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Technical Report

[White Paper] Which Key UHPLC Characteristics Are Required for a High Throughput LC/MS Assay?

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Abstract:

LC/MS/MS is one of the most powerful techniques for a quantitative assay due to its superior selectivity and sensitivity. For the laboratory to be efficient in sample throughput and have the ability to extract the most information possible from each sample, it is essential to consider certain characteristics of the front-end UHPLC system. This paper introduces some critical features, along with an advanced configuration, of a front-end UHPLC that enable higher throughput.

Keywords: HPLC, UHPLC, LC/MS front, solvent delivery pump, autosampler, carryover, throughput, multiplex analysis

Which Characteristics Are Required for an LC/MS Assay?

With high sensitivity and selectivity, LC/MS is a workhorse technique for quantitative assays.
Mass spectrometry (MS) detection allows simultaneous identification and quantitative analysis of multiple compounds, even if the chromatographic separation is imperfect. This ability dramatically improves an assay's throughput for multiple component methods.
To achieve an ultra-fast UHPLC/MS assay, the analytical cycle time is the most critical consideration. Higher resolution columns with sub-2µm particles or superficially porous particles, allowing for a shorter column length, contribute to shorter analytical cycle times. For this reason, these columns have become indispensable in laboratories that have hundreds to thousands of samples every day, such as DMPK and clinical testing labs.

However, ultra-high throughput is not achieved simply by using the fastest columns. First and foremost, a rapid injection speed and high sample capacity are needed to fulfill the ultra-high throughput demand. Secondly, the system must reduce other external factors like rinsing time. If the target compound(s) has a strong adsorption property, the LC system should be rinsed carefully after every injection to avoid carryover that causes quantitative errors. This rinsing phase can increase the analytical cycle time. Thirdly, we need to manage "non-data-acquisition time" for washing the column and system equilibration.

In this white paper, we introduce the key front-end UHPLC competencies required for a high-throughput LC/MS assay.

1. Injection Speed and Sample Capacity

Injection speed plays a pivotal role in achieving a high-throughput assay, as the duration of the injection sequence is simply added to the analytical cycle time. For the purposes of this paper, the injection sequence is defined as the time required for the autosampler to inject a sample – not including post-injection rinsing. It is not smart if an injection sequence adds 30 seconds to a one-minute analytical cycle time. In such an instance, for every 100 samples analyzed, one would experience over 50 minutes of "non-data-acquisition time". This is not conducive to a high-throughput assay.
The Nexera SIL-40 series autosamplers offer the fastest injection speed, as shown in Fig. 1. The 6.7 second injection cycle does not impede a rapid analysis assay. For the same 100 samples with a one-minute analytical cycle time noted above, the Nexera series would yield only eleven minutes of "non-data acquisition time". That time savings would allow the laboratory to run an additional 35 samples in the same time period.

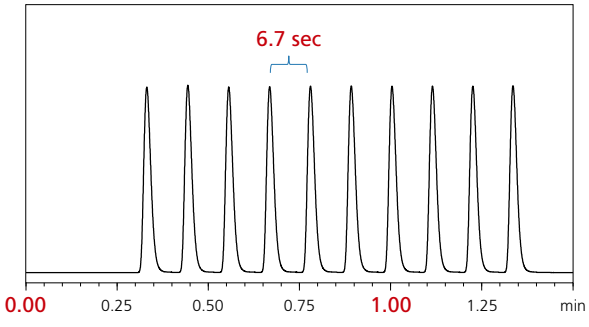

Fig. 1 UV chromatogram of caffeine obtained with the SIL-40

In order to evaluate the effectiveness of sample analysis with this ultra-fast injection sequence, we carried out an ultra-fast analysis of drugs in blood plasma utilizing a triple quadrupole mass spectrometer, the Shimadzu LCMS-8050[1]. The plasma samples, spiked with verapamil and its isotopically labelled analog, were injected on a 2.1 mm I.D.× 5 mm L column every 18 seconds and separated by isocratic elution (Fig. 2).

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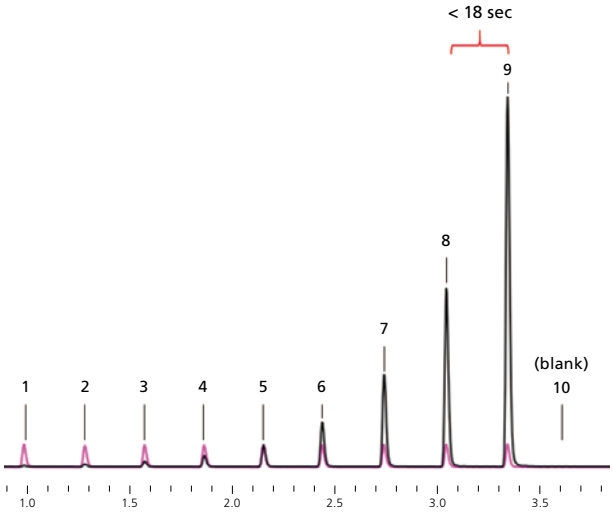


Fig. 2 Linearity over the bioanalytically relevant concentration range. Black: Verapamil chromatogram. Pink: Verapamil-D6 chromatogram. 1: STD 1 (0.39 µg/L), 2: STD 2 (0.78 µg/L), 3: STD 3 (1.56 µg/L), 4: STD 4 (3.12 µg/L), 5: STD 5 (6.25 µg/L), 6: STD 6 (12.5 µg/L), 7: STD 7 (25 µg/L), 8: STD 8 (50 µg/L), 9: STD 9 (100 µg/L), 10: Blank.

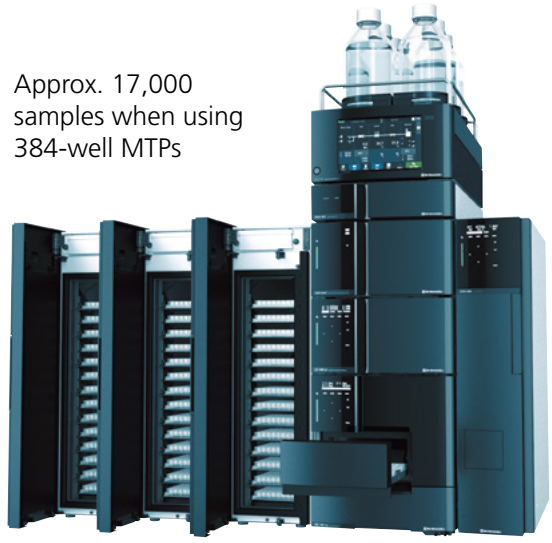


Fig. 4 Nexera series platform with multiple Plate Changers

Incredibly, even after 300 injections, the analytical stability remained high (Fig. 3). The %RSD of the internal standard (shown in blue) was 2.4%, stable even under ultra-fast injection conditions with a cycle time of less than 18 seconds. This confirms the ability to measure a large number of samples with an ultra-fast injection sequence.

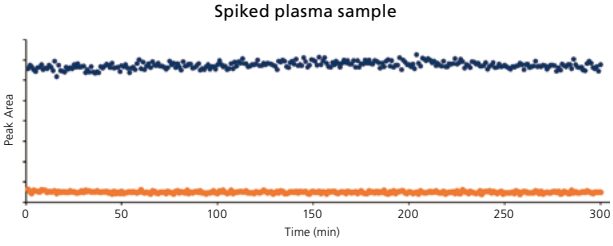


Fig. 3 Spiked plasma sample (0.39 µg/L), 300 consecutive injections. RSD% peak area without any smoothing: 2.4% for isotopically labeled analog (blue), 9.3% for verapamil (orange).

The sample capacity of the UHPLC platform also plays a critical role in enabling high-throughput analysis. Sample capacity allows performing long analytical sequences with little human intervention. Not only is the autosampler's capacity itself important, but also the availability of supporting devices to tailor the instrument to a laboratory's specific needs. The SIL-40 series autosampler has a capacity of three vial plates or well plates. The Plate Changer unit, which has a capacity of 14 standard well plates, can be connected to the SIL-40 series. In fact, up to three Plate Changers can be connected to a SIL-40 autosampler, as shown in Fig. 4.

The ease of adding samples to the running system is another important characteristic for high-throughput devices. Nexera's open-access design adds samples to the device and analytical sequence as sample preparation is completed without interrupting ongoing analyses.

2. Low-Carryover and Rinsing Function

Due to the ultra-high sensitivity of LC/MS/MS systems, they can detect trace levels of compounds remaining in the system (Fig. 5). This carryover can have a major negative impact on LC/MS analysis. Carryover is also a serious issue from a throughput perspective because it requires additional rinsing phases within the analytical sequence, which, in turn, leads to longer cycle times. In the worst-case scenario, the washing duration exceeds the data acquisition period and adds to the "non-data-acquisition time". The most significant sources of carryover often derive from the autosampler and column. A compound, which possesses adsorption properties due to a specific chemical interaction with the surface materials of the LC system or column, may remain adsorbed and possibly elute in subsequent injections. Column-based carryover is best dealt with during method development – making sure that the mobile phase/gradient conditions are sufficient to remove any trace amounts of the analytes. For the "LC System"-derived carryover, solutions are not quite so easy. "LC System"-based carryover can arise from many different sources, such as inappropriate vial/plate closures, imperfect tubing connections/fittings, and surface material interactions. For the purpose of this white paper, we will investigate carryover sources relating to the autosampler and its operation and ways to minimize them. There are two ways to prevent carryover as it relates to the autosampler: 1. Innovative design, and 2. Effective countermeasures.

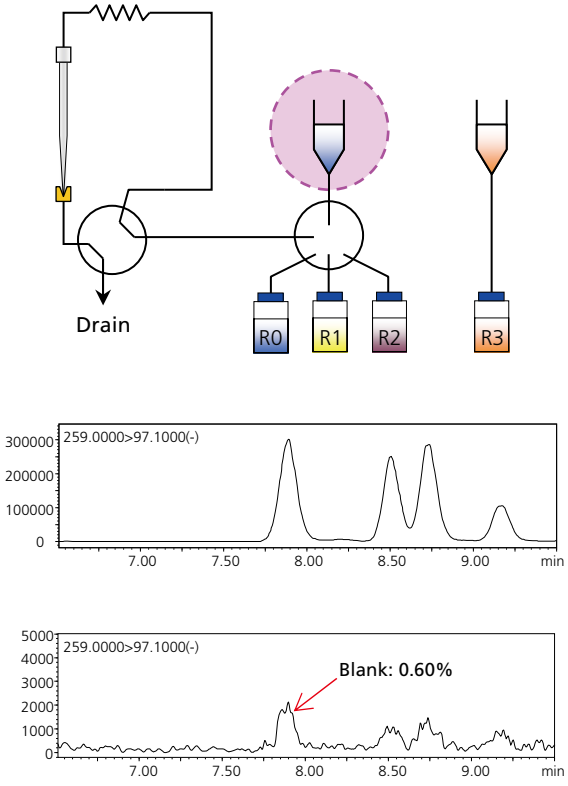


Fig. 5 Example of carryover (with the dipping rinse); Standard solution; Glucose 6-phosphate (1 µmol/L), Blank; water. ESI (-). Detailed analytical conditions are described in [4].

Innovative Design:

The SIL-40 series of autosamplers have a needle-in-the-flow path or total-volume-injection design. In this design, the sample is aspirated into the needle and the total volume is swept directly onto the system. The "sample loop" is actually part of the flow path and is continually washed with the method gradient, aiding in reducing chemical adsorption. This design eliminates the intermediate step of loading a separate sample loop and the required valve openings and closings, which can trap the sample and lead to carryover. The total injection method is faster and cleaner – a necessity for high-throughput LC/MS analysis.

With each design iteration, we made subtle but effective changes to obtain the world's best carryover performance without the need for rinsing. Simple changes like reducing the contact surface area between the needle and needle seal; less area means less chance for material sticking. The flow characteristics through the injection port and high-pressure valve have been optimized to reduce eddies or stagnant areas that can trap small amounts of the injected sample. The internal and external surfaces of the needle are finely polished and treated to minimize active adsorption sites. The sample loop and needle in the autosampler are connected with the "zero-dead-volume" design. These and other proprietary design characteristics allow the SIL-40 to claim the world's best carryover performance of 0.0015% without rinsing (under specific analytical conditions). [4]

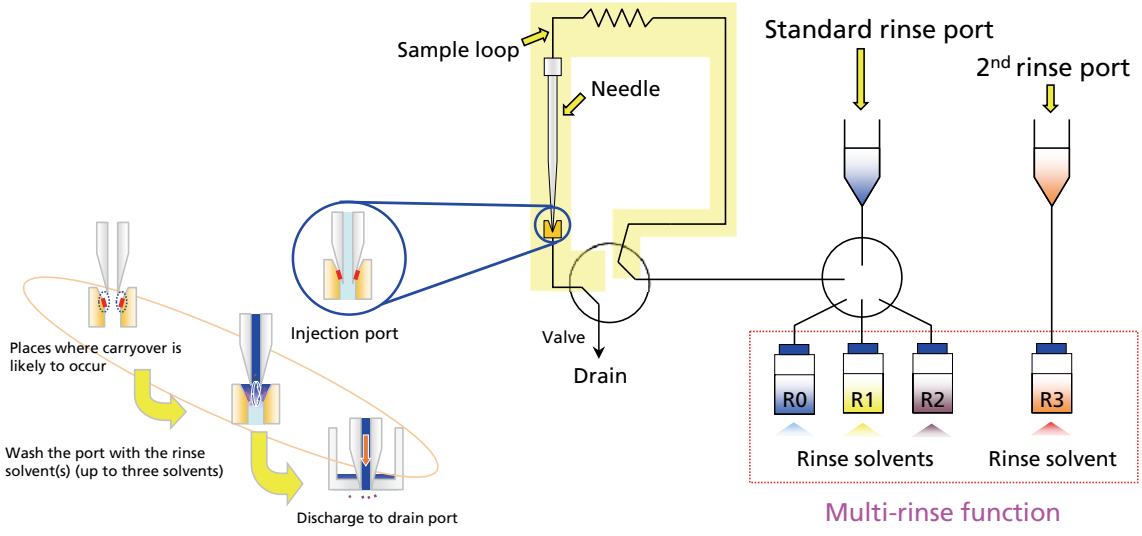


Fig. 6 Various rinsing methods and multiple rinsing solutions.

Effective Countermeasures:

Of course, not all molecules that we want to analyze will allow us to skip post-sample injection cleanup. So, we need other methods to remove any remaining compounds. Typical areas of concern are the injection port and the autosampler needle. Rinsing the needle is one of the most effective solutions to flush out the compounds on the surface of the needle. Most of the latest autosamplers have rinsing functions that allow dipping the needle into a washing solution. But it's important to note that not only the outer but also the internal surface of the needle is exposed to a risk of adsorption. Additionally, the inside wall of the injection port can also be contaminated if the needle is not perfectly clean. Compounds may accumulate around these parts and become a source of carryover.

Overcoming this issue requires a well-designed washing program. Various rinsing methods and multiple rinsing solvents are helpful to address a wide range of chemical properties of the target compounds. Fig. 6 illustrates the parts to be taken care of with multiple rinsing solutions; inside and outside of the needle, the sample loop, and the inside and surface of the injection port. By carefully choosing rinsing solutions (R0, R1, and R2), one can design a wash routine to eliminate any carryover – strong organic wash, acidic or basic wash, ionic wash – whatever is needed for the class of compound in question.

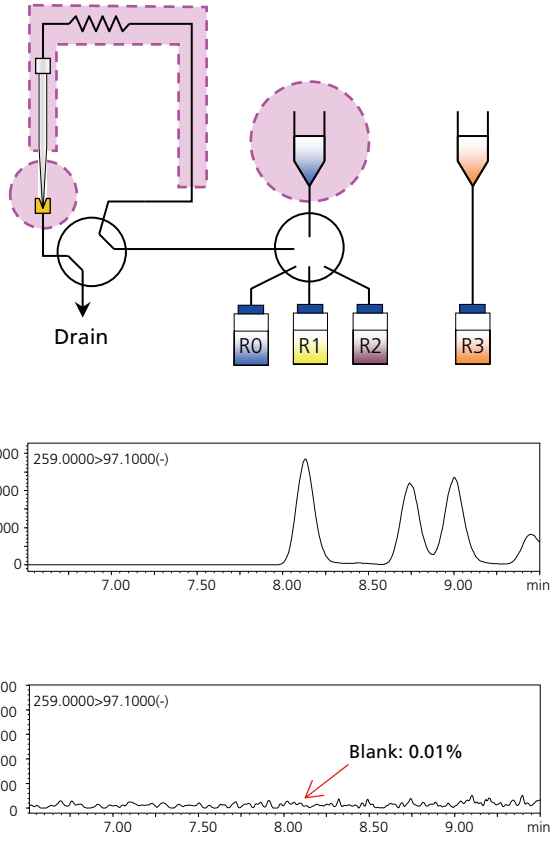


Fig. 7 Improved carryover property; Standard solution; Glucose 6-phosphate (5 µmol/L). Blank; water. ESI (-). Detailed analytical conditions are described in [4].

Fig. 7 shows that an internal rinse of the needle and injection port reduced the carryover significantly compared to what's shown in Fig. 5 when only a needle dip was utilized. Within the wash program, you can customize when and how rinsing takes place to optimize cleaning ability and instrument performance. Fig. 8 shows what can be obtained by utilizing the internal rinsing options.

Of course, any wash/rinse routine will add time to the total autosampler sequence. And this may have an impact on the analytical sequence time. However, with the many options and flexibility available with the SIL-40 series, coupled with the built-in innovative design characteristics, the effects will be minimal.

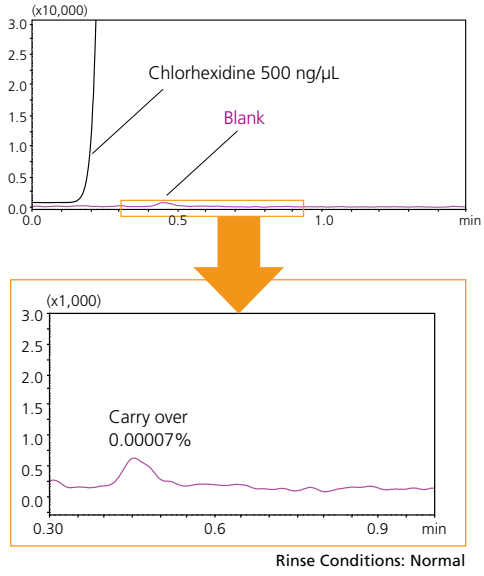
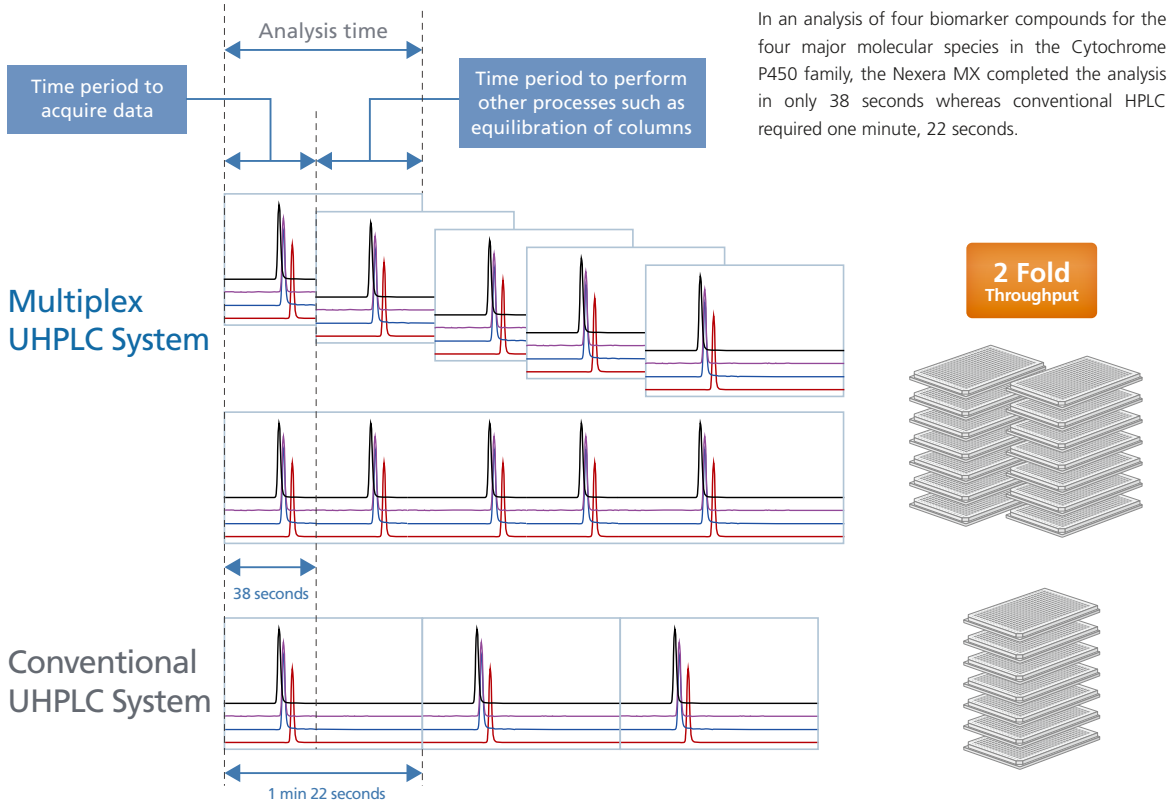


Fig. 8 Improvement in carryover property due to internal rinsing with multiple solutions.

3. Maximize Throughput with a Multiplex Configuration

Conventional analysis requires performing various processes, such as column washing, equilibration at initial mobile phase concentrations, and preparation for the next sample injection, within one cycle. These are essential processes to obtain reliable data; however, these actions result in more "non-data-acquisition time" that impacts a throughput. An LC/MS platform that reduces this "non-data-acquisition time" would increase throughput and, as a result, lead to higher laboratory productivity. One solution to improve throughput is to overlap the data acquisition time and washing/equilibration phase by switching between two streams in one LC/MS (Fig. 9). The Nexera MX system with MX Dual Stream Technology (MX-DST) incorporates a special flow line structure and instrument control system. It uses two alternating sample introduction streams to enable overlapping control of sample injections. Immediately after Stream 1 completes data acquisition, Stream 2 starts data acquisition. During Stream 2's data acquisition, Stream 1 performs its washing/re-equilibration routine. Using this configuration, there is very little "non-data-acquisition time" (Fig. 10).[3]



In an analysis of four biomarker compounds for the four major molecular species in the Cytochrome P450 family, the Nexera MX completed the analysis in only 38 seconds whereas conventional HPLC required one minute, 22 seconds.

Fig. 9 Example of multiplex analysis for Cytochrome P450 assay

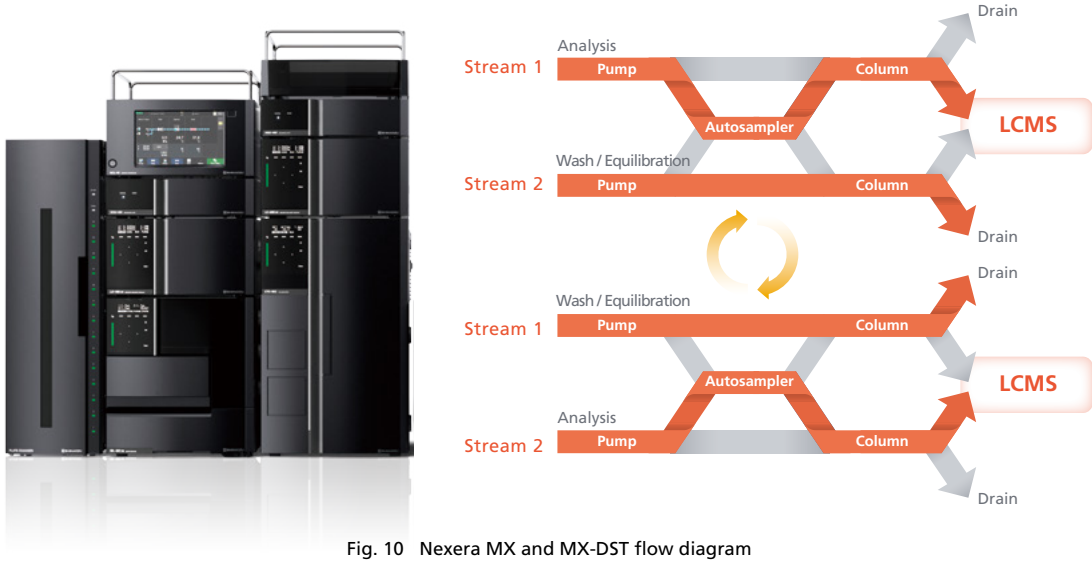


Fig. 10 Nexera MX and MX-DST flow diagram

Conclusions

Throughout this paper, we have presented several characteristics needed to optimize a UHPLC system for use in a high-throughput laboratory. We have shown that the Shimadzu Nexera LC-40 Series meets these criteria with no exceptions. Coupled with LabSolutions software and a Shimadzu triple quadrupole LC/MS/MS, the Shimadzu solutions for high-throughput analysis are unmatched. Of course, we realize that purchasing a mass spectrometer requires a significant investment that cannot be changed easily. Therefore, Shimadzu works hard to benefit the entire scientific community by making our instrument control available to any and all Mass Spec vendors so that our UHPLC systems can be used to their full potential.

Reference

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