

## MSMS Methods from MW Lists

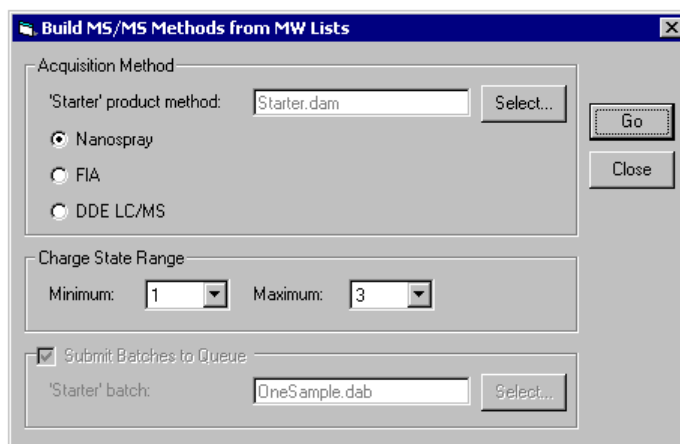
This section describes the operation of a program for using lists of molecular weights obtained from text files as the basis for creating a series of MS/MS acquisition methods. One acquisition method is created for each file containing a mass list.

The intention is that, for example, a series of protein digests are analysed using a MALDI-TOF instrument. For those samples for which the resulting list of peptide molecular weights is insufficient to locate the protein in a database, the next step is to obtain a series of MS/MS spectra using the QStar. Given each list of molecular weights obtained from the MALDI experiments, the program automatically creates the necessary acquisition method. Depending on the options you select, the acquisition method may be for use with nanospray or FIA, in which case the method is set-up to acquire a product spectrum for each of the molecular weights specified. Alternatively, the acquisition method may be set-up to perform LC/MS using the list of molecular weights as an inclusion list for a data dependent experiment (DDE).

### Operation

To use the program, select it from Analyst's 'Script' menu – the dialog shown in the figure below is presented. The program requires that you specify a 'starter' acquisition method which is used as a template for building the output acquisition methods, the type of experiment which you wish to perform, and the charge state range to consider. The program also allows you to optionally submit each sample to the Analyst queue for subsequent acquisition. These options are explained in detail below.

Once you have filled-in the dialog, click the 'Go' button to complete the process. As also described below, the program will ask you to select a directory containing the molecular weight lists. All 'txt' files contained in this directory are assumed to contain valid lists and are processed accordingly. In addition to the list of molecular weights, these files may also contain certain sample-related information which is used if you are submitting batches to the queue – for a detailed description of these files, see the 'Format of the Molecular Weight Lists' section below.



Build MS/MS Methods from MW Lists

The dialog contains the following items:

*'Starter' product method: Select* – You must specify a 'starter' acquisition method which is used as a template for constructing each output MS/MS acquisition method. All MS parameters, with the sole exception of the actual precursor m/z values, are taken from this method. This 'starter' method also optionally defines the methods for external devices such as LC pumps and autosamplers which, if present, are used unchanged. The exact format of this method depends on whether or not you are using the 'DDE LC/MS' option – details are given in the next few paragraphs below.

*Nanospray* – Select this option if you wish to create MS/MS acquisition methods compatible with nanospray. In this case, the ‘starter’ acquisition method must define a single period containing one or more ‘Product’ experiments. The output method created by the program contains clones of this period, one for each precursor m/z value. If, for example, 10 m/z values are used, the total duration of the output method will be 10 times longer than the ‘starter’ method. By selecting a ‘starter’ method which contains more than one ‘Product’ experiment, you can acquire product spectra for each m/z value using different instrument parameters, most likely different collision energies.

*FIA* – Select this option if you wish to create MS/MS acquisition methods compatible with flow injection analysis. As far as the format of the output acquisition methods is concerned, this option is identical to the ‘Nanospray’ option with the exception of the timing of the method. In this case, the total duration of the ‘starter’ method is preserved. For example if the one-and-only period in the ‘starter’ method has a duration of 120 sec and 10 periods are created (*i.e.* 10 different m/z precursor values), each period will have a duration of 12 sec.

*DDE LC/MS* – Select this option if you wish to create LC/MS methods which take advantage of the inclusion list feature of data dependent experiments. This will acquire product spectra of the m/z values of interest, provided that they are actually present in the data. In this case, the ‘starter’ acquisition method must define a valid DDE method – see the *Analyst User Guide* for details as to how this is done. In addition, all of the default DDE Criteria specified using the ‘DDE Selection Criteria’ script are used – again, please see the *User Guide*. The program will add the required m/z values to the inclusion list for each method, leaving all other parameters unchanged.

*Charge State Range: Minimum and Maximum* – Since you supply the program with molecular weight lists, most likely obtained from MALDI experiments, you need to tell the program which charge states it should consider when building the API acquisition method. Many peptides will exist predominantly in the +2 charge state for an API experiment, however depending on a number of factors other charge states are also expected. The program assumes that the charging agent is always hydrogen: if the ‘starter’ acquisition method defines a positive mode experiment, the program assumes that one hydrogen atom is gained for each charge, otherwise that one hydrogen is lost for each charge.

When building a ‘FIA’ or ‘DDE LC/MS’ experiment, the program creates one period in the output acquisition method for each charge state for each molecular weight. When building a ‘DDE LC/MS’ acquisition method, the program adds each of these m/z values to the inclusion list. Using the data shown in the figure above, if the text file specifies a molecular weight of 400 amu, m/z values of 401, 201 and 134.33 are used (assuming a positive mode experiment).

*Submit Batches to Queue* – Select this option if you wish to automatically submit each sample to the Analyst queue for acquisition. Each sample uses the corresponding acquisition method which was created for it. Note that in order for the samples submitted to the queue to be actually acquired, you must ensure that the queue is ‘Ready’ and ‘Started’; you can do this either before or after running this utility program. This option only makes sense if you are using an autosampler with either the ‘FIA’ or ‘DDE LC/MS’ option.

*‘Starter’ batch: Select* – If you have selected the ‘Submit Batches to Queue’ option, you must specify a ‘starter’ acquisition batch which defines exactly one sample. This batch is created and saved using Analyst’s ‘Batch Editor’ in the usual way. Several of the fields defined by the batch such as the ‘Sample Name’, the ‘Data File’ name and optionally the position of the sample in the autosampler are overridden by the program as defined in the ‘Format of Mass List File’ section below. The main reason why you must specify this ‘starter’ batch is that it defines the autosampler type, rack and plate types, *etc.*

*Go* – Click this button to start building the acquisition methods. The program asks you to select the directory containing the mass list files. All files contained in this directory with the ‘txt’ file extension are processed; other files in this directory are ignored. The exact format of these text files is described in the next section.

*Close* – Click this button to dismiss the dialog, either to cancel the operation or after the acquisition methods have been created.

### **Format of the Molecular Weight Lists**

The section describes the exact format of the text file for specifying the list of molecular weights and other optional information. The molecular weights are specified one per line (separated by an end-of-line character); it is important to note that the program expects molecular weights to be specified, *not* MH masses.

If you are using the 'Submit Batches to Queue' option, you may specify additional information in the text file to define the name of the sample, the name of the output data file to acquire, its position in the autosampler, *etc.* In this case, the corresponding line of the text file should consist of an identifier (for example 'Sample Name' *without* the quotation marks), followed by a tab character, followed by the actual value, finally followed by an end-of-line character. The table below lists the various identifiers which may be used along with their descriptions. Note that all of these fields are optional and that reasonable defaults are used if a value is not specified, *except* that the 'Vial Position' must be specified if you are using the 'Submit Batches to Queue' option.

Identifier	Description
Sample Name	The name of the sample. If not specified, this field defaults to the name of the mass list text file (without the 'txt' extension).
Sample ID	An identifier for the sample. If not specified, this field defaults to the name of the mass list text file (without the 'txt' extension).
Data File	The name of the data file to create. The file is always created in the 'Data' directory of the project associated with the starter batch. If not specified, this field defaults to the name of the mass list text file (with the 'txt' extension removed and a 'wiff' extension added).
Rack Position	For multi-rack autosamplers only, this field specifies the rack number which contains the sample. If not specified, the value from the starter batch is used directly.
Plate Position	For multi-plate autosamplers only, this field specifies the plate number which contains the sample. If not specified, the value from the starter batch is used directly.
Vial Position	This field specifies the position of the sample in the autosampler. This field is required if you are submitting samples to the Analyst queue.

The following is an example of a mass list text file. The positions of the tab and end-of-line characters are explicitly shown.

Sample Name	tab	MySample	end-of-line
Data File	tab	MyDataFile	end-of-line
Vial Position	tab	5	end-of-line
423.2343	end-of-line		
819.1345	end-of-line		
1022.3765	end-of-line		