April 17, 2017

Dr. Michael Shapiro
Acting Assistant Administrator
Office of Water
U.S. Environmental Protection Agency
Ariel Rios Building (MD 4101M)
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

RE: Minimum Criteria for Selected Ion Monitoring (SIM) Methods

Dear Dr. Shapiro,

During the August 2014 face-to-face meeting, the Environmental Laboratory Advisory Board (ELAB or Board), a standing Federal Advisory Committee Act board that advises the U.S. Environmental Protection Agency (EPA or Agency), was asked by a public request to investigate issues related to selected ion monitoring (SIM) gas chromatography/mass spectrometry (GC/MS) methods. ELAB discussed this request and wrote to EPA's Forum on Environmental Measurements (FEM) on October 22, 2014, suggesting that there were several potential SIM issues regarding technology and quality control measures. You, as FEM Chair, responded to ELAB on February 18, 2015, indicating an extreme interest in pursuing this with ELAB.

ELAB voted to form a task group to formulate a list of minimum criteria for SIM GC/MS methods. During the course of several meetings, the task group gathered information on SIM criteria in existing EPA and other GC/MS methods, discussed the issue with several SIM experts, and formulated a proposed list of minimum SIM criteria. This list was presented for review to the full Board, which voted to submit the list to EPA for consideration.

ELAB appreciates the opportunity to provide this information to the Agency in support of setting minimum criteria for SIM methods that will achieve harmonization among similar EPA methods and be achievable by many laboratories performing analyses in support of EPA regulations.

The Board looks forward to working with EPA on this topic.

Respectfully,

Henry Leibovitz, Ph.D.
Chair, Environmental Laboratory Advisory Board

cc: ELAB members
Lara Phelps, ELAB Designated Federal Official

Attachment: *Minimum Criteria for Selected Ion Monitoring (SIM) Methods*

Minimum Criteria for Selected Ion Monitoring (SIM) Methods

Background/Assumptions. Traditionally, identification and quantitation of trace organic constituents in environmental samples using gas chromatography/mass spectrometry (GC/MS) is accomplished using full-scan mode, in which all masses are monitored in each scan. This facilitates obtaining full mass spectra for compound identification as each organic compound elutes from the GC column. The sensitivity of GC/MS analyses generally can be increased by using the selected ion monitoring (SIM) mode, in which a small number of masses are monitored for each target analyte. The masses that are monitored are carefully chosen to be significant contributors to the mass spectrum of the eluting target compounds. The set of masses is changed in timed sequence throughout the chromatographic run to correlate with the characteristic ions of the analytes, surrogate standards and internal standards as they elute from the chromatographic column. Sensitivity is increased because more time is spent measuring the masses present in the eluting compounds rather than measuring all masses.

Most full-scan GC/MS methods may be conducted in SIM mode. Further, certain MS systems are capable of running full scan and SIM at the same time. This document details the suggested minimum requirements for using SIM reliably. Without the benefit of a full mass spectrum, in SIM mode care must be taken to balance the chance of false negatives (not detecting a compound this is actually present) and false positives (detecting a compound that is actually absent). The minimum criteria presented here are considered a reasonable balance between false positives and false negatives because the criteria are tight enough to require fairly strong analytical signals without being so restrictive as to demand perfection.

These minimum criteria will likely need to be tailored to the specific data quality objectives (DQOs) of the project. SIM is primarily used to increase sensitivity. Qualitative identification is secondary. There is a tradeoff between excluding background interferences versus unambiguous identification. The judgment of experienced analysts should be allowed to include data qualifiers or narration when needed to annotate results.

Note that using SIM to push an analysis to the highest sensitivity on complex environmental samples will require a significant amount of experienced analyst judgment in applying these minimum criteria.

This document is focused on high-resolution GC combined with low-resolution MS. It is not intended to nor does it address high-resolution MS, tandem MS (MS-MS or MS-MS-MS), or high-performance liquid chromatography.

The Board has used the imperative “must” for requirements, “should” for recommendations, and “may” for optional alternatives.

Definitions. Terminology may vary among various instrument manufacturers and MS types. These differences should not preclude a laboratory from configuring a SIM method that meets these minimum criteria. A SIM profile, or acquisition profile, is a list of retention time windows

during the GC run. During each retention time window, the MS moves from mass to mass, acquiring the detector signal at each mass for a specified "dwell time," generally in milliseconds. "Scan descriptors" is the set of masses and dwell times. The "cycle time" is the amount of time it takes to cycle through the scan descriptors. An example of a minimum definition of scan descriptors is shown here:

GROUP	START TIME (min)	Selected Ions	DWELL TIME (millisecond)
1	14.00	188, 190, 222, 224	175
2	18.92	256, 258, 289.90, 291.90	175

The initial demonstration of capability (IDC) is performed by the laboratory to show that its instrumentation, personnel and implementation of the method meet the quality control (QC) requirements specified in the method.

Minimum SIM Criteria. This is a minimum set of SIM criteria that should be considered when operating a GC/MS in SIM mode.

a. **Personnel.** Configuration of the instrument—including acquisition parameters, dwell times and retention times; operation of the GC/MS instrument; and interpretation of the associated data—should be performed by analysts skilled in SIM. This should include at least 6 months of supervised experience running SIM analyses. Certain aspects of SIM analyses require analyst interpretation. The analysts who are going to perform the instrument analyses and process SIM data must perform the IDC specified in the method so that they can demonstrate their ability to correctly generate results in the matrices of interest, considering the possibility of matrix specific co-eluting concomitant ions. Specifically, when a SIM variation of a method is used, the laboratory should ensure that the IDC is performed using its SIM procedure.

b. **Method flexibility.** Method modifications that are precluded by the applicable regulation should not be made. In recognition of technological advances in analytical systems and techniques, however, the laboratory is permitted to modify the GC inlet, inlet conditions, column, injection parameters, and other GC and MS conditions, where these modifications are not precluded. Initial sample size and final extract volume may be altered as long as the target detection limits/reporting limits are met. Otherwise, changes may not be made to sample collection, preservation, sample preparation, or to the QC requirements. In all cases where method modifications are proposed, the analyst must perform the procedures outlined in the IDC and verify that all QC acceptance criteria in the method are met, including QC method performance for spiked samples.

c. **Type of MS.** Any type of MS can be used, provided it is capable of meeting the data quality requirements of the full-scan version of the method (e.g., quadrupole, ion trap, time of flight). When an ion trap MS is used, SIM mode is referred to as selected ion storage (SIS). For the purpose of this document, SIM and SIS are interchangeable.

d. **MS tuning criteria.** Use a consistent, reliable tuning procedure that can be detailed in the standard operating procedures. This may include maximizing the sensitivity for target analytes or using full-scan MS tuning criteria (e.g. BFB, DFTPP, PFTBA) from the method. Perform the tune check in full-scan mode if there is a need for SIM ion ratios to agree with full scan ion ratios or if the MS will alternate between full and SIM scans. Use the same tune criteria for all calibration, QC and sample analyses.

e. **Number of scans per peak.** Longer dwell times lead to better sensitivity but also longer cycle times resulting in fewer scans across each chromatographic peak. A minimum of five scans per chromatographic peak should be used, adjusting dwell times as required. This is considered sufficient to allow an accurate measurement of peak area. Alternating full and SIM scan modes may be used as long as all criteria in this document are met.

f. **Number of scan descriptors.** The use of a large number of descriptors with longer dwell times can limit scans per peak. A minimum of one quantitation and two qualifying ions for each analyte should be used, unless fewer than three ions with intensity greater than 30 percent of the base peak are available. Compounds with fewer than three ions greater than 30 percent of the base peak require lower abundance peaks to be present to confirm positive identifications. Consider using a high-mass abundant ion for quantitation because this should give better selectivity and/or sensitivity.

g. **SIM acquisition parameters.** The chromatogram may be divided into time windows, also known as segments or periods, with different acquisition parameters for each time window. This includes beginning and ending retention time of the window, masses to be monitored, dwell time for each mass (milliseconds), and scan time (seconds/scan). SIM time windows should be set to allow full baseline-to-baseline elution of peaks within the designated time window. Analyze a mid- to high-concentration standard in full scan mode. Select one primary quantitation ion (QI) and at least two confirmation ions. Verify that the QI ion is free from interferences resulting from an identical fragment ion in any overlapping peak(s). Selection of the QI should be based on the best compromise between the intensity of the signal for that ion and the likelihood and intensity of interferences. Interfering ions need to be considered as a major source of error when using SIM. The most intense ion might not be the best QI. Adjust the cycle time to measure at least five spectra during the elution of each GC peak.

h. **Sensitivity.** To demonstrate instrument sensitivity, all ions used for qualitative determination must be present at their expected relative abundances in the lowest calibration standard, and the quantitation ion must meet initial and continuing calibration criteria.

i. **Retention time windows.** Establish an appropriate (e.g., ±3 standard deviation) retention time window for each analyte, internal standard and surrogate analyte to identify them in QC and sample chromatograms. Base the retention time window on measurements of actual retention time variation for each compound in standard solutions collected over the course of time. The suggested variation is plus or minus three times the standard deviation of the retention time for each compound for at least five injections. The injections from the initial calibration and from the initial demonstration of capability may be used to calculate the size of the retention window. Retention time in the sample should match within 0.06 relative retention time (commonly known as RRT) units of an authentic standard analyzed under identical conditions. Matrix interferences can cause minor shifts in retention time and may be evident as shifts in the retention times of the internal standards. The experience of the analyst should weigh heavily on the determination of an appropriate retention window size.

j. **Identification/identification verification criteria (e.g., ion ratios).** To confirm identification, the chromatographic peaks for each ion should maximize within two scans of each other. Assessing this at low concentrations or for narrow peaks may require some analyst judgment. A confirmed detection needs a detectable peak for the quantitation ion and all identification ions. The ratio of each of the two qualifying ions to the quantitation ion must be evaluated and should agree with the ratio observed in an authentic standard within ±20 percent (absolute). For example, if the base peak is used for quantitation (100%), and the ratio of the secondary ion in the standard is 45 percent, then the ratio of this ion in the sample must be between 25 and 65 percent.

   Interfering ions need to be considered as a major source of error when using SIM. Analyst judgment must be applied to the evaluation of ion ratios for complex matrices because the ratios can be affected by co-eluting compounds present in the sample matrix. Analysts should be encouraged to use qualifiers or narration to distinguish between qualified and confirmed detections (i.e., near hits/near misses). Also, additional confirmations may be advisable depending on project DQOs, such as using peak shapes or additional confirmatory or deuterated standards.

k. **Automated peak detection.** Software for detection of peaks can be used to avoid manual peak detection and integration. Care is needed to make sure that integration parameters are set to successfully agree with analyst judgment.

l. **Other criteria that have not been mentioned.** Other GC/MS requirements in the method, which have not been mentioned here, however named or described, should be followed as detailed in the method. This includes, but may not be limited to, internal standards, surrogate standards, initial and continuing calibrations, laboratory control samples, matrix spike/matrix spike duplicate samples, minimum reporting limits/detection limits, and initial/ongoing QC requirements. QC spiking concentrations should be adjusted to be within the calibration ranges expected with the use of SIM.