Why Instrument Detection Limit (IDL) is a Better Metric for Determining The Sensitivity of Triple Quadrupole LC/MS Systems

Technical Overview

Authors

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Customer Checklist

- ✓ Do you report your analysis results as detected amount instead of signal-to-noise?
- ✓ Do you report the limit of detection (LOD) and limit of quantitation (LOQ) of your analysis?
- ✓ Do you use RSD to check the precision and repeatability of your quantitative analysis?
- ✓ Is precision and repeatability critical to your analysis results?
- ✓ Is precision at low concentration import to you?
- ✓ Do you want a sensitivity specification that is more aligned with your daily analysis?

Then IDL based on standard deviation (RSD) of area response is for you.

Introduction

Assessing the sensitivity of LC/MS instruments has relied on comparison of signal-to-noise (S/N) values for various compounds including reserpine (positive ion mode) and chloramphenicol (negative ion mode). While S/N is a useful metric, the calculated values depend on the manner in which noise is determined, including whether post acquisition filtering or smoothing is used to reduce the numerical value of noise (N) and, therefore, produce S/N values which do not reflect the ability of an instrument to detect low levels of target analytes.

To provide a better sensitivity performance assessment, Agilent is leading the way with an additional metric that provides more accurate and reliable criteria for triple quadrupole performance, **Instrument Detection Limit (IDL)**. Although S/N is meaningful for LC/MS triple quadrupole applications where solvent or matrix sources of baseline noise can be easily measured, IDL is a better, more rigorous, and statistically valid measurement that eliminates the uncertainty and variability associated with S/N calculations. The IDL specification is based on statistical analysis of precision that provides customers with a practical means of evaluating triple quadrupole LC/MS sensitivity for their intended quantitative applications.

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IDL – How is it Determined?

The IDL is the minimum amount of analyte required to produce a signal that is statistically distinguishable from the background noise level within a specified confidence level. IDL is related to the standard deviation (SD) of the measured area response of the replicate injections and the statistical confidence factor *t* by:

IDL = *t* × SD, where the SD and IDL are expressed in area counts

The confidence factor *t* is determined using Student t-distribution with a 99 % confidence level and n–1 (number of replicate injections minus 1) degrees of freedom. For data analysis software that reports relative standard deviation (RSD = SD/Mean Value), IDL can be determined in units (for example, femtogram) of the amount of the standard injected using the following formula:

IDL = *t* × (RSD/100 %) × amount measured

For example, the IDL measurement for the Agilent 6490 Triple Quadrupole LC/MS System in positive mode is calculated using 10 replicate injections of 5 fg of reserpine (Figure 1). In this case, the *t* value is 2.821 with a 99 % confidence level and 9 degrees of freedom (n = 10). The peak area precision (RSD) is 7.2 % and the value of IDL is:

IDL = 2.821 × (7.2 %/100 %) × 5 fg = 1.02 fg

Thus, an amount of reserpine greater or equal to 1.02 fg is detectable and distinguishable from the background noise with a 99 % probability.

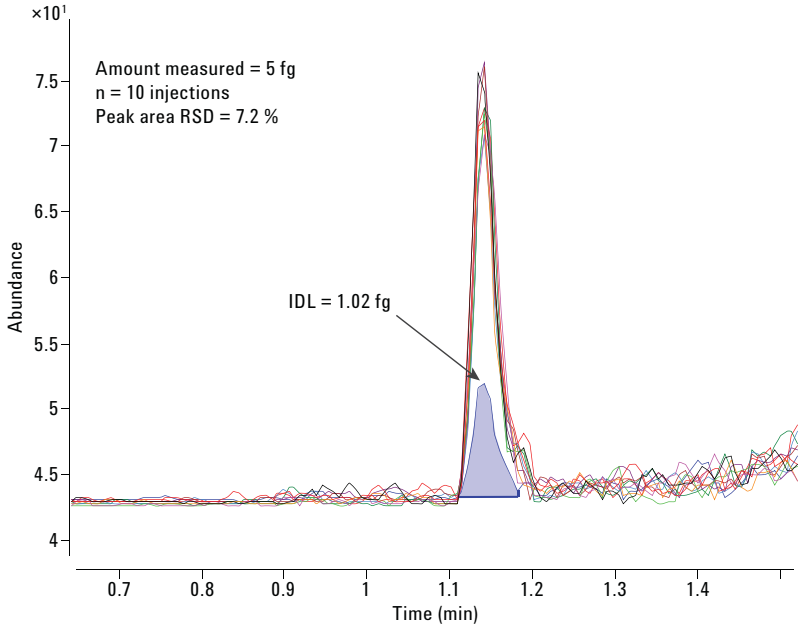

Figure 1. An example for one set of MRM chromatograms of *m/z* 609 to 195 from 5 fg of reserpine; 10 replicate injections using the Agilent 6490 Triple Quadrupole LC/MS System.

Figure 2 shows that the IDL of the 6490 Triple Quadrupole LC/MS instrument in negative mode was measured using 10 replicate injections of 5 fg of chloramphenicol. The peak area precision (RSD) was 8.9 %, so the value of IDL is:

IDL = 2.821 × (8.9 %/100 %) × 5 fg = 1.25 fg

Thus, an amount of chloramphenicol equal to, or greater than 1.25 fg is detectable and easily distinguishable from the background noise with a 99 % probability.

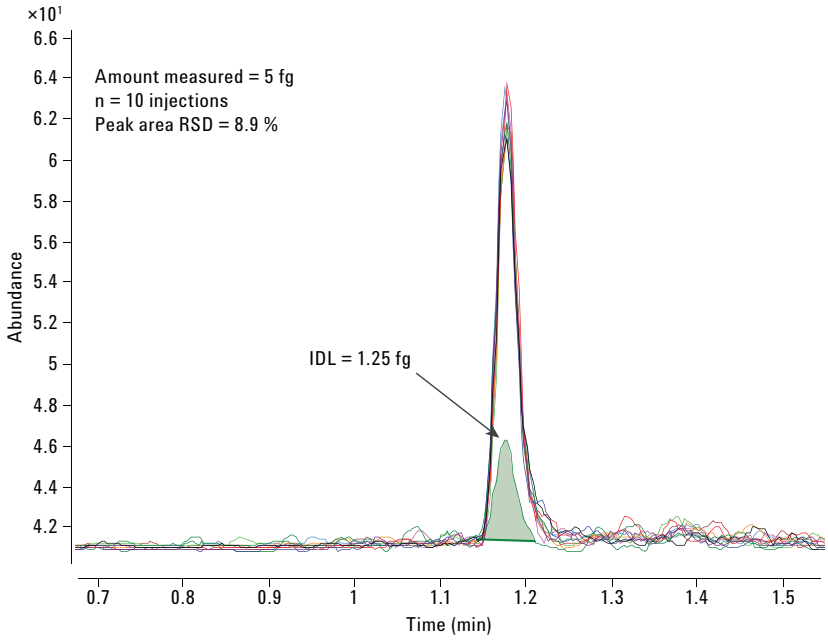

Figure 2. An example for one set of MRM chromatograms of *m/z* 321 to 152 from 5 fg of chloramphenicol; 10 replicate injections using the Agilent 6490 Triple Quadrupole LC/MS System.

Why IDL is a Better Metric for Determining Sensitivity

- **IDL follows the guidelines of many respected organizations.** IUPAC, EPA, and many other organizations use similar calculations to determine detection limits for analytical methods.

- **IDL is based upon a well-established statistical formula.** Using a 99 % confidence interval, Student t-distribution is applied to the area precision (RSD) from 10 consecutive replicate injections to calculate IDL.

- **IDL assesses typical performance from a series of replicate injections, not a single manual injection.** A series of 10 consecutive measurements yields a more representative view of system performance. IDL is based on standard deviation of area responses and demonstrates the precision and repeatability that you need for your instruments and methods.

- **IDL is not biased by clever manipulation of baseline noise.** Today's high-performance triple quadrupole LC/MS systems use checkout chemicals, for example, 1 pg of reserpine in positive ion mode and 1 pg of chloramphenicol in negative ion mode, to perform S/N measurements. Noise measurements of these chemical standards are not consistent, and the calculated S/N values have questionable validity. IDL eliminates this source of ambiguity.

- **IDL is measured at realistic low analytical levels.** The amount of analyte used to measure IDL is near the expected limit of detection (LOD).

- **IDL confirms performance of every component in the LC/MS system.** A series of automated injections confirms the precision of every component in the triple quadrupole LC/MS system from the autosampler, to the HPLC, and mass spectrometer. All instrument components used in your methods are tested as a completely integrated system.

- Learn more about the science and statistics concerning IDL here: www.agilent.com/chem/IDLtechnicaloverview

Summary

Historically, S/N of a chromatographic peak determined from a single measurement has been used as a convenient standard to compare the sensitivity performance of two LC/MS systems. The measured S/N can vary significantly with different selected baseline regions and different software algorithms used to determine baseline noise. The choice of where and how to measure S/N in an LC/MS chromatogram is subjective, and often fails to estimate the LOD, especially when a relatively high level of analyte is used for S/N measurement. From the analytical perspective, there is a significant benefit to having a performance metric that offers a realistic and meaningful assessment of the LOD and the system precision near the LOD. IDL is an objective performance metric based on the multi-injection statistical methodology and precision of the replicate injections. This metric provides the customers with a useful means of evaluating the sensitivity of triple quadrupole LC/MS systems.

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