



Maximizing Triple Quadrupole Mass Spectrometry Productivity with the Agilent StreamSelect LC/MS System

Application Note

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Abstract

This application note demonstrates how the Agilent StreamSelect LC/MS system increases the throughput of traditional LC/MS analyses. By mirroring an LC method on two HPLC systems and coordinating the use of a single triple quadrupole mass spectrometer, up to twice the throughput can be achieved. Agilent MassHunter StreamSelect software requires minimal user input and is compatible with existing LC/MS methods. Based on the parameters provided, the software determines the most efficient way of injecting and analyzing samples automatically.

A previously developed analytical method for the analysis of 25-OH vitamin D2 and D3 by LC/MS/MS used a single high-performance liquid chromatography (HPLC) stream¹. The method was implemented on a StreamSelect LC/MS system to demonstrate an increase in LC/MS productivity while maintaining excellent robustness and reliability.



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Introduction

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 (gradient, flow rate, column packing, and so forth), a user is often only interested in a portion of the total data collected by a mass spectrometer. Typically, there is time during each chromatographic separation where no compounds of interest are being analyzed by the mass spectrometer, leaving the instrument underutilized for a large period of time.

This application note describes the ability to increase mass spectrometer productivity through the use of the Agilent StreamSelect LC/MS system with an online sample cleanup option. The Agilent MassHunter StreamSelect control software is capable of orchestrating the timing of all HPLC components and coordinating the analytical usage of the mass spectrometer.

In a previous application note¹, an analytical method was developed for the rapid, sensitive and accurate determination of 25-OH vitamin D2 and D3 in serum on the Agilent 6460 Triple Quadrupole LC/MS system equipped with Agilent JetStream (AJS) technology. This method was transferred to a StreamSelect LC/MS system, allowing for twice the sample throughput without any additional method development.

Experimental

Reagents and Standards

Stock solutions of 25-OH D2, 25-OH D3, and their deuterated internal standards (IsoSciences) were prepared at 10 µg/mL in methanol (Honeywell) and stored at -20 °C. Calibration standard solutions were prepared from these stock solutions at 1, 25, 75, 125, and 250 ng/mL in pooled serum. Deuterated 25-OH D2 and D3 internal standards were diluted to 1,000 ng/mL with methanol.

Instrumentation

The StreamSelect LC/MS system is completely integrated and comprised of a triple quadrupole mass spectrometer coupled to two HPLC systems, all controlled by a single software application. The HPLC systems used here were configured for online sample cleanup using one quaternary and one binary pump per system. The samples were loaded onto a trapping column where the analytes were retained and washed using the quaternary pump. The wash was sent to waste, reducing the amount of matrix introduced into the mass spectrometer. Shortly before the analytes eluted from the trapping column, a valve was switched and the binary pump was used to elute the analytes onto an analytical column where further chromatographic separation was performed. To set up the system, a previously collected standard data

file was loaded into the MassHunter StreamSelect software. The data acquisition method was extracted from the data file and a window of interest was specified using the data file's chromatogram. Based on that information, the software automatically coordinated all timing related to running the HPLC system.

Quaternary pump gradient

Time (min)	% B
0.00	50
1.00	90
2.30	90
2.40	98
3.90	98
3.91	50

Binary pump gradient

Time (min)	% B
0.00	85
3.20	85
3.21	98
3.80	98
3.81	85

Valve timing

Time (min)	Position
0.00	1
1.90	2
2.30	1

LC conditions	
Trapping column	Agilent Eclipse Plus C18 Guard Column, 2.1 × 12.5 mm, 5 µm
Analytical column	Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm
Column temperature	50 °C
Injection volume	10 µL
Needle wash	Flush port, 50:25:25 IPA:MeOH:H ₂ O, 5 seconds
Mobile phase A	H ₂ O + 0.1 % formic acid
Mobile phase B	MeOH + 0.1 % formic acid
Flow rate	0.5 mL/min
Stop time	5.0 minutes

MS/MS configuration and conditions

Configuration

Agilent 6460 Triple Quadrupole Mass Spectrometer equipped with Agilent JetStream	
Ion mode	Positive
Drying gas temperature	275 °C
Drying gas flow	5 L/min
Nebulizer pressure	45 psi
Sheath gas temperature	325 °C
Sheath gas flow	11 L/min
Capillary voltage	5,000 V
ΔEMV	200 V

MRM transitions

Compound	Precursor	Product	Frag (V)	Dwell (msec)	CE (V)
25-OH Vitamin D3	401.3	383.2	106	50	4
25-OH Vitamin D3	401.3	365.3	106	50	4
25-OH Vitamin D2	413.3	395.3	106	50	4
25-OH Vitamin D2	413.3	355.2	106	50	4
25-OH Vitamin D3-d3	404.3	386.3	106	50	4
25-OH Vitamin D2-d3	416.4	398.3	106	50	4

Sample preparation

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

Results and Discussion

In the previously developed analytical method, which used a single HPLC stream, it was observed that more than 50 % of the data collected by the mass spectrometer was not needed since the analytes of interest eluted during a narrow retention time window. The StreamSelect system mirrors components of a single stream system to provide a second stream, operating in parallel to the first. By loading the standard LC/MS method and specifying when the analytes of interest elute, the MassHunter StreamSelect software is able to determine the most efficient method of injecting and analyzing a batch of samples. By staggering injections on parallel streams and switching between the two streams at the appropriate time, the StreamSelect system can achieve up to twice the throughput of a standard LC/MS system (Figure 1).

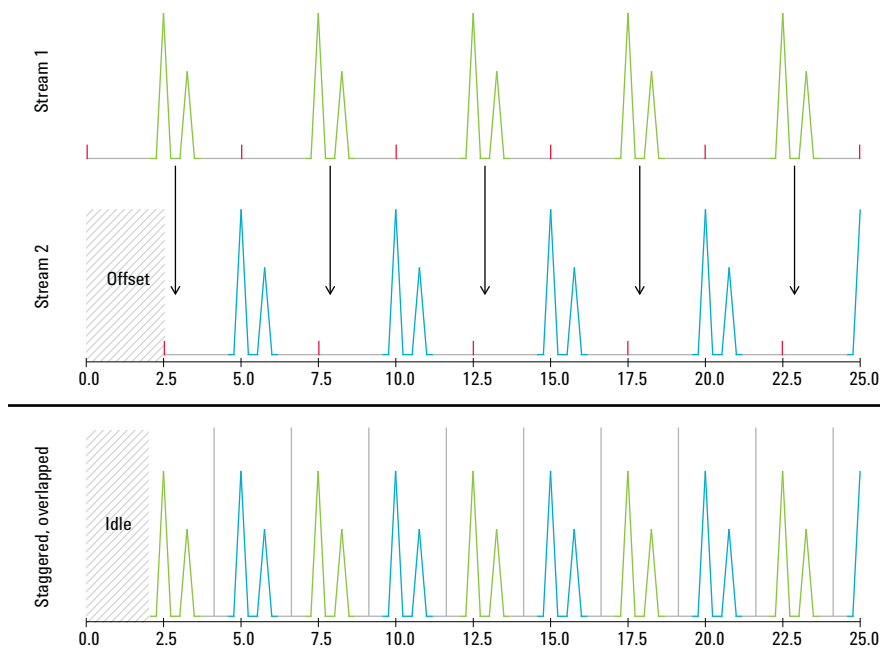


Figure 1. The Agilent StreamSelect LC/MS System maximizes MS usage by running staggered analyses on two parallel LC systems and automatically coordinating MS analysis.

The two parallel LC systems displayed excellent agreement when comparing quantitative results (Figure 2) with an $R^2 > 0.999$ for both 25-OH vitamin D2 and D3. Deviations in retention time between the two streams were also minimal - well below 10 % RSD (Figure 3).

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

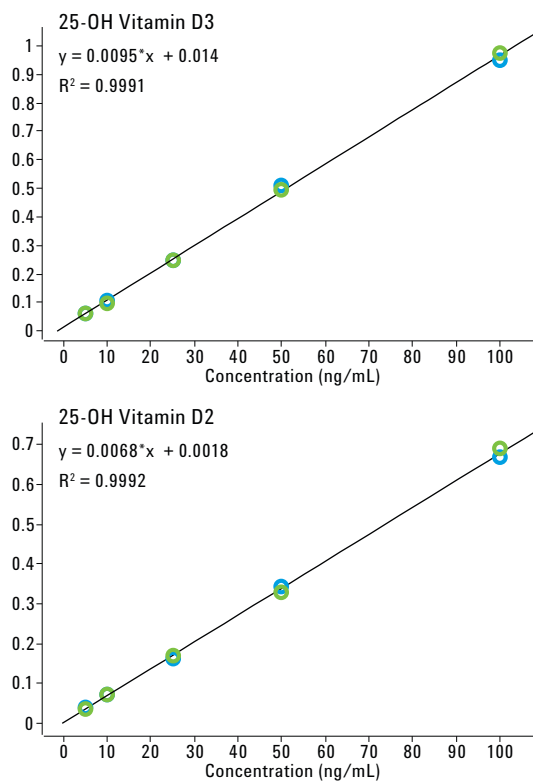


Figure 2. Combined calibration curves for 25-OH vitamin D2 and D3 across both LC systems; Stream 1 (green) and Stream 2 (blue).

Conclusions

The Agilent StreamSelect LC/MS system was evaluated using a previously developed analytical method for the measurement of 25-OH vitamin D2 and D3 in serum. MassHunter StreamSelect software coordinates a completely integrated LC/MS system consisting of two parallel HPLC streams and a single triple quadrupole mass spectrometer. No special method development is required as the user selects a standard LC/MS method, defines when the analytes of interest elute, and the software automatically coordinates the analysis by setting all necessary timing. The use of the StreamSelect LC/MS system not only provides equivalent sensitivity, linearity, and reproducibility when compared to the previously developed method, but also increases productivity of a single triple quadrupole mass spectrometer. This application note illustrates the potential of the StreamSelect LCMS System as a solution for enhanced productivity in a wide range of LC/MS analyses.

Reference

1. R. Doyle, A. Szczesniewski, and K. McCann, "Rapid Analysis of 25-OH Vitamin D in Serum Using an Agilent Triple Quadrupole LC/MS System with Automated Online Sample Cleanup", Agilent Publication 5991-2035EN (2013).

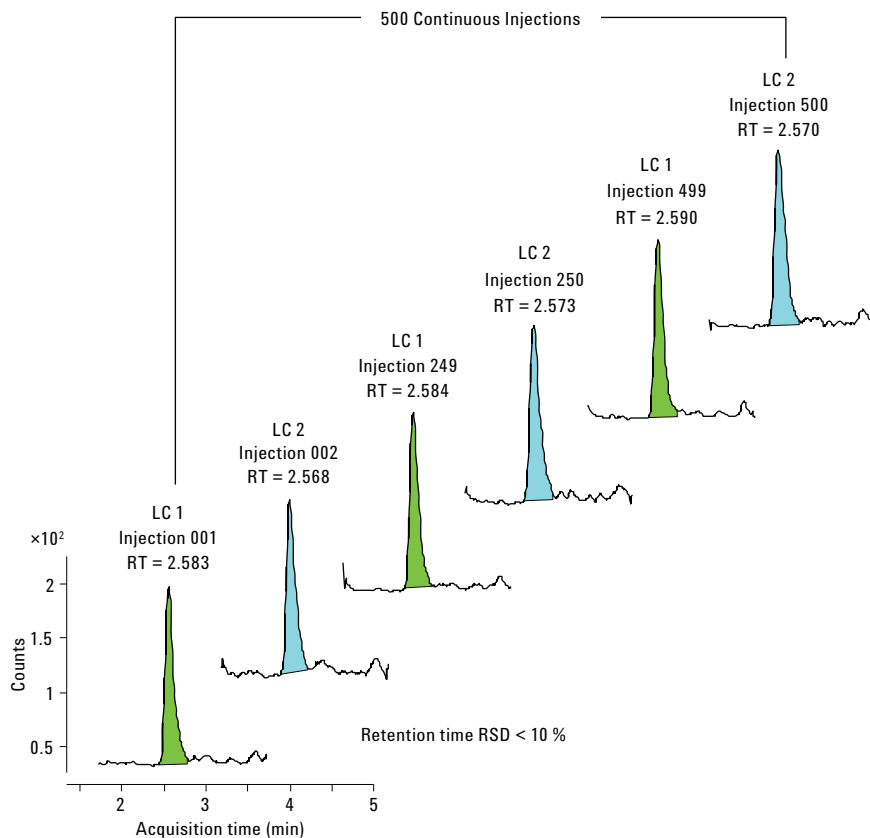


Figure 3. Retention time reproducibility of 25-OH Vitamin D3 over 500 injections across both LC systems; Stream 1 (green) and Stream 2 (blue).

www.agilent.com/chem/streamselect

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