Recommended First Experiment for LC and GC Systems

It is **highly** recommended that when evaluating MassWorks™ for use with your instrument(s), the first experiment be simple and well controlled. While more complex experiments can be done later on, performing a simple infusion experiment can be done quickly and easily and will reveal the potential of using MassWorks with your instruments(s).

The Design of an Infusion Experiment for LC Systems

A simple mixture of a pure analyte compound for analysis and 1 or 2 pure standards should be made at concentrations levels of about 10-20 µM that give good mass spectral signal-to-noise for both the analyte and the calibration standard ion(s). Serving as a starting point, these levels of concentrations should be adjusted based on the nature of compounds and instrument conditions. The desired signal to noise for the mass spectral response of both the analyte and standard ions is 100:1 or higher after necessary averaging. It is important that the standards are no more than 50Da away from the sample. Ideally, the two standards should bracket the analyte. It is also important that both the analyte and the standard be free of any interference. If you are submitting your data to Cerno for purposes of evaluation, it is fine to submit the data as a blind test by simply leaving out the identity of the analyte compound and supplying the elemental composition(s) of just the standard ion(s).
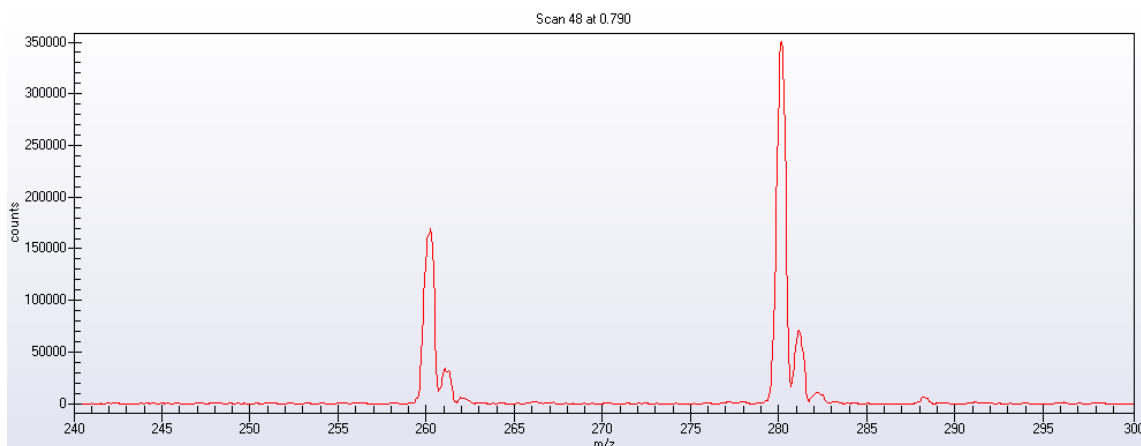

Figure 1. A single MS scan from a typical infusion experiment with the analyte (260Da) and a single calibration standard (280Da). Note that the spectrum is clean and free from any interferences and the standard ion is about 20Da from the analyte. While no special mass spectral tuning is required, it is highly desirable for this version of MassWorks to have clearly observable M+1 peak next to its monoisotopic counterpart as shown in the above example.

The Design of an External Calibration Experiment for GC Systems

While an infusion-like experiment can be run with GC/MS systems, it is easiest to use the standard calibration samples common in GC/MS run as an external standard. Many

GC/MS systems have a build in calibration gas which can be easily introduce into the systems for calibration. The commonly used PFTBA (PC-43, $(CF_3(CF_2)_3)_3N)$) provides a usable mass range for calibration of about 50-500Da for EI and 200-700Da for CI.

First run the calibration standard using following the guidelines below in "Acquiring the Data" and run the system for 1-2 minutes while introducing the calibration standard. Immediately after flushing the system of any calibrant, run the analyte using your normal GC methods.

Acquiring the Data

First, and foremost, the data must be acquired in Profile (also sometimes called continuum, scan or raw) mode.
MASSWORKS CANNOT CALIBRATE USING CENTROID DATA!
In addition, it is important to set the Ion Threshold to zero or to as small a value as possible. The acquisition rate (also called step size or interval) should be set to achieve at least 5 samples across the monoisotopic peak (FWHM). For example, for a typical quadrupole instrument with FWHM=0.5Da, the sampling interval should be set to 0.1Da or slightly less. Any filter parameters should be set to apply the minimum amount of filtering. An example of instrument settings for an Agilent LC MSD is shown below.
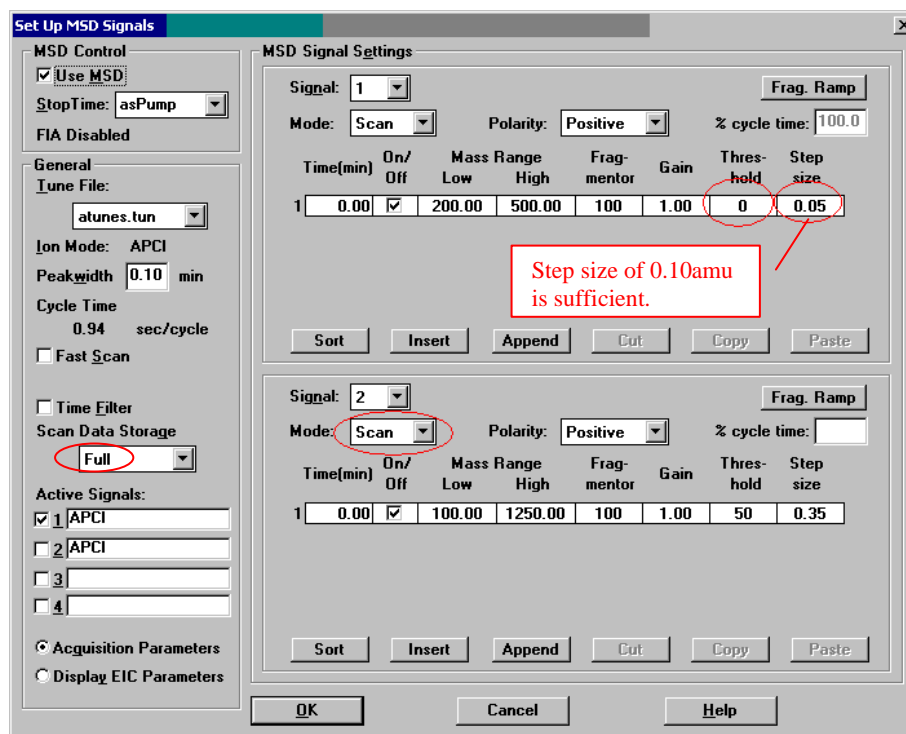

Figure 2. Typical parameter settings for the Agilent LC MSD for collecting data for MassWorks. Note that the Threshold is set to zero, this is critical for Agilent or else key data points will be dropped. Also note the step size for appropriate peak sampling and the Mode is set to Scan to acquire data in profile mode. Note the Scan Data Storage is set to Full to have full MS scan data available for MassWorks' post processing.

Infuse the sample and adjust ionization conditions to obtain stable signals, and then acquire the data for at least 1 to 2 minutes or otherwise to achieve 100:1 signal to noise in the averaged mass spec. This may either involve a good number of quality scans to be acquired and averaged for analysis. Once the data has been acquired, inspect the data for any signs of detector saturation in either the analyte or standard signals. This is easily identified by inspecting the ratio of the M and M+1 peaks. If the relative abundance of M+1 peak is larger than its theoretically expected value, you likely are saturating the detector. Also, a flat top or inverted peak on the monoisotopic peak is an indication of saturation.

IF THE SIGNAL IS SATURATED, POOR RESULTS WILL BE OBTAINED!

Dilute the sample if necessary and repeat the run until no saturation is observable. As a precaution, it is always a good idea to perform the infusion experiment at different concentration levels by diluting the sample. Cerno can then inspect the run and use the one that exhibits good signal-to-noise without exhibiting saturation.
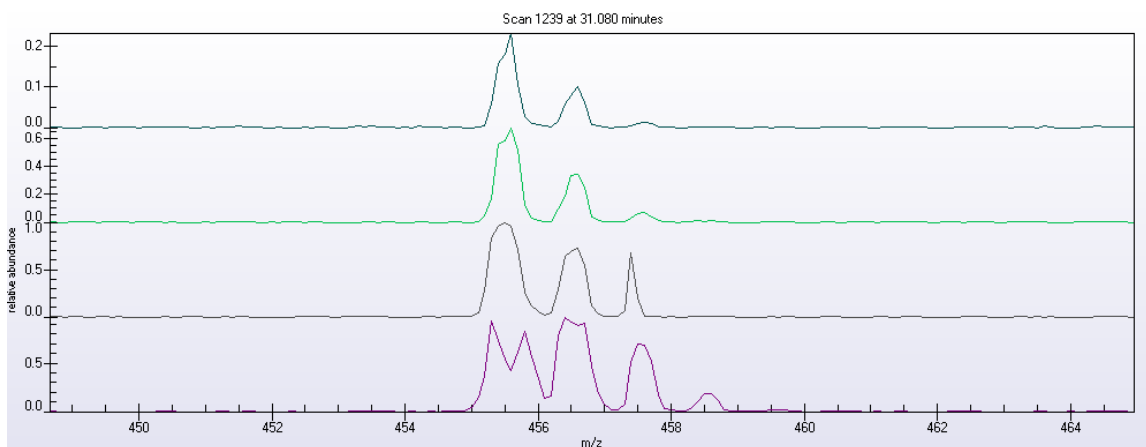

Figure 3 A signal shown with no saturation to extreme saturation. From top to bottom, no saturation, moderate saturation, high saturation, extreme saturation.

A Note on Ion Traps

Unlike quadrupoles and TOF instruments, the ability to attain high mass accuracy is fundamentally limited on some Ion Trap instruments. Despite the comprehensive calibration of MassWorks, space charge effects typically limit the achievable mass accuracy to about 30 to 80 mDa on the classical ion traps, e.g., those from Thermo. The accuracy is a function of the trap geometry and the ion concentration and chemical composition in the trap. Newer trap designs with larger volumes are designed to alleviate this problem somewhat but still perform almost a factor of 10 worse than typical quadrupole designs. On linear traps with large internal volumes such as Q TRAP from ABI/Sciex, the space charge effect is in better control, allowing for smaller intrinsic mass error of less than 10-20mDa with some care in experimentation. Another approach to control the intrinsic mass errors associated with ion traps is to avoid infusion experiments where all ions are trapped at the same time, and instead perform well separated LC/MS experiments where different ions are trapped at different retention time points.

Despite the space charge limitation, Cerno has found that the elemental composition of unknowns can still be attained in many cases despite the poor mass accuracy. This is accomplished by using the calibrated lineshape that MassWorks provides to match the isotope profile of the unknown. The calibrated spectrum provides a well defined isotope profile which is a distinctive signature for a given composition. This approach, called CLIPS for <u>C</u>alibrated <u>L</u>ineshape <u>I</u>sotope <u>P</u>rofile <u>S</u>earch, has shown good results in situations where mass accuracy is limited. Naturally, it is also applicable to cases where high mass accuracy is attainable to further enhance the selectivity in elemental composition determination to, for example, arrive at **<u>unique</u>** formula through the use of qTOF or higher resolution systems.

Uploading the Data for Evaluation

Once the data has been acquired, contact Cerno and we will provide information on uploading your data for evaluation. In general, due to the file size, it is necessary to upload via FTP. However, many companies block uploading to FTP sites. If the files are small enough, you can send them as an email attachment. Alternatively, you can try to upload them from a location outside of your company's computer firewalls. For some instruments, (Agilent and Waters) you need to send the entire experiment folder. For some ABI Sciex experiments, you only need to sent the *.wiff file and the *.wiff.scan file (if it exists). For Thermo files, only the *.raw file is required. If you have run a GC experiment with an external calibration, be sure to include both the calibration run and the analyte run. The easiest way to send multiple files is to zip them into a single compressed file. You can do this in Windows XP by selecting the files or folder, right clicking the mouse, and selecting "Send to compressed (zipped) folder". Attach the resulting *.zip file to your email or upload to the Cerno assigned FTP location.

Along with the data, please provide the following information:

- The elemental composition for the standard ion(s)
- The nominal mass(es) of the analyte(s) for which high mass accuracy is sought and/or elemental compositions are to be determined
- The make and model of the instrument

If any additional information is needed, we will contact you.