



Setting up a SIM Acquisition Method MS ChemStation

Selected Ion Monitoring (SIM) allows the mass spectrometer to detect specific compounds with very high sensitivity. In SIM mode, the instrument is set to gather data at masses of interest, instead of stepping the mass filter over a wide range of masses.

Since the mass spectrometer collects data at only the masses of interest, it responds only to those compounds that possess the selected mass fragments. In essence, the instrument is focused on only the compounds of interest. Also, because only a few masses are monitored, much more time may be spent looking at these masses, with the attendant increase in sensitivity.

SIM also allows the collection of more points across a chromatographic peak, thus enhancing the accuracy and precision of quantitative results.

To achieve the potential benefits of SIM, careful attention must be paid to the acquisition parameters. A complete discussion of SIM setup is beyond the scope of this document, but it is covered in detail in the various GC-MSD Instrument and ChemStation Operation courses offered by Agilent Technologies.

The following outline includes the most important considerations in SIM Method setup

- ✓ Be certain the instrument is tuned and calibrated before gathering data and making decisions on Method setup.
- ✓ Select appropriate ions to monitor for each target compound.
- ✓ Group signals to be monitored into time programmed SIM groups to minimize the number of ions acquired at any one time.
- ✓ Select a dwell time for each group that yields 15-20 cycles across a peak.

Selecting appropriate ions to monitor

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It is common practice is to monitor 3 ions per compound. One ion signal is used to quantitate, and the others are used for qualitative information. Since, by definition, internal standards are present in every sample, analysts will frequently monitor only two ions for the internal standard--the quant ion and one qualifier.

Determination of the SIM ions is of utmost importance. Unless you are following a pre-determined Method, generally you will need a scan-mode spectrum of the compounds you plan to analyze by SIM. When examining the spectrum for candidate SIM ions, consider ions that are:

- Unique to the compound (not common in a wide range of compound spectra). If selecting a derivatized compound, do not select ions that represent only the portion of the molecule added by the derivatizing step.
- Higher in mass (usually more specific and separated from interference). Work by McLafferty has shown that ions of higher mass to charge are more unique.
- Abundant (easy detect at low concentration). If you chose an ion of very low abundance (albeit very unique), this compound may not be found at low concentrations.
- Characteristic of the compound class (in some cases). For example, if you are looking at aromatic hydrocarbons, the molecular ion is typically very abundant (possibly the base peak). This would be an excellent choice for a quant ion or a qualifier.

Once you have chosen the fragments to be monitored, it is important to determine the accurate mass (± 0.1 AMU) for the fragments you will monitor. The fragment labeled m/z 371 may be more accurately measured as 371.2 or 371.3. This deviation from whole numbers is due to the mass defect of certain elements, and the measured mass is called the **mass centroid**. For optimum sensitivity and stability, you should monitor the mass centroid in your SIM Method to ± 0.1 AMU.

There are two ways to determine the Mass Centroid:

The validity of these measurements depends on an accurately calibrated mass axis. Insure the mass axis is accurately calibrated before proceeding.

1. **Tabulate a full scan spectrum.** Prepare a standard sample of sufficient concentration to obtain scan spectra of the compounds of interest. Inject the standard sample, then tabulate the mass spectrum for several data points across the apex of the peak in the resulting data file. (Select **Spectrum ... Tabulate**). Use

the m/z value shown in the tabulation (to the nearest 0.1 AMU) for the SIM acquisition mass. If necessary, take the mathematical average of several scans across the apex to the nearest 0.1 AMU.

2. **Perform a dynamic SIM calibration.** Set up a SIM acquisition for several incremental masses around the ion of interest. For example, if you are looking for the 76 and 91 ions in toluene, do a SIM acquisition looking for the 75.9, 76.0, 76.1, 76.2, 90.9, 91.0, 91.1 and 91.2 ions. Evaluate the mass spectrum and use the strongest ion for each of these fragments in your SIM Method.

Of these Methods, the dynamic SIM calibration should be the most accurate, the scan tabulation may be somewhat easier in many cases.

Grouping ions into SIM groups

To gain the benefits of SIM, one must limit the number of ions being monitored at any one time. Although the ChemStation allows 50 simultaneous ions, monitoring more than 10-15 ions at the same time defeats the purpose of SIM and should be avoided.

Most SIM analyses monitor 3 to 8 ions simultaneously in a given time period or range. If more ions are needed to do your analysis, try to separate them into SIM groups.

A SIM group is a group of simultaneously monitored ions. Only one group can be monitored at a given time, and the start time for group 2 becomes the end time for group 1. Using SIM groups, a Method can monitor the important ions for a list of compounds by switching from one group to another at the appropriate time during the chromatographic run. You would only look at the ions of interest, in the time range of interest.

For additional details on SIM parameters, including groups and group start time, consult the on-line help by selecting the **Help** button on the **MS SIM/Scan Parameters** window.

Determining an appropriate dwell time

Once the fragments are chosen, the mass centroids are determined, and the ions grouped into SIM groups, you will need to set the dwell time for each group.

If your groups contain 2-5 ions, try 50 sec as a first value for dwell time. To determine what adjustment needs to be made in the dwell time setting, you will need to run the Method once and examine the resulting data.

The central goal in adjusting the dwell time is to optimize cycle time to get 15 to 20 points or scans across your chromatographic peak(s). We will evaluate the data acquired with the dwell time set to 50 msec, and determine what adjustment, if any, is required.

1. Load the data file acquired with your 50 msec Method.

2. Determine the number of SIM cycles across your peak as follows:
 - Use the mouse to select a spectrum from the start of the peak (where the signal leaves the baseline).
 - Note and record the scan number of this spectrum (listed in the header info at the top of the spectrum display).
 - Repeat for the end of the peak (where the signal approaches the base line)
 - The difference in these numbers is the number of SIM cycles over the peak.
3. If the number of cycles is less than 10, the dwell time needs to be reduced. If more than 25, increase the dwell time. Your goal should be about 15-20 points across most peaks.

There are subtleties and intricacies in the set up of a proper SIM Method. The GC-MSD and ChemStation Operation courses offered by Agilent provides an excellent way to gain knowledge and experience in this area. For further information on the availability and scheduling of these courses, visit the Agilent web site at www.chem.agilent.com, and select **Education.**