

Considerations when using LC-MS/MS Systems with Fast and High Resolution Liquid Chromatography

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Overview

This application note describes the benefits of using Fast and High resolution HPLC systems with small particle size columns and mass spectrometric detection. Requirements for HPLC and MS/MS systems are discussed to enable quantitation with highest throughput, sensitivity and/or resolution.

Introduction

The advent of small particle size High Performance Liquid Chromatography (HPLC) columns has caused a renewed focus on the front end separation technology leading into Mass Spectrometry (MS) systems. This is due to both the development in small particle manufacturing processes as well as the development of commercially available HPLC systems that can operate at high backpressures in order to provide the flows required for efficient separation. The benefit of such technology is that it can be used to either increase resolution or maintain resolution at faster run times or a combination of both depending on the column length and particle size chosen.

The practical benefits to LC-MS/MS include the ability to increase sample throughput by reducing run times, the ability to increase sensitivity 2-3 times by generating more concentrated sharper peaks, and the ability to utilize enhanced resolution to better separate targeted analytes and matrix ions in complex matrices that may lead to ion suppression effects.

The downside being that high pressure HPLC systems are both more expensive to purchase as well as maintain. Also, that the use of small particle size columns may also require more extensive sample preparation and filtering to avoid column clogging.

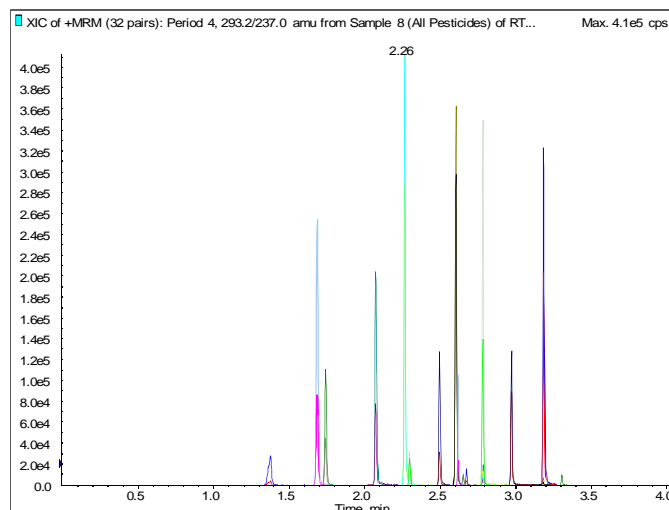


Figure 1. Detection of 16 priority pesticides using a fast gradient and a 1.8 µm particle size column – dwell times of all 32 MRM transitions were adjusted to give a minimum of 15 data points per peak

The following example in Figure 2 depicts the advantages of Fast HPLC utilizing small particles size columns, namely the ability to use the combination of faster gradients and higher flow rates to provide sharper HPLC peaks, thus providing better sensitivity, while reducing run time.

A mixture of 17 pesticides was analyzed by LC-MS/MS using a 3200 QTRAP[®] LC/MS/MS System with Turbo V[™] Source and Electrospray Ionization (ESI) probe. All analytes were detected using two Multiple Reaction Monitoring (MRM) transitions. Separation was performed on a 2.5 µm column with a mobile phase of water/methanol + 5 mM ammonium formate. The chromatograms in Figure 2 show clearly the sensitivity enhancements and reduced run times using higher flow rates and fast gradients.

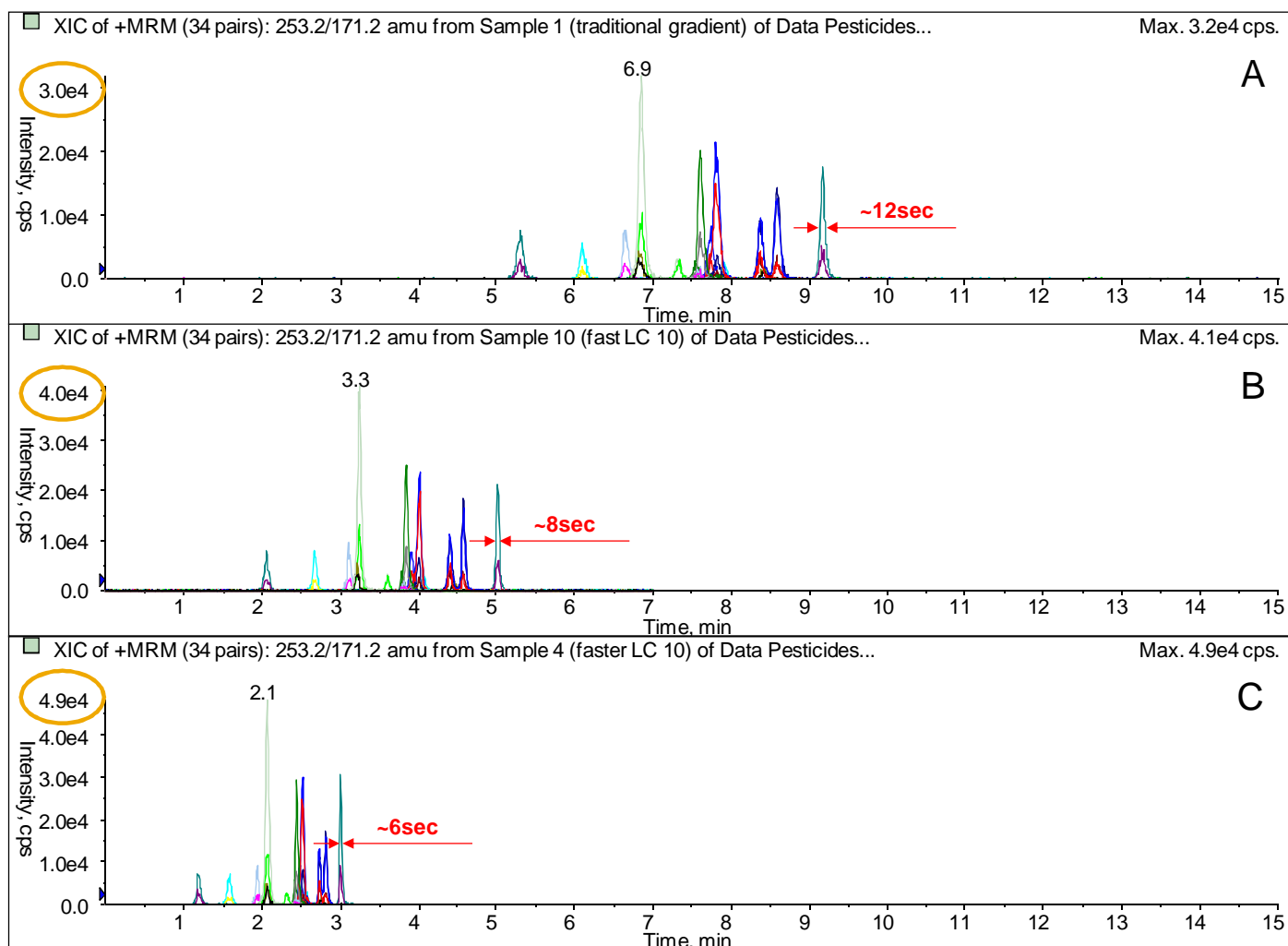


Figure 2. Use of small particle size column with fast gradients to increase sensitivity and throughput (LC-MS/MS detection of 17 pesticides in Multiple Reaction Monitoring with A) 8 min gradient at 250 μ L/min B) 5 min gradient at 500 μ L/min C) 2 min gradient at 500 μ L/min on a Phenomenex Synergi Fusion-RP 50x2 mm column with 2.5 μ m particles)

HPLC Requirements

In order to fully utilize small particle size columns, the HPLC system used requires the following attributes:

- Accurate and reproducible HPLC gradient pumps that operate from 0.1 to 1.5mL/min at high back pressure with a minimum delay volume
- An autosampler with a fast cycle time to reduce the overall run time and also, with low carry-over in order to avoid contamination between injections
- A column oven to accurately control the column temperature and to reproducibly elevate the column temperature in order to reduce solvent viscosity and backpressure

It should be noted that the Waters Acquity UPLC system, the Agilent 1200 Series Rapid Resolution LC system, the Dionex Ultimate 3000 LC system, and the Shimadzu Prominence UFLC_{XR} system are all designed to be used with regular as well as small particle size columns to provide fast chromatography. Furthermore, that all of these systems are fully controlled by Analyst[®] Software to provide fast chromatography when used in conjunction with AB SCIEX Mass Spectrometers. However, it should be noted that when choosing a fast HPLC system, the performance as well as cost of purchase and maintenance should all be considered prior to purchase.

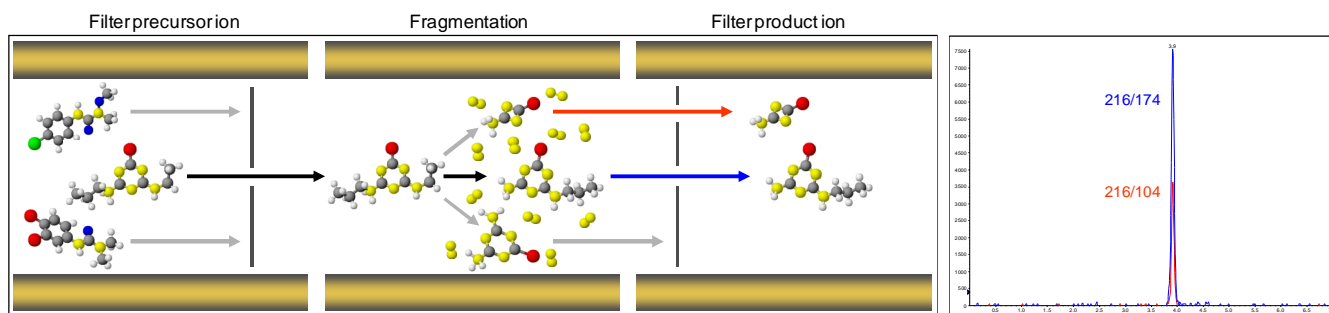


Figure 3. Schematics of a triple quadrupole mass spectrometer operating in Multiple Reaction Monitoring (MRM) with a resulting chromatogram

MS/MS Requirements

In order for a triple quadrupole LC-MS/MS operating in MRM mode (Figure 3) to be compatible with Fast HPLC, it requires the following:

- An ionization source with excellent ionization efficiency at flow rates between 0.1 and 1.5 mL/min

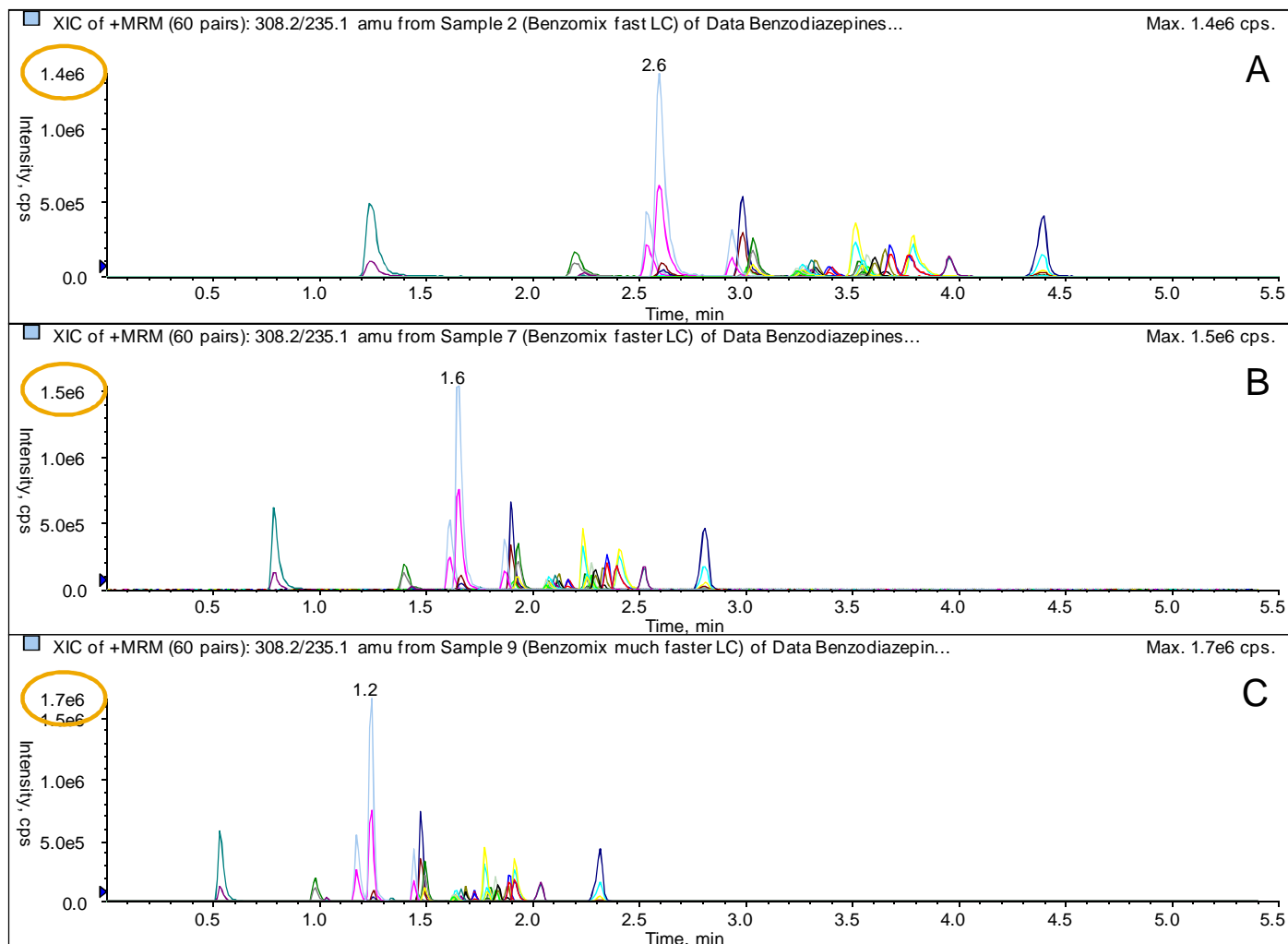


Figure 4. Use of Turbo V™ Source at high flow rates with small particle size column (LC-MS/MS detection of 30 benzodiazepines in Multiple Reaction Monitoring with A) 0.6 mL/min and 500°C B) 1 mL/min and 600°C C) 1500 mL/min and 700°C on a Zorbax SB-C18 50x4.6 mm column with 1.8 μm particles)

- A fast and efficient collision cell that can operate at low dwell and pause times to enable the acquisition of enough data points across narrow LC peaks with a minimal loss in sensitivity and no cross-talk

The AB SCIEX industry standard Turbo V™ Source with orthogonal spray and optimized flow dynamics provides excellent performance across a broad flow rate range without splitting or a roll off in sensitivity, thus providing the ability to utilize both standard Fast LC flow rates as well as to go up to 1.5 mL/min, to maximize throughput without sacrificing sensitivity.

The example chromatograms shown in Figure 4 illustrates the advantages of Turbo V™ Source operating using ESI in combination with Fast HPLC at higher flow rates without loss in sensitivity due to optimized ion source temperature.

A mixture of 30 benzodiazepines was analyzed by LC-MS/MS using a 3200 QTRAP® LC/MS/MS System with Turbo V™ Source and ESI probe. All compounds were detected in MRM mode. Separation was performed on a 1.8 µm column with a mobile phase of water/acetonitrile + 0.2% formic acid and 2 mM ammonium formate at different flow rates. The ion source temperature was optimized for complete evaporation of solvents depending on the flow rate.

In terms of quantitation, the amount of MRM transitions and thus compounds that can be monitored in a given run is directly dependent on the ability of the collision cell to use short dwell and pause times to provide enough, typically 10-15, data points across a peak for good quantitative reproducibility of less than 10% while still maintaining sensitivity and avoiding cross-talk.

The industry standard AB SCIEX Linear Accelerator (LINAC®) Collision Cell provides the ability to analyze hundreds of compounds in a set period of time, with a single time window while utilizing dwell and pause times on the order of a few milliseconds as shown in Figure 5.

Scan Speed and Dwell Time

Given the general confusion about scan speed versus dwell time, it should be noted that scan speed is only applicable to full scan qualitative experiments like a Q1, Product Ion, Precursor Ion, and Neutral Loss scan where the quadrupole is set to scan over a range of masses in a set period of time in order to determine what compounds may be present in the unknown sample.

However, when performing a MRM experiment for targeted quantitation where Q1 and Q3 are set to filter specific ions as they pass through the ion optics, it is the dwell and the pause times and the number of MRM transitions monitored that determine the cycle time and thus the number of data points obtained across a given HPLC peak.

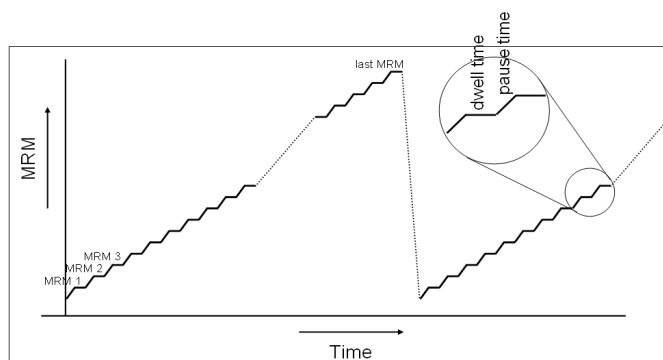


Figure 6. Illustration of Dwell and Pause times during an MRM experiment. The MRM experiment contains dwell time to detect ion of interest and pause time to avoid cross talk: Total cycle time = number of MRM transitions multiplied with (dwell + pause); Total cycle time must give 10-15 data points over HPLC peak to allow accurate quantitation.

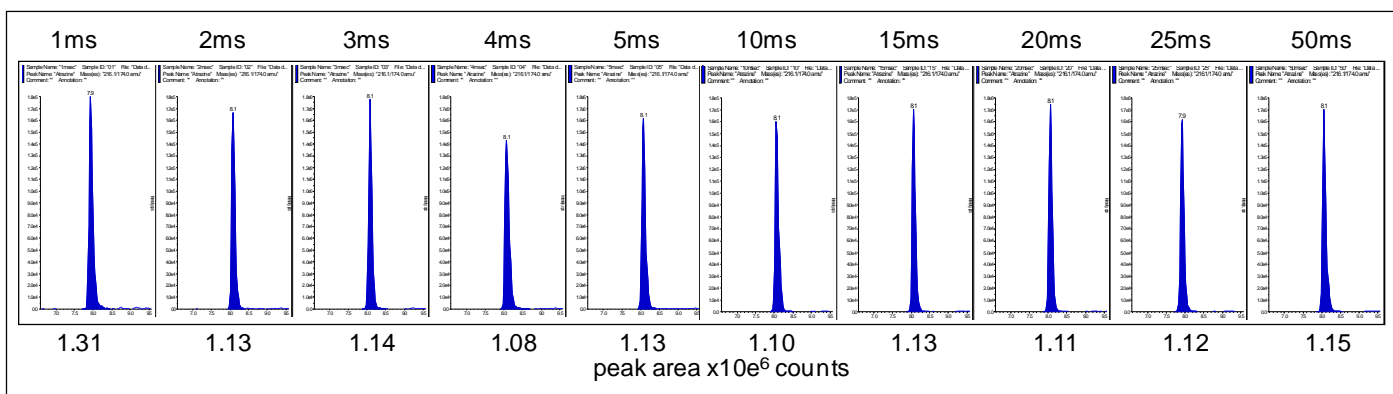


Figure 5. Sensitivity in Multiple Reaction Monitoring depending on dwell time in milliseconds

The amount of compounds that can be analyzed in a set period of time is determined by the cycle time and the HPLC peak width. As an example, the monitoring of 100 MRM transitions with a dwell time and pause time of 5ms each would have a total cycle time of 1 second, thus providing 15 data points across a 15 second wide HPLC peak. If a larger number of analytes has to be monitored and/or the peak width is narrower because of the use of fast HPLC the MRM dwell time has to be adjusted accordingly.

An example of detecting 16 priority pesticides using 2 MRM transitions and a fast HPLC gradient over 3 min is presented in Figure 1 on page one. The use of a 1.8 μm particle size column and a flow rate of 2 mL/min resulted in a typical peak width of 2.5 s. Dwell times of all MRM transitions were adjusted to allow the collection of sufficient data points over each peak.

Summary

In summary, small particle size columns can be used in conjunction with triple quadrupole LC-MS/MS systems to increase throughput, sensitivity and/or resolution for targeted quantitative applications.

AB SCIEX LC-MS/MS systems, with the patented Turbo V™ Source and LINAC® Collision Cell can fully utilize Fast and High Resolution HPLC systems, such as the Waters Acquity UPLC system, the Agilent 1200 Series Rapid Resolution LC system, the Dionex Ultimate 3000 LC system, and the Shimadzu Prominence UFLC_{XR} system.

However, when doing so, peak width, dwell and pause time all need to be considered in order to provide the best LC-MS/MS performance while providing enough data points across a peak for accurate and reproducible quantitation.

While Fast HPLC can provide the enhancements discussed, the results obtained are still dependent on the same factors that govern traditional HPLC separation, namely, stationary phases, particle size, solvent systems, and column temperature.

Because AB SCIEX mass spectrometers and a range of HPLC systems can be fully controlled with Analyst® Software, the best combination of HPLC system and MS/MS detector can be chosen to meet specific needs.

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Publication number: 1282110-01