# Achieving Ultra-High-Speed Analysis with LC/MS/MS

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## 1. Can a Conventional LC/MS/MS System Achieve Ultra-High-Speed Analysis Solely by Employing a Sub-2 μm Column?

Since analysis times can be accelerated at least 10-fold compared with conventional systems, systems that provide ultra-high-speed analysis can improve analysis operations. Although it may seem that conventional LC/MS/MS systems could achieve ultra-high-speed analysis simply by switching to a sub-2 μm column, is it really so?

This report addresses the problems associated with conventional LC/MS/MS systems in achieving ultra-high-speed analysis, and introduces a resolution to these problems.

## 2. The Problem of Separation Deterioration

The first consideration is the LC instrument. Shortened analysis times can be achieved by (1) reducing the column volume (e.g., shortening the length), or (2) increasing the mobile phase flow rate. However, separation will deteriorate with a typical column. A better approach is to reduce the column particle size and increase the number of theoretical plates. Sub-2 μm particle sizes are now being used worldwide. And since there is no change in theoretical plate height when the flow rate is increased, separation is maintained (see Fig. 1).

However, if a sub-2 μm column is installed in an LC instrument not initially designed for ultra-high-speed analysis, instead of behaving according to theory, the separation deteriorates notwithstanding the shortened analysis time. This results from the relative increase in diffusion within the LC instrument flow line.

In some cases, maximized LC separation is not necessary because MS/MS selectivity is high; however, separation from impurities, etc. is frequently required in order to ensure data reliability. It is therefore preferable to maximize the resolution of any LC separation.

However, associated with sub-2 μm separations is a significant increase in pressure. Since the pressure tolerance will be exceeded in LC instruments not designed for ultra-high speeds, sub-2 μm columns cannot, in fact, be used.

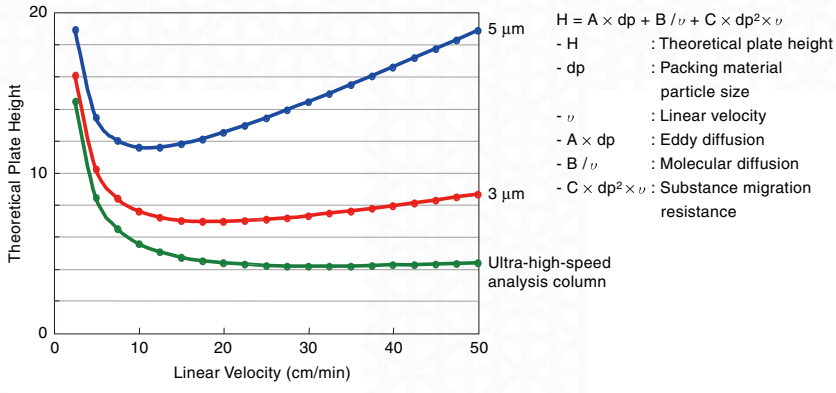


Fig. 1 van Deemter Theory

## 3. The Problem of Reduced Number of Acquired Points

When ultra-high-speed analysis is conducted using UHPLC (Ultra High Performance LC), peaks entering the MS are extremely narrow. However, with conventional MS/MS instruments that cannot handle ultra-high speeds, duty cycles are long, and acquisition rates are slow, resulting in a reduced number of points across each peak. These MS/MS instruments squander time while changing analysis conditions, such as positive - negative ion switching or monitoring multiple MRM transitions, reducing the time available for the real work of acquiring data (see Fig. 3).

For instance, when switching from one channel to another on a television using a remote control, program viewing is momentarily unavailable. Similarly, no ionization is conducted during positive - negative ion switching time in an MS/MS. Also, when compound B is to be measured following compound A, measurement does not take place during the MRM switching operation (Pause Time). Therefore, the longer the time required for switching, the fewer the number of points acquired.

The number of data points acquired has a great bearing on the accuracy of the peak shapes generated. A smaller number of points results in non-gaussian, non-repeatable peak shapes. Fig. 2 shows the relationship between the number of acquired points and chromatogram shape, and it is clear that at least 10 data points per peak are required to obtain reproducible, smooth peak shapes. When the number of acquired points per peak is only 4 or 5, there is a large divergence from a true peak shape. Not only does peak shape distortion occur, but the repeatability of the chromatogram itself is diminished, which adversely affects the quantitation results and sensitivity.

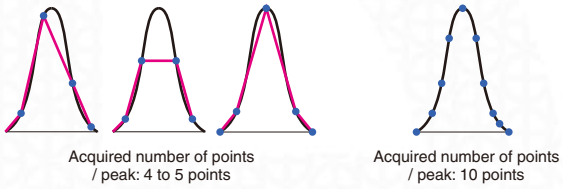


Fig. 2 Relationship Between Acquired Point Number and Chromatogram Peak Shape

## 4. The Problem of Increased Cross Talk

In conventional MS/MS instruments that cannot support ultra-high speed, the compound ions stall and lose speed in the collision cell, which increases cross talk.

The product ions generated through collision with the inert gas in the collision cell travel toward the exit of the collision cell. But, since ions slow down during this process, part of this migration toward the exit is still ongoing even after the next Dwell Time (measurement) starts. When these residual product ions pass Q3 and reach the detector, they are erroneously detected as product ions of the ongoing measurement. This phenomenon is referred to as cross talk, and adversely affects the qualitative and quantitative results, as well as sensitivity. In instruments not designed for high-speed analysis, cross talk is amplified as the duty cycle decreases.

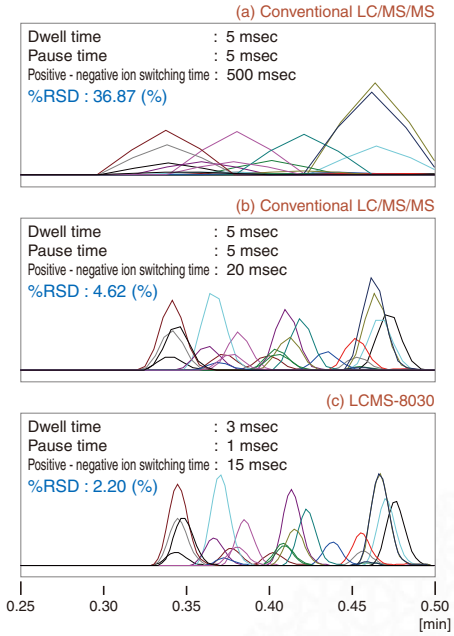


Fig. 4 Positive - Negative Ion Switching + MRM Measurement Ultra-High-Speed Test

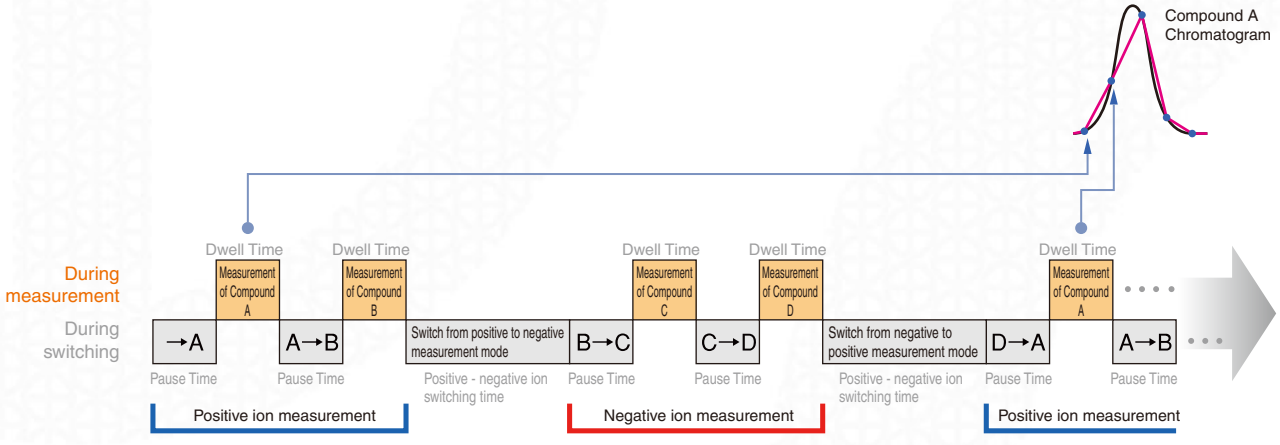


Fig. 3 Example of MRM Measurement While Positive - Negative Ion Switching Between 4 Compounds A - D

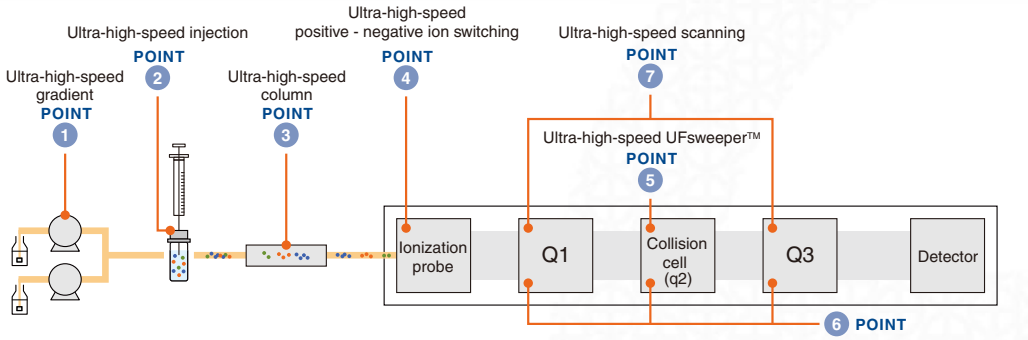


Fig. 5 Ultra-High-Speed LC/MS/MS System with LCMS-8030 & Nexera™


## 5. Next Generation LC/MS/MS: LCMS-8030

In response to the widespread use of UHPLC together with demand for ultra-high-speed LC/MS/MS, an MS/MS instrument that demonstrates ultra-high-speed performance in combination with UHPLC has been designed. That instrument is the LCMS-8030 triple quadrupole mass spectrometer. As shown in Fig. 4, the LCMS-8030 achieves ultra-high-speed analysis that has not been possible with previous LC/MS/MS instruments.
Below are seven requirements for an ultra-high-speed LC/MS/MS; these are achieved with Shimadzu's Nexera™ UHPLC in combination with the LCMS-8030.

**Point 1** : Ultra-High-Speed Gradient
Ultra-high-speed, high-accuracy gradient delivery is achieved with a solvent delivery pump which is able to deliver up to 3 mL/min at its maximum pressure rating (130 MPa: 0.0001–3 mL/min, 80 MPa: 3.0001–5 mL/min) together with a 20 µL ultra-high efficiency mixer.

**Point 2** : Ultra-High-Speed Injection
A system which can merely complete a single analysis in a short time cannot be said to support true ultra-high-speed analysis. The total cycle time must be considered. The SIL-30AC autosampler is equipped with a newly improved injection unit which achieves low carryover even without time-consuming needle rinsing. With an injection taking barely 10 seconds, ultra-high-speed analysis is possible because long autosampler cycle times are eliminated.

**Point 3** : Ultra-High-Speed Columns
The Shimadzu Shim-pack XR Series ODS (octadecyl), C8 (octyl), and Phenyl (phenylpropyl) are examples of the abundant array of ultra-high-speed columns available today.

**Point 4** : Ultra-High-Speed Positive - Negative Ion Switching
Instantaneous switching between positive ionization and negative ionization is required to ionize compound molecules into anions and cations at ultra-high-speed. At barely 15 msec, the LCMS-8030 achieves the world's fastest polarity switching speed.

**Point 5** : Ultra-High-Speed UFsweeper™
The LCMS-8030 UFsweeper™ (patent pending) collision cell technology accelerates product ions out of the collision cell. The result is higher CID efficiency and ultra-fast ion transport to reduce the sensitivity losses and cross talk that are observed on other systems.

**Point 6** : Ultra-High-Speed Pause Time
The LCMS-8030 achieves a Pause Time of just 1 msec. Because this allows a proportionally longer Dwell Time, fast analysis with greater reproducibility is possible.

**Point 7** : Ultra-High-Speed Scanning
The LCMS-8030 adopts the ultra-high-speed scanning technology which has been highly successful in Shimadzu's GC-MS "GCMS-QP2010 Ultra" and LC-MS "LCMS-2020." A scan speed of 15,000 u/sec, not yet attained by other companies, is achieved.

## 6. Combination of LCMS-8030 and Shimadzu LC

Connecting an existing LC system to the LCMS-8030 allows quick conversion to an LC/MS/MS system. Connecting the Shimadzu Prominence or LC-VP HPLC will provide a general-purpose LC/MS/MS system, and if the Nexera™, Prominence UFLCXR, or Prominence UFLC is connected, an ultra-high-speed LC/MS/MS system will be created. In any of these configurations, the resulting system can be controlled by "LabSolutions LCMS," software that provides full operational compatibility with LCsolution. In addition, it is also possible to upgrade from LCsolution to LabSolutions LCMS. Thus, the LCMS-8030 is a universal LC/MS/MS system that can handle a full range of analyses from conventional to ultra-high-speed analyses.

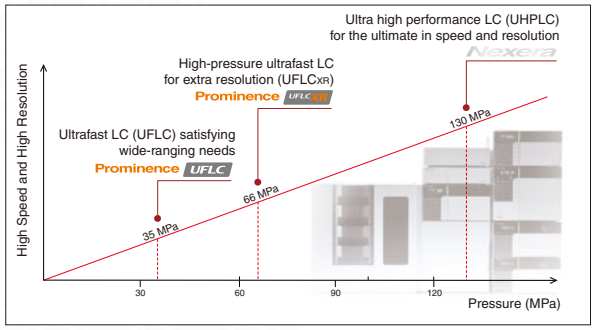


Fig. 6 Shimadzu UHPLC System Lineup

## 7. Necessity of Ultra-High-Speed Performance for Qualitative Analysis

If ultra-high speed is to be applied to qualitative analysis, an LC/MS/MS system capable of ultra-high-speed performance is required.

## 7-1. Scan Speed and Sensitivity

Scan speed is an important factor in the various scan modes, including product ion, precursor ion, and the constant neutral loss scan modes. Scan speed is very important in ultra-high-speed analysis in order to acquire the greatest amount of information possible.
However, if the scan speed is increased, the total number of compound ions that can pass through Q1 or Q3 within one scan operation is reduced, thereby resulting in reduced sensitivity. On the other hand, if the scan speed is relaxed, even though it is possible to increase the number of ions detected, ultra-high-speed analysis is sacrificed.

## 7-2. Why Is There a Sensitivity Loss for High-Mass Ions?

Compound ions of varying mass enter Q1 (or Q3), but in scan mode, only the compound ions within the specified range of *m/z* values pass through Q1 (or Q3). In this case, the time it takes to pass through is not uniform for all ions. According to this principle, the greater the *m/z* value, the slower the passage speed. For this reason, sensitivity decreases markedly for ions with large *m/z* values in ultra-high-speed scanning measurement (see Fig. 7).

## 7-3. Scan Speed and Sensitivity

In the LCMS-8030, a new technology (patent pending) is adopted which automatically optimizes the voltage of Q1 or Q3 depending on the *m/z* value and the scan speed. The LCMS-8030 not only features an ultra-high scan speed of 15,000 u/sec, but also allows ultra-high-speed scan measurement while maintaining high sensitivity (see Fig. 8).

As described above, the LCMS-8030 is a universal LC/MS/MS system with capabilities for a full range of analyses from conventional all-purpose to ultra-high-speed analyses. Moreover, it is an LC/MS/MS system that delivers ultra-high-speed performance in qualitative as well as quantitative analysis.

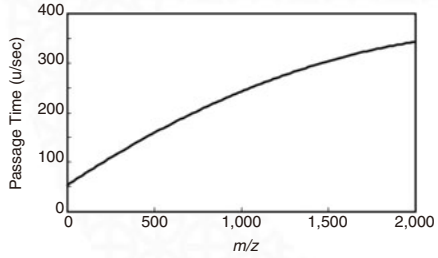


Fig. 7 Relationship of Passage Time Through Q1 (or Q3) and *m/z*

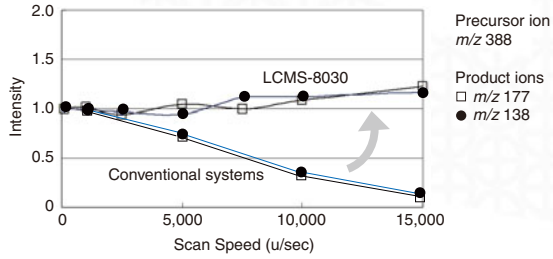


Fig. 8 Achieving Both Scan Speed and Sensitivity
(Example of Product Ion Scan)

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- Technical Report Vol. 7: Migration Points to Fast Analysis; Optimization of the System and Analysis Conditions
- Technical Report Vol. 17: HPLC Techniques to Improve LCMS Performance and Productivity
- Technical Report Vol. 19: LCMS-2020 - Reduces Limitations of HPLC Analysis and UFLC Analysis
- Technical Report Vol. 24: Reduced Solvent Consumption and Operational Costs with Ultra High Speed Analysis


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