

PeakTrak[®] Ion Finder

keywords: Ion Finder, PeakTrak, mass spectrometry

Abstract

The PeakTrak Ion Finder makes it easy to find ions associated with the compound you want to purify and to add them to the signals to be detected by PeakTrak. Many compounds are detected as fragments, adducts, and multiply charged ions. PeakTrak automatically searches the mass spectrum for possible ions based on the molecular weight of the compound. As the Ion Finder has no knowledge of the compound, some of the suggested ions will not be associated with the compound to be purified.

How does it work?

After entering a molecular weight, PeakTrak looks for masses in the spectrum that match loss of various fragments and additions of various solvent compounds. The software also searches for multimers, solvent adducts, and charge carriers that replace protons such as sodium and potassium. The program also identifies any possible multiply charged ions. The Ion Finder can use live data from the **MS Method Development** window or from previously saved data.

Fragments, adducts, and multimers

After being charged, ions minimize their energy in many ways. The ions can rearrange by losing neutral fragments such as water, carbon dioxide, or ammonia. Molecules can also lose labile groups once ionized; this is common with protecting groups (like BOC and others).

Another way to stabilize a charged species is to “share the charge” with a solvent molecule (from the dissolution solvent, MS-carrier solvent, or even from the chromatography elution solvent) to make an adduct or with another molecule to make a dimer or a larger multimer. Molecules, particularly in positive ionization, may be charged by another ion than a proton. Sodium and potassium are commonly detected. These ions may come from glassware or sample work-up—from salting-out an extraction, for example.

Multimers can be minimized by injecting samples with a low concentration. For small molecules (smaller than ~600 Da), a concentration of 1 mg dissolved in 20 mL is appropriate. For a reaction mixture, this is about 1 drop in a 20 mL scintillation vial filled to its shoulder.

Fragments (loss of group)	Calculation
Water	$M+H-H_2O$
Ammonia	$M+H-NH_3$
BOC protecting group	$M+H-BOC$
Carbon dioxide	$M-H-CO_2$
Adducts (addition of molecule)	Calculation
Methanol	$M+H+MeOH$
Acetonitrile	$M+H+ACN$
Water	$M+H+H_2O$
DMSO	$M+H+DMSO$
Multimer	Calculation
Dimer	$2M+H$
Dimer	$2M-H$
Charge carriers (adducts)	Calculation
H	$M+H$
Loss of H	$M-H$
Na	$M+Na$
K	$M+K$
Ammonia	$M+H+NH_3$

Table 1. Partial list of fragments and adducts listed in the PeakTrak Ion Finder.

To use the Ion Finder, inject the sample from the **MS Method Development** window, then click the **Ion Finder** button to open the **Ion Finder** window. Enter the molecular mass of your target compound, and the Ion Finder will display any detected ions that match the data in Table 1.

Click the button next to the ion to add it to the ions to be detected. Figure A shows an injection of salicylic acid listing both the (M-H) and (M-H-CO₂) ions.

Ion finder limitations

Because the Ion Finder has no chemical knowledge, some of the results will be impossible for your compound or only applicable to the injection or carrier solvent. For example, a sample dissolved in water may show a water adduct during direct injection that will not be present if run on a silica column eluted with hexane and ethyl acetate with a methanol carrier solvent. While running reverse phase with an acidic solvent modifier, a compound injected as a sodium salt may be converted to the acid form.

The Ion Finder only acts on the ten most intense peaks in the display. Depending on the impurities, some of the desired compounds' mass peaks may not be noted by the Ion Finder. Rescaling the mass scale can eliminate unwanted intense peaks and allow detection of the desired compound.

Changing the **Ion Settings** in the MS Method Development screen will change the ions seen. Remember that the **Ion Settings** choice does not transfer to the detection options for the mass spectrometer in the **Method Editor**—the ion settings that gave a good result in the **MS Method Development** window needs to be chosen!

Compounds outside the mass range and their multiply-charged species

Although the mass range of the Purlon only goes to 2000 m/z for the L model (1200 m/z for the S), compounds (like peptides and others) with molecular mass greater than this can still be detected by looking at their multiply-charged species. Multiply charged ions tend to uniquely identify a compound. Multiply charged ions are identified with the equation

$$\frac{(M + nH)}{n}$$

where n is the number of protons added during the ionization process. Due to Coulombic repulsion, there are generally at least three residues between charges.ⁱ The Ion Finder will consider ions within ± 2 Da of the calculated multiply charged ion to be a valid match from the entered ion because the results depend on the entered molecular weight, which depends on assumptions about the isotopic distribution used to calculate the molecular weight of the compound.

ⁱ Douglass, K.A.; Andre R. Venter, A.R. Predicting the Highest Intensity Ion in Multiple Charging Envelopes Observed for Denatured Proteins during Electrospray Ionization Mass Spectrometry by Inspection of the Amino Acid Sequence. *Anal. Chem.* **2013**, 85, 8212–8218

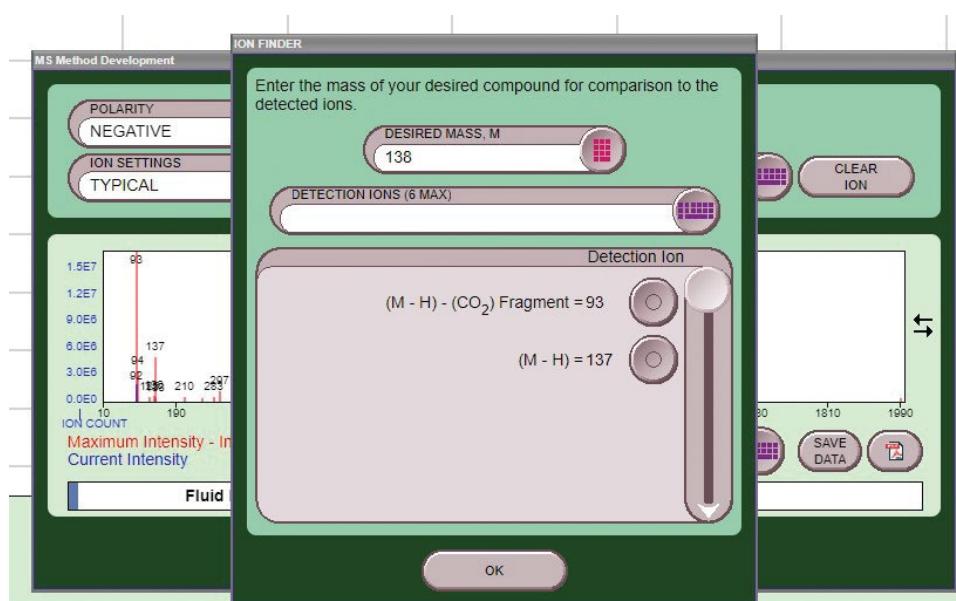


Figure 1. Ion Finder of salicylic acid with negative ion mass spectrum in the background.

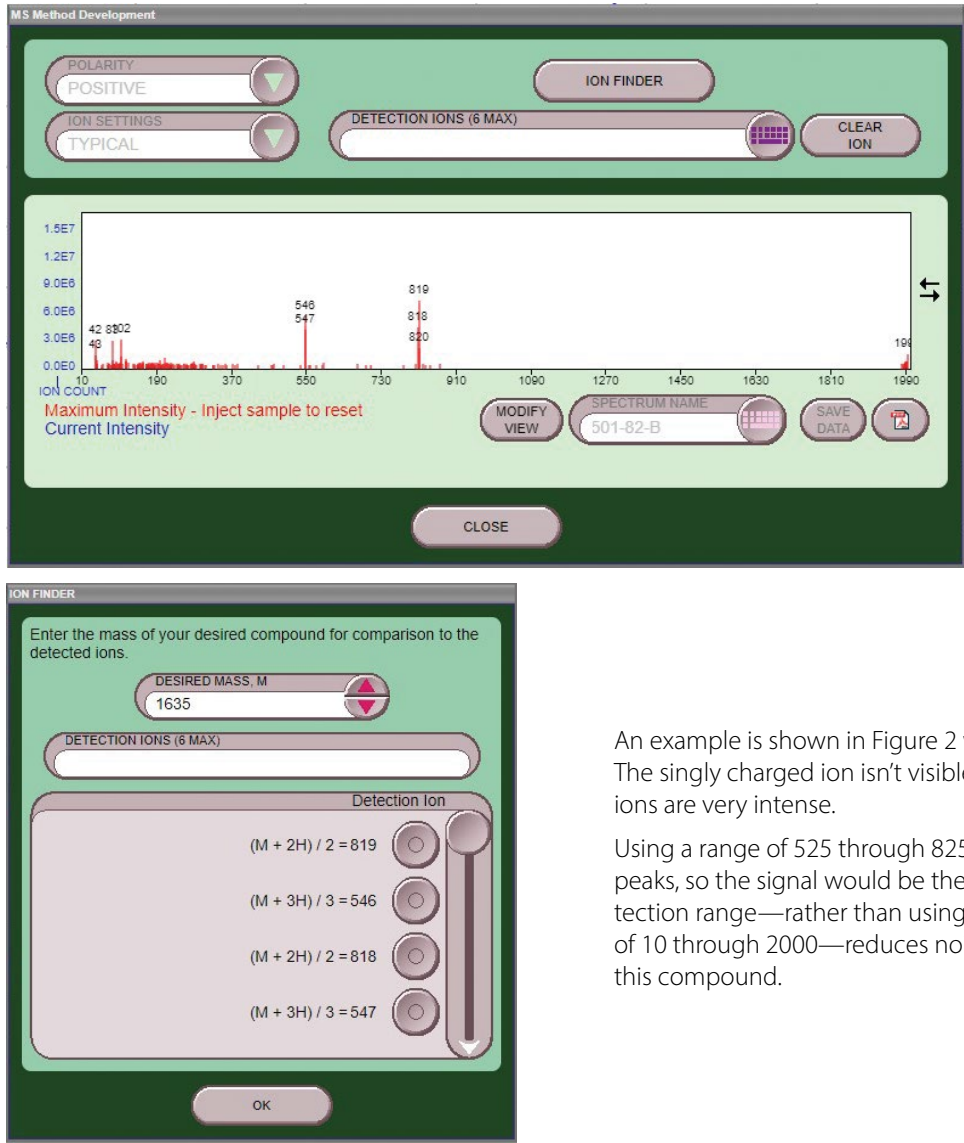


Figure 2. Multiply charged species of a peptide EGFRvii.

An example is shown in Figure 2 where EGFRvii was detected. The singly charged ion isn't visible, but the $[M+2]^{2+}$ and $[M+3]^{3+}$ ions are very intense.

Using a range of 525 through 825 encompasses both those peaks, so the signal would be the sum of both. Limiting the detection range—rather than using the entire spectrometer range of 10 through 2000—reduces noise, improving the detection of this compound.