Application Note

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# Signal, Noise, and Detection Limits in Mass Spectrometry


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## Abstract

In the past, the signal-to-noise of a chromatographic peak determined from a single measurement has served as a convenient figure of merit used to compare the performance of two different MS systems. Design evolution of mass spectrometry instrumentation has resulted in very low noise systems that have made the comparison of performance based upon signal-to-noise increasingly difficult, and in some modes of operation impossible. This is especially true when using ultra-low noise modes such as high resolution mass spectrometry or tandem MS; where there are often no ions in the background and the noise is essentially zero. Statistical methodology commonly used to establish method detection limits for trace analysis in complex matrices as a means of characterizing instrument performance is shown to be valid for high and low background noise conditions.

## Introduction

Trace analysis in analytical chemistry generally requires establishing the limit of detection (LOD, or simply detection limit), which is the lowest quantity of a substance that can be distinguished from the system noise absent of that substance (a blank value). Mass spectrometers are increasing used for trace analysis, and an understanding of the factors that affect the estimation of analyte detection limits is important when using these instruments. There are a number of different detection limits commonly used. These include the instrument detection limit (IDL), the method detection limit (MDL), the practical quantification limit (PQL), and the limit of quantification (LOQ). Even when the same terminology is used, there can be differences in the LOD according to nuances of what definition is used and what type of noise contributes to the measurement and calibration. There is much confusion regarding figures of merit for instrument performance such as sensitivity, noise, signal-to-noise ratio and detection limits. An understanding of the factors that contribute to these figures of merit and how they are determined is important when estimating and reporting detection limits. Modern mass spectrometers, which can operate in modes that provide very low background noise and have the ability to detect individual ions, offer new challenges to the traditional means of determining detection limits.

### Terminology

- **Instrument background signal**—The signal output from the instrument when a blank is measured; generally a voltage output that is digitized by an analog to digital converter.
- **Noise (N)**—The fluctuation in the instrument background signal; generally measure as the standard deviation of the background signal.
- **Analyte signal (S)**—The change in instrument response to the presence of a substance.
- **Total instrument signal**—The sum of the analyte signal and the instrument background signal.
- **Signal-to-noise ratio (S/N)**—The ratio of the analyte signal to the noise measured on a blank.
- **Sensitivity**—The signal response to a particular quantity of analyte normalized to the amount of analyte giving rise to the response; generally determined by the slope of the calibration curve. Sensitivity is often used interchangeably with terms such as S/N and LOD. For the purpose of this document, sensitivity will only apply to this analytical definition.

### Instrument detection limit (IDL)

Most analytical instruments produce a signal even when a blank (matrix without analyte) is analyzed. This signal is referred to as the instrument background level. Noise is a measure of the fluctuation of the background level. It is generally measured by calculating the standard deviation of a number of consecutive point measurements of the background signal. The instrument detection limit (IDL) is the analyte concentration required to produce a signal that is distinguishable from the noise level within a particular statistical confidence limit. Approximate estimate of LOD can be obtained from the signal-to-noise ratio (S/N) as described in this document.

### Method detection limit (MDL)

For most applications, there is more to the analytical method than just analyzing a clean analyte. It might be necessary to remove unwanted matrix components, extract and concentrate the analyte, or even derivatize the analyte for improved chromatography or detection. The analyte may also be further diluted or concentrated prior to analysis on an instrument. Additional steps in an analysis add additional opportunities for error. Since detection limits are defined in terms of error, this will increase the measured detection limit. This detection limit (with all steps of the analysis included) is called the MDL. An approximate estimate of LOD can often be obtained from the S/N of the analyte measured in matrix.

### Limit of quantification (LOQ) and practical limit of quantification (PQL)

Just because we can tell something from noise does not mean that we can necessarily know how much of the material there actually is with a particular degree of certainty. Repeated measurements of the same analyte under the same conditions, even on the same instrument, give slightly different results each time due to variability of sample introduction, separation, and detections processes in the instrument. The LOQ is the limit at which we can reasonably tell the difference between two different values of the amount of analyte. The LOQ can be drastically different between labs so another detection limit referred to as the Practical Quantitation Limit (PQL) is commonly used. There is no specific mathematical relationship between the PQL and the LOQ based on statistics. The PQL is often practically defined simply as about 5 to 10 times the MDL.

## Estimating IDL and MDL using signal-to-noise

Mass spectrometry measurements of an analyte generally use a chromatograph as a means of sample introduction. The resulting analyte signal from a chromatograph will have an approximately Gaussian shaped distribution as a function of time (Figure 1). In the case of chromatographic analyte introduction into the MS instrument, the signal is not constant and does not represent the same analyte amount at all points of the sample set. For the purpose of estimating detection limits by using signal-to-noise ratios, the measurement of the signal is generally accepted to be the height of the maximum of the chromatographic signal (S in Figure 1) above the baseline ($\overline{X}_B$), and an estimate of the background noise under the peak must be made. A standard for estimating the noise is to measure the peak-to-peak (minimum to maximum) value of baseline noise, away from the peak tails, for 60 seconds before the peak (Figure 1) or 30 seconds before and after the peak. With the advent of modern integrator and data systems, the baseline segments for estimation of noise are auto-selected, and noise is calculated as the standard deviation (STD) or root-mean-square (RMS) of the baseline over the selected time window. However, it will be shown that the single measurement S/N approach fails in many cases.

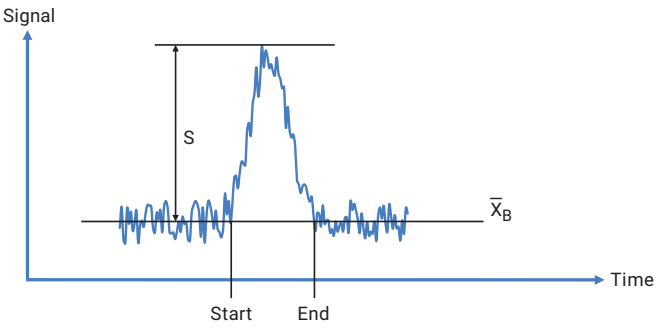


**Figure 1.** Analyte signal as a function of time for a chromatographic peak, demonstrating the time dependence in the amount of analyte present.

When a quantitative measurement of the amount of analyte is made, the signal is the total integrated signal of the Gaussian peak from start to end with the background subtracted. This area is a single sampling measurement and is a single point estimator for the true analyte amount in the population (that is, the amount of analyte in the original sample). The amount of analyte as a function of time can be expressed as: $C(t) = KC_0F(t)$; where $C_0$ is the amount of analyte in the test sample, F(t) is the shape of the chromatographic peak (amplitude versus time), and K is a calibration factor. If the analyte profile is reproducible and the response is linear in analyte amount, the area of the peak can be used as a measure of the original amount of analyte since the constant of proportionality, K, can be determine by calibration using known amounts of analyte. Repeated measurements (areas) of the same sample will yield a set of somewhat different responses that are normally distributed about the true value representing the population. The variance in the measured set of signals for both the sample and the background are due to a variety of factors: (1) variances in the amount injected, (2) variances in the amount of sample transferred onto the GC column, (3) variances in the amount of background, (4) variances in the ionization efficiency, (5) variances in the ion extraction from the ion source and transmission through the mass analyzer, and (6) variances in the recorded detection signal representing the number of ions measured. The latter factor at low ion fluxes will occur even if the number of ions striking the ion detector were the same, the output signal will be slightly different since the ion detector response depends on where the ion strikes it. A significant contributor to variance will be the determination of the area of the chromatographic peak. Variations in the determination of peak start, end and area below the background all contribute errors. Collectively, these variances can be viewed as sampling noise. That is, variations in the output signal due to the collective sampling and detecting processes. These variances are in addition to the normal variations due to measuring a finite number of ions (that is, the ion statistics).

Modern mass spectrometer systems are capable of operating in a variety of modes that can make the background nearly zero. MS/MS, negative ion chemical ionization, and high resolution mass scanning can often have a near zero system background signal, particularly when the background from a chemical matrix is absent. Figure 2 shows a GC/MS extracted ion chromatogram for a clean standard of octafluoronaphthalene (OFN) in a clean system with a very low background. Each point in an extracted ion chromatogram represents the intensity of a centroided mass peak. If there are not enough ions for a particular mass to be centroided, the resulting intensity value may be reported as zero. It is possible with very clean systems operating in the MS/MS or negative chemical ionization mode to have no observable background ions and a zero calculated noise. This situation can be made more severe by increasing the threshold for ion detection. Under these circumstances, it is possible to increase the ion detector gain, and the signal level, without increasing the background noise. The signal of the analyte increases, but the noise does not. This is misleading and unacceptable.

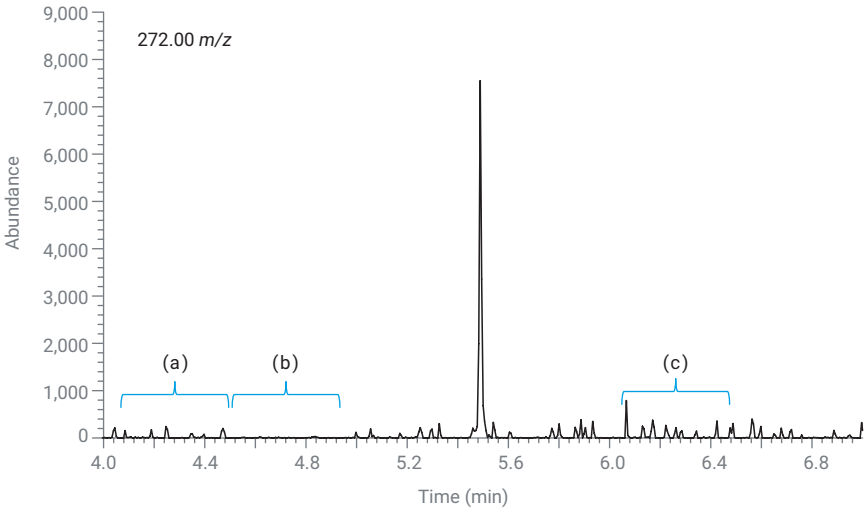


**Figure 2.** EI full scan extracted ion chromatogram of *m/z* = 272 from 1 pg OFN exhibiting very small chemical ion noise.

The result is two practical problems: (1) What region of the baseline should be selected to estimate the background noise, and (2) although the signal increased, there was no increase in the number of ions detected and therefore no change to the real detection limit. Unlike the high background case, when the background is very low, but not zero, the measured noise will depend strongly on where the noise is measured. The regions of the background labeled (a), (b), and (c) in Figure 2 have measured RMS noise values that are 54, 6, and 120 respectively. The resulting S/N values can differ by a factor of 20 in this example due exclusively to the large variation in where the noise is measured. Therefore, the use of S/N as an estimate of the detection limit will clearly fail to produce usable values when there is low and highly variable ion noise. The situation becomes even more indeterminate when the background noise is zero as shown in the MS/MS chromatogram in Figure 3. In this case, the noise is zero and the S/N becomes infinite. The only noise observed in Figure 3 is due to the electronic noise, which is several orders of magnitude lower than noise due to the presence of ions in the background.

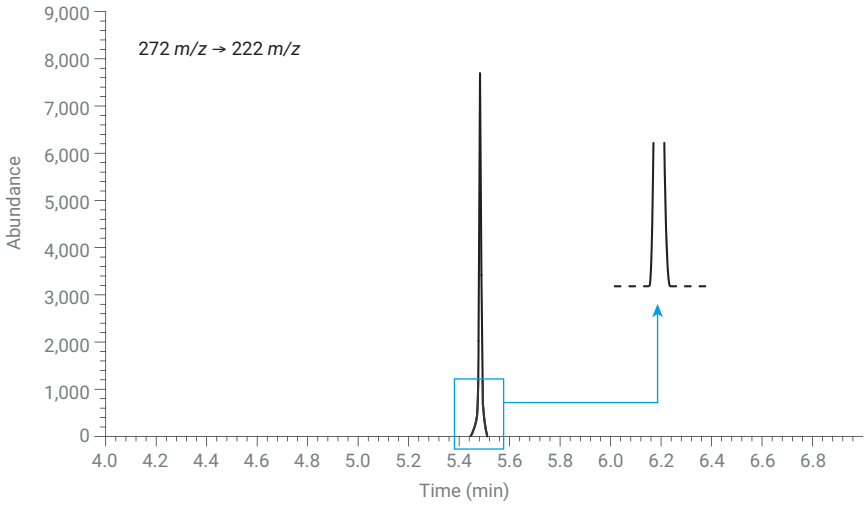


**Figure 3.** EI MS/MS extracted ion chromatogram of *m/z* 222.00 from 100 fg OFN exhibiting no chemical ion noise.

## Alternate methods to estimating IDL and MDL

There are many alternative methods to estimate the IDL and MDL that produce more reliable estimates[2–7] for analytes introduced by a chromatograph. For the USA, the most common is the recommended EPA Guidelines Establishing Test Procedures for the Analysis of Pollutants.[2] A commonly used standard in Europe is found in The Official Journal of the European Communities, Commission Decision of 12 August 2002; Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.[4]

Both of these methods are similar and require injecting multiple duplicate standards to assess the uncertainty in the measuring system. A small number of identical samples having a concentration near the expected limit of detection (within 5 to 10 times the noise level) are measured along with a comparable number of blanks. The average blank value is subtracted from each of the analyte measurements to remove the area contribution from any constant background if necessary. Often, because of the specificity of mass spectrometry detection, the contribution from the blank is negligible and is excluded once the significance of the contribution has been confirmed. The standard deviation of the set of measured analyte signals (that is, integrated areas of the baseline subtracted chromatographic peaks) is then determined. Since the variance in the peak areas includes both the analyte signal noise, the background noise, and the variance from injection to injection; the statistical significance of a single measurement being distinguishable from the system and sampling noise can be established with a known confidence level (that is, a known probability that the measured area is statistically different from the system noise). The use of signal-to-noise from a single sample measurement as an estimate of IDL does not capture the sample-to-sample variation or sampling noise that causes multiple measurements of the same analyte to be somewhat different.

When the number of measurements is small (that is n <30), the one-sided Student t-distribution[1] is used to determine the test statistic $t_\alpha$. In the case of chromatographic peaks, modern data systems report the area of the peak above the baseline, (that is, the background is subtracted, but not the contribution to the variance of the signal). The IDL or MDL is determined as the amount of analyte $\overline{X}$ that gives a signal (peak area) statistically greater than the population mean value of zero ($\mu = 0$) as detailed in Appendix I.

$$IDL = \overline{X} - \mu = \overline{X} = t_\alpha \sigma_{\overline{X}} = t_\alpha S_{\overline{X}}$$

Where the value of $t_\alpha$ comes from a table of the Student t-test using n – 1 (number of measurements minus one) as the degrees of freedom, 1 – α is the probability that a measurement is greater than zero, and the standard deviation of the set of measurements $S_{\overline{X}}$ is used as an estimate of the true standard deviation of the distribution of sample means. This discussion eatablishes that at least two or more measurements are required in order to estimate the standard deviation and estimate the sampling noise. The larger the number of measurements n, the smaller is the value of $t_\alpha$ and less the uncertainty in the estimate of the IDL or MDL.

Figure 4 shows that for a given amount of standard, $C_{STD}$, replicate measurements produce a distribution of measured values centered about the mean value $\overline{X}_{STD}$. The standard deviation is a measure of the width of this distribution. The IDL is amount of sample, $C_{IDL}$, corresponding to a mean measured value, $\overline{X}_{IDL}$ , that allows 1 – α of the measurements to be greater than zero; where α is the percentage or probability that a measured value is equal or less than zero. Figure 5 shows the effect of a smaller standard deviation (greater precision) for the same α and the same instrument sensitivity. A smaller standard deviation of the measurements, keeping α the same, results in a smaller IDL since the mean value of the measured distribution is moved to smaller values in order to keep the same percentage (that is, same α) of the new distribution greater than zero.

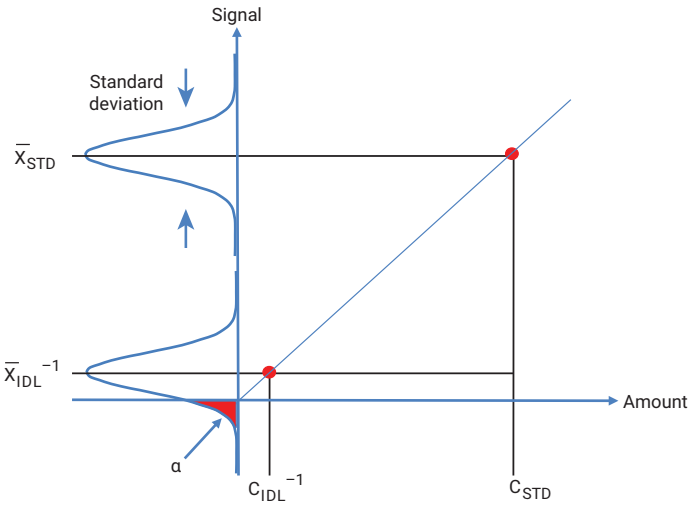


**Figure 4.** Instrument detection limit–amount of analyte with signal that is statistically >0. Large variance in measured values.

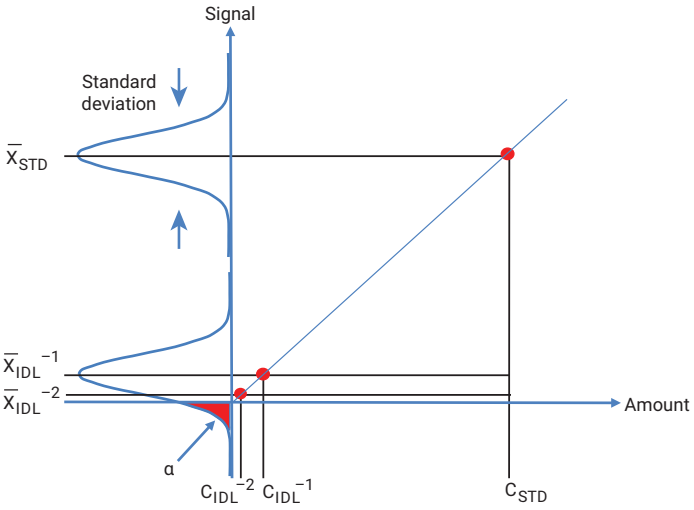


**Figure 5.** Instrument detection limit–amount of analyte with signal that is statistically >0. Small variance in measured values.

As an example, for the eight replicate injections in Figure 6 (seven degrees of freedom; n = 7) and a 99% (1 – α = 0.99) confidence interval, the value of the test statistic ($t_\alpha$) is 2.998. Since the instrument detection limit is desired, a population mean of zero is assumed (that is 99% probability that a single measurement of $\overline{X}_A$ (a single area measurement) will result in a signal statistically greater than zero with a probability of 1 – α). For eight samples, the mean value of the area is 810 counts, the standard deviation is 41.31 counts, the relative standard deviation is 5.1% and the value of the IDL is:

$IDL = t_\alpha S_{\overline{X}}$

$\overline{X}_A$ = (2.998)(41.31) = 123.85 counts. Since the calibration mean for 200 fg was 810 counts, the IDL is: (123.85 counts) (200 fg)/(810 counts) = 30.6 fg.

For data systems reporting relative standard deviation (RST) the IDL is:

IDL = ($t_\alpha$)(RSD)(amount standard)/100%. In the previous example the IDL = (2.998)(5.1%)(200 fg)/100% = 30.6 fg

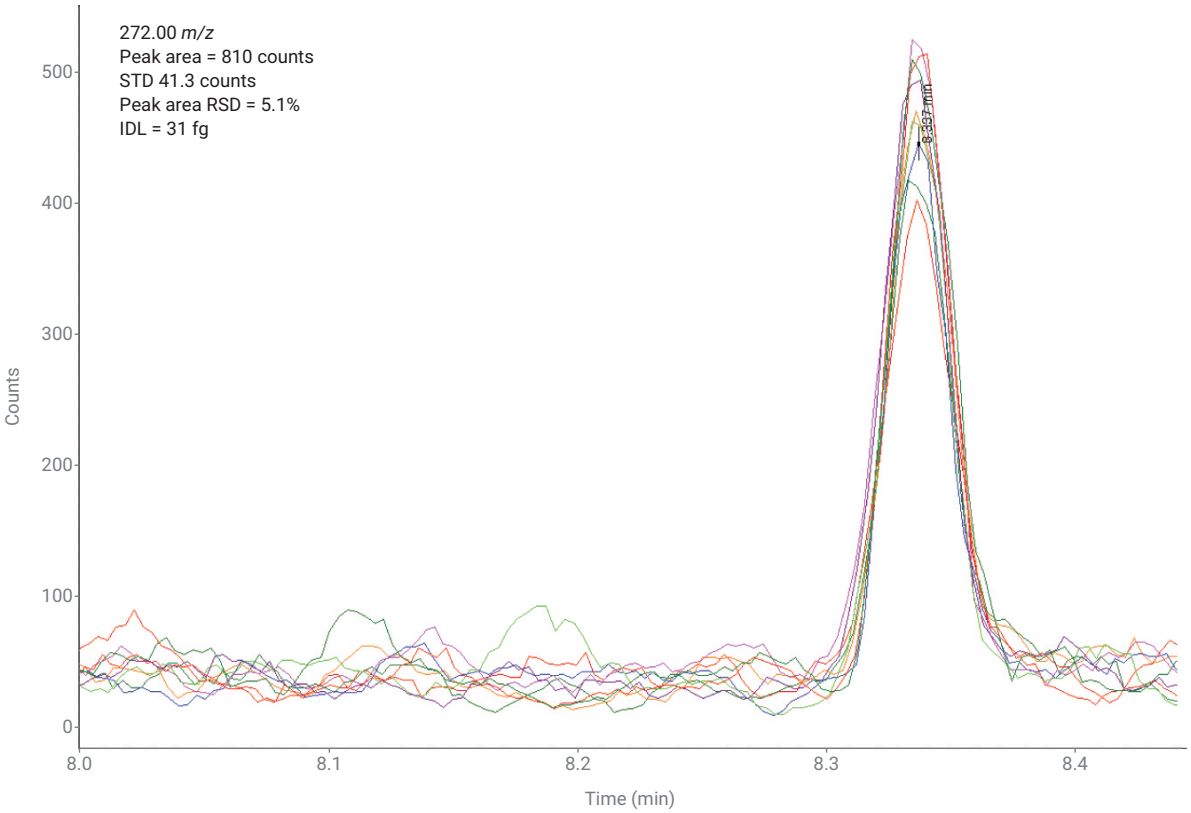


**Figure 6.** EI full scan extracted ion chromatograms of *m/z* = 272 from 200 fg OFN; eight replicate injections.

## Conclusion

The historical use of the signal-to-noise ratio of a chromatographic peak determined from a single measurement is a convenient means of estimating IDL in many cases. A more practical means of estimating the IDL and MDL is to use the multi-injection statistical methodology commonly used for trace analysis in complex matrices. Using the mean value and standard deviation of replicate injections provides a way to estimate the statistical significance of differences between low level analyte responses and the combined uncertainties in both the analyte and background measurement, and the uncertainties in the analyte introduction or sampling process. This is especially true for modern mass spectrometers for which the background noise is nearly zero. The multi-injection method of estimating instrument and method detection limits is rigorously and statistically valid for both high and low background noise conditions.

## References


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## Appendix I

### How a signal is distinguished from noise

The estimation of detection limits depends on how a signal from an analyte is distinguished from the background noise. The methodology for this estimate is rooted in statistical hypothesis testing. Hypothesis testing is similar to a criminal trial. In a criminal trial, the assumption is that the defendant is innocent. The null hypothesis $H_0$, expresses an assumption of innocence. The opposite of the null hypothesis is $H_a$, the alternative hypothesis – it expresses an assumption of guilt. The hypothesis for a criminal trial would be written:

$H_0$: The defendant is innocent

$H_a$: The defendant is guilty

To test these competing statements, or hypothesis, a trial is held. The evidence presented at trail provides the sample information. If the sample information is not inconsistent with the assumption of innocence, the null hypothesis that the defendant is innocent cannot be rejected. However, if the sample information is inconsistent with the assumption, the null hypothesis must be rejected and the alternative hypothesis can be accepted.

In the case of chromatographic peaks, modern data systems report the area of the peak above the baseline (that is, the constant background is subtracted, but not the contribution to the variance of the signal). The mean value of the population $\mu_A$ of the analyte is the true value of the amount of analyte stored in the analyte collection vessel. A measurement of a sample aliquot is a single-point estimation of the population mean. The mean value $\overline{X}_A$ of a set of replicate sample measurements is an approximation to the population mean $\mu_A$. The question to be answered: Is the sample set mean value statistically greater or equal to the mean population within some specified confidence level? Since the IDL is desired, the population mean is assumed to be zero. In this case the rejection criterion is:

- $H_0$: $\mu_A \leq 0$ The estimate of the signal is not different from zero within the stated confidence or significance limit.
- $H_a$: $\mu_A > 0$ The estimate of the signal is different from zero within the stated confidence or significance limit.
- Accept $H_0$ if $\mu_A \leq 0$ and reject $H_0$ if $\mu_A > 0$ and accept the alternative hypothesis $H_a$.

The statistical criteria for testing the hypothesis is: if the test statistic $t_\alpha$ is less than a value that is obtained from a probability table. The table value depends on the number of measurements (n) that contributed to obtaining the mean value and the probability that the mean value is greater than the population mean.

$$t_\alpha = \frac{\overline{X} - \mu}{\sigma_{\overline{X}}} < \text{table value}$$

Where:

$\overline{X}_A$ is the mean value of the set of sample measurements

$\mu_A$ is the true value of the population

$\sigma_{\overline{X}}$ is the standard deviation of the set of sample measurements

$t_\alpha$ is the test statistic

$1 - \alpha$ is the probability that the sample set mean is different from the population mean

The value of the test statistic t depends on the number of degrees of freedom which is defined as the number of measurements minus one. For small numbers of measurements (n <30), the value of $t_\alpha$ comes from a table of the Student t-test using n – 1 (number of measurements minus one). For example, for seven measurements (six degrees of freedom) and a confidence level of 99% ($\alpha = 0.01$ which is a 99% probability that the value is different from zero; $\mu = 0$) the table value is 3.143. Therefore, if:

$$\frac{\overline{X} - \mu}{\sigma_{\overline{X}}} = \frac{\overline{X}}{S_{\overline{X}}} = t_\alpha < 3.143$$

accept the null hypothesis, and if this is greater than $t_\alpha$, the null hypothesis must be rejected and the alternative hypothesis (that is, the background corrected analyte signal is statistically different from zero) must be accepted with a $1 - \alpha$ probability of being correct. The value of the standard deviation of the population $\sigma_{\overline{X}}$ is usually approximated by the standard deviation of the sample set $S_{\overline{X}}$. For four samples (three degrees of freedom) and a 97.5% confidence level, the value of the test statistic is 3.18. For eight samples (seven degrees of freedom) and a 99.0% confidence level, the value of the test statistic is 2.90. The average value is approximately 3. Therefore, the common rule for estimating the IDL or MDL is: $\overline{X}_{IDL} = 3\, S_{\overline{X}}$.

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DE16430639



Printed in the USA, January 6, 2023
5990-7651EN

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