

Improvements to the Deconvolution Algorithms in MassHunter Qualitative Analysis rev. B.03.01 Service Pack 3 (SP3)

The new Maximum Entropy algorithm in MassHunter Qualitative Analysis rev. B.03.01 SP3 (Build 346.14) made improvements in deconvolution performance and speed. These have particular applicability when used with data from the 6538/40 UHD Accurate Mass LC/MS Q-TOF models.

How to use Maximum Entropy for high-resolution data

Deconvolute (MS): Maximum Entropy

For high-resolution instruments (G6540A, G6538A) data, if you want to see the isotope resolved deconvoluted spectra for small proteins < 30 kDa, set “Mass step” to be 0.1 Da as the following:

Method Editor: Deconvolute (MS): Maximum Entropy

Deconvolution Results Advanced

Mass range: 5000.00-30000.00 Daltons

Mass step: 0.1000 Daltons

S/N threshold: 30.0

☐ Use limited m/z range

600.0000-5000.0000 m/z

Adduct: Proton

Average mass: 50 % peak height

Isotope width: Automatic 20.0000 Daltons

Compound filters

Minimum consecutive charge states: 5

Minimum protein fit score: 8

The average mass of the deconvoluted protein is determined and labeled with a red diamond as shown in the figure below. The average mass is also listed in the compound table and is used to match the theoretical value of the target protein.

Maximum Entropy deconvolution result for high-resolution data

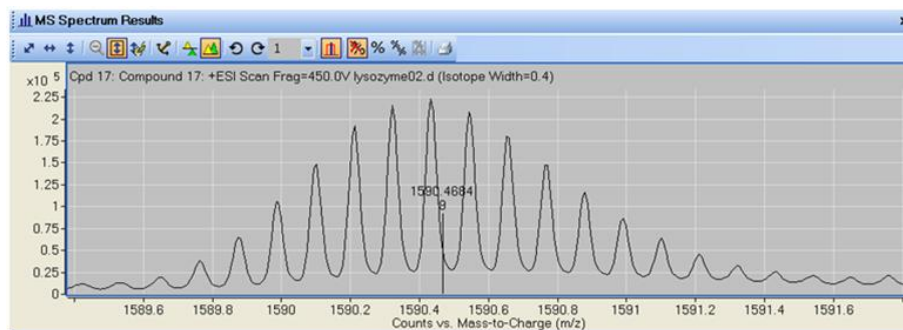


Figure 1. Raw spectrum overlaid with ion sets; 1590.4684 is charge 9 ion's average MW.

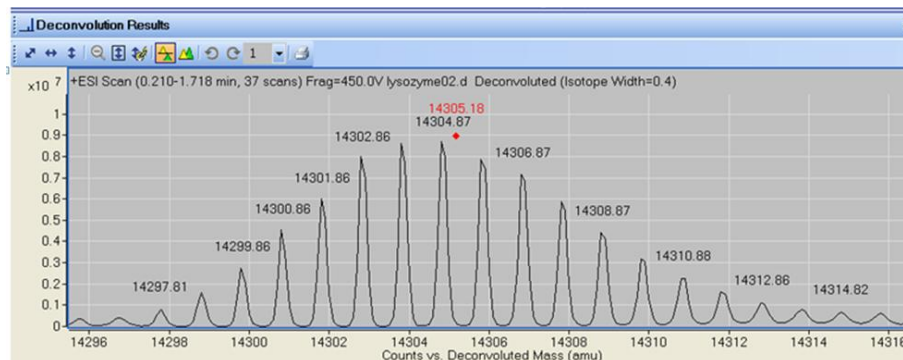


Figure 2. Deconvoluted spectrum—the red label with diamond represents isotope cluster's average MW; Peaks with black labels are isotopes.

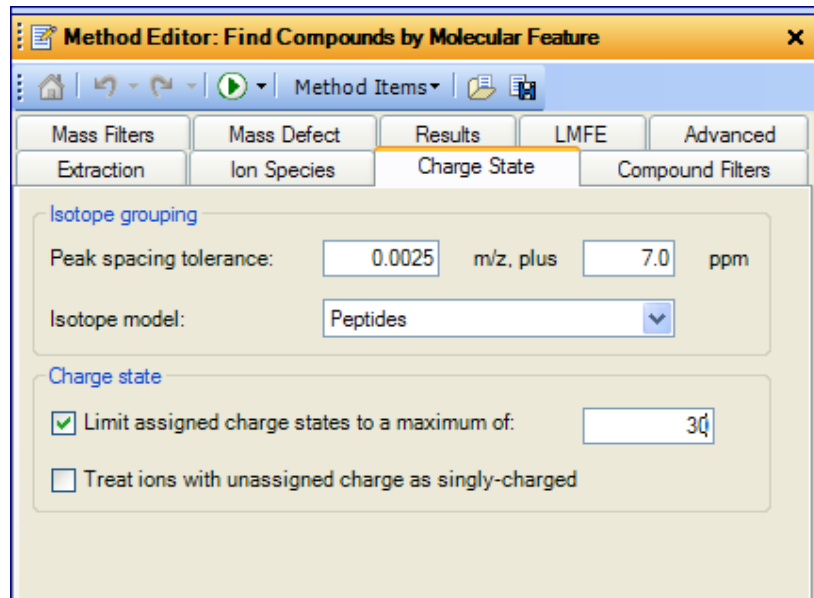
Compound List											
Show/Hide	File	RT	Mass	Fit Score	Min Z	Max Z	Vol	Height	Area	Hits (
<input checked="" type="checkbox"/>	lysozyme02.d		14305.1768	10	5	13		2181269			
<input checked="" type="checkbox"/>	lysozyme02.d		14367.7479	9	5	11		632479			
<input checked="" type="checkbox"/>	lysozyme02.d		14287.4368	10	5	30		413184			
<input checked="" type="checkbox"/>	lysozyme02.d		14322.5368	9	5	13		307133			

Figure 3. Compound table—displays protein average MW.

MFE and LMFE

Find Compounds by Molecular Feature

For high-resolution instruments (G6540A, G6538A) data, if you want to obtain the monoisotopic mass for the isotope resolved small proteins < 20 kDa, set maximum charge state to be 30 as the following:



The screenshot shows the 'Method Editor: Find Compounds by Molecular Feature' dialog box. The 'Charge State' tab is selected. Under the 'Isotope grouping' section, 'Peak spacing tolerance' is set to 0.0025 m/z, plus 7.0 ppm, and 'Isotope model' is set to Peptides. Under the 'Charge state' section, the checkbox 'Limit assigned charge states to a maximum of:' is checked, and the value 30 is entered in the adjacent text box. The checkbox 'Treat ions with unassigned charge as singly-charged' is unchecked.

Section	Parameter	Value
Isotope grouping	Peak spacing tolerance	0.0025 m/z, plus 7.0 ppm
	Isotope model	Peptides
Charge state	Limit assigned charge states to a maximum of:	30
	Treat ions with unassigned charge as singly-charged	Unchecked

For high-resolution instruments (G6540A, G6538A) data, if you want to use LMFE to obtain the average mass for the proteins, set “Smooth peaks” in Spectrum filtering to be 0.7 times peak width as following:

The screenshot shows the 'Method Editor: Find Compounds by Molecular Feature' dialog box. The 'LMFE' tab is selected. Under 'Spectrum filtering', the 'Smooth peaks' checkbox is checked and set to 0.700 times peak width. Other settings include 'Typical RT peak width' at 0.200, 'Smoothing' at 0.200 times peak width, 'Remove baseline' at 10.000 times peak width, 'Remove spikes' at 0.250 times peak width, and 'Remove wide peaks' at 10.000 times peak width.

Category	Option	Value	Unit
Chromatogram filtering	Typical RT peak width	0.200	
	Smoothing	0.200	times peak width
Spectrum filtering	Smooth peaks	0.700	times peak width
	Remove baseline	10.000	times peak width
	Remove spikes	0.250	times peak width
	Remove wide peaks	10.000	times peak width

Resolved Isotope

Deconvolute: Resolved Isotope

For high-resolution instruments (G6540A, G6538A) data, if you want to use Resolved Isotope algorithm for isotope resolved small proteins < 20k Da, set the maximum charge state to be 30 as the following:

The screenshot shows the 'Method Editor: Deconvolute: Resolved Isotope' dialog box. The 'Charge State' tab is selected. Under 'Isotope grouping', 'Peak spacing tolerance' is set to 0.0025 m/z, plus 7.0 ppm, and 'Isotope model' is set to 'Peptides'. Under 'Charge state', 'Limit assigned charge states to a maximum of' is set to 30, and 'Treat ions with unassigned charge as singly-charged' is checked.

Category	Option	Value	Unit
Isotope grouping	Peak spacing tolerance	0.0025	m/z, plus 7.0 ppm
	Isotope model	Peptides	
Charge state	Limit assigned charge states to a maximum of	30	
	Treat ions with unassigned charge as singly-charged	<input checked="" type="checkbox"/>	

