analysis times can be shortened through appropriate LC method choices, a user is often only interested in a portion of the total data collected by an LC/MS system. Typically, there is time during each chromatographic separation where no compounds of interest are being analyzed by the mass spectrometer, leaving the instrument under-utilized for a large period of time.

This work explores the ability to increase mass spectrometer productivity through the automated use of an expanded dual channel high performance liquid chromatography (HPLC) system with an online sample cleanup option. New system control software is capable of orchestrating the timing of all HPLC components and coordinating the analytical utilization of the mass spectrometer.



25-OH Vitamin D2 25-OH Vitamin D3

Figure 1. Structures of 25-OH Vitamin D_2 and D_3

Sample Preparation

1. Combine 150µL of sample with 15µL of 1,000 ng/ml internal standard solution, and 150µL of acetonitrile in an extraction tube.
2. Vortex for 30 seconds and let stand for 15 minutes at room temperature.
3. Add 750µL of heptane and vortex for 30 seconds.
4. Centrifuge at 13,000 rpm for 5 minutes.
5. Transfer organic layer (top) to a clean extraction tube.
6. Evaporate to dryness with nitrogen at room temperature.
7. Reconstitute in 200µL of 75:25 methanol:0.1% formic acid in water.
8. Transfer samples to a 96-well plate for analysis.

Instrumentation

The complete, integrated LC/MS/MS system is comprised of a triple quadrupole mass spectrometer coupled to a configurable HPLC system, all controlled by a single software application. For the purposes of this work, the expanded HPLC system consists of a high-capacity autosampler, four binary pumps, four HPLC columns, two temperature-controlled column compartments and three switching valves. To operate the system, a standard data file collected by LC/MS/MS is loaded into the software. The data analysis method is extracted from the data file and a window of interest is specified using the data file's chromatogram. Based on that information, the software automatically coordinates all timing related to running the HPLC system.

Trapping Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 12.5 mm, 5 µm
Analytical Column	Poroshell 120 EC-C18 2.1 x 50mm, 2.7µm
Injection Volume (µl)	10
Column Temp (°C)	50
Flow Rate (ml/min)	0.5
Mobile Phase A	Water +0.1% Formic Acid
Mobile Phase B	Methanol +0.1% Formic Acid

Table 1. LC parameters

Results and Discussion

A previously developed LC/MS method for the analysis of these analytes was used for testing the capabilities of this new solution. The standard method uses an autosampler, two binary pumps, two HPLC columns, one temperature-controlled column compartment and one switching valve to perform online sample cleanup during the analysis. With a runtime of 5 minutes, the analytes of interest reach the mass spectrometer between approximately 2 minutes to 4 minutes; more than 50% of the data collected by the mass spectrometer is of no interest. The standard method utilizes what is considered a single HPLC stream. The expanded HPLC system mirrors certain components of this single stream system to provide a second stream, operating in parallel to the first. By loading the standard method and window of interest into the automation software, the software is able to determine the most efficient method of injecting and analyzing a list of samples. By staggering injections on parallel streams and switching between the two streams at the appropriate time, throughput of the integrated expanded system can double the throughput achieved with the standard method.

Figure 2. Agilent StreamSelect LC/MS Solution with Online Sample Cleanup

The two parallel LC systems displayed excellent agreement when comparing quantitative results (figure 3) with an $R^2 > 0.999$ for both 25-OH vitamin D_2 and D_3. Deviations in retention time between the two streams were also minimal at ≤ 10% RSD (figure 4).

Since both LC systems and the MS are controlled by a single piece of integrated automation software, excellent reliability and robustness is also observed. If a system error, such as a leak, occurs while a batch is running, the system will automatically reschedule samples to run on a single LC system without loosing any samples.

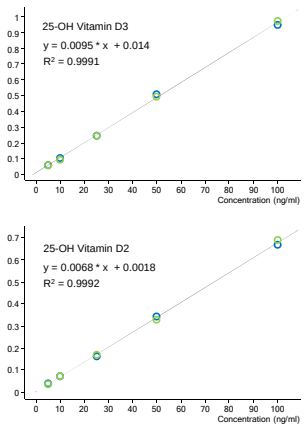


Figure 3. Combined calibration curves for 25-OH vitamin D_2 and D_3 across both LC systems.

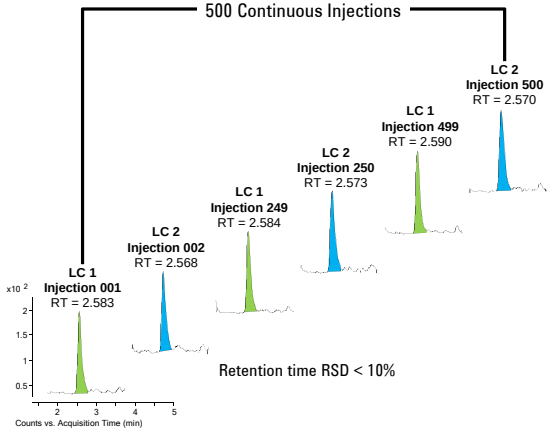


Figure 4. Retention time reproducibility of 25-OH Vitamin D_3 over 500 injections across both LCs.

Conclusions

Fully automated software controlling a completely integrated LC/MS system consisting of two parallel LC streams has been developed. No special method development is required; the user supplies a standard method and defines a window of interest, allowing the software to determine all necessary timing and coordination of the analysis.