A Practical Strategy for Determining and Verifying Detection Limits

By Thomas Georgian, Ph.D. and Kenneth E. Osborn

Detection limits are statistical estimates used to evaluate analytical data for operational, regulatory and scientific purposes. In particular, detection limits are required for laboratories performing analytical work under the federal Clean Water Act (CWA) or the Safe Drinking Water Act (SDWA). Regulations may require the use of the U.S. Environmental Protection Agency (EPA) Method Detection Limit (MDL) defined in 40 *CFR* Part 136, Appendix B. In accordance with certification requirements and EPA guidelines, laboratory quality assurance (QA) programs typically specify determinations of detection limits annually and when a significant change has been made to a method or an instrument. Unfortunately, detection limit determinations at the bench can be time consuming and can give rise to contentious results (e.g., since detection limits are instrument and matrix-dependent). A simple and practical approach for determining and verifying detection limits is proposed.

Detection Defined by EPA

The MDL is defined in the *Federal Register* (40 *CFR* Part 136, Appendix B) as "the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero."[1] Operationally, the MDL is determined by analyzing seven replicates of a sample containing the analyte of interest at a concentration between one to five times the expected detection limit. Typically, for aqueous methods, multiple reagent water blanks are fortified at suitable concentrations and subsequently processed through the entire analytical procedure (e.g., both the preparatory and instrumental portions of the method). The MDL is primarily calculated by multiplying the standard deviation for a set of at least seven replicate measurements by the appropriate Student's t value (which is equal to about three for seven replicates and the 99% confidence level).

Expense of Determination and an Alternative

Because laboratory QA programs may require that this process be repeated on an annual basis and whenever major changes are made to an analytical procedure, detection limit determinations can be time consuming and expensive. The use of preexisting quality control standards, such as laboratory control standards (LCSs) or method blanks to estimate detection limits can dramatically reduce both time and expense.[2, 3] For example, if the standard deviation is not strongly dependent upon analyte concentration, then detection limits estimated from reagent blanks should be approximately equal to those estimated from fortified LCSs. This strategy may also result in more accurate detection limits since detection limit estimates tend to be more reliable when a large number of replicates are available (e.g., at least 20 or 30 replicates are frequently used to establish control chart using in-house quality control data). However, it should also be noted that these approaches take only random error into account; systematic error or method bias is not included.

It should also be noted that the detection limit is a statistical quantity that minimizes false positives and does not address false negatives. In other words, it can be concluded that an analyte is present in a sample with at least 99% confidence when an analytical reading obtained is equal to or greater than the detection limit. However, when analyte is actually present in the sample at a level equal to the detection limit, the probability that the analyte will be detected will be only about 50% and not 99%! When the standard deviation is relatively constant at low concentrations (e.g., one to five times the detection limit), the probability that the analyte will be detected will be approximately 99% when the analyte is present in sample at twice the detection limit.

Equivalently, when an analyte is reported as "not detected," or is reported as < DL, the analyte may be present at a concentration as high as two times the detection limit. Two times the detection limit will be referred to as the "reliable detection limit" and is recommended as the lowest "reporting limit" for non-

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detects. Note that, as the term is being used here, the "reporting limit" is the lowest concentration at which a result can be confidently reported as "not detected" and is not equal to the quantitation limit, which is the lowest concentration at which a quantitatively reliable result can be reported.

Proposed Verification

After the detection limit study is initially performed (e.g., after method development using the 40 *CFR* method), verify the detection limit using a "detection limit check sample." Spike a method blank at two to three times the detection limit and process it through the entire analytical method. If the analyte can be definitively detected (e.g., the visual observation of a "peak" greater than baseline noise), then the detection limit check sample has verified the detection limit. Unless the signal is much greater than baseline noise, it is recommended that this be done at least two times to demonstrate that the detection limit check sample can be consistently detected, or process a check sample at a slightly higher concentration.

The lowest reporting limit for non-detects is equal to the concentration of the detection limit check sample. If the analyte cannot be detected–for example, because of low analyte recovery or because the detection limit is erroneously too low–then process the check sample at a higher concentration (e.g., four times the detection limit) and repeat the process until the analyte is detected. Set the reporting limit for non-detects at the concentration of the check sample analysis that results in detection.

Note that if the analyte is not detected at twice the calculated detection limit, then the actual detection limit may be estimated to be equal to or less than about one-half of the concentration of the check sample that gives rise to a definitive detection.

Once an initial detection limit study is completed, an ongoing verification of the detection limit can be accomplished by including a detection limit check sample with routine analytical batches or by periodically verifying the detection limit. A new detection limit study is unnecessary as long as the analyte can be detected in the check sample. A check sample should be analyzed or a new detection limit study should be performed when there is a significant change in the analytical methodology, such as when a new gas chromatography column is installed. Note that the detection limit check sample takes both random and systematic error into account. For example, if the detection limit is too small or there is significant analyte loss during sample preparation, then the analyte will not be detected in the check sample.

The U.S. Army Corps of Engineers has been using this approach when contracting for laboratory analytical services for several years. A number of environmental testing laboratories have either implemented this strategy or have expressed approval for the overall approach. It is particularly cost-effective for multicomponent analytes such as Aroclors. For example, if method detection limits were required for all of the Aroclors in Method 8082 of SW-846, then a laboratory would need to process and analyze a set of seven replicates for each of the seven Aroclors listed in the method, a total of 49 analyses. (The number of replicates can be reduced by simultaneously spiking Aroclors such as 1016 and 1260 into a blank, but aside from this, each method blank for the MDL study would be spiked with only one type of Aroclor because of overlapping chromatographic peaks.) If a laboratory were to perform both water and soil analyses, then about 100 replicates would be needed. If multiple preparatory methods were performed for either the water or soil analyses (e.g., separatory funnel and continuous liquid-liquid extractions [LLE] for the aqueous analyses), then at least twice this number of analyses would be required–a market value of tens of thousands of dollars of analytical work. Using the strategy above, a total of about seven replicates would be required to verify the detection limits for each preparatory method.

Conclusion

Detection limit studies are time consuming and expensive, and can produce ambiguous or erroneous results such as those arising from low method bias. A practical strategy based upon empirical demonstration of detection capability using check samples is proposed. In particular, analytical capability (i.e., sensitivity) can be verified by routinely or periodically including check samples at the reliable detection limit (two times the detection limit) with batches of samples, avoiding the need to annually repeat method detection limit studies, which frequently entails a significant investment of analytical resources.

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