

Collecting MS Data for use with MassWorks™

Data Acquisition for MassWorks

Since MassWorks performs a comprehensive calibration on mass spectral data both in terms of m/z and peak shape functions, MS scan data needs to be in an unprocessed form as much as possible. Specifics include:

1. Ion threshold, where applicable, should be set to zero or as low as allowable.
2. Any mass spectral domain or chromatographic domain filtering (such as Chrom filters) should be turned off or with the parameters set to levels where no or minimal processing is performed on the data stream. For example,
 - a. In the case of box-car averaging, set the window size to 1.
 - b. In the case of Savitzky-Golay filtering, set the order of polynomial to be one less than the number of data points involved.
3. The mass spectral scan data should be acquired in the continuous or profile mode with enough number of points sampled across a mass spectral peak. Typically, a minimum of at least 5 points need to be samples across the FWHM (Full Width at Half Maximum) of a monoisotopic peak. For example, for a unit mass resolution instrument with FWHM = 0.50Da, a sampling interval of less than 0.1Da is needed.
4. While centroid data are typically acquired for routine operation of a mass spectrometer, this type of data is not amenable for MassWorks processing, as all important information has been lost in the system's centroiding process.
5. On some systems, due to the limitations on the detector or electronics associated with it, the mass spectral response may saturate at higher end of concentration levels. This will cause systematic error for MassWorks calibration and should be avoided by either working with a lower concentration sample or with the rising/declining part of a chromatographic peak where applicable.

Acquiring Calibration Data

There are two types of calibration data that could be acquired:

1. External calibration data with a set of known ions covering the mass range of interest. For GC/MS systems equipped with an on-board calibration mixture, such as perfluorotributylamine, one can acquire repeated mass spectral scans of this calibration mixture for about 5-10min. The settings for mass spectral scans, such as mass range, scan rate, settle time after each scan etc, need to be the same as those for GC/MS or LC/MS. Other parameters such as the mass spectral tuning parameters should also be kept the same as the GC/MS or LC/MS runs. For LC/MS, one can acquire the calibration data through continuous infusion or loop injection of either poly-alanine or a drug mixture. Multiple (5-10) loop injections are preferred over infusion as it involves the same mobile phases as those for LC/MS runs. Cerno Bioscience has found the following drug mixture work well for small molecule applications:

Compound Name	Exact Mass (m/z)
Erythromycin	734.4685
Reserpine	609.2806
Tyr-Tyr-Tyr	508.2084
Loperamide	477.2309
Terfenadine	472.3216
Buspirone	386.2556
Promethazine	285.1425
Acetaminphen	152.0712

2. Internal calibration through one or few known ions. The internal standard can be either physically introduced through post-column infusion/dual spraying or naturally occurring as is the case for drug metabolism study where the parent drug can serve as the sole internal calibration ion.

Internal standard, though optional, is required to achieve the highest possible mass accuracy on systems that show significant short term variations.

Acquiring GC/MS or LC/MS Data

Under the same instrument tuning and mass spectral scanning conditions, perform the profile mode data acquisition with no or minimal data preprocessing or on-board filtering (see section above on Data Acquisition for MassWorks).

It should be noted that the mass standard error is inversely proportional to the square root of the ion signals. Therefore, a higher ion signal (when attainable) will always help with the mass accuracy.