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Mass spectrometry

# Sensitivity and acquisition speed with triple quadrupole technology

## What is important to determine effectiveness and how to effectively measure performance


### Authors

Alan Atkins[1], Cristina Jacob[2], Charles Maxey[2], Neloni Wijeratne[2], Claudia Martins[2], and Mary Blackburn[2]; [1]Thermo Fisher Scientific, Hemel Hempstead, UK, [2]Thermo Fisher Scientific, San Jose, CA, USA



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### Introduction

Ultra-high performance liquid chromatography (UHPLC) systems, when combined with triple quadrupole mass spectrometers (MS) with selected reaction monitoring (SRM) analysis, have become widely accepted and utilized for the detection and quantitation of analytes in a wide range of matrices and many different application areas.[1,2] Also known as tandem mass spectrometry, or LC-MS/MS, the adoption of the technique has been driven by its sensitivity, selectivity, and high throughput when compared to other instrumentation, such as UV detection.

When considering the performance characteristics of an LC-MS/MS system, the primary parameter evaluated normally is sensitivity. However, evaluating the sensitivity of an LC-MS/MS system is not a straightforward task, and sensitivity is not the only consideration when selectivity and throughput must be factored in as well. Sensitivity is often defined in terms of a specific analyte and its signal-to-noise ratio (S/N) (e.g., reserpine in positive ionization mode and chloramphenicol in negative ionization mode). However, for most applications, multiple analytes are required to be analyzed in each sample. Even when using chromatography to separate analytes via their retention time (RT), multiple analytes may need to be detected concurrently. The detection speed of the

mass spectrometer becomes important to ensure sufficient data sampling in cases of narrow chromatographic peak widths and/or large SRM transition lists in quantitative LC-MS. For the purpose of this document, dwell time is the time spent by the MS transmitting and detecting the precursor-to-product ion beam. The time needed to acquire an SRM transition is the cumulation of the dwell time and interscan delay time. The interscan delay comprises switching RF and DC voltage, evacuating the Q2 collision cell, polarity switching if applicable, and the time needed to write the data to file. Therefore, the acquisition speed, SRMs/second, is different than the scan rate as defined as Daltons (Da)/second.

We will discuss how sensitivity can be defined and the different ways that it can be evaluated. We will also discuss the need for high SRMs/s rates for multi-analyte analysis and why the scan rate (Da/s) is largely irrelevant for SRM analysis.

## Sensitivity

The strict IUPAC definition of sensitivity is simply Analyte Response/Analyte Concentration. The greater the signal intensity, for a given amount of analyte, the more sensitive the detector. However, when using quadrupole MS to detect lower and lower amounts of analyte, background noise and interferences become the limitation for low-level compound detection. Selective ion monitoring (SIM) uses a single mass filter to isolate a precursor ion, then measures the intensity of the precursor ion. SRM uses two mass filters and a collision cell: first to isolate a precursor ion, fragment it into multiple product ions, then sequentially isolate and measure the intensity of the product ions. For the same analyte at the same concentration, SIM mode will give a higher signal for the precursor than the corresponding SRM mode will give for the product ion. This is because the fragmentation efficiency of the precursor ion and transmission of the product ion are less than 100%. However, in SRM mode, by fragmenting the precursor ion into a product ion, an increase in the selectivity is achieved by significantly reducing the background noise and interference. The reduction in background noise and interferences is far greater than the loss in analyte signal. This means that SRM benefits from much higher S/N ratios and therefore can detect analytes at much lower concentrations. Thus, to detect low amounts of analyte, the ratio of S/N is more important than just the analyte response. For similar reasons, even when evaluating SRM performance, just measuring peak intensity or peak area cannot be used to compare or evaluate a system. Different MS instruments and different vendors may use different scaling factors for their detectors, hence the need to avoid comparing signal counts as a measure of sensitivity.

## Signal-to-noise (S/N)

Signal-to-noise remains an important parameter when evaluating the limit of detection (LoD) for a specific analyte using a specific method. LoD is defined as an amount of analyte that produces a signal that can be distinguished from the background with an error of probability.[3] LoD may be broadly defined as 3 × S/N, but this tends to relate historically from LC-UV detection, where the background noise is more consistent compared to LC-MS/MS systems. Depending on the nature of the noise, an LoD on a modern LC-MS/MS system may be lower than 3 × S/N.

The main issue with S/N as a parameter for measuring sensitivity on MS systems is that it can be very hard to accurately and reproducibly measure. Determination of baseline noise can be variable due to different software algorithms and different methods for determining noise, such as peak-to-peak or root mean square (RMS) (Figure 1). The region of baseline chosen to evaluate the noise contribution, both the position and the width, can also significantly affect noise calculations (Figure 2). It is certainly not possible to measure the noise directly beneath the peak, so users arbitrarily chose a region on either side of the peak. The acquisition rate (scan speed) can also affect S/N calculations; a low sampling rate has the potential to artificially enhance S/N by having sufficient data points to determine the apex of the chromatographic peak, and so determine the signal intensity, but inadequate data points to accurately determine the true baseline noise; a low sampling rate effectively averages out some of the noise (Figure 3).

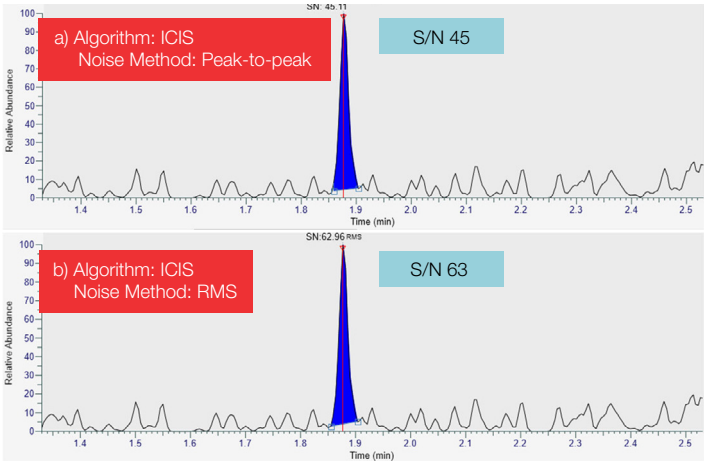


**Figure 1. Comparison of S/N calculations using the same algorithm but using different methods to determine noise: a) peak-to-peak and b) root-mean-square (RMS). For the same integrated peak, S/N is 40% higher using RMS.**

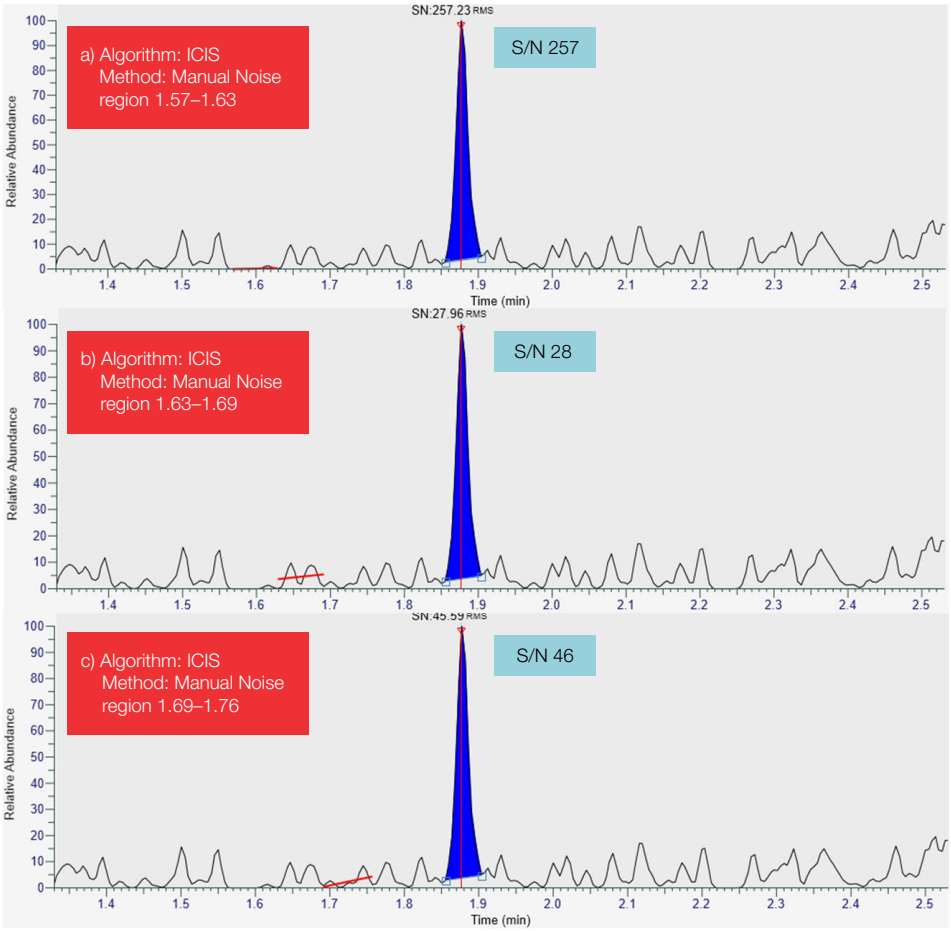


**Figure 2. Comparison of S/N calculations using a manually selected noise region. The noise region, indicated by a red bar, is approximately the same width as the peak. Depending on whether a low noise region (a), a high noise region (b), or medium noise region (c) is selected, S/N values can be as much as 10-fold different.**

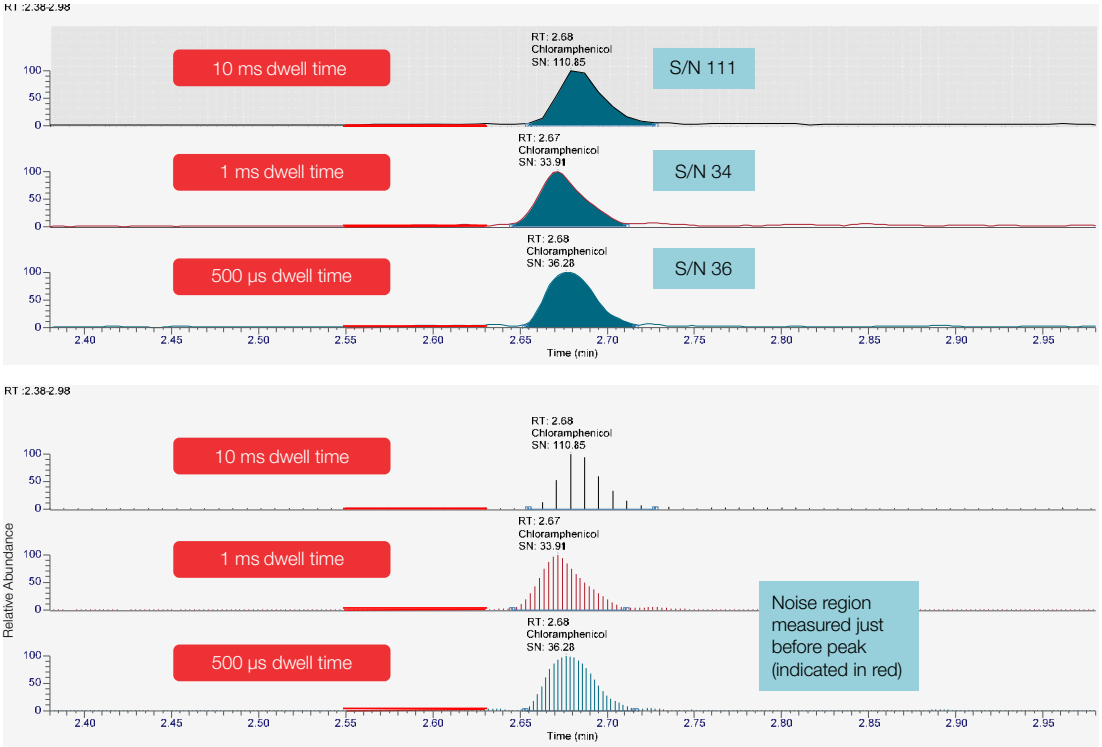


**Figure 3. Comparison of the same analyte at the same concentration, measured with different dwell times. Top traces show the peak-to-peak trace; the bottom traces show the individual scans at the different dwell times. Peak intensity stays the same even at dwell time below 1 ms. Signal-to-noise is improved at 10 ms, largely due to the averaging effect of longer dwell times.**

An increasingly common occurrence is that there is no detectable noise in the baseline. Great steps have been made in reducing the amount of noise measured in the LC-MS/MS systems. These include higher purity water, solvents and additives, better ion source design, improved ion optics, and enhancements to reduce the transmission of neutral species. Increasing mass resolution, whether by highly selective reaction monitoring (H-SRM) on triple quads, or high-resolution, accurate mass (HRAM) detectors such as the Thermo Scientific™ Orbitrap Exploris™ range of mass spectrometers, can again reduce chemical background noise. The introduction of orthogonal techniques such as field asymmetric ion mobility spectrometry (FAIMS) contributes orthogonal selectivity to liquid chromatography and mass spectrometry, increasing selectivity and reducing background noise. All of these advancements can reduce noise to undetectable levels. When there is no detectable noise, S/N becomes infinite and therefore irrelevant.

### Instrument detection limits

Instrument detection limits (IDL) have been proposed as an alternative method to S/N, for evaluating system performance. The IDL is based on method detection limits (MDL) but limited to only testing the performance of the instrument, rather than the complete method, which would include sampling, sample prep., etc. Both MDL and IDL are based on replicate measurements ($n > 6$) of an analyte, spiked into a reagent blank at a concentration 2–5 × higher than the LoD. The MDL is based on a statistical model as used by U.S. Environment Protection Agency[4] using a Student's *t*-test. The MDL is determined as part of a full method validation. Therefore, an LoD is experimentally defined, reagent blanks are analyzed and the response subtracted from the analyte response, and the determination of the MDL is an iterative process (i.e., it is repeated with different spike levels until a consistent MDL is achieved).

Although based on an MDL, the IDL is often determined without defining the LoD; therefore, the analyte amount spiked can be too high or too low. As stated in the EPA method, the amount spiked must not be greater than 10 × MDL (and therefore this should be true for the IDL, too). Another assumption with IDL is that because the chromatographic peak is integrated above baseline noise, it is assumed that the noise contribution is zero, but this is rarely true. IDL is based only on precision. It does not account for the accuracy of the measurement. In addition, the IDL value, an amount estimated through a statistical model, may not be detectable via LC-MS analysis.

### Limits of quantitation

A limit of quantitation (LoQ) is a better measure of analytical performance, as it is determined by both precision and accuracy. The FDA, EPA, and EURL regulatory agencies[5-7] define an LoQ as precision (% CV) and accuracy (% bias) to be both less than 20%. Precision is important to determine that the measurement is reproducible and reliable. Accuracy is important to determine that the measurement is a true indication of the amount in a sample. As the amount in a sample decreases, the accuracy will eventually deviate from the true value, either because there is no longer enough signal to detect it or the contribution from the background noise increases. Evaluating system performance in terms of precision and accuracy, at low analyte concentrations, is therefore a better indication of system performance, which also reflects the real-world situation where both precision and accuracy are required.

### What defines that an assay is fit-for-purpose?

So far, we have discussed S/N, LoDs, IDLs, and LoQs in terms of how sensitive an LC-MS/MS system may be. Often, these are defined by a single analyte (e.g., reserpine or chloramphenicol), but increasingly, the demand is to measure more analytes with greater confidence and with much higher throughput.[8] Therefore, sensitivity for multiple analytes, along with selectivity and throughput, needs to be considered to determine how successful the fit-for-purpose assay is for the laboratory.

Precision and accuracy are both improved with longer dwell times. The longer the instrument can measure the SRM ion beam, the more consistent the data will be. Baseline noise also becomes more consistent at higher dwell times, so that the contribution of noise is reduced, improving accuracy. Adding more analytes to an established method requires shorter dwell times per SRM transition within the acquisition/cycle time, as defined by the time taken to acquire all SRM transitions. The cycle time needs to be short enough that each chromatographic peak can be adequately characterized (typically 10–15 scans across a peak). As chromatographic peaks become sharper, this requires a corresponding reduction in cycle time. To improve specificity, addition of confirming SRMs for each analyte may be required, as well as isotopically labelled internal standards. Isotopically labelled standards may also be required to correct for recovery and/or matrix effects. Using scheduled windows for analysis, where the analyte is only detected within a specific time window across the chromatographic run, can significantly reduce the number of measured analytes within any cycle time, enabling longer dwell times per SRM transition. This can be further

improved by using longer columns and shallower LC gradients, though this then comes at the expense of sample throughput. As increasing sample throughput is probably the biggest driver of productivity in most analytical labs, there are limitations to how much chromatographic separation can be extended. Therefore, having an MS with fast acquisition rates is desirable to maintain quantitative performance and increase selectivity (by increasing number of confirming ions) without having to compromise throughput.

### SRM acquisition rates compared to scan rate

The SRM acquisition rate (SRMs per second) can easily be confused with scan rate (Da per second). The Thermo Scientific™ TSQ™ Plus portfolio of triple quadrupole mass spectrometers can acquire 600 SRMs per second with a pos/neg switching time of only 5 ms. This fast SRM acquisition rate has been enabled by reducing the interscan delay between SRMs, allowing more dwell time for the analytes of interest. Even when dwell times are below 1 ms, good quantitative performance can be achieved due to the enhanced transmission of ions (Figure 3). This means that hundreds of analytes, with multiple SRMs per compound, can be analyzed in a single run,[9] or a multi-component assay can be run with injection-to-injection times of 2 minutes or less[10].

Scan rate (Da/s), on the other hand, is the speed at which the quadrupoles can scan across the user-defined mass range. A high scan rate on triple quadrupole mass spectrometers is important for Full Scan, Product Ion Scan, Precursor Ion Scan, and Neutral Loss Scan techniques because the cycle time can be maintained at higher rates. However, this is at the expense of sensitivity as the faster the instrument scans the user-defined mass range, the less time the detector has to measure a specific *m/z* ion population. Any time scanning across the mass range is time not transmitting the maximum ion flux.

Experiments using SRM mode are the most sensitive and therefore the most widely used data acquisition mode on triple quadrupole mass spectrometers. The quadrupole mass analyzers do not scan but instead are set to transmit the maximum ion flux for the precursor ion (Q1) and the resultant product ion (Q3). The maximum ion flux is at the *m/z* apex of the specific SRM transition being measured. For multiple SRMs, reducing the interscan delay time is most important factor in increasing data acquisition rate, not how fast the quadrupoles can scan the *m/z* range.

## Summary and conclusions

Although sensitivity is a very important parameter for any analytical instrument, it is not the only consideration for whether that instrument is suitable for a particular application or workflow. The SRM acquisition parameters per compound are critical for sensitivity but also must ensure selectivity relative to the matrix, and adequate cycle time for the overall method to acquire the desired number of data points for the chromatographic separation. In addition, flexibility, robustness, and ease-of-use should also be assessed and determined whether maximizing any of these attributes is at the expense of another. Acquisition speed that is reliable, accurate, and precise at low dwell times is an increasingly important feature on triple quadrupole mass spectrometer systems. As the number of measured analytes increase, MS selectivity needs to be maintained so that laboratory throughput needs can be realized.

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