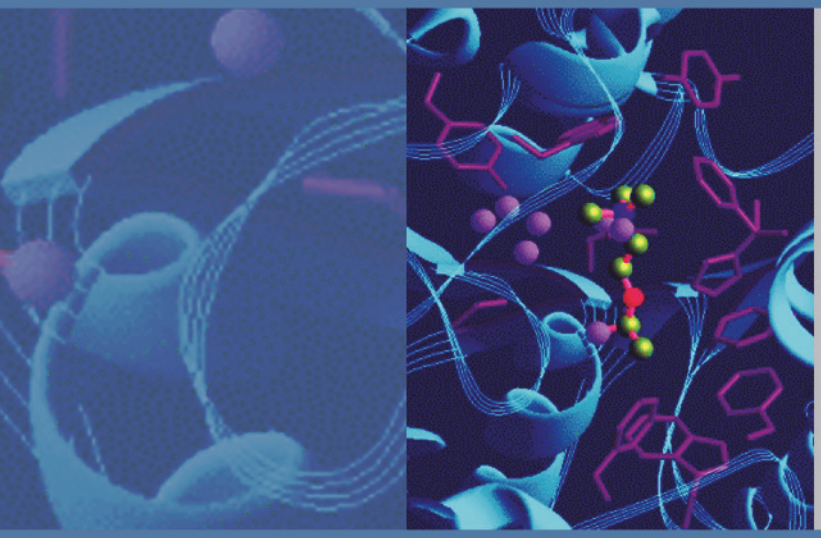

Thermo

# Protein Deconvolution

Version 1.0

## User Guide

XCALI-97400 Revision A October 2011

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# Contents


**Preface** . . . . . **v**
Related Documentation . . . . . v
System Requirements . . . . . vi
Activating a Protein Deconvolution License . . . . . vi
Safety and Special Notices . . . . . viii
Contacting Us . . . . . viii

**Chapter 1 Introduction** . . . . . **1**
Features . . . . . 1
Xtract Algorithm . . . . . 2
ReSpect Algorithm . . . . . 2
Workflow . . . . . 3
Inputs and Outputs . . . . . 5
Inputs . . . . . 5
Outputs . . . . . 5
Starting the Protein Deconvolution Application . . . . . 5
Specifying the Default RAW File Directory . . . . . 6
Exiting the Protein Deconvolution Application . . . . . 7

**Chapter 2 Deconvolving Isotopically Resolved Mass Spectra with the Xtract Algorithm9**
Setting Up an Xtract Protein Deconvolution . . . . . 9
Method Selection Page Parameters . . . . . 11
Creating an Xtract Parameter Set . . . . . 12
Parameters Page Parameters for the Xtract Algorithm . . . . . 18
Selecting the Spectrum to Deconvolve . . . . . 21
Chromatogram Page Parameters . . . . . 27
Chromatogram Pane Shortcut Menu . . . . . 29
Chromatogram Pane Header . . . . . 30
Source Spectrum Pane Shortcut Menu . . . . . 30
Source Spectrum Pane Header . . . . . 30
Deconvolving the Spectrum . . . . . 31
Process and Review Page Parameters for the Xtract Algorithm . . . . . 38

Displaying a Deconvolution Report . . . . . 40
Reporting Page Toolbar . . . . . 42
Sample Information Section . . . . . 43
Chromatogram Parameters Section . . . . . 44
Source Chromatogram Section . . . . . 44
Source Spectrum Section . . . . . 45
Main Parameters (Xtract) Section . . . . . 46
Advanced Parameters (Xtract) Section . . . . . 46
Deconvolved Spectrum Section . . . . . 47
Xtract Masses Table Section . . . . . 47
Source Spectrum Evidence Section . . . . . 48
Loading Saved Results . . . . . 50

**Chapter 3 Deconvolving Isotopically Unresolved Mass Spectra with the ReSpect Algorithm51**

Setting Up a ReSpect Protein Deconvolution . . . . . 51
Method Selection Page Parameters . . . . . 53
Creating a ReSpect Parameter Set . . . . . 54
Parameters Page Parameters for the ReSpect Algorithm . . . . . 60
Selecting the Spectrum to Deconvolve . . . . . 66
Obtaining the Best Results with the ReSpect Algorithm . . . . . 72
Chromatogram Page Parameters . . . . . 73
Chromatogram Pane Shortcut Menu . . . . . 75
Chromatogram Pane Header . . . . . 75
Source Spectrum Pane Shortcut Menu . . . . . 76
Source Spectrum Pane Header . . . . . 76
Deconvolving the Spectrum . . . . . 76
Process and Review Page Parameters for the ReSpect Algorithm . . . . . 83
Displaying a Deconvolution Report . . . . . 84
Reporting Page Toolbar . . . . . 86
Sample Information Section . . . . . 86
Chromatogram Parameters Section . . . . . 87
Source Chromatogram Section . . . . . 88
Source Spectrum Section . . . . . 89
Main Parameters (ReSpect) Section . . . . . 90
Advanced Parameters (ReSpect) Section . . . . . 90
Deconvolved Spectrum Section . . . . . 91
ReSpect Masses Table Section . . . . . 92
Source Spectrum Evidence Section . . . . . 92
Loading Saved Results . . . . . 94

**Index . . . . . 95**

# Preface

This guide describes how to use the Thermo Protein Deconvolution application to deconvolve mass spectra.

**Contents**


- Related Documentation
- System Requirements
- Activating a Protein Deconvolution License
- Safety and Special Notices
- Contacting Us


## Related Documentation

The Protein Deconvolution application includes Help and these manuals as PDF files:

- *Protein Deconvolution User Guide*
- *Protein Deconvolution Quick Start Guide*

### ❖ To view product manuals

- Go to **Start > All Programs > Thermo Protein Deconvolution > Manuals.**
- From the Protein Deconvolution window, choose **Help > Manuals**.

### ❖ To open Help

- From the Protein Deconvolution window, choose **Help > Protein Deconvolution Help**.
- If available for a specific window or dialog box, click **Help** or press F1 for information about setting parameters.

# System Requirements

The Protein Deconvolution application requires a license. In addition, your system must meet these minimum requirements.

| System | Requirements |
|---|---|
| Hardware | • 2 GHz processor with 1 GB RAM<br>• CD-ROM drive<br>• Video card and monitor capable of 1280 × 1024 resolution (XGA)<br>• 75 GB available on the C: drive<br>• NTFS format |
| Software | • Microsoft™ Windows™ 7<br>• Microsoft .NET Framework 4<br>• Microsoft Visual C++™ 2010 Redistributable package (x86)<br>• (Optional) Thermo Xcalibur™ 2.2 SP1 |

# Activating a Protein Deconvolution License

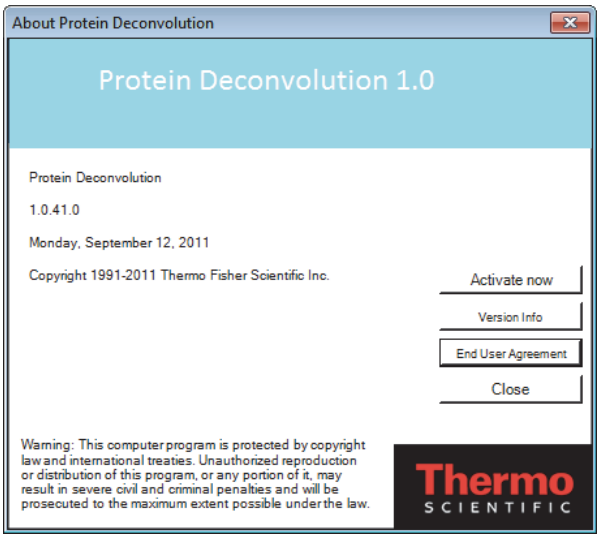
You must activate a Protein Deconvolution license before you can use the application to deconvolve a spectrum.

### ❖ To activate a Protein Deconvolution license

1. Choose **Help > About Protein Deconvolution** in the upper right corner of the Protein Deconvolution window.

   The About Protein Deconvolution dialog box opens, as shown in Figure 1.

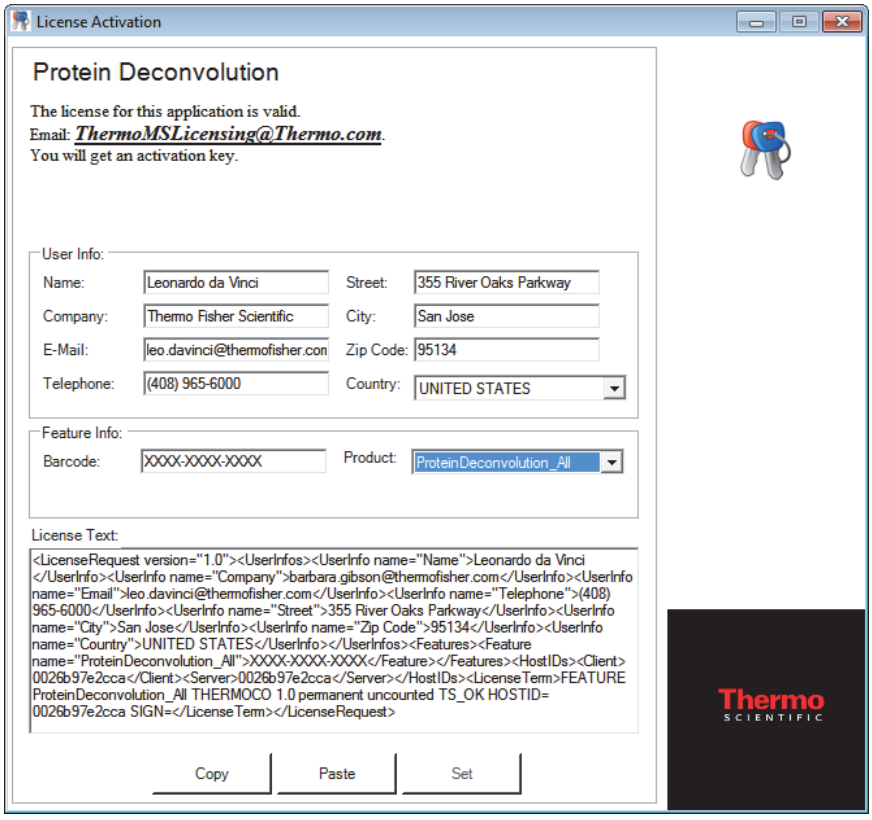
**Figure 1.** About Protein Deconvolution dialog box



2. Click **Activate Now**.

   The License Activation dialog box opens, as shown in Figure 2.

**Figure 2.** License Activation dialog box




3. Complete all the boxes in the User Info area.
4. In the Barcode box, type the bar code, which you can find on the DVD.
5. In the Product list, select **ProteinDeconvolution_All**.

   After you complete the information in the User Info and Feature Info areas, code similar to that shown in Figure 2 appears in the License Text box.

> **Note** Check to be sure there are no spaces in the License Text code.

6. Select the text in the License Text box, click **Copy**, paste the copied text into an e-mail, and send it to ThermoMSLicensing@Thermo.com.

   You will receive a license e-mail within two business days.

7. When you receive your license e-mail, copy the text from the body of the e-mail, place the cursor in the License Text box of the License Activation dialog box, and click **Paste**.

   The Set button now becomes available.

8. Click **Set**.

# Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

**CAUTION** Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

**IMPORTANT** Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

**Note** Highlights information of general interest.

**Tip** Highlights helpful information that can make a task easier.

# Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ **To contact Technical Support**

| | |
|---|---|
| Phone | 800-532-4752 |
| Fax | 561-688-8736 |
| E-mail | us.techsupport.analyze@thermofisher.com |
| Knowledge base | www.thermokb.com |

Find software updates and utilities to download at mssupport.thermo.com.

❖ **To contact Customer Service for ordering information**

| | |
|---|---|
| Phone | 800-532-4752 |
| Fax | 561-688-8731 |
| E-mail | us.customer-support.analyze@thermofisher.com |
| Web site | www.thermo.com/ms |

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Go to www.thermoscientific.com/wps/portal/ts/contactus.

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Go to mssupport.thermo.com, agree to the Terms and Conditions, and then click **Customer Manuals** in the left margin of the window.

### ❖ To suggest changes to documentation or to Help

- Fill out a reader survey online at www.surveymonkey.com/s/PQM6P62.
- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

# Introduction

This chapter describes the purpose, features, workflows, inputs, and outputs of the Protein Deconvolution application. It also explains how to start and exit the application, activate a Protein Deconvolution license, and specify a default RAW file directory.

**Contents**


- Features
- Workflow
- Inputs and Outputs
- Starting the Protein Deconvolution Application
- Specifying the Default RAW File Directory
- Exiting the Protein Deconvolution Application


## Features

Electrospray ionization (ESI) of intact peptides and proteins produces mass spectra that contain series of multiply charged ions with associated mass-to-charge (*m/z*) ratios. The resulting spectrum is complex and difficult to interpret, requiring mathematical algorithms for the analysis of the data. Through a process called deconvolution, the Protein Deconvolution application uses such algorithms to transform a charge state series into a molecular mass. The application identifies multiple peaks in the mass spectrum associated with different charge states of the same component and displays information about the masses and abundance of that component. For example, peaks at 1000 *m/z*, 1111 *m/z*, and 1250 *m/z* might be the charge states 10, 9, and 8 for a protein with a mass of 10 000 Da.

The Protein Deconvolution application includes two independent deconvolution algorithms for mass spectral data:

- Xtract, which deconvolves isotopically resolved mass spectra, that is, spectra in which it is possible to distinguish separate peaks for different isotopic compositions of the same component
- ReSpect™, which deconvolves isotopically unresolved (or unseparated) mass spectra, that is, spectra in which it is not possible to distinguish the separate peaks for different isotopic compositions of the same component

Whether mass spectra are isotopically resolved or unresolved depends not on the specific instrument model but on the resolution of the instrument, the mass of the compounds involved, and the details of the experiment run.

You can produce more than one deconvolved spectrum for any given mass spectrum.

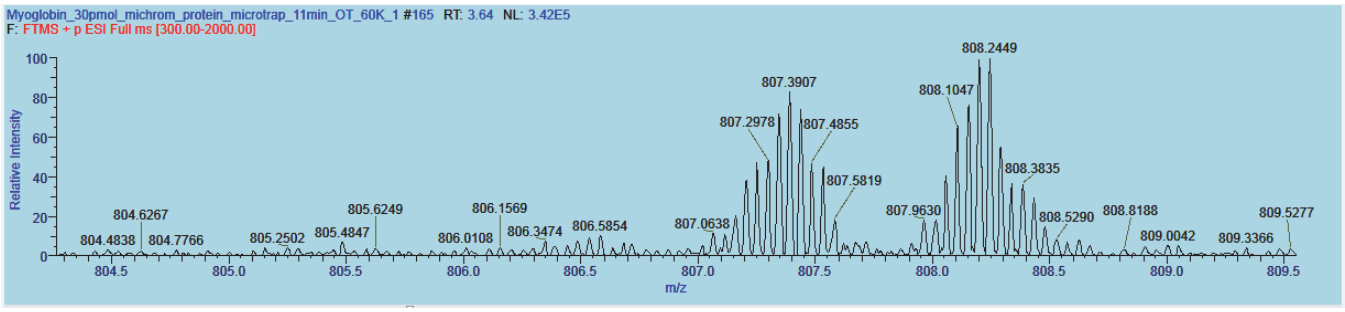
## Xtract Algorithm

The Xtract algorithm deconvolves isotopically resolved mass spectra of peptides and proteins. It simultaneously combines all observed charge states into a single isotopic cluster with charge state 1 or zero (uncharged). The Xtract algorithm always deisotopes the isotopic patterns, and the resulting deconvolved spectrum only shows the monoisotopic mass.

When used properly, the Xtract algorithm reduces spectral noise and provides a high-intensity mass spectrum of monoisotopic peaks. You can use the results table, called the monoisotopic mass list, of the deconvolved mass-spectral peaks or the extracted spectra (not the original MS/MS spectra) as the input to various search engines.

Figure 3 shows an isotopically resolved mass spectrum.

**Figure 3.** Isotopically resolved mass spectrum




## ReSpect Algorithm

The ReSpect algorithm from Positive Probability, Ltd. (PPL) is a robust and efficient data-fitting method that deconvolves isotopically unresolved complex mass spectra from biomolecules, such as small and large proteins, to the neutral average mass of each molecule. It determines the mass-to-charge ratio (*m/z*) of every peak in an ESI mass spectrum and evaluates all possible charge states for any particular peak as determined by the mass ranges. Given a theoretical model, it returns the least-squares best fit to the data by adjusting the model parameters. It fits the data only within the data's errors or noise level to avoid overfitting. For realistic solutions, the ReSpect algorithm constrains the parameters to be non-negative. It also provides artifact-free charge deconvolution and deisotoping analysis.

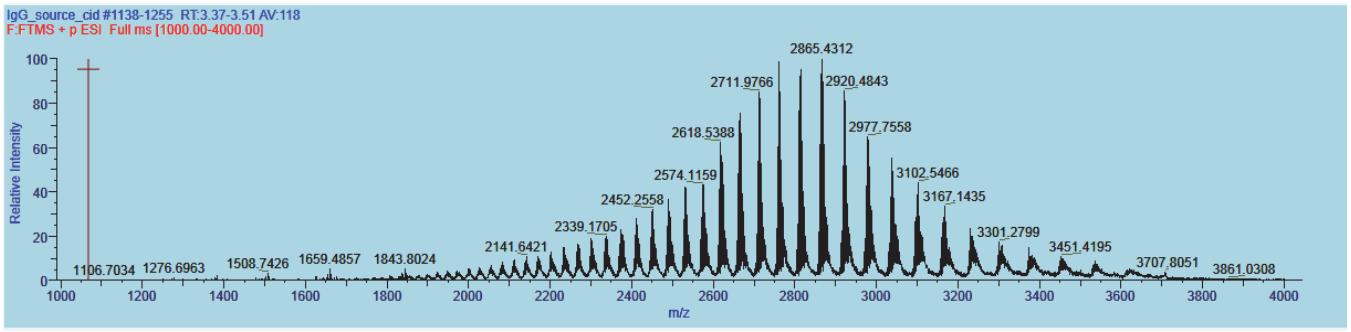
For analyzing spectra, the ReSpect algorithm includes an optional spectrum preconditioning method, including automated baseline subtraction, and a number of automated and semiautomated peak-modeling facilities.

The ReSpect algorithm first performs a baseline subtraction. Next it performs a peak deconvolution to produce a list of peaks, and then it filters these peaks. Lastly, it performs a charge deconvolution to convert the remaining peaks from a mass-to-charge spectrum to a mass spectrum. The ReSpect algorithm uses spacing patterns, which are indicative of mass, to determine what the average mass should be.

The ReSpect algorithm can accommodate both low-charge-state spectra and data with a low signal-to-noise ratio, so it does not require high-quality data to produce meaningful results. You can use it to confirm molecular masses of peptides.

Figure 4 shows an isotopically unresolved mass spectrum.

**Figure 4.** Isotopically unresolved mass spectrum

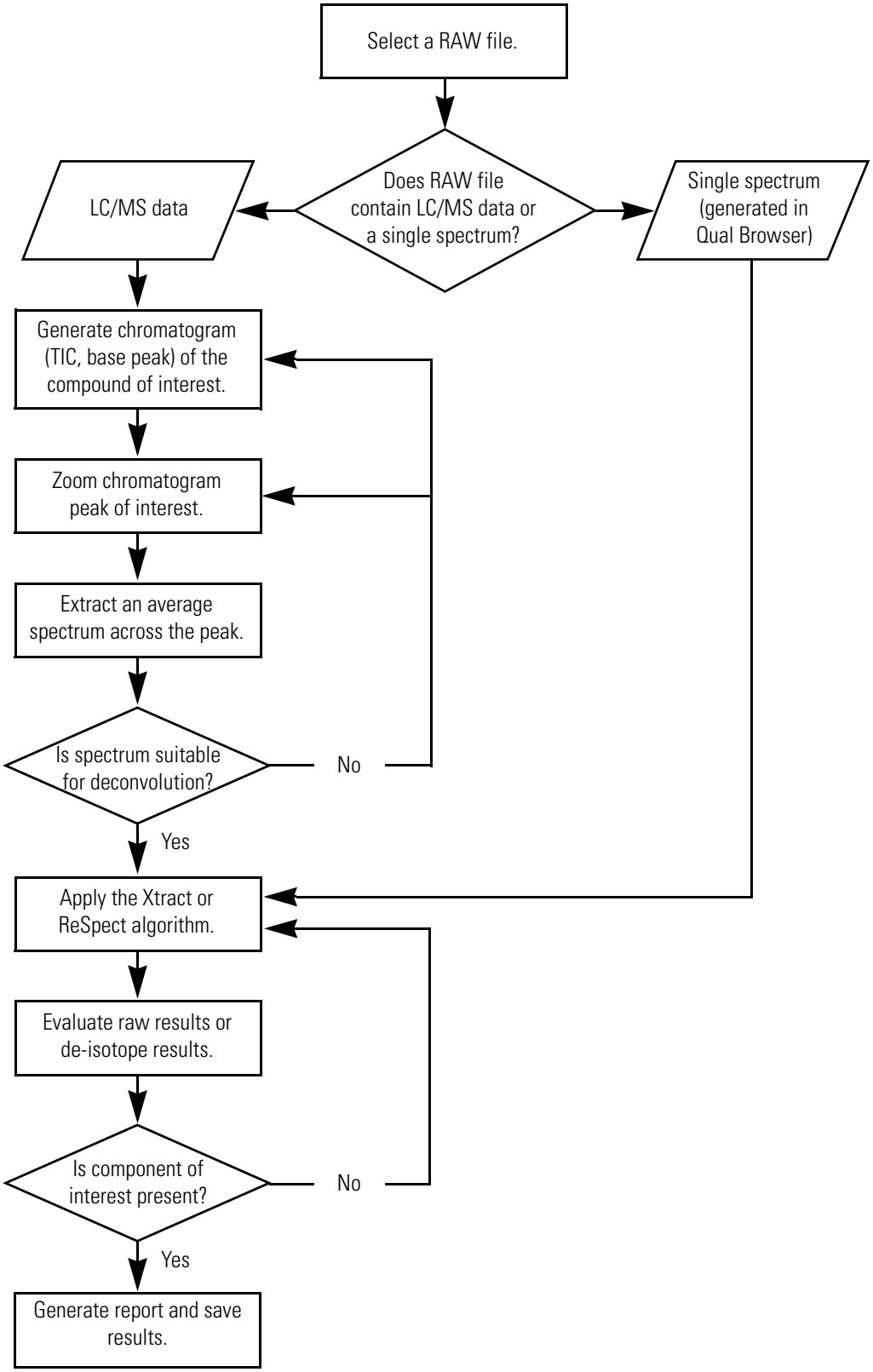



# Workflow

Figure 5 shows the workflow to follow to deconvolve mass spectra with the Protein Deconvolution application. The workflow is nearly the same for the Xtract algorithm as it is for the ReSpect algorithm.

You can select the chromatogram to deconvolve either in the Protein Deconvolution application or in the Qual Browser utility, which is part of the Xcalibur data system. When transferring data—for example, in an e-mail—to submit to the Protein Deconvolution application, you might want to reduce the amount of data to transfer by using Qual Browser to select a single spectrum. For instructions on using Qual Browser to select a chromatogram, refer to the *Thermo Xcalibur Qualitative Analysis User Guide*.

**Figure 5.** Protein Deconvolution workflow



# Inputs and Outputs

The Protein Deconvolution application can accept the following input and generate the following output.

## Inputs

The input file to the Protein Deconvolution application is the RAW file from a mass spectrometry experiment. The RAW file can contain LC/MS data or a single spectrum.

You can use the Protein Deconvolution application with the data from Exactive™, Orbitrap™, and FTMS mass spectrometers.

You cannot use the Protein Deconvolution application with LCQ, LTQ, TSQ, or Velos data.

## Outputs

As output, the Protein Deconvolution application produces a deconvolved spectrum and peak information that you can view on the Reporting page of the Protein Deconvolution window. You can save this report to a PDF file.

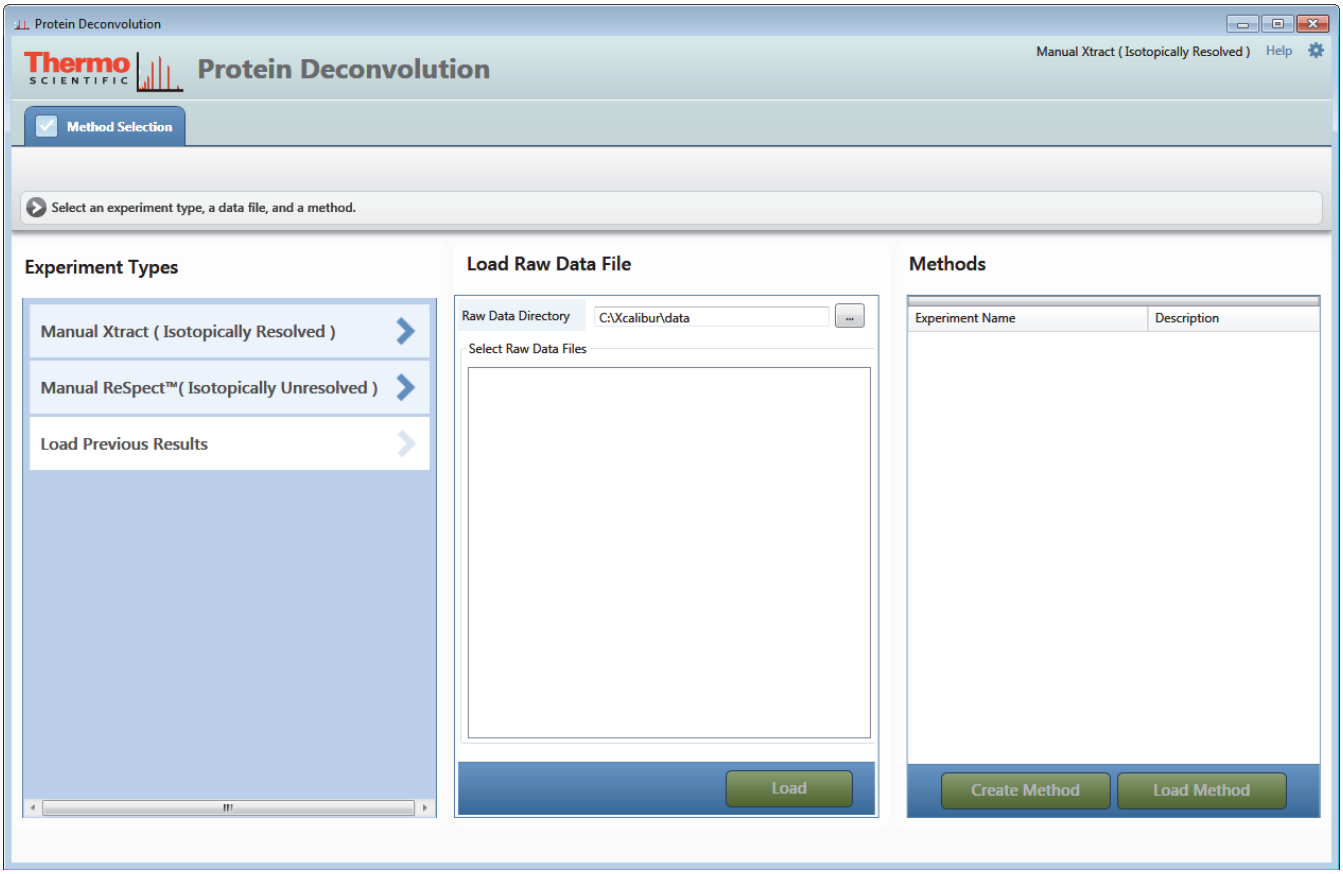
# Starting the Protein Deconvolution Application

❖ **To start the Protein Deconvolution application**

- Choose **Start > All Programs > Thermo Protein Deconvolution** or click the **Protein Deconvolution** icon,  .

  The Protein Deconvolution window appears, as shown in Figure 6.

**Figure 6.** Method Selection page of the Protein Deconvolution window



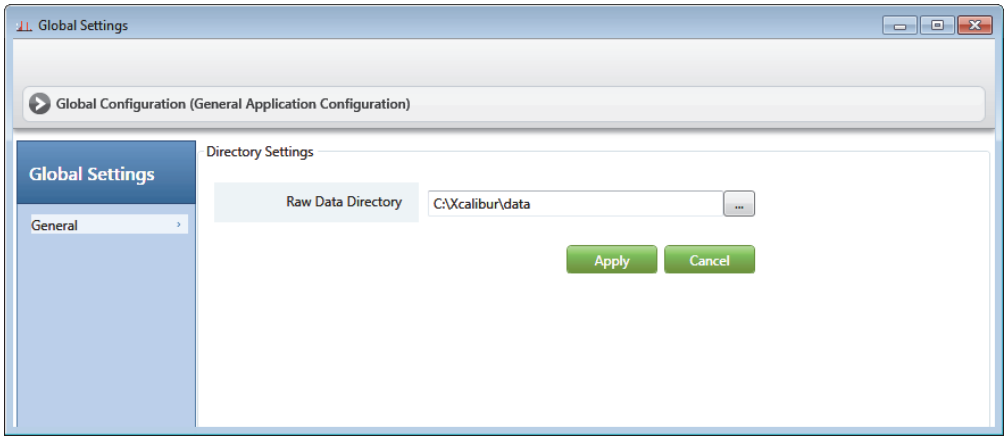

# Specifying the Default RAW File Directory

You can specify the default directory where you want to store your RAW files.

### ❖ To specify the default directory for your RAW files

1. Click the **Global Settings** icon, , in the upper right corner of the Protein Deconvolution window, to activate the Global Settings dialog box, shown in Figure 7.

**Figure 7.** Global Settings dialog box



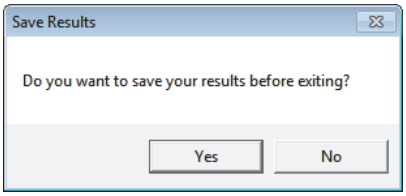

2. In the Raw Data Directory box, click the browse button (...) to browse to the appropriate directory.
3. Click **Apply**.

# Exiting the Protein Deconvolution Application

❖ **To exit the Protein Deconvolution application**

1. Click the **Close** button,  , in the upper right corner of the Protein Deconvolution window.
2. If you have clicked the Process button to produce deconvolution results and have not saved them, the following prompt box appears:

**Figure 8.** Prompt to save results before exiting




3. Click **Yes** or **No**.

# Deconvolving Isotopically Resolved Mass Spectra with the Xtract Algorithm

This chapter explains how to deconvolve isotopically resolved mass spectra with the Xtract algorithm.

**Contents**


- Setting Up an Xtract Protein Deconvolution
- Creating an Xtract Parameter Set
- Selecting the Spectrum to Deconvolve
- Deconvolving the Spectrum
- Displaying a Deconvolution Report
- Loading Saved Results


When you generate a deconvolved spectrum from an isotopically resolved protein mass spectrum, the source MS spectrum can be a single spectrum from an LC/MS data file, an averaged spectrum from an LC/MS data file, or a single spectrum from a RAW file containing only that spectrum. The Xtract algorithm transforms this source spectrum into a mass spectrum and displays it in a new pane labeled with mass units rather than with the mass-to-charge ratio (*m/z*) on the *x* axis. For information on the Xtract algorithm, see "Xtract Algorithm" on page 2.

## Setting Up an Xtract Protein Deconvolution

First select the Xtract deconvolution algorithm, a RAW file, and a parameter set.

❖ **To set up a protein deconvolution with the Xtract algorithm**

1. Click the **Method Selection** tab if it is not already selected.

   The Method Selection page contains three panes:

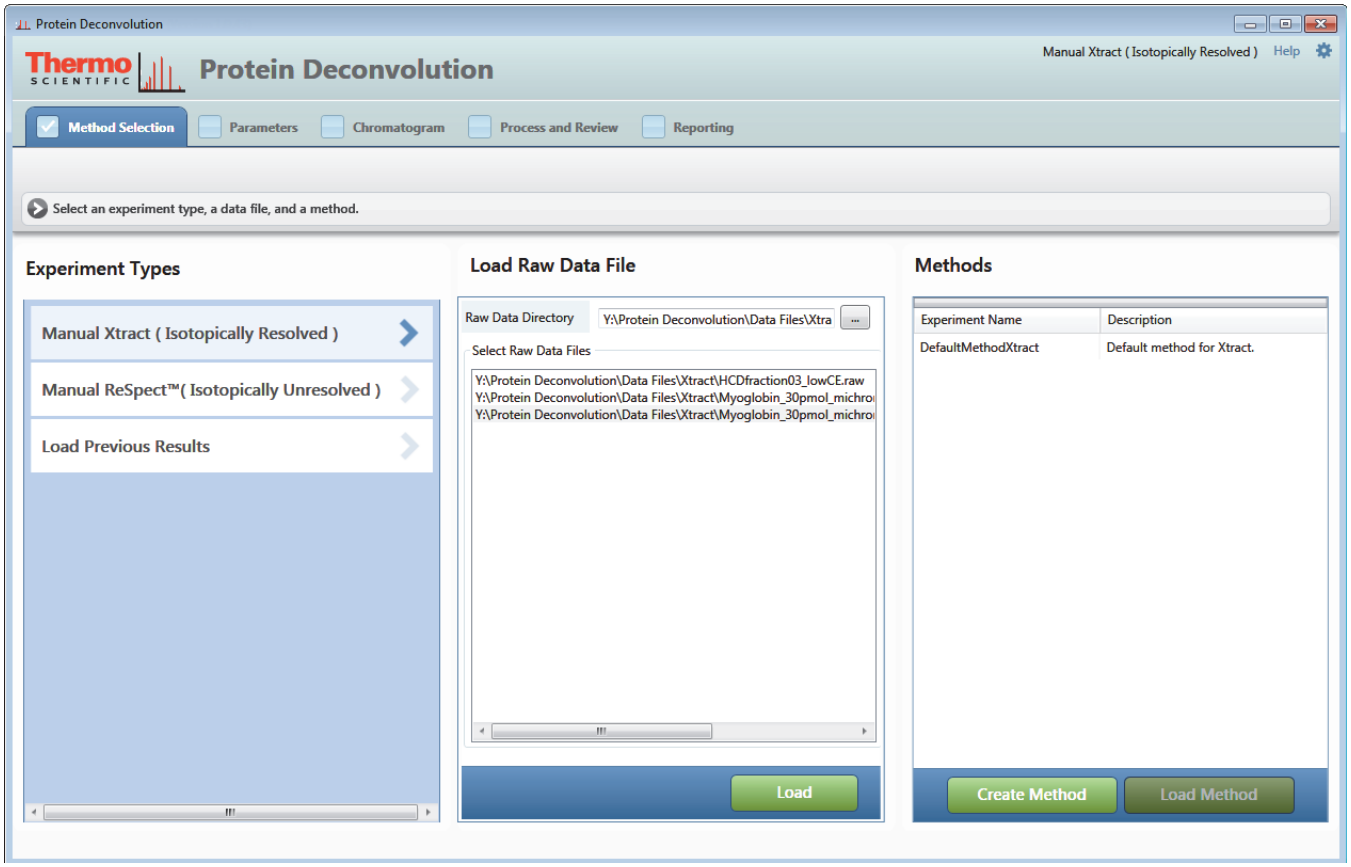
   - Experiment Types pane: Displays the available deconvolution algorithms and a command that you can use to load the saved results of previous deconvolutions.

- Load Raw Data File pane: Displays the list of the available RAW files for the selected algorithm.
- Methods pane: Displays the available parameter sets.

2. In the Experiment Types pane, click **Manual Xtract (Isotopically Resolved)**.
3. In the Load Raw Data File pane, select the RAW data file that contains the spectral data for your sample:
   a. In the Directory box, type the path of the RAW file or click the Browse button (...) to browse to the directory containing the file.
   b. In the Select Raw Data Files box, click the name of the RAW file.
   c. Click **Load**.

   Several more tabs appear along the top of the Protein Deconvolution window, and the available parameter sets appear in the Methods pane, as shown in Figure 9.

**Figure 9.** Method Selection page after the RAW file is loaded for Xtract deconvolution



4. Specify the extraction parameter set to use by doing one of the following in the Methods pane:

   - If one of the existing parameter sets contains the appropriate parameters, select the name of the parameter set of interest and click **Load Method**. The Protein Deconvolution application automatically transfers you to the Chromatogram page if there is a chromatogram and to the Process and Review page if there is a single spectrum. To use the Chromatogram page, follow the instructions in "Selecting the Spectrum to Deconvolve" on page 21. To use the Process and Review page, follow the instructions in "Deconvolving the Spectrum" on page 31.
   - If the existing parameter sets do not contain the appropriate extraction parameters or if there are no existing parameter sets, click **Create Method** to create a new parameter set. The Protein Deconvolution application automatically transfers you to the Parameters page. Follow the instructions in "Creating an Xtract Parameter Set" on page 12.

## Method Selection Page Parameters

The Method Selection page consists of the Experiment Types, Load Raw Data File, and Methods panes. The parameters in these panes are the same for Xtract and ReSpect deconvolutions.

### Experiment Types Pane Parameters

Table 1 lists the parameters in the Experiment Types pane of the Method Selection page.

**Table 1.** Experiment Types pane parameters

| Parameter | Description |
|---|---|
| Manual Xtract (Isotopically Resolved) | Deconvolves an isotopically resolved mass spectrum with the Xtract algorithm. |
| Manual ReSpect (Isotopically Unresolved) | Deconvolves an isotopically unresolved mass spectrum with the ReSpect algorithm. |
| Load Previous Results | Loads the saved results of a previous deconvolution. |

### Load Raw Data File Pane Parameters

Table 2 lists the parameters in the Load Raw Data File pane of the Method Selection page.

**Table 2.** Load Raw Data File pane parameters

| Parameter | Description |
|---|---|
| Raw Data Directory | Specifies the directory where the RAW file containing the spectrum to deconvolve is located. |
| Select Raw Data Files | Specifies the name of the RAW file containing the spectrum to deconvolve. |
| Load | Loads the specified RAW file. |

### Methods Pane Parameters

Table 3 lists the parameters in the Methods pane of the Method Selection page.

**Table 3.** Methods pane parameters

| Parameter | Description |
|---|---|
| Experiment Name | Specifies the name of the parameter set to use in the deconvolution. |
| Description | Briefly describes the parameter set to use in the deconvolution. |
| Create Method | Activates the Parameters page so that you can specify the parameters for a new parameter set. |
| Load Method | Loads the specified existing parameter set. |

# Creating an Xtract Parameter Set

When you click Create Method in the Methods pane of the Method Selection page, the Protein Deconvolution application automatically transfers you to the Parameters page.

The Parameters page features two panes containing parameters that control the deconvolution:

- Main Parameters (Xtract): Displays basic parameters that might change often. These parameters also appear on the Process and Review page.
- Advanced Parameters (Xtract): Displays parameters that only infrequently need changing. Only experienced users should change these parameters.

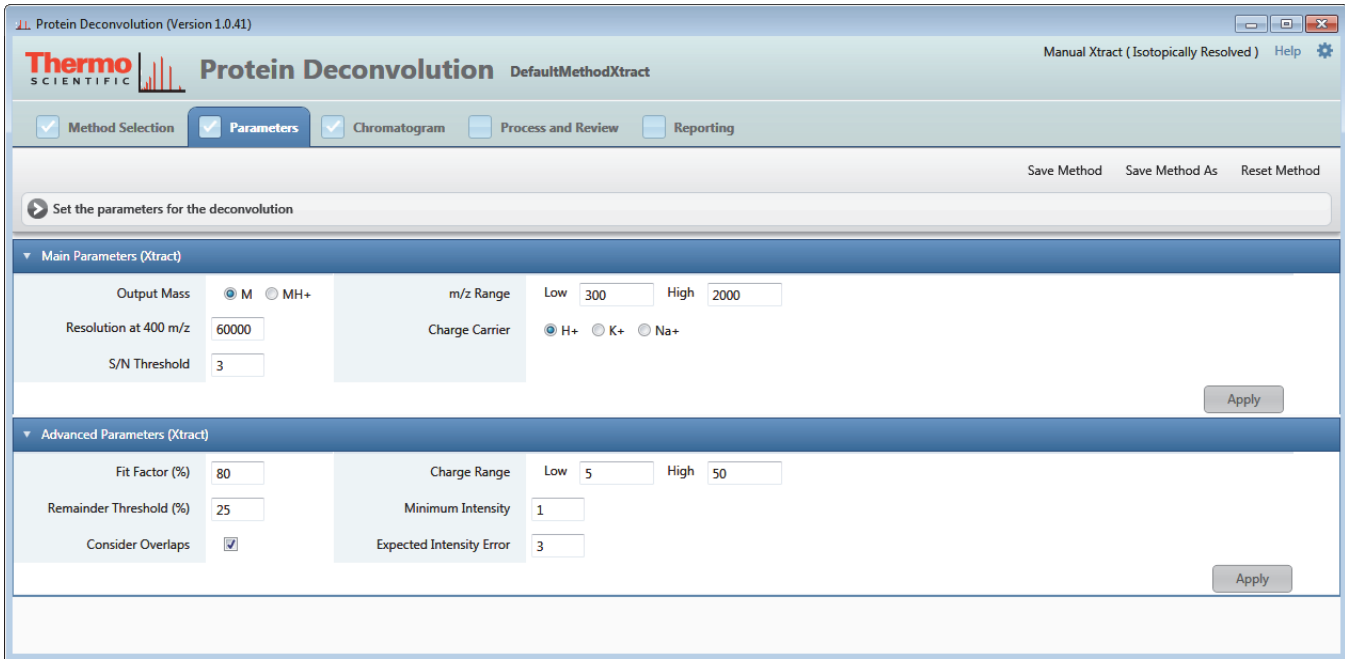
For detailed descriptions of these parameters, see Table 4 on page 18.

### ❖ To create an Xtract parameter set

1. Click the **Parameters** tab if it is not already selected.

   The default settings for the Xtract algorithm automatically populate the parameter boxes on the Parameters page, as shown in Figure 10.

**Figure 10.** Parameters page for Xtract deconvolution




**Note** The Xtract default parameter settings provide a good balance between sensitivity and report size by detecting all of the significant components while excluding low-intensity noise peaks that might inflate the final report to an excessive length.

2. (Optional) Change the appropriate parameters in the Main Parameters (Xtract) pane:

   - Output Mass: Determines whether the Xtract algorithm returns a single peak at either the monoisotopic mass or the monoisotopic MH+ mass.
     - (Default) M: Specifies that the results file contains a single peak for the monoisotopic mass. This option generates masses without adducts.
     - MH+: Specifies that the results file contains a monoisotopic MH+ mass. This option generates masses with adducts.

- Resolution at 400 m/z: Defines the resolution of the source spectrum at an *m/z* value of 400. This parameter is not needed if the Xtract algorithm deconvolves FTMS, Orbitrap, or Exactive data, because the data contains the information in the spectrum. You must set this parameter for all other spectrum types. The default is not a fixed number but varies from RAW file to RAW file. In cases where the mass spectrometer measured the resolution in the RAW file at an *m/z* value other than 400, the Xtract algorithm scales it as follows to account for the variation in instrument resolution versus *m/z*:

  $$R_{converted} = R_{measured} \times \sqrt{\frac{M_{measured}}{400}}$$

  where:

  – $R_{converted}$ is the resolution to be converted.

  – $R_{measured}$ is the measured mass-to-charge ratio (*m/z*).

  – $M_{measured}$ is the measured mass-to-charge ratio (*m/z*) other than 400.

- S/N Threshold: Specifies a signal-to-noise (S/N) threshold, *x*, above which the Xtract algorithm considers a measured peak to be a real (accepted) peak. The Xtract algorithm ignores peaks below this threshold.

  Any spectral peak must be *x* times the intensity of the calculated noise for that spectrum before the Xtract algorithm considers it.

  The minimum value is 1, and there is no maximum.

  The default is 3.

- m/z Range: Specifies the portion of the input spectrum that the Xtract algorithm processes.

  – Low: Specifies the lowest end of the input spectrum.

  – High: Specifies the highest end of the input spectrum.

  For example, if the total mass range of the spectrum is mass 100 to 2000, a setting of 300 to 500 for the m/z Range parameter means that the Xtract algorithm processes only peaks with masses between 300 and 500 *m/z*.

  The default is the complete mass range of the processed spectrum. This number is not a fixed number but varies from RAW file to RAW file.

- Charge Carrier: Specifies the adduct ions used during ESI processing. Adduct ions bring the charge to the molecule, and this charge converts it to an ion.

  – (Default) H+: Specifies that the adduct is hydrogen.

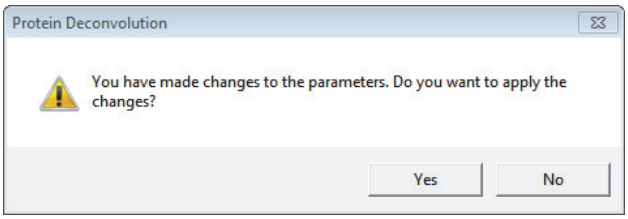
  – K+: Specifies that the adduct is potassium.

  – Na+: Specifies that the adduct is sodium.

3. If you want to return the parameters in the Main Parameters (Xtract) pane to their original default settings, click **Reset Method**; otherwise, click **Apply**.

   If you make changes to the parameters on this pane but do not apply them and then click another tab, the message box shown in Figure 11 appears. Click **Yes** to apply the parameter changes or **No** to restore the parameter defaults.

   **Figure 11.** Reminder to apply parameters

4. (Optional) If you are an experienced user, change the appropriate parameters in the Advanced Parameters (Xtract) pane:

   - Fit Factor (%): Measures the quality of the match between a measured isotope pattern and an averagine distribution of the same mass, as a percentage. Enter a value between 0 and 100%.
     - 0% requires a low fit only.
     - 100% means that the measured isotope profile is identical to the theoretical averagine isotope distribution.

     The default is 80%.

   - Remainder Threshold (%): Specifies the height of the smaller overlapping isotopic cluster, as a percentage, with respect to the height of the most abundant isotopic cluster when the Xtract algorithm attempts to resolve overlapping isotopic clusters. For example, if one isotopic cluster in a spectrum has an abundance of 100 and you set the Remainder Threshold parameter to 30%, the Xtract algorithm ignores any overlapping clusters with an abundance less than 30.

     The Remainder Threshold parameter is a percentage value that lies between 0 and 100%.

     The default is 25%.

   - Consider Overlaps: Determines whether the Xtract algorithm is more tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster.
     - (Default) Selected: The Xtract algorithm is more tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster. Because this option can lead to increased false positives, select it only in cases where you expect overlapping isotopic clusters in a data set.
     - Unselected: The Xtract algorithm is less tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster.

- Charge Range: Specifies the lowest and highest charge state to be deconvolved.
  - Low: Specifies the lowest charge state.
  - High: Specifies the highest charge state.

  For example, if you set this parameter range from 1 through 5, the Xtract algorithm considers only charge states 1 through 5 for deconvolution. It ignores charges 6 and higher.

  The default range is 5 through 50.
- Minimum Intensity: Specifies a minimum intensity threshold to filter out possible background noise, even when you set the S/N Threshold parameter to zero.

  The minimum value is 0, and there is no maximum. The default value is 1.
- Expected Intensity Error: Specifies the permissible percentage of error allowed in calculating the ratio of the most abundant isotope to the next isotope higher in mass in the isotope series.

  The default is 3%.

5. If you want to return the parameters in the Advanced Parameters (Xtract) pane to their original default settings, click **Reset Method**; otherwise, click **Apply**.

   If you change any parameters in this pane but do not click Apply and then click another tab, the message box shown in Figure 11 on page 15 appears. Click **Yes** to apply the parameter changes or **No** to restore the parameter defaults.

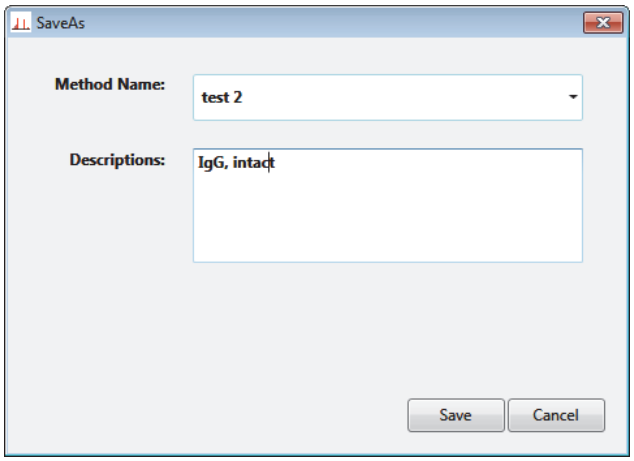
6. Click **Save Method** or **Save Method As** to save the parameter set and give it a name.

   The Save Method command saves current parameter values to the existing method, overwriting any previous values. The Save Method As command saves parameter values to a new method.

7. In the Save or SaveAs dialog box, do the following:
   a. In the Method Name box, type a name for the parameter set.
   b. In the Description box, briefly describe the parameter set. For example, you might want to describe the sample and the proteins analyzed.

      Figure 12 shows a completed dialog box.

**Figure 12.** Completed SaveAs dialog box




c. Click **Save**.

**Note** The Protein Deconvolution application automatically saves all parameter sets that you create to the database in C:\ProgramData\Thermo\ProteinDeconvolution\methods.sqlite. You cannot save individual method files to a directory that you choose.

The next time that you access the Method Selection page and click Manual Xtract (Isotopically Resolved), you will see the name of the parameter set that you saved in the Methods pane.

**Note** You cannot delete existing parameter sets from the Methods pane except by deleting the entire file from the C:\ProgramData\Thermo\ProteinDeconvolution\methods.sqlite database.

The application transfers you to the Chromatogram page so that you can select the spectrum to deconvolve. For information on this process, see "Selecting the Spectrum to Deconvolve" on page 21.

## Parameters Page Parameters for the Xtract Algorithm

Table 4 describes the parameters on the Parameters page for an Xtract deconvolution.

**Table 4.** Parameters page parameters for Xtract deconvolution (Sheet 1 of 4)

| Parameter | Description |
|---|---|
| **Main Parameters (Xtract) pane** | Displays basic parameters that might change often. These parameters also appear on the Process and Review page. |
| Output Mass | Determines whether the Xtract algorithm returns a single peak at either the monoisotopic mass or the monoisotopic MH+ mass.<br>• (Default) M: Specifies that the results file contains a single peak for the monoisotopic mass. This option generates masses without adducts.<br>• MH+: Specifies that the results file contains a monoisotopic MH+ mass. This option generates masses with adducts. |
| Resolution at 400 m/z | Defines the resolution of the source spectrum at an *m/z* value of 400. This parameter is not needed if the Xtract algorithm extracts FTMS, Orbitrap, or Exactive data, because the data contains the information in the spectrum. You must set this parameter for all other spectrum types. In cases where the mass spectrometer measured the resolution in the RAW file at an *m/z* value of other than 400, the Xtract algorithm scales it as follows to account for the variation in instrument resolution versus *m/z*:<br>$R_{converted} = R_{measured} \times \sqrt{\frac{M_{measured}}{400}}$<br>where:<br>• $R_{converted}$ is the resolution to be converted.<br>• $R_{measured}$ is the measured mass-to-charge ratio (*m/z*).<br>• $M_{measured}$ is the measured mass-to-charge ratio (*m/z*) other than 400.<br>The default is not a fixed number but varies from RAW file to RAW file. |

**Table 4.** Parameters page parameters for Xtract deconvolution (Sheet 2 of 4)

| Parameter | Description |
|---|---|
| S/N Threshold | Specifies a signal-to-noise (S/N) threshold, *x*, above which the Xtract algorithm considers a measured peak to be a real (accepted) peak. The Xtract algorithm ignores peaks below this threshold.<br><br>Any spectral peak must be *x* times the intensity of the calculated noise for that spectrum before the Xtract algorithm considers it.<br><br>Range: 1 to No maximum. Recommended value is 2 to *n*.<br><br>Default: 3 |
| m/z Range | Specifies the portion of the input spectrum that the Xtract algorithm processes.<br><br>• Low: Specifies the lowest end of the input spectrum.<br><br>• High: Specifies the highest end of the input spectrum.<br><br>For example, if the total mass range of the spectrum is mass 100 to 2000, a setting of 300 to 500 for the m/z Range parameter means that the Xtract algorithm processes only peaks with masses between 300 and 500 *m/z*.<br><br>The default is the complete mass range of the processed spectrum. This number is not a fixed number but varies from RAW file to RAW file. |
| Charge Carrier | Specifies the adduct ions used during ESI processing. Adduct ions bring the charge to the molecule, and this charge converts it to an ion.<br><br>• (Default) H+: Specifies that the adduct was hydrogen.<br><br>• K+: Specifies that the adduct was potassium.<br><br>• Na+: Specifies that the adduct was sodium. |
| Apply | Implements the parameter settings that you selected. This button is only available if you have changed any parameter settings in the Main Parameters (Xtract) pane. |

**Table 4.** Parameters page parameters for Xtract deconvolution (Sheet 3 of 4)

| Parameter | Description |
|---|---|
| **Advanced Parameters (Xtract) pane** | Displays parameters that only infrequently need changing. Only experienced users should change these parameters. |
| Fit Factor (%) | Measures the quality of the match between a measured isotope pattern and an averagine distribution of the same mass. Enter a value between 0 and 100%.<br>• 0% requires a low fit only.<br>• 100% means that the measured isotope profile is identical to the theoretical averagine isotope distribution.<br>Default: 80% |
| Remainder Threshold (%) | Specifies the height of the smaller overlapping isotopic cluster, as a percentage, with respect to the height of the most abundant isotopic cluster when the Xtract algorithm attempts to resolve overlapping isotopic clusters. For example, if one isotopic cluster in a spectrum has an abundance of 100 and you set the Remainder Threshold parameter to 30%, the Xtract algorithm ignores any overlapping clusters with an abundance less than 30.<br>Range: 0–100%<br>Default: 25% |
| Consider Overlaps | Determines whether the Xtract algorithm is more tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster.<br>• (Default) Selected: The Xtract algorithm is more tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster. Because this option can lead to increased false positives, select it only in cases where you expect overlapping isotopic clusters in a data set.<br>• Unselected: The Xtract algorithm is less tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster. |

**Table 4.** Parameters page parameters for Xtract deconvolution (Sheet 4 of 4)

| Parameter | Description |
|---|---|
| Charge Range | Specifies the lowest and highest charge state to be deconvolved.<br>• Low: Specifies the lowest charge state.<br>• High: Specifies the highest charge state.<br>Default range: 5 through 50<br>As an example, if you set this parameter range from 1 through 5, the Xtract algorithm considers only charge states 1 through 5 for deconvolution. It ignores charges 6 and higher. |
| Minimum Intensity | Specifies a minimum intensity threshold to filter out possible background noise, even when you set the S/N Threshold parameter to zero.<br>Range: 0 to No maximum<br>Default: 1 |
| Expected Intensity Error | Specifies the permissible percentage of error allowed in calculating the ratio of the most abundant isotope to the next isotope higher in mass in the isotope series.<br>Default: 3% |
| Apply | Implements the parameter settings that you selected. This button is only available if you have changed any parameter settings in the Advanced Parameters (Xtract) pane. |

# Selecting the Spectrum to Deconvolve

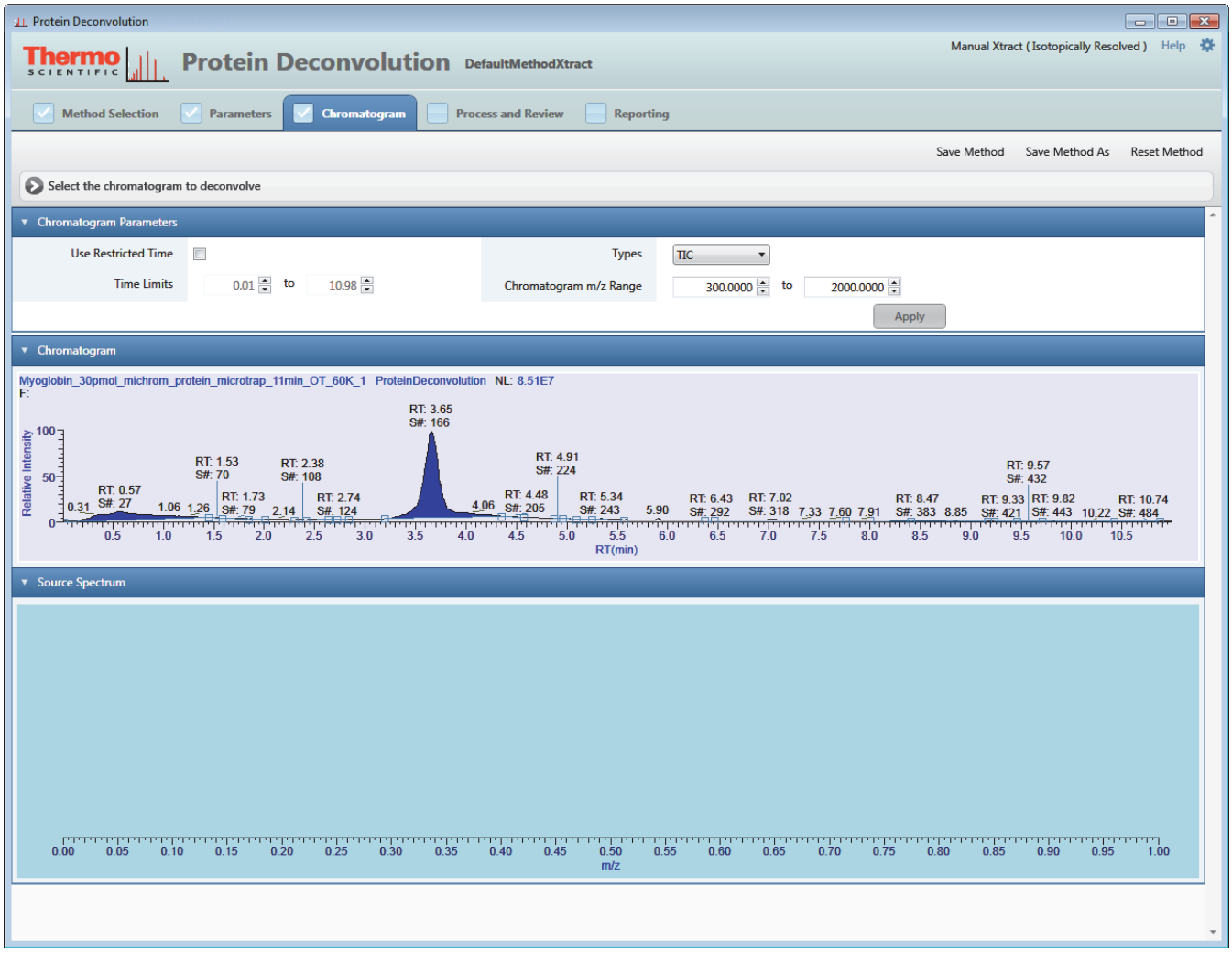
When you click Save Method or Save Method As after you have set the parameters on the Parameters page or when you load an existing parameter set and click Load Method in the Method Selection page, the Protein Deconvolution application automatically transfers you to the Chromatogram page. Use the Chromatogram page to select the best possible spectrum for the target protein for deconvolution.

### ❖ To select the spectrum to deconvolve

1. Click the **Chromatogram** tab if it is not already selected.

   Figure 13 shows the Chromatogram page.

**Figure 13.** Chromatogram page for Xtract deconvolution




The Chromatogram page displays three panes:

- Chromatogram Parameters: Contains parameters that you can use to adjust the view in the Chromatogram pane.
- Chromatogram: Displays a chromatogram of the data in the RAW file. A chromatogram view shows the intensities of one or more masses as a function of time. By default, the Chromatogram pane displays a TIC chromatogram, as shown in Figure 13. The chromatogram is fully magnified. You can use the zooming and averaging functions in this pane to generate a spectrum (for instructions, see step 4).
- Source Spectrum: Displays the spectrum to deconvolve, either single-scan or averaged, that you selected in the Chromatogram pane. The mass spectrum in this pane is empty until you select a region in the chromatogram.

2. (Optional) Use the parameters in the Chromatogram Parameters pane to adjust the chromatogram displayed in the Chromatogram pane:

   - Use Restricted Time: Determines whether the Protein Deconvolution application zooms the part of the chromatogram that you designate with the Time Limits parameters.
     - (Default) Unselected: Displays the entire chromatogram.
     - Selected: Zooms the designated part of the chromatogram.
   - Time Limits: Specifies the beginning and the end of the chromatogram that you want to zoom.

     The default values for both limits depend on the data in the RAW file.

     This parameter is only available when you select the Use Restricted Time check box.
   - Types: Specifies the type of chromatogram to display in the Chromatogram pane:
     - (Default) TIC: Displays a total ion current chromatogram.
     - BPC: Displays a base peak chromatogram. The base peak is the largest peak in a spectrum.

     For information on these types of chromatograms, see Table 5 on page 27.
   - Chromatogram m/z Range: Specifies the range of mass-to-charge (*m/z*) values used to create the chromatogram. You can use this parameter to select a narrower range. The Xtract algorithm ignores the portions of the spectrum outside this range.

     You might want to create a narrower range because the intact proteins are usually at a higher *m/z,* and any small molecule contaminants and background are below 600 *m/z*. Instead of creating a TIC using the full *m/z* range, the Xtract algorithm calculates a TIC by summing those protein peaks within the narrower *m/z* range. The resulting TIC is basically an extracted ion chromatogram (XIC). The Xtract algorithm redraws the BPC with the most intense peak within the selected *m/z* range rather than the whole spectrum. In both cases, any peaks for background components generally disappear from the chromatogram, and the only peak left is for the target proteins.

     The default values for both limits depend on the data in the RAW file. The default is the entire chromatogram for the given spectrum.

3. If you want to return the parameters in the Chromatogram Parameters pane to the original default settings, click **Reset Method**; otherwise, click **Apply**.

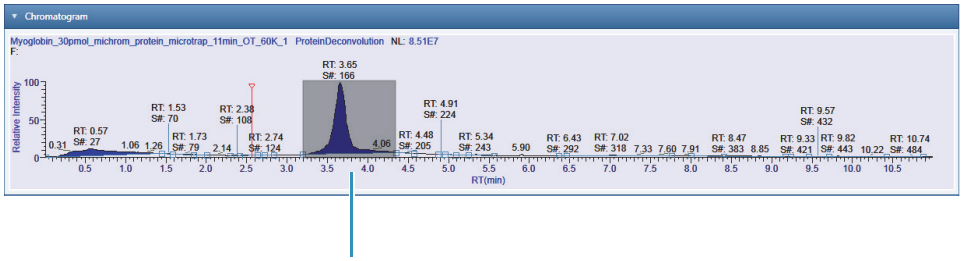
   If you change any parameters in this pane but do not click Apply and then click another tab, the message box shown in Figure 11 on page 15 appears. Click **Yes** to apply the parameter changes or **No** to restore the parameter defaults.

4. (Optional) Adjust the view in the Chromatogram pane:

   - To enlarge the view to see more detail, do one of the following:

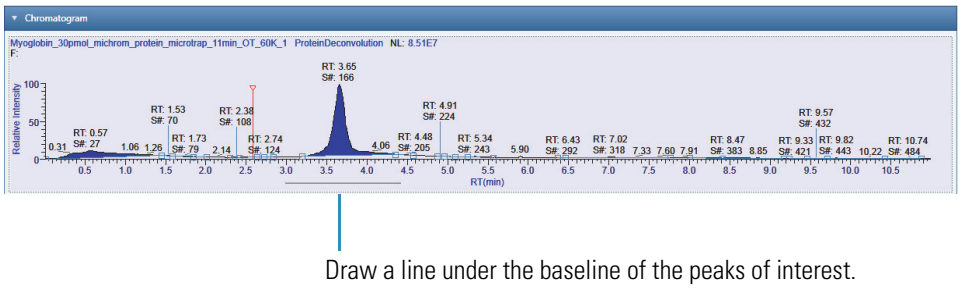
     – Right-click and choose **Mode > Auto Zooming** from the shortcut menu if it is not already selected, and drag the red cross-shaped cursor over the peak or peaks of interest to form a box, as shown in Figure 14.

     **Figure 14.** Enlarging an area by drawing a box around the peaks of interest

     – Keeping the left mouse button pressed, draw a line beneath the baseline of the peaks of interest, as shown in Figure 15.

     **Figure 15.** Enlarging an area by drawing a line beneath the baseline of the peaks of interest

     – Right-click and choose **Zoom In** from the shortcut menu to zoom the entire chromatogram.

     If there is no obvious chromatographic peak, change the limits of the m/z Range parameter in the Parameters page to find it.

   - To shrink the view of the entire spectrum, right-click and choose **Zoom Out**.

   - To reset the view to the original spectrum, right-click and choose **Reset Scale**.

5. Create a spectrum in the Source Spectrum pane by doing one of the following:

   - In the Chromatogram pane, place the red cross-shaped cursor on the chromatogram to select a single scan and to display the associated mass spectrum at that time point, as shown in Figure 16. You can use the left and right arrow keys to move to the previous or next time point in the chromatogram. The spectrum window automatically updates.

   –or–

- Select a region of the chromatogram to display an averaged spectrum for all the scans in the selected region in the Source Spectrum pane:

  i. Right-click and choose **Mode > Averaging**.

  ii. Drag the red cross-shaped cursor across the area of interest.

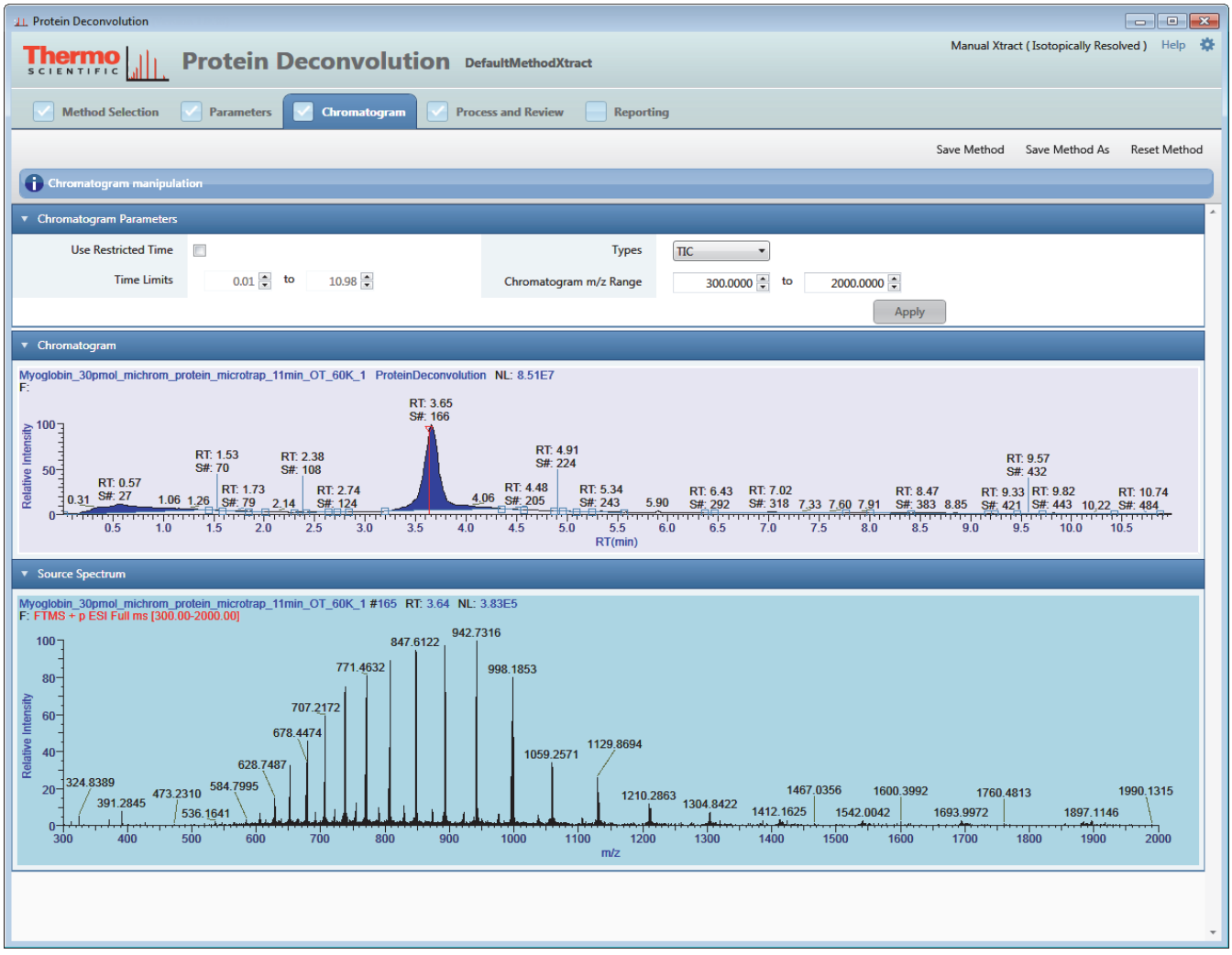
  The horizontal line of this cursor aids in assessing peak height. The Protein Deconvolution application calculates an average spectrum for this interval.

  The averaging method is better suited to complex data than the single-scan method.

**Tip** (Optional) You can perform step 1 through step 5 in Qual Browser in the Xcalibur data system, and then right-click and choose **Export > Write to RAW File** so that you can import the file into the Protein Deconvolution application.

The spectrum appears in the Source Spectrum pane of the Chromatogram page, as shown in Figure 16.

**Figure 16.** Spectrum in the Source Spectrum pane for Xtract deconvolution

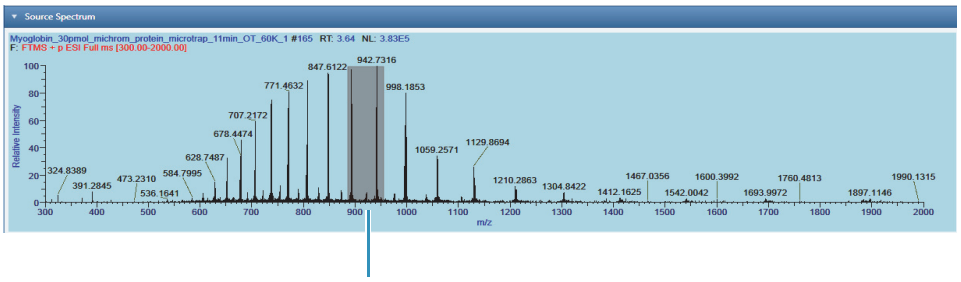


The Source Spectrum pane shows the actual spectrum, either single-scan or averaged, to be deconvolved. This spectrum also appears in the Process and Review pane for deconvolution. The Xtract algorithm can deconvolve centroid spectra and profile spectra. The Protein Deconvolution application automatically chooses the appropriate type of spectrum. The Source Spectrum pane displays profile information if it is available and centroid information if it is not.

- Centroid data represents mass spectral peaks in terms of two parameters: the centroid (the weighted center of the mass) and the intensity (the normalized area of the peak). The data is displayed as a bar graph.
- Profile data represents the entire spectrum as a succession of points in *m/z* and intensity. The data is displayed as a line.

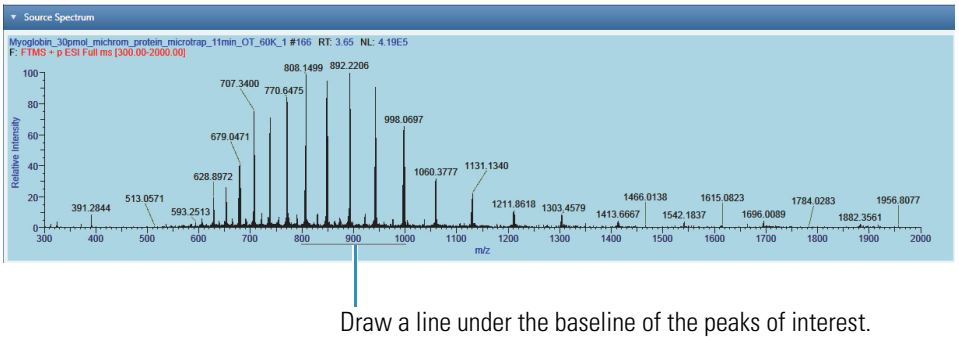
6. (Optional) Adjust the view in the Source Spectrum pane if necessary.
   - To enlarge the view to see more detail, do one of the following:
     – Drag the red cross-shaped cursor over the peak or peaks of interest to form a box, as shown in Figure 17.

**Figure 17.** Enlarging an area by drawing a box around the peaks of interest




     – Keeping the left mouse button pressed, draw a line beneath the baseline of the peaks of interest, as shown in Figure 18.

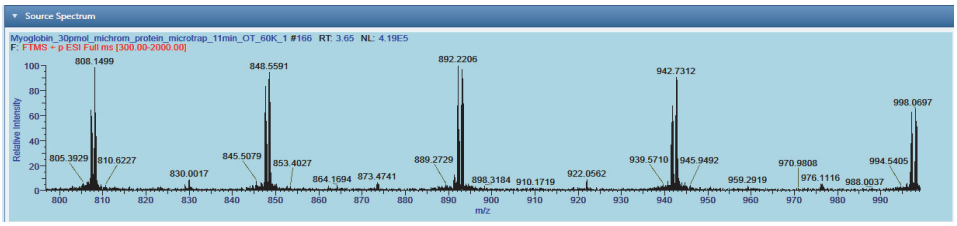
**Figure 18.** Enlarging an area by drawing a line beneath the baseline of the peaks of interest




     – Right-click and choose **Zoom In** to enlarge the view of the entire spectrum.

Figure 19 gives an example of the enlarged isotopic clusters.

**Figure 19.** Enlarged peaks in the Source Spectrum for Xtract deconvolution




- To shrink the view of the entire spectrum, right-click and choose **Zoom Out**.
- To reset the view to the original spectrum, right-click and choose **Reset Scale**.

Unlike adjustments in the Chromatogram pane, which you use to select a spectrum for processing, adjustments in the Source Spectrum pane do not affect the spectrum that the Protein Deconvolution application deconvolves. In particular, they do not change the *m/z* range that the deconvolution algorithm uses.

7. When the spectrum is suitable for Xtract processing, click the **Process and Review** tab, and follow the instructions in "Deconvolving the Spectrum" on page 31.

## Chromatogram Page Parameters

Table 5 lists the parameters that are available on the Chromatogram page for Xtract deconvolutions.

**Table 5.** Chromatogram page parameters for Xtract deconvolution (Sheet 1 of 3)

| Parameter | Description |
|---|---|
| **Chromatogram Parameters pane** | Displays the parameters that govern the appearance of the chromatogram in the Chromatogram pane. |
| Use Restricted Time | Determines whether the Protein Deconvolution application zooms the part of the chromatogram that you define with the Time Limits parameters.<br>• Selected: Zooms the specified part of the chromatogram.<br>• (Default) Unselected: Displays the entire chromatogram. |
| Time Limits | Specifies the beginning and the end of the chromatogram that you want to zoom.<br>The default values for both limits depend on the data in the RAW file.<br>This parameter is only available when you select the Use Restricted Time check box. |

**Table 5.** Chromatogram page parameters for Xtract deconvolution (Sheet 2 of 3)

| Parameter | Description |
|---|---|
| Types | Determines the type of chromatogram displayed in the Chromatogram pane:<br>• (Default) TIC: Displays a total ion current chromatogram, which shows the summed intensity across the entire range of masses being detected at every point in the analysis. The range is typically several hundred mass-to-charge units or more. In complex samples, the TIC chromatogram often provides limited information as multiple analytes elute simultaneously, obscuring individual species.<br>• BPC: Displays a base peak chromatogram, which shows only the most intense peak in each spectrum. This means that the base peak chromatogram represents the intensity of the most intense peak at every point in the analysis. Base peak chromatograms often have a cleaner look and are therefore more informative than TIC chromatograms because the background is reduced by focusing on a single analyte at every point. |
| Chromatogram m/z Range | Specifies the range of mass-to-charge (*m/z*) values used as input to the chromatogram. The Xtract algorithm ignores the portions of the spectrum outside this range.<br>The default values for both limits depend on the data in the RAW file. The default is the entire chromatogram for the given spectrum. |
| Apply | Implements the parameter settings that you selected. This button is only available if you have changed any parameter settings in the Chromatogram Parameters pane. |
| **Chromatogram pane** | Displays the chromatogram contained in the RAW file. |
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| RT (min) (*x* axis) | Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column. |

**Table 5.** Chromatogram page parameters for Xtract deconvolution (Sheet 3 of 3)

| Parameter | Description |
|---|---|
| **Source Spectrum pane** | Displays the spectrum that you selected. |
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| m/z (*x* axis) | Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion. |

## Chromatogram Pane Shortcut Menu

When you right-click in the Chromatogram pane, a shortcut menu appears that contains the commands listed in Table 6.

**Table 6.** Chromatogram page shortcut menu

| Parameter | Description |
|---|---|
| Mode | Determines whether dragging the cursor zooms or selects a range of scans to average.<br>• Averaging: Averages the spectra for all the scans in the region that you drag the cursor over in the Chromatogram pane and displays them in the Source Spectrum pane.<br>• (Default) Auto Zooming: Enlarges the area that you drag the cursor over in the Chromatogram pane without changing the view displayed in the Source Spectrum pane. |
| Scale | Restores the original chromatogram that first appeared in the Chromatogram pane. |
| Copy | Copies the view in the Chromatogram pane. |
| Zoom Out | Shrinks the view in the Chromatogram pane two times. |
| Zoom In | Enlarges the view in the Chromatogram pane two times. |

## Chromatogram Pane Header

The header in the Chromatogram pane displays the following information. The example values come from Figure 16 on page 25.

- The name of the RAW file, for example, Myoglobin_30pmol_microm_protein_microtrap_11min_OT_60K_1
- The name of the product, Protein Deconvolution
- NL: The intensity of the most abundant peak in the entire LC/MS run, for example, 8.51E7
- F: The scan filter used during the LC/MS run. The scan filter indicates the type of mass analyzer used to acquire the data in the raw data file and the ionization technique used. If this field is blank, no scan filter was used.

## Source Spectrum Pane Shortcut Menu

When you right-click in the Source Spectrum pane, a shortcut menu appears that contains all of the commands in Table 6 except Mode, but they apply to the Source Spectrum pane rather than the Chromatogram pane. For information on these commands, see Table 6 on page 29.

## Source Spectrum Pane Header

The header in the Source Spectrum pane displays the following information. The example values come from Figure 16 on page 25.

- Name of the RAW file, for example, Myoglobin_30pmol_microm_protein_microtrap_11min_OT_60K_1
- Scan number or range of scan numbers, for example, #165
- RT: Retention time, which is the time in the mass chromatogram when any particular precursor ion is observed, for example, 3.64
- NL: The the intensity of the most abundant peak in the entire LC/MS run, for example, 3.83E5
- F: The scan filter used during the LC/MS run, for example, FTMS + p ESI Full ms [300.00–2000.00]. The scan filter indicates the type of mass analyzer used to acquire the data in the raw data file and the ionization technique used. If this field is blank, no scan filter was used.

# Deconvolving the Spectrum

When you arrive at the Process and Review page, shown in Figure 20, you have already selected the chromatogram and source spectrum on the Chromatogram page. You can zoom in and out of these views, but you cannot change them on the Process and Review page. You must manually navigate back to the Chromatogram pane to change these views.

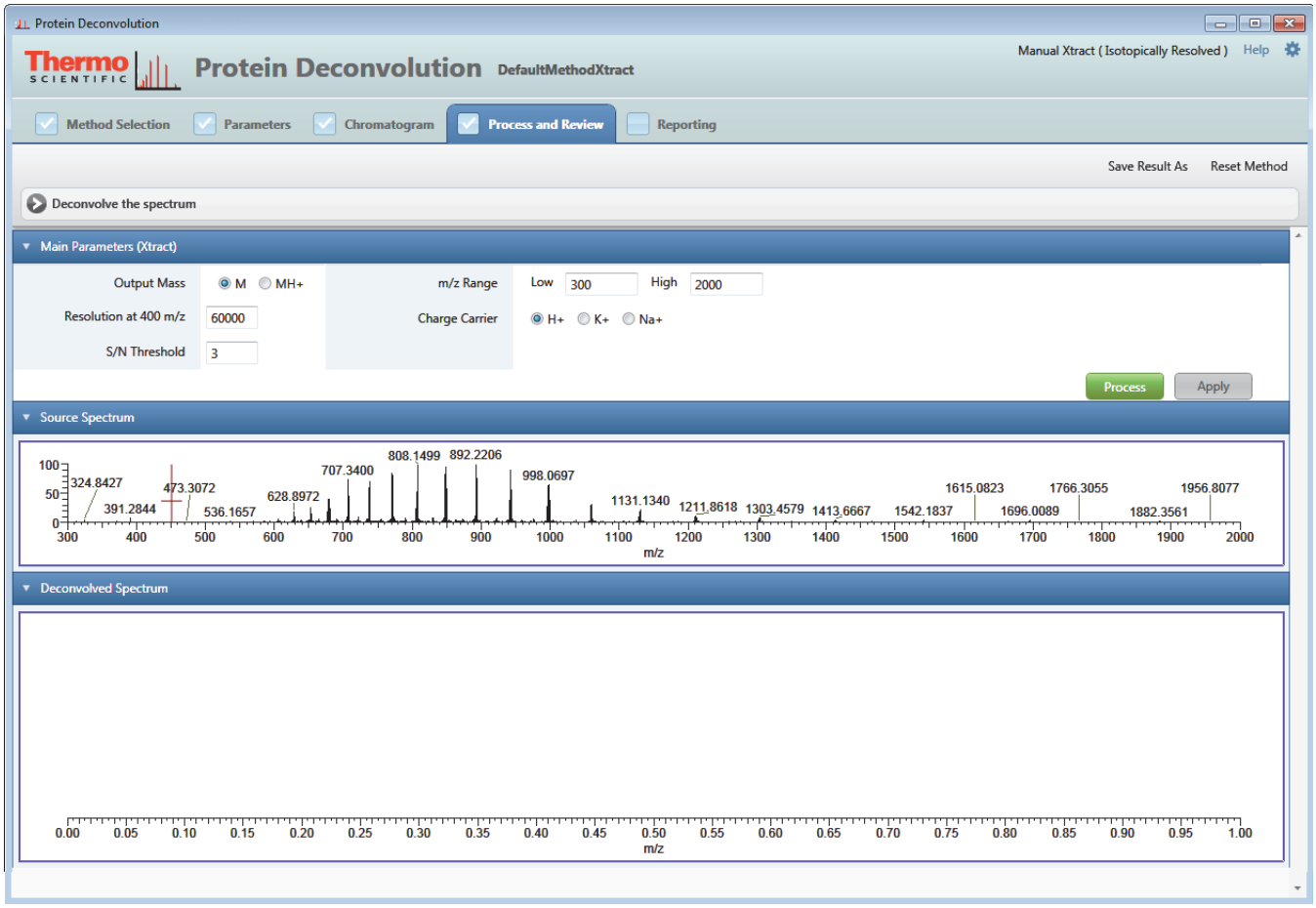
Use the Process and Review page to deconvolve the selected spectrum and to view the resulting data to ensure that the results make sense. You can also export the data into a Microsoft Excel™ spreadsheet for use in other applications.

### ❖ To deconvolve the spectrum

1. If you are not already on the Process and Review page, click the **Process and Review** tab.

   Figure 20 shows the initial Process and Review page.

**Figure 20.** Initial Process and Review page for Xtract deconvolution



The initial Process and Review page displays three panes:

- Main Parameters (Xtract): Displays the same parameters as those in the Main Parameters (Xtract) pane on the Parameters page so that you can adjust them without returning to the earlier page.
- Source Spectrum: Displays the spectrum that you selected in the Source Spectrum pane of the Chromatogram page.
- Deconvolved Spectrum: Displays the deconvolved spectrum resulting from applying the Xtract algorithm.

2. (Optional) Adjust any parameters in the Main Parameters (Xtract) pane. For information on these parameters, see Table 4 on page 18.
3. Click **Process** in the Main Parameters (Xtract) pane.

   The Xtract algorithm finishes processing, producing a deconvolved spectrum and a list of the components that it detected.

The Protein Deconvolution application displays the output spectrum in the Deconvolved Spectrum pane as a set of peaks in mass and intensity. It displays the component list in the Results pane as a table of masses, intensities, charge state information, and quality scores. You can expand each entry in this table to display detailed information about the individual charge states that the entry contains.

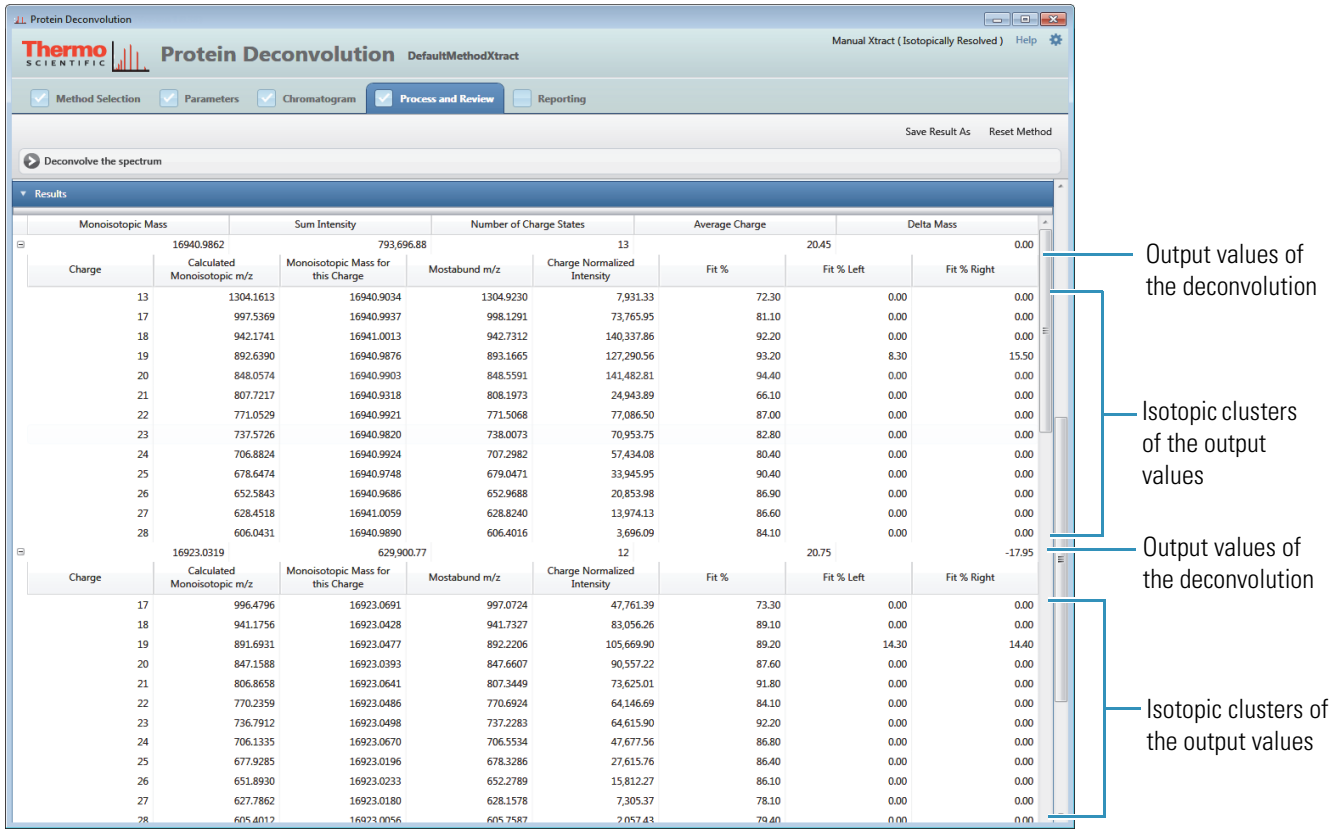
As shown in Figure 21, the peaks in the Monoisotopic Mass, Sum Intensity, Number of Charge States, Average Charge, and Delta Mass columns represent the outputs of the deconvolution. Each peak in the Results table is composed of isotopic clusters. Each isotopic cluster in the original spectrum provides evidence for the cluster in the deconvolved spectrum.

**Figure 21.** Deconvolved spectrum in the Process and Review page for Xtract deconvolution


You can sort the data in each column of the peak table from lowest to highest or highest to lowest by clicking the column header. For example, click the Number of Charge States column header. The Protein Deconvolution application, which initially displays the number of charge states in this column in order from lowest to highest, now displays the number of charge states from highest to lowest. Click again to display the numbers from lowest to highest.

Click the plus sign (+) to the far left of a row in the peak table. As shown in Figure 22, eight new columns appear: Charge, Calculated Monoisotopic m/z, Monoisotopic Mass for This Charge, Mostabund m/z, Charge Normalized Intensity, Fit%, Fit% Left, and Fit% Right. These values are the isotopic clusters that constitute the peaks shown in the five output columns.

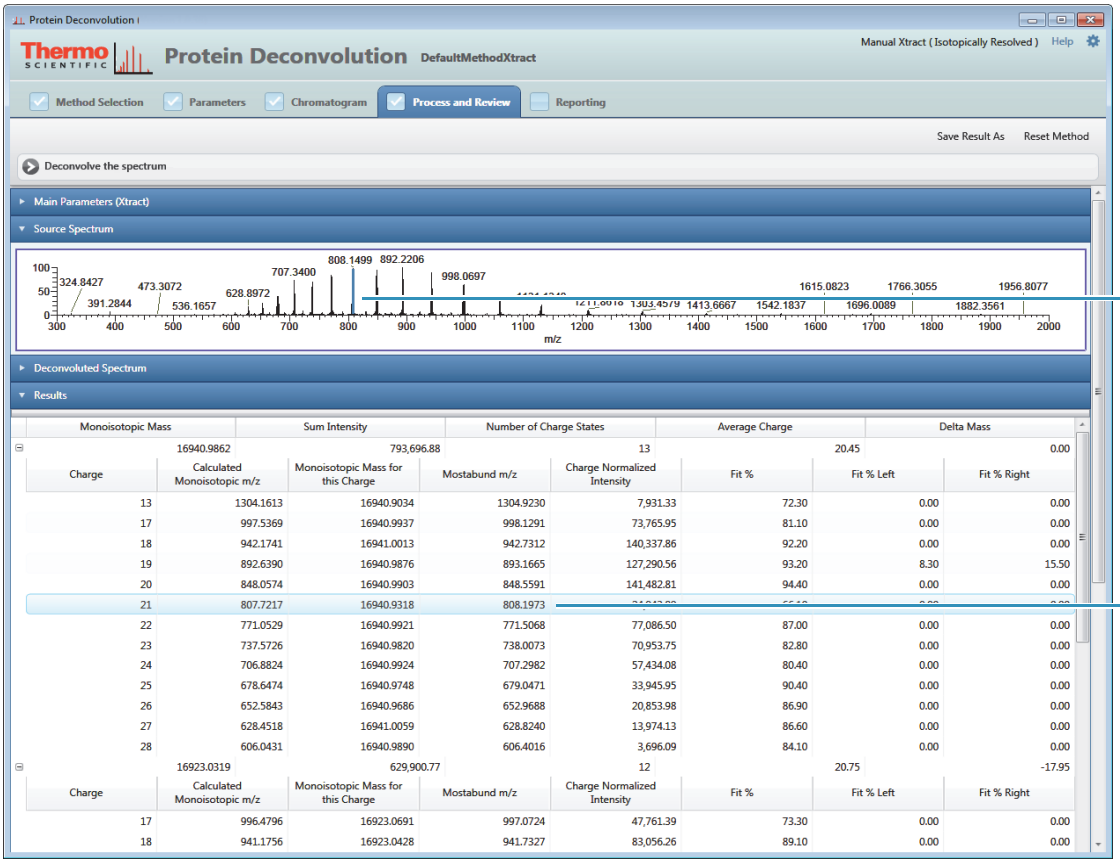
**Figure 22.** Hierarchical table in Results pane for Xtract deconvolution




In addition, the peak table includes the following features:

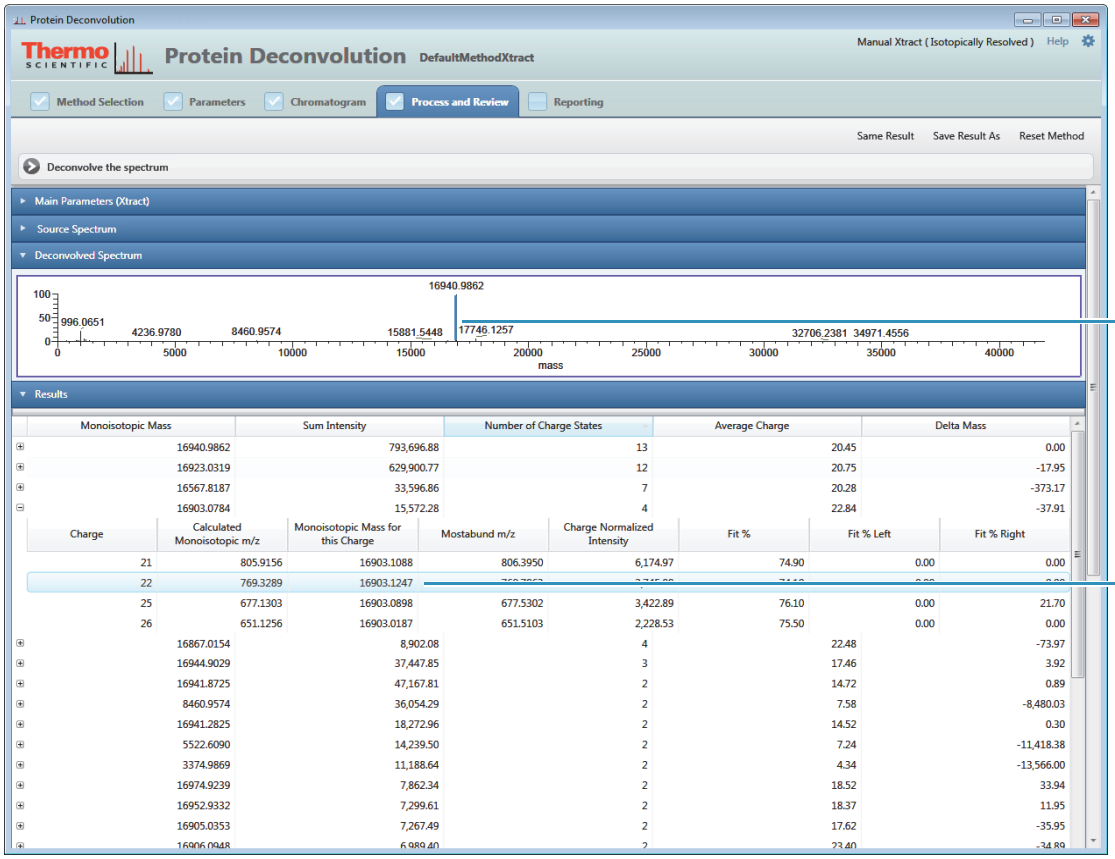
- The Charge column lists the charges of the individual isotopic clusters that comprise the total number shown in the Number of Charge States column.
- The Mostabund m/z column displays the mass-to-charge ratio of the most abundant isotope, or the height of the tallest peak in the isotopic distribution. When you click on a value in this column, the application automatically highlights the corresponding spectral line in the Source Spectrum pane, as shown in Figure 23. You might have to right-click and choose Reset to Scale to see the spectral line. In Figure 23, a blue spectral line marks the selected value of 808.1973 in the Mostabund m/z column.

**Figure 23.** Highlighted spectral line corresponding to a selected value in the Mostabund m/z column




- The Monoisotopic Mass for This Charge column displays the calculated monoisotopic mass for each charge state. When you click on a value in this column, the application automatically zooms the mass-to-charge ratio (*m/z*) of the selected charge state in the Deconvolved Spectrum pane, as shown in Figure 24, highlighting it with a blue line. You might have to zoom in to see the highlighted line; for instructions, see "Selecting the Spectrum to Deconvolve" on page 21.

**Figure 24.** Highlighted mass-to-charge ratio of the selected charge state




Spectral line corresponding to selected value in the Monoisotopic Mass for This Charge column.

Selected value in the Monoisotopic Mass for This Charge column (16903.1247)

- The Fit values are scores given as a confidence value for the listed isotopic clusters.

For more information on the columns in the Results table, see Table 7 on page 38.

### ❖ To adjust the Xtract deconvolution results

1. If you are not satisfied with the deconvolution results, do the following:
   - Adjust the parameters in the Main Parameters (Xtract) pane on either the Process and Review page or the Parameters page, and click **Apply**.

   –or–

   - Return to the Parameters page, adjust the parameters in the Advanced Parameters (Xtract) pane, and click **Apply**.
2. When you finish adjusting the parameters, click **Process** on the Process and Review page again.

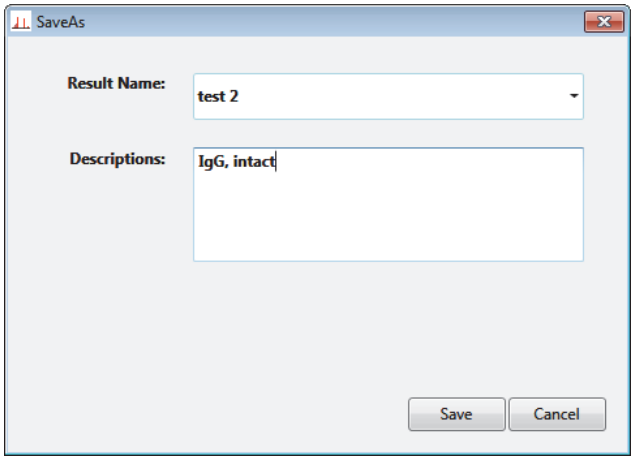
If you are satisfied with the results, you can save them by using the following procedure.

### ❖ To save the results of the deconvolution

1. Click **Save Result As**.
2. In the SaveAs dialog box, do the following:
   a. In the Result Name box, type the name of the results file.
   b. In the Description box, type a brief description of the results.

      The dialog box should resemble that shown in Figure 25.

      **Figure 25.** SaveAs dialog box

   c. Click **Save**.

   The Protein Deconvolution application saves the results of the deconvolution in a file with an .sqlite suffix in the same directory where you stored the RAW files.

   You can also copy and paste any one of the views in this window to a Microsoft PowerPoint™ presentation file.
3. If you want to analyze another averaged spectrum from the same LC/MS data file, navigate back to the Chromatogram pane and follow the instructions in "Selecting the Spectrum to Deconvolve" on page 21.

### ❖ To export the results of the deconvolution

1. Double-right-click anywhere in the Results pane.
2. Choose **Export**.
3. In the Save As dialog box, browse to or type the name of the file to store the results in.
4. Click **Save**.

The Protein Deconvolution application stores the data in the Results pane in an Excel file with an .xls suffix.

# Process and Review Page Parameters for the Xtract Algorithm

The Process and Review page displays parameters that you can set for the protein deconvolution, the source spectrum, the deconvolved spectrum, and a table showing the results of the deconvolution.

Table 7 describes the types of information available on the Process and Review page for Xtract deconvolutions.

**Table 7.** Process and Review page information for Xtract deconvolution (Sheet 1 of 2)

| Parameter | Description |
|---|---|
| **Main Parameters (Xtract) pane** | The parameters in the Main Parameters (Xtract) pane are the same as those in the Main Parameters (Xtract) pane of the Parameters page. For information on the parameters on this page, see Table 4 on page 18. |
| Process | Deconvolves the spectrum with the Xtract algorithm. |
| Apply | Implements the parameter settings that you selected. |
| **Source Spectrum pane** | Displays the selected spectrum before deconvolution. |
| m/z (*x* axis) | Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion. |
| **Deconvolved Spectrum pane** | Displays the deconvolved spectrum. |
| Abundance (*y* axis) | Displays the peak height. |
| Mass (*x* axis) | Displays the actual mass of an ion in atomic mass units. |
| **Results pane** | Displays the masses and intensities of the peaks that the Xtract algorithm detected during the deconvolution, along with their quality scores. |
| Monoisotopic Mass | Displays a weighted average of the monoisotopic masses of each charge state:<br>$Monoisotopic\ Mass = \frac{\sum_{1}^{i}(Monoisotopic\ Mass\ of\ This\ Charge \times Charge\ Normalized\ Intensity)}{Sum\ Intensity}$<br>where *i* is the sequential order of the charge in the Charge column. |
| Sum Intensity | Displays the sum of the intensities of the isotopic clusters in a charge state. |
| Number of Charge States | Displays the number of detected isotopic clusters for a given deconvolved mass. |

**Table 7.** Process and Review page information for Xtract deconvolution (Sheet 2 of 2)

| Parameter | Description |
|---|---|
| Average Charge | Displays the average of the charge numbers in the Charge column. |
| Delta Mass | Displays the difference between the mass of a specific compound and the mass of the highest-intensity compound. |
| Charge | Displays the imbalance between the number of protons (in the nuclei of the atoms) and the number of electrons that a molecular species (or adduct ion) possesses. If the species possesses more protons than electrons, its charge state is positive. If it possesses more electrons than protons, its charge state is negative. |
| Calculated Monoisotopic m/z | Displays the mass-to-charge ratio of the calculated monoisotopic mass for a specific charge state. |
| Monoisotopic Mass for This Charge | Displays the detected monoisotopic mass for a specific charge state. |
| Mostabund m/z | Displays the mass-to-charge ratio of the most abundant isotope, or the height of the tallest peak in the isotopic distribution. |
| Charge Normalized Intensity | Displays the quotient of the intensity divided for this charge by the relevant charge. |
| Fit% | Displays the quality of the match between a measured isotope pattern and an averagine distribution of the same mass. This column displays a value between 0 and 100%.<br>• 0% requires only a poor fit between the measured pattern and the averagine pattern.<br>• 100% requires a very good (even though not exact) fit between the measured pattern and the averagine pattern.<br>A fit factor of 100% means that the measured isotope profile is absolutely identical with a theoretical averagine distribution. |
| Fit% Left | Displays the quality of the match between a measured isotope pattern and an averagine distribution that is one dalton smaller than the calculated monoisotopic mass. |
| Fit% Right | Displays the quality of the match between a measured isotope pattern and an averagine distribution that is one dalton larger than the calculated monoisotopic mass. |

# Displaying a Deconvolution Report

When you click Process on the Process and Review page, the Protein Deconvolution application generates a report displaying several aspects of the deconvolution so that you can track the progression of the data. You can view this report on the Reporting page. You can also save this report as a PDF file.

**Note** The default Xtract parameter settings provide a good balance between sensitivity and report size. If you adjust these parameters so that a report becomes filled with a large number of low-intensity noise peaks, a system without sufficient memory might hang. If your system hangs, restart the application and rerun it with a more restrictive set of parameters.

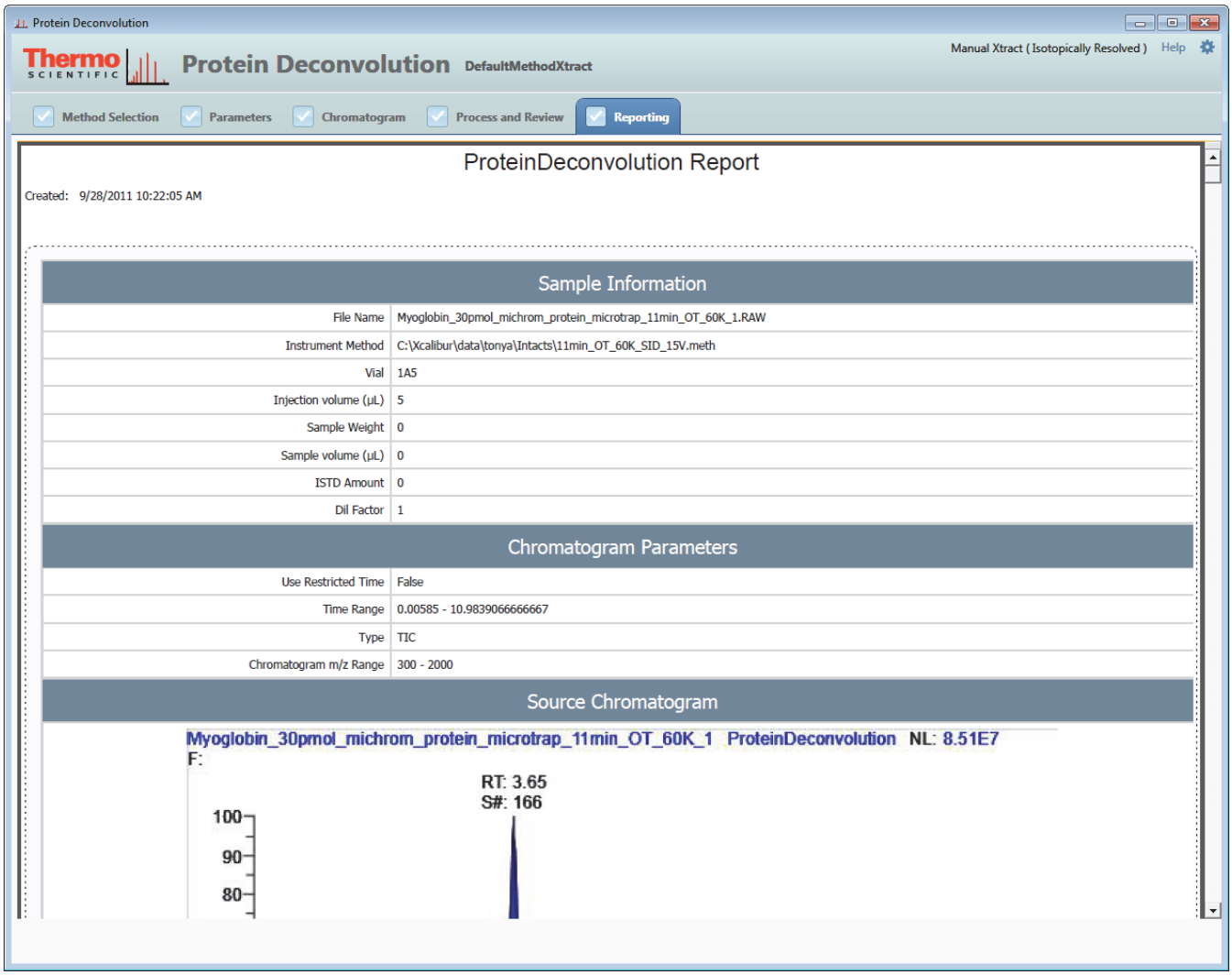
### ❖ To display a report

- Click the **Reporting** tab when you have finished analyzing the data.

  The Reporting page, partially shown in Figure 26, displays a summary of all results for a given data file. It contains the following sections:

  - Sample Information Section
  - Chromatogram Parameters Section
  - Source Chromatogram Section
  - Source Spectrum Section
  - Main Parameters (Xtract) Section
  - Advanced Parameters (Xtract) Section
  - Deconvolved Spectrum Section
  - Xtract Masses Table Section
  - Source Spectrum Evidence Section

**Figure 26.** Partial view of the Reporting page for Xtract deconvolution




### ❖ To save the report in a PDF file

1. Move the cursor near the bottom of the screen.

   The Reporting page toolbar shown in Figure 27 appears.

2. Click the **Show Acrobat** icon,  .

   The Adobe™ Acrobat™ application toolbar appears at the top of the screen.

3. On the Adobe toolbar, click the **Save File** icon,  .

   The Save a Copy dialog box opens.

4. Specify the path and name of a PDF file to store the reports in, and click **Save**.

### ❖ To print a report

1. Hover the cursor near the bottom of the screen.
2. Click the **Print File** icon,  , on the Reporting page toolbar shown in Figure 27.
3. In the Print dialog box, set the appropriate printing parameters, and click **OK**.

## Reporting Page Toolbar

You can activate the Reporting page toolbar, shown in Figure 27, by hovering the cursor near the bottom of the screen.

**Figure 27.** Reporting page toolbar


This toolbar contains the following icons.

**Table 8.** Icons on the Reporting page toolbar

| | |
|---|---|
|  | Opens the Save a Copy dialog box so that you can save the report in a PDF file. |
|  | Opens the Print dialog box so that you can print the reports. |
|  | Displays the previous page. |
|  | Displays the next page. |
|   | Displays the current page, followed by the total number of pages in the report. To move to a different page, double-click the current page, type the new page number, and press ENTER. |
|  | Enlarges the view. |
|  | Shrinks the view. |
|  | Activates an Adobe Acrobat application toolbar so that you can perform the functions available in an Acrobat file. |

## Sample Information Section

The Sample Information section of the report, shown in Figure 28, displays information about the sample that the spectrum was taken from.

**Figure 28.** Sample Information pane for Xtract deconvolution

| Sample Information | |
|---|---|
| File Name | Myoglobin_30pmol_michrom_protein_microtrap_11min_OT_60K_1.RAW |
| Instrument Method | C:\Xcalibur\data\tonya\Intacts\11min_OT_60K_SID_15V.meth |
| Vial | 1A5 |
| Injection volume (µL) | 5 |
| Sample Weight | 0 |
| Sample volume (µL) | 0 |
| ISTD Amount | 0 |
| Dil Factor | 1 |

Table 9 lists the parameters in the Sample Information section. All the parameters in this section are read-only.

**Table 9.** Sample Information section parameters for Xtract deconvolution

| Parameter | Description |
|---|---|
| File Name | Displays the name of the RAW file. |
| Instrument Method | Displays the name of the instrument method file. |
| Vial | Displays the position number of the sample in the autosampler. |
| Injection Volume (µL) | Displays the injection volume of the sample to be injected, in microliters. |
| Sample Weight | Displays the amount of a component in the sample. |
| Sample Volume (µL) | Displays the volume of a component in the sample. |
| ISTD Amount | Specifies the correction for the internal standard amount. If the value in this box is not 0.000, the value is used in an algorithm to correct for a case when any internal standard amounts specified in the active instrument method are correct, but when the amount of internal standard actually in one or more samples is different than the amount specified in the instrument method.<br><br>This correction eliminates the necessity of remaking any samples to the internal standard concentrations or amounts specified in the instrument method and rerunning the samples. |
| Dil Factor | Specifies the dilution factor that was used to prepare the sample. |

## Chromatogram Parameters Section

The Chromatogram Parameters section, shown in Figure 29, displays the settings that you chose in the Chromatogram Parameters pane of the Chromatogram page. For more information on these parameters, see Table 5 on page 27.

**Figure 29.** Chromatogram Parameters section for Xtract deconvolution

| Chromatogram Parameters | |
|---|---|
| Use Restricted Time | False |
| Time Range | 0.00585 - 10.9839066666667 |
| Type | TIC |
| Chromatogram m/z Range | 300 - 2000 |

## Source Chromatogram Section

The Source Chromatogram section, shown in Figure 30, displays the chromatogram contained in the RAW file. It is the same chromatogram that appears in the Chromatogram pane of the Chromatogram page.

**Figure 30.** Source Chromatogram section for Xtract deconvolution

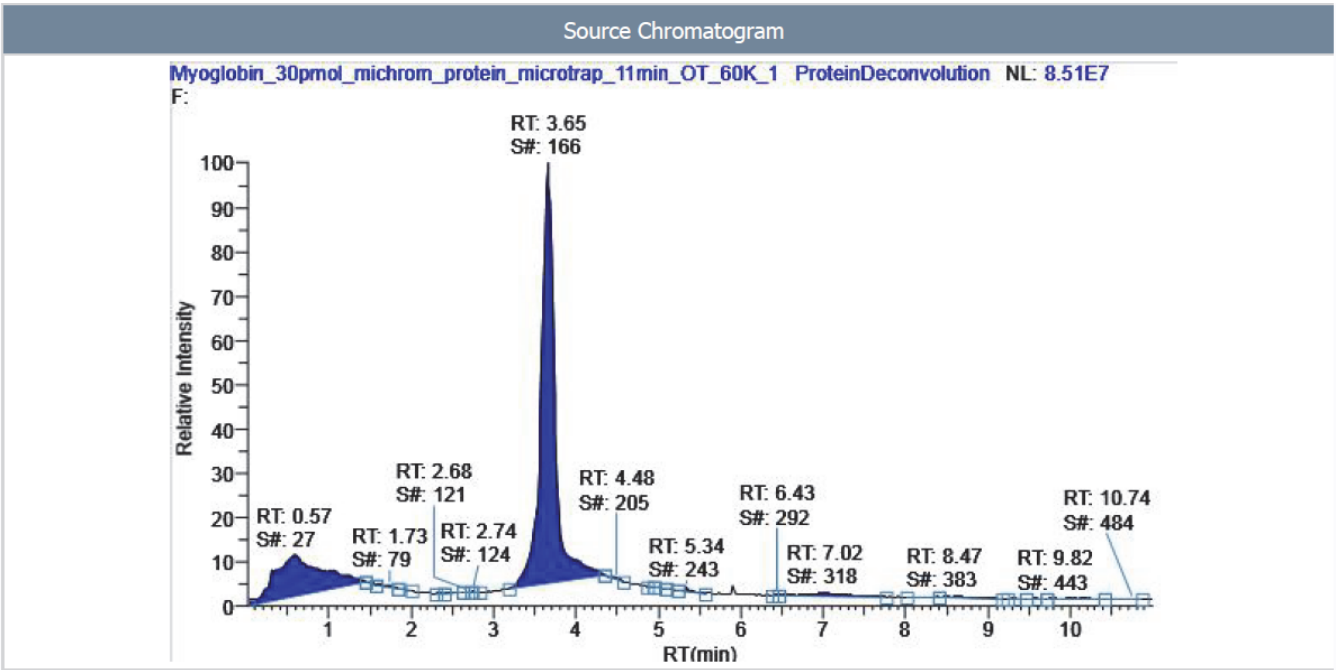


Table 10 lists the parameters in the Source Chromatogram section.

**Table 10.** Source Chromatogram section parameters for Xtract deconvolution

| Parameter | Description |
|---|---|
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| RT (min) (*x* axis) | Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column. |

## Source Spectrum Section

The Source Spectrum section, shown in Figure 31, displays the spectrum that you selected in the Source Spectrum pane of the Chromatogram page.

**Figure 31.** Source Spectrum section for Xtract deconvolution

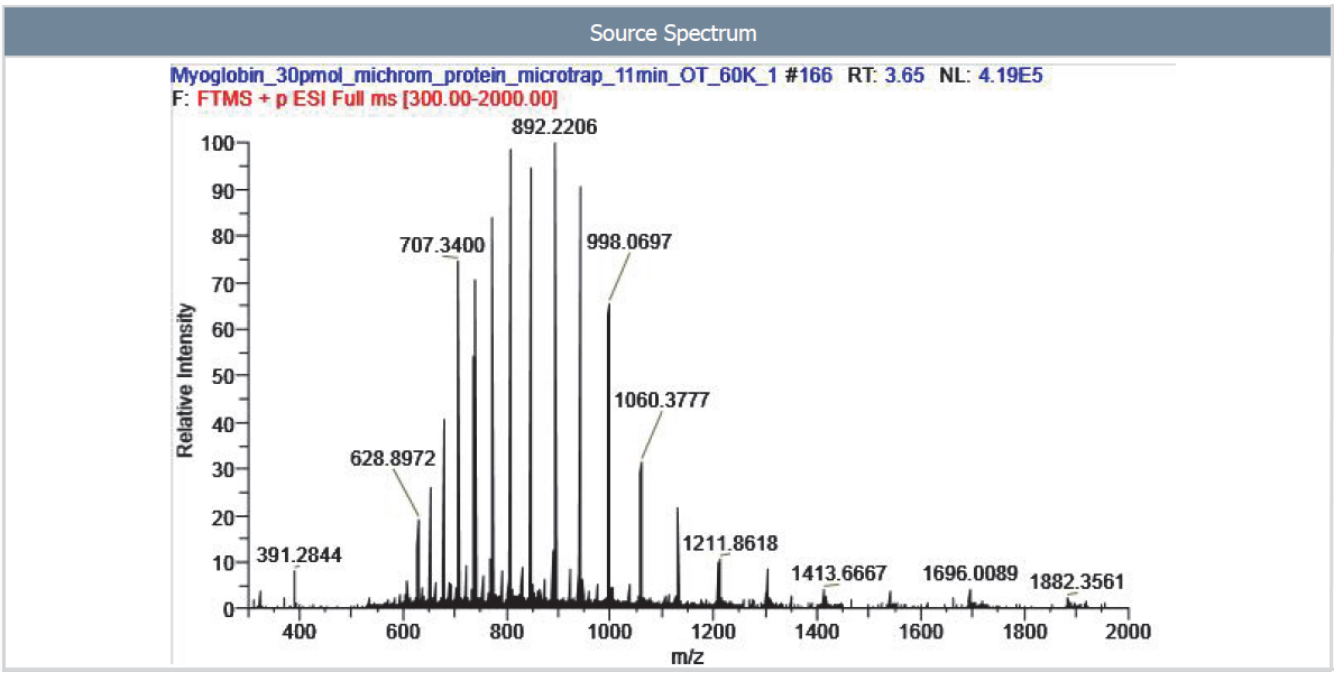


Table 11 lists the parameters in the Source Spectrum section.

**Table 11.** Source Spectrum section parameters for Xtract deconvolution

| Parameter | Description |
|---|---|
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| m/z (*x* axis) | Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion. |

## Main Parameters (Xtract) Section

The Main Parameters (Xtract) section, shown in Figure 32, displays the parameter settings that you selected on the Parameters page for the deconvolution. For information on these parameters, see Table 4 on page 18.

**Figure 32.** Main Parameters (Xtract) section for Xtract deconvolution

| Main Parameters (Xtract) | |
|---|---|
| Output Mass | M |
| Resolution at 400 m/z | 60000 |
| S/N Threshold | 3 |
| m/z Range | 300 - 2000 |
| Charge Carrier | H |

## Advanced Parameters (Xtract) Section

The Advanced Parameters (Xtract) section, shown in Figure 33, displays the parameter settings that you selected in the Advanced Parameters (Xtract) pane of the Parameters page for the deconvolution. For information on these parameters, see Table 4 on page 18.

**Figure 33.** Advanced Parameters (Xtract) section for Xtract deconvolution

| Advanced Parameters (Xtract) | |
|---|---|
| Fit Factor (%) | 44 |
| Remainder Threshold (%) | 25 |
| Consider Overlaps | True |
| Charge Range | 1 - 50 |
| Minimum Intensity | 1 |
| Expected Intensity Error | 3 |

## Deconvolved Spectrum Section

The Deconvolved Spectrum section, shown in Figure 34, displays the same information that appears in the Deconvolved Spectrum pane of the Process and Review page.

**Figure 34.** Deconvolved Spectrum section for Xtract deconvolution

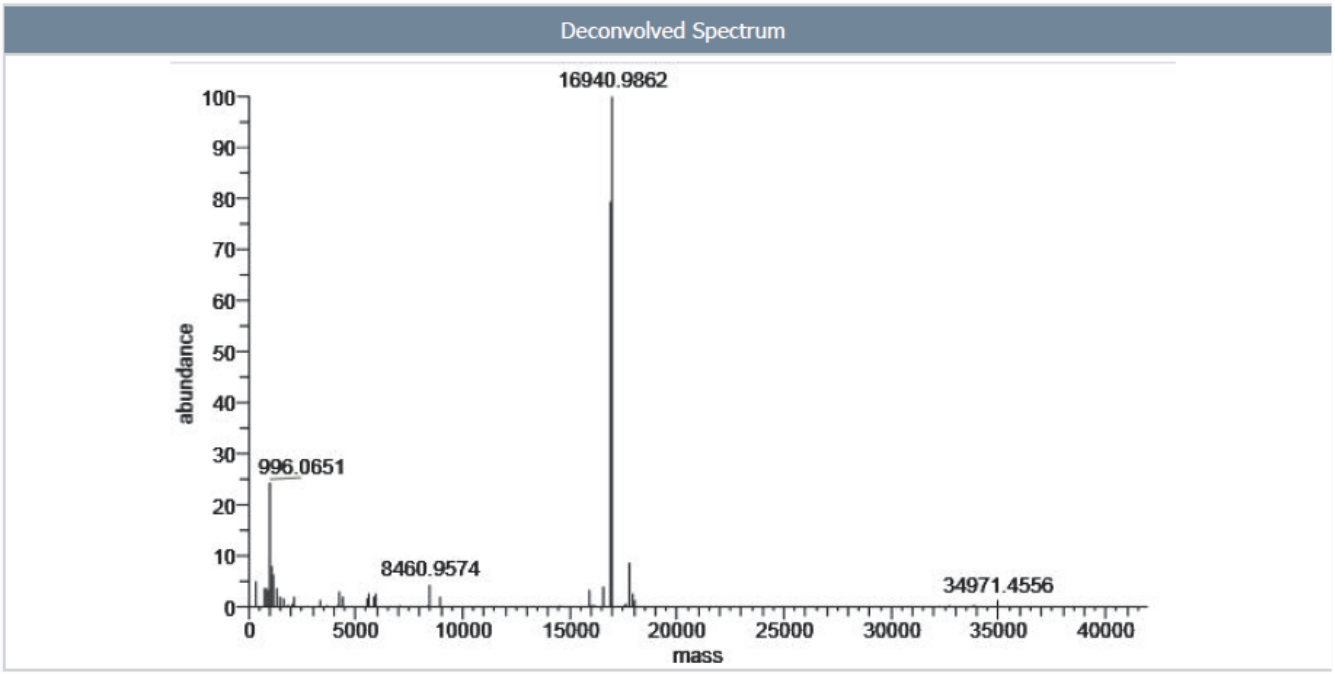



Table 12 lists the parameters in the Deconvolved Spectrum pane.

**Table 12.** Deconvolved Spectrum section parameters for Xtract deconvolution

| Parameter | Description |
|---|---|
| Abundance (*y* axis) | Displays the peak height. |
| Mass (*x* axis) | Displays the actual mass of an ion in atomic mass units. |

## Xtract Masses Table Section

The Xtract Masses Table section, shown in Figure 35, displays the results of the deconvolution. It contains the same columns as those on the Results pane on the Process and Review page. For information on the columns in this table, see Table 7 on page 38.

**Figure 35.** Xtract Masses Table section for Xtract deconvolution

| Xtract Masses Table | | | | |
|---|---|---|---|---|
| Monoisotopic Mass | Sum Intensity | Number of Charge States | Average Charge | Delta Mass |
| 16941.0072 | 840507.53 | 12 | 20.43 | 0.0000 |
| 16923.0527 | 581164.67 | 11 | 20.51 | -17.9545 |
| 1128.9263 | 113721.91 | 1 | 1.00 | -15812.0809 |
| 1128.8614 | 106507.24 | 1 | 1.00 | -15812.1458 |
| 4235.9808 | 55973.63 | 1 | 4.00 | -12705.0264 |
| 390.2771 | 49361.78 | 1 | 1.00 | -16550.7301 |
| 4231.9814 | 42729.39 | 1 | 4.00 | -12709.0258 |
| 1128.7929 | 41047.99 | 1 | 1.00 | -15812.2143 |
| 16940.8478 | 39447.12 | 4 | 13.93 | -0.1594 |
| 892.5224 | 38140.05 | 1 | 1.00 | -16048.4848 |
| 5892.3163 | 36742.61 | 1 | 8.00 | -11048.6909 |
| 706.5789 | 35678.18 | 1 | 1.00 | -16234.4283 |
| 1128.7260 | 35332.31 | 1 | 1.00 | -15812.2812 |
| 16922.8757 | 33654.34 | 2 | 15.13 | -18.1315 |
| 847.8949 | 30735.29 | 1 | 1.00 | -16093.1123 |
| 993.8789 | 25902.67 | 1 | 1.00 | -15947.1283 |
| 810.1378 | 25346.32 | 1 | 1.00 | -16130.8694 |
| 17727.3653 | 21977.45 | 1 | 22.00 | 786.3581 |
| 773.3179 | 21080.95 | 1 | 1.00 | -16167.6893 |
| 803.6185 | 20019.82 | 1 | 1.00 | -16137.3887 |
| 16567.8786 | 18596.26 | 6 | 21.11 | -373.1286 |
| 33882.4087 | 17867.76 | 1 | 30.00 | 16941.4015 |

## Source Spectrum Evidence Section

The Source Spectrum Evidence section, shown in Figure 36, displays a table and an accompanying graph for every scan in the sample. The table shows all the charge states that the Protein Deconvolution application detected. It displays the same parameters as those displayed in the Results pane on the Process and Review page. For information on these parameters, see Table 7 on page 38. The graph shows the isotopic clusters that are associated with a particular component.

The table shown in Figure 36 shows only a partial list of values.

**Figure 36.** Source Spectrum Evidence section for Xtract deconvolution

| Monoisotopic Mass:16940.9831 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Charge | Calculated Monoisotopic m/z | Monoisotopic Mass for this Charge | Mostabund m/z | Charge Normalized Intensity | Fit % | Fit % Left | Fit % Right |
| 17 | 997.5369 | 16940.9937 | 998.1291 | 73809.4153 | 81.10 | 0.00 | 0.00 |
| 18 | 942.1741 | 16941.0013 | 942.7312 | 140337.8561 | 92.20 | 0.00 | 0.00 |
| 19 | 892.6390 | 16940.9876 | 893.1665 | 127290.5611 | 93.20 | 8.30 | 15.50 |
| 20 | 848.0574 | 16940.9903 | 848.5591 | 141482.8145 | 94.40 | 0.00 | 0.00 |
| 21 | 807.7217 | 16940.9657 | 808.1973 | 89391.6805 | 87.10 | 0.00 | 0.00 |
| 22 | 771.0529 | 16940.9921 | 771.5068 | 77086.5009 | 87.00 | 0.00 | 0.00 |
| 23 | 737.5726 | 16940.9820 | 738.0073 | 70953.7535 | 82.80 | 0.00 | 0.00 |
| 24 | 706.8824 | 16940.9924 | 707.2982 | 57434.0825 | 80.40 | 0.00 | 0.00 |
| 25 | 678.6474 | 16940.9748 | 679.0471 | 33945.9472 | 90.40 | 0.00 | 0.00 |
| 26 | 652.5843 | 16940.9686 | 652.9688 | 20853.9762 | 86.90 | 0.00 | 0.00 |
| 27 | 628.4518 | 16941.0059 | 628.8240 | 13975.7511 | 86.60 | 0.00 | 0.00 |
| 28 | 606.0431 | 16940.9890 | 606.4016 | 3696.7739 | 84.10 | 0.00 | 0.00 |

Source Spectrum Evidence

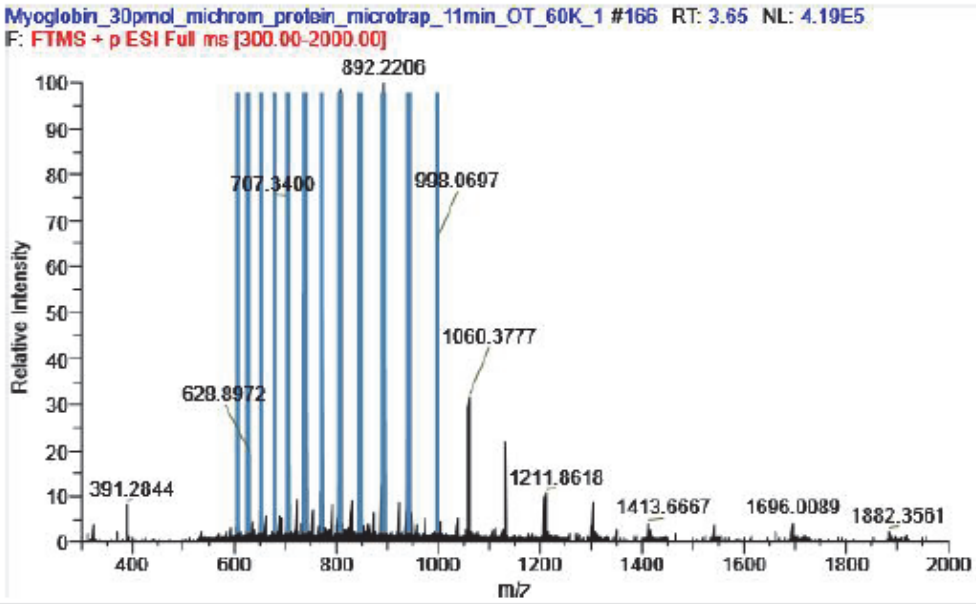



Table 13 lists the parameters in the Source Spectrum Evidence section.

**Table 13.** Source Spectrum Evidence section parameters for Xtract deconvolution

| Parameter | Description |
|---|---|
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| m/z (*x* axis) | Displays the mass-to-charge ratio. |

# Loading Saved Results

If you saved the results of a deconvolution, you can reload them at a later time.

### ❖ To load saved results

1. Click the **Method Selection** tab.
2. In the Experiment Types pane, click **Load Previous Results**.
3. In the Raw Data Directory of the Load Result File pane, type the path and name of the file containing the saved results or click the **Browse** button (...) to browse to the location of the file.
4. In the Select Result Files box, select the name of the .sqlite file containing the results, and click **Load**.

# Deconvolving Isotopically Unresolved Mass Spectra with the ReSpect Algorithm

This chapter explains how to deconvolve isotopically unresolved mass spectra with the ReSpect algorithm.

**Contents**


- Setting Up a ReSpect Protein Deconvolution
- Creating a ReSpect Parameter Set
- Selecting the Spectrum to Deconvolve
- Deconvolving the Spectrum
- Displaying a Deconvolution Report
- Loading Saved Results


When you generate a deconvolved spectrum from an isotopically unresolved intact protein mass spectrum, the source MS spectrum can be a single spectrum from an LC/MS data file, an averaged spectrum from an LC/MS data file, or a single spectrum from a RAW file containing only that spectrum. The ReSpect algorithm then transforms this source spectrum into a mass spectrum and displays it in a new pane labeled with mass units rather than with the mass-to-charge ratio (*m/z*) on the *x* axis. For information on the ReSpect algorithm, see "ReSpect Algorithm" on page 2.

## Setting Up a ReSpect Protein Deconvolution

First select the ReSpect deconvolution algorithm, a RAW file, and a parameter set.

❖ **To set up a protein deconvolution with the ReSpect algorithm**

1. Click the **Method Selection** tab if it is not already selected.

   The Method Selection page contains three panes:

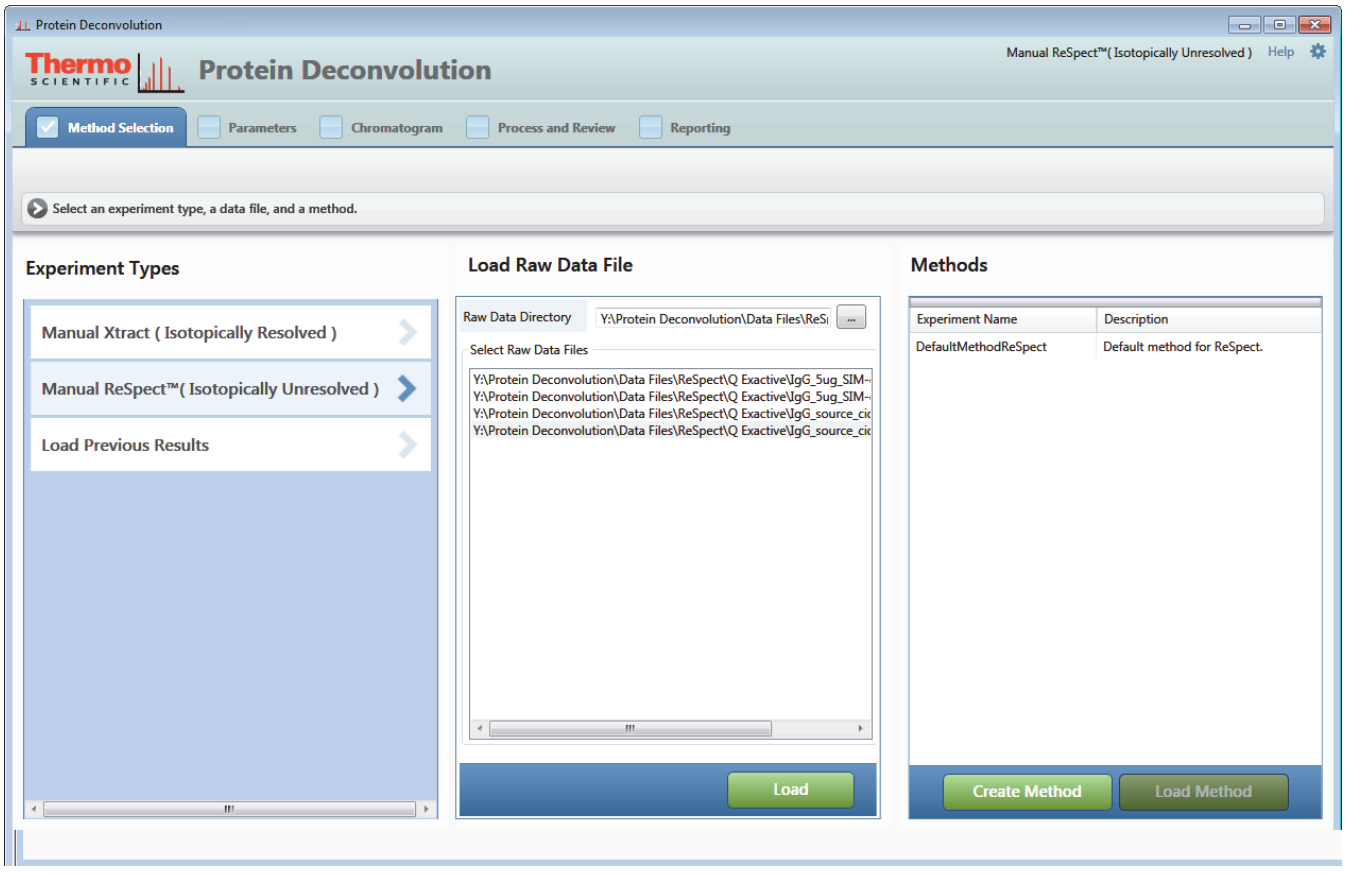
   - Experiment Types pane: Displays the available deconvolution algorithms and a command that you can use to load the saved results of previous deconvolutions.

- Load Raw Data File pane: Displays the list of the available RAW files for the selected algorithm.
- Methods pane: Displays the available parameter sets.

2. In the Experiment Types pane, click **Manual ReSpect (Isotopically Unresolved)**.
3. In the Load Raw Data File pane, select the RAW data file that contains the spectral data for your sample:
   a. In the Directory box, type the path of the RAW file or click the Browse button (...) to browse to the directory containing the file.
   b. In the Select Raw Data Files box, click the name of the RAW file.
   c. Click **Load**.

   Several more tabs appear along the top of the Protein Deconvolution window, and the available parameter sets appear in the Methods pane, as shown in Figure 37.

**Figure 37.** Method Selection page after the RAW file is loaded for ReSpect deconvolution



4. Specify the extraction parameter set to use by doing one of the following in the Methods pane:

   - If one of the existing parameters sets contains the appropriate extraction parameters, select the name of the parameter set of interest and click **Load Method**. The Protein Deconvolution application automatically transfers you to the Chromatogram page if there is a chromatogram and to the Process and Review page if there is a single spectrum. To use the Chromatogram page, follow the instructions in "Selecting the Spectrum to Deconvolve" on page 66. To use the Process and Review page, follow the instructions in "Deconvolving the Spectrum" on page 76.
   - If the existing parameter sets do not contain the appropriate extraction parameters or if there are no existing parameter sets, click **Create Method** to create a new parameter set. The Protein Deconvolution application automatically transfers you to the Parameters page. Follow the instructions in "Creating a ReSpect Parameter Set" on page 54.

## Method Selection Page Parameters

The Method Selection page consists of the Experiment Types, Load Raw Data File, and Methods panes. The parameters in these panes are the same for Xtract and ReSpect deconvolutions.

### Experiment Types Pane Parameters

Table 14 lists the parameters in the Experiment Types pane of the Method Selection page.

**Table 14.** Experiment Types pane parameters

| Parameter | Description |
|---|---|
| Manual Xtract (Isotopically Resolved) | Deconvolves an isotopically resolved mass spectrum with the Xtract algorithm. |
| Manual ReSpect (Isotopically Unresolved) | Deconvolves an isotopically unresolved mass spectrum with the ReSpect algorithm. |
| Load Previous Results | Loads the saved results of a previous deconvolution. |

### Load Raw Data File Pane Parameters

Table 15 lists the parameters in the Load Raw Data File pane of the Method Selection page.

**Table 15.** Load Raw Data File pane parameters

| Parameter | Description |
|---|---|
| Raw Data Directory | Specifies the directory where the RAW file containing the spectrum to deconvolve is located. |
| Select Raw Data Files | Specifies the name of the RAW file containing the spectrum to deconvolve. |
| Load | Loads the specified RAW file. |

### Methods Pane Parameters

Table 16 lists the parameters in the Methods pane of the Method Selection page.

**Table 16.** Methods pane parameters

| Parameter | Description |
|---|---|
| Experiment Name | Specifies the name of the parameter set to use in the deconvolution. |
| Description | Briefly describes the parameter set to use in the deconvolution. |
| Create Method | Activates the Parameters page so that you can specify the parameters for a new parameter set. |
| Load Method | Loads the specified existing parameter set. |

# Creating a ReSpect Parameter Set

When you click Create Method in the Methods pane of the Method Selection page, the Protein Deconvolution application automatically transfers you to the Parameters page.

The Parameters page features two panes containing parameters that control the deconvolution:

- Main Parameters (ReSpect): Displays basic parameters that might change often. These parameters also appear on the Process and Review page.
- Advanced Parameters (ReSpect): Displays parameters that only infrequently need changing. Only experienced users should change these parameters.

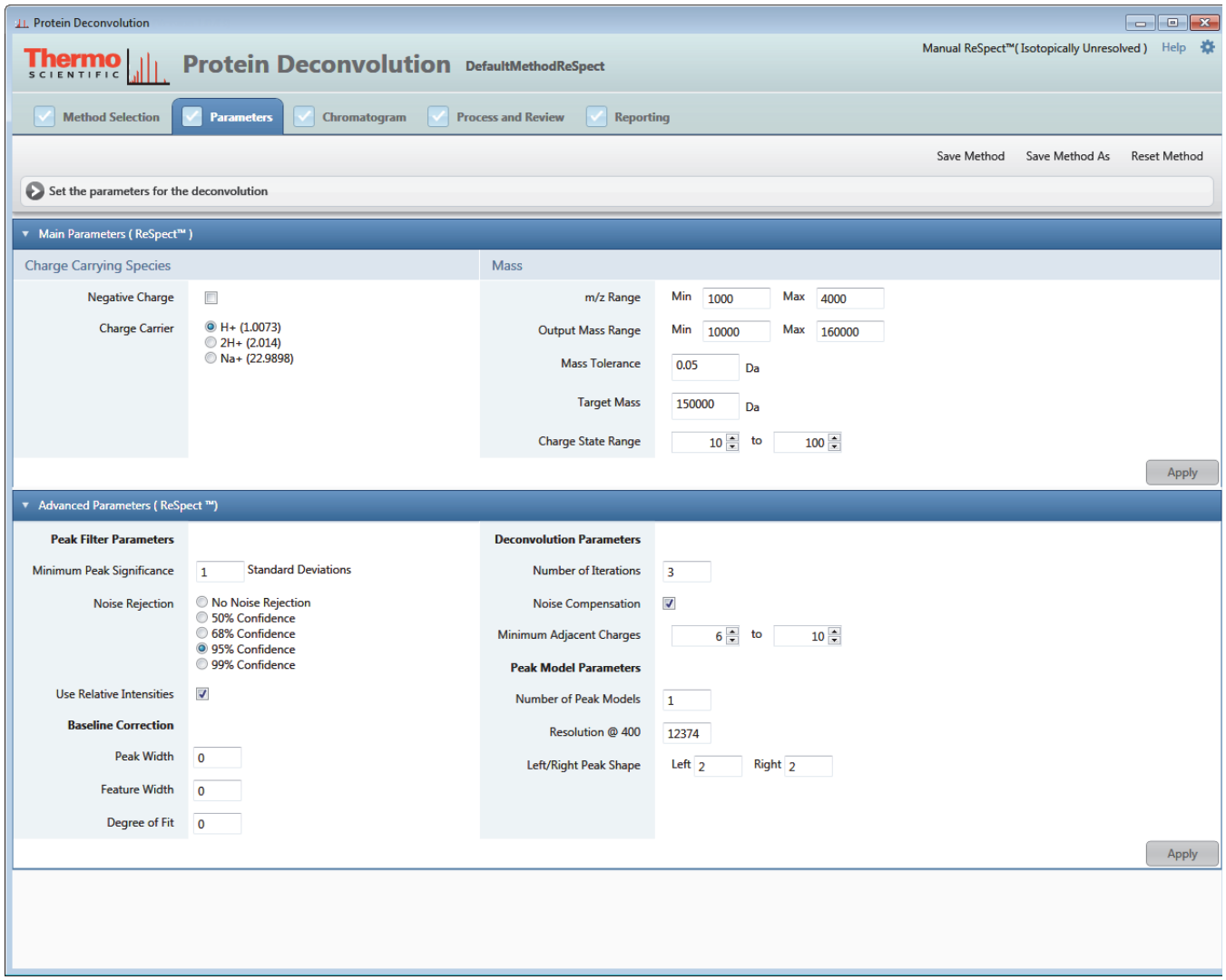
For detailed descriptions of these parameters, see Table 17 on page 60.

### ❖ To create a ReSpect parameter set

1. Click the **Parameters** tab if it is not already selected.

The default settings for the ReSpect algorithm automatically populate the parameter boxes on the Parameters page, as shown in Figure 38.

**Figure 38.** Parameters page for ReSpect deconvolution




2. (Optional) Change the appropriate parameters in the Main Parameters (ReSpect) pane:
   - Negative Charge: Specifies whether the data was acquired in positive charge mode (through protonation) or negative charge mode (through deprotonation) during the ESI process:
     - (Default) Unselected: The data was acquired in positive charge mode.
     - Selected: The data was acquired in negative charge mode.
   - Charge Carrier: Specifies the adduct ions used during ESI processing. Adduct ions bring the charge to the molecule that converts it to an ion.
     - (Default) H+ (1.0073): Specifies that the adduct was hydrogen.

  - 2H+ (2.014): Specifies that the adduct was deuterium.
  - Na+ (22.9898): Specifies that the adduct was sodium.
- m/z Range: Specifies the portion of the input spectrum that the ReSpect algorithm processes.
  - Min: Specifies the lowest end of the input spectrum.
  - Max: Specifies the highest end of the input spectrum.

  The default range is 1000 through 4000.
- Output Mass Range: Specifies the required output mass range.
  - Min: Specifies the lowest end of the mass range.
  - Max: Specifies the highest end of the mass range.

  The default range is 10 000 through 16 000.
- Mass Tolerance: Specifies the global allowable error for the mass-to-charge (*m/z*) values of peaks in a charge state series as they appear in the input spectrum. This parameter compensates for calibration errors and the effects of local noise, peak overlaps, and other sources of mismatches between the model and the actual peak profiles.

  The default is 0.05 Da/charge number.
- Target Mass: Specifies an expected target mass, in daltons, to use in calculating the peak model. This parameter is critical but does not have to be exact; a value within 5 and 10% of the actual target is sufficient for best performance. For samples where the range of masses is broad, choose a mass somewhere in the middle of the range. For example, if the IgG light (~20 kDa), heavy chains (~50 kDa), and the intact antibody are found in the same sample, choose 75 kDa as the target mass.

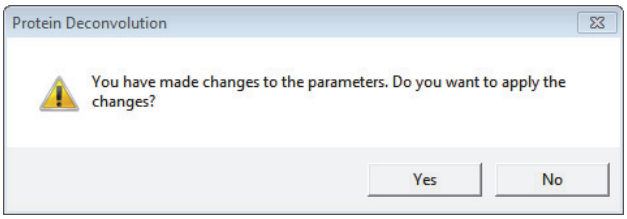
  The default is 150 000 Da.
- Charge State Range: Sets the allowable range for the number of charge states that must appear for a component to be recognized. The ReSpect algorithm rejects potential components with fewer than the minimum or greater than the maximum number of charge states.

  The default range is 10 through 100.

3. If you want to return the parameters in the Main Parameters (ReSpect) pane to their original default settings, click **Reset Method**; otherwise, click **Apply**.

   If you make changes to the parameters on this pane but do not apply them and then click another tab, the message box shown in Figure 39 appears. Click **Yes** to apply the parameter changes or **No** to restore the parameter defaults.

**Figure 39.** Reminder to apply parameters




4. (Optional) If you are an experienced user, change the appropriate parameters in the Advanced Parameters (ReSpect) pane:

   - Minimum Peak Significance: Specifies a significance level, in standard deviations, that determines whether the ReSpect algorithm discards a peak as a noise feature or retains it as a legitimate peak. The ReSpect algorithm retains peaks equal to or greater than this selected significance level. The higher the significance level, the more stringent this filtering is.
   - Noise Rejection: Removes noise and irrelevant features from the list of peaks.
     - No Noise Rejection: Retains all peaks and features.
     - 50% Confidence: Rejects all features up to a significance corresponding to 0.7 standard deviations.
     - 68% Confidence: Rejects all features up to a significance corresponding to 1 standard deviation.
     - (Default) 95% Confidence: Rejects all features up to a significance corresponding to 2 standard deviations.
     - 99% Confidence: Rejects all features up to a significance corresponding to 3 standard deviations.
   - Use Relative Intensities: Determines whether the Protein Deconvolution application calculates the intensity of each peak relative to the noise level of the spectrum in the vicinity of the peak. For more information on this option, see Table 17 on page 60.
     - (Default) Selected: Calculates the intensity of each peak relative to the noise level of the spectrum in the vicinity of the peak.
     - Unselected: Calculates global noise, that is, the noise across the entire spectrum.

     This option is particularly useful with spectra where the noise level varies significantly across the spectrum, especially for highly complex spectra for 150 kDa antibodies. This “noise” is not a product of the instrument but is instead due to the high complexity of the sample.
   - Peak Width: Specifies the half-height width of a typical peak, in data intervals. This value does not need to be exact. The default is 0, which causes the ReSpect algorithm to calculate a peak width automatically.

- Feature Width: Specifies how wide the feature of the baseline should be, in data points. The default is 0, which causes the ReSpect algorithm to calculate a feature width automatically.
- Degree of Fit: Specifies the offset by which to lower or raise the baseline height. This adjustment is non-linear so that its effect is proportional to the underlying noise amplitude. Set Degree of Fit to 0 for automatic computation. Set Degree of Fit to 1 for manual computation. The range is 0.0–2.0. The default is 0.

  Values less than 1 lower the computed baseline, and values greater than 1 raise it.
- Number of Iterations: Specifies how far the deconvolution is to proceed.
  - 0: Use when a crude deconvolution is sufficient. Use only for data with a large variation in peak width or where speed is important.
  - 1: Use where there is a substantial change in peak width across the data to be processed.
  - 2: Use if there is a significant change in peak width across the data or where it is not possible to accurately model the peak profile.
  - (Default) 3: Use when a high-quality deconvolution is required, and there is only a small change in peak width across the region to be processed.
  - 4: Use when extreme deconvolutions are required. This value is only relevant for good signal-to-noise data where there is only a very small change in peak width across the region to be processed. The designed model must also be a good fit to the peak profiles.

  The range is 0 through 4. Non-integer values are allowed. Negative values indicate an absolute number of iterations to perform.

  Decreasing the value for this parameter reduces the computation time. Starting at 0, the computation time roughly doubles for each integer increase.
- Noise Compensation: Determines whether the ReSpect algorithm improves signal detection where the noise level varies across the data.
  - (Default) Selected: Improves signal detection where the noise level varies across the data. The Noise Compensation option is selected by default.
  - Unselected: Does not improve signal detection where the noise level varies across the data.
- Minimum Adjacent Charges: Specifies the minimum number of peaks in a row that must appear at the low and the upper end of the input spectrum if the ReSpect algorithm is to recognize a component as real. The ReSpect algorithm rejects potential components with fewer than this number of adjacent peaks.

  The minimum values for this parameter are 1 and 1, and there is no maximum value. The default values are 6 and 10.

- Number of Peak Models: Controls the resolution of the peak modeling process by dividing the observed *m/z* range into a uniformly spaced set of regions equal to this number. The Protein Deconvolution application generates a single peak model for each of these regions on the basis of the observed *m/z* value and instrument resolution at the midpoint of each region.

  The default is 1.

- Resolution @ 400: Defines the resolution at mass 400. This parameter is read-only.
- Left/Right Peak Shape: Control the sharpness of a peak.

  The default for both the left and the right peak shape is 2.

5. If you want to return the parameters in the Advanced Parameters (ReSpect) pane to their original default settings, click **Reset Method**; otherwise, click **Apply**.

   If you change any parameters in this pane but do not click Apply and then click another tab, the message box shown in Figure 39 on page 57 appears. Click **Yes** to apply the parameter changes or **No** to restore the parameter defaults.

6. Click **Save Method** or **Save Method As** to save the parameter set and give it a name.

   The Save Method command saves current parameter values to the existing method, overwriting any previous values. The Save Method As command saves parameter values to a new method.

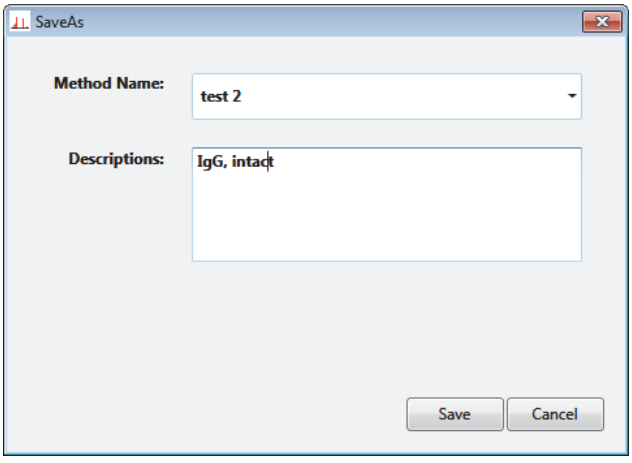
7. In the Save or SaveAs dialog box, do the following:

   a. In the Method Name box, type a name for the parameter set.

   b. In the Description box, briefly describe the parameter set. For example, you might want to describe the sample and the proteins analyzed.

      Figure 40 shows a completed dialog box.

      **Figure 40.** Completed SaveAs dialog box

   c. Click **Save**.

**Note** The Protein Deconvolution application automatically saves all parameter sets that you create to the database in C:\ProgramData\Thermo\ProteinDeconvolution\methods.sqlite. You cannot save individual method files to a directory that you choose.

The next time that you access the Method Selection page and click Manual ReSpect (Isotopically Unresolved), you will see the name of the parameter set that you saved in the Methods pane.

**Note** You cannot delete existing parameter sets from the Methods pane except by deleting the entire file from the C:\ProgramData\Thermo\ProteinDeconvolution\methods.sqlite database.

The application transfers you to the Chromatogram page so that you can select the spectrum to deconvolve. For information on this process, see "Selecting the Spectrum to Deconvolve" on page 21.

## Parameters Page Parameters for the ReSpect Algorithm

Table 17 describes the parameters on the Parameters page for a ReSpect deconvolution.

**Table 17.** Parameters page parameters for ReSpect deconvolution (Sheet 1 of 7)

| Parameter | Description |
|---|---|
| **Main Parameters (ReSpect) pane** | Displays basic parameters that might change often. These parameters also appear on the Process and Review page. |
| **Charge Carrying Species** | Displays parameters that control information about the adduct: what it is and whether it is a plus or minus charge. |
| Negative Charge | Indicates whether the data was acquired in positive charge mode (through protonation) or negative charge mode (through deprotonation) during the ESI process:<br>• Selected: The data was acquired in negative charge mode.<br>• (Default) Unselected: The data was acquired in positive charge mode. |
| Charge Carrier | Specifies the adduct ions used during ESI processing. Adduct ions bring the charge to the molecule that converts it to an ion.<br>• (Default) H+ (1.0073): Specifies that the adduct was hydrogen.<br>• 2H+ (2.014): Specifies that the adduct was deuterium.<br>• Na+ (22.9898): Specifies that the adduct was sodium. |

**Table 17.** Parameters page parameters for ReSpect deconvolution (Sheet 2 of 7)

| Parameter | Description |
|---|---|
| **Mass** | Displays parameters that control the input and output mass-to-charge ratio (*m/z*), mass, and charge state ranges. |
| m/z Range | Specifies the portion of the input spectrum that the ReSpect algorithm processes.<br>• Min: Specifies the lowest end of the input spectrum.<br>Default: 1000<br>• Max: Specifies the highest end of the input spectrum.<br>Default: 4000 |
| Output Mass Range | Specifies the required output mass range:<br>• Min: Specifies the lowest end of the mass range.<br>Default: 10 000<br>• Max: Specifies the highest end of the mass range.<br>Default: 160 000 |
| Mass Tolerance | Specifies the global allowable error for the mass-to-charge (*m/z*) values of peaks in a charge state series as they appear in the input spectrum. This parameter compensates for calibration errors and the effects of local noise, peak overlaps, and other sources of mismatches between the model and the actual peak profiles.<br>Default: 0.05 Da/charge number |
| Target Mass | Specifies an expected target mass, in daltons, to use in calculating the peak model. This parameter is critical but does not have to be exact; a value within 5 and 10% of the actual target is sufficient for best performance. For samples where the range of masses is broad, choose a mass somewhere in the middle of the range. For example, if the IgG light (~20 kDa), heavy chains (~50 kDa), and the intact antibody are found in the same sample, choose 75 kDa as the target mass.<br>Default: 150 000 Da |
| Charge State Range | Sets the allowable range for the number of charge states that must appear for a component to be recognized. The ReSpect algorithm rejects potential components with fewer than the minimum or greater than the maximum number of charge states.<br>Default range: 10 through 100 |

**Table 17.** Parameters page parameters for ReSpect deconvolution (Sheet 3 of 7)

| Parameter | Description |
|---|---|
| **Advanced Parameters (ReSpect) pane** | Displays parameters that only infrequently need changing. Only experienced users should change these parameters. |
| **Peak Filter Parameters** | Displays parameters that control how potential peaks in the spectrum that might be associated with compounds are identified and which ones are excluded as being too small. The Protein Deconvolution application applies these parameters after it applies the Baseline Correction parameters. |
| Minimum Peak Significance | Specifies a significance level, in standard deviations, that determines whether the ReSpect algorithm discards a peak as a noise feature or retains it as a legitimate peak. The ReSpect algorithm retains peaks equal to or greater than this selected significance level. The higher the significance level, the more stringent this filtering is.<br><br>Default: 1 |
| Noise Rejection | Removes noise and irrelevant features from the list of peaks.<br>• No Noise Rejection: Retains all peaks and features.<br>• 50% Confidence: Rejects all features up to a significance corresponding to 0.7 standard deviations.<br>• 68% Confidence: Rejects all features up to a significance corresponding to 1 standard deviation.<br>• (Default) 95% Confidence: Rejects all features up to a significance corresponding to 2 standard deviations.<br>• 99% Confidence: Rejects all features up to a significance corresponding to 3 standard deviations. |

**Table 17.** Parameters page parameters for ReSpect deconvolution (Sheet 4 of 7)

| Parameter | Description |
|---|---|
| Use Relative Intensities | Calculates the intensity of each peak relative to the noise level of the spectrum in the vicinity of the peak. The ReSpect algorithm performs this calculation automatically when you select the Noise Compensation option, which is selected by default.<br><br>The relative intensity reflects the signal-to-noise ratio of a feature and informally attempts to capture its reliability in the deconvolution results. Relative intensity is more effective than intensity if the noise level varies appreciably in different regions of the spectrum, as is often the case in mass spectrometry.<br><br>The Use Relative Intensities parameter mainly acts as a confidence filter to distinguish the peaks in a peak table that are likely to be true signals from those likely to be noise artifacts. For this purpose, relative intensities perform at least as well as intensities and sometimes better.<br><br>Relative intensities are not suitable for quantitative comparisons of signal strengths, so intensities should be used instead. The Use Relative Intensities parameter is therefore not the default option for deisotoping.<br><br>This option is particularly useful with spectra where the noise level varies significantly across the spectrum, especially for highly complex spectra for 150 kDa antibodies. This “noise” is not a product of the instrument but is instead due to the high complexity of the sample.<br><br>• (Default) Selected: Calculates the intensity of each peak relative to the noise level of the spectrum in the vicinity of the peak.<br><br>• Unselected: Does not calculate the intensity of each peak relative to the noise level of the spectrum in the vicinity of the peak. |

**Table 17.** Parameters page parameters for ReSpect deconvolution (Sheet 5 of 7)

| Parameter | Description |
|---|---|
| **Baseline Correction** | Displays parameters that identify and remove a baseline from the spectrum. They perform three functions on a RAW data file:<br>• Remove unwanted positive intensity that is not part of the useful signals.<br>• Compute the noise compensation, which can improve the separation of noise from signals in subsequent processing.<br>• Compute noise estimates for the noise-compensated data.<br>The Protein Deconvolution application applies these parameters before performing any other processing operations. It must perform these functions on raw, unfiltered data. |
| Peak Width | Specifies the half-height width of a typical peak, in data intervals. This value does not need to be exact. The default is 0, which causes the ReSpect algorithm to calculate a peak width automatically.<br>Default: 0 |
| Feature Width | Specifies how wide the feature of the baseline should be, in data points. The default is 0, which causes the ReSpect algorithm to calculate a feature width automatically.<br>Default: 0 |
| Degree of Fit | Specifies the offset by which to lower or raise the baseline height. This adjustment is non-linear so that its effect is proportional to the underlying noise amplitude. Set Degree of Fit to 0 for automatic computation. Set Degree of Fit to 1 for manual computation.<br>Range: 0.0–2.0<br>Default: 0<br>Values less than 1 lower the computed baseline, and values greater than 1 raise it. |

**Table 17.** Parameters page parameters for ReSpect deconvolution (Sheet 6 of 7)

| Parameter | Description |
|---|---|
| **Deconvolution Parameters** | Displays parameters that control the operation of the deconvolution itself. |
| Number of Iterations | Specifies how far the deconvolution is to proceed.<br>• 0: Use when a crude deconvolution is sufficient. Use only for data with a large variation in peak width or where speed is important.<br>• 1: Use where there is a substantial change in peak width across the data to be processed.<br>• 2: Use if there is a significant change in peak width across the data or where it is not possible to accurately model the peak profile.<br>• (Default) 3: Use when a high-quality deconvolution is required, and there is only a small change in peak width across the region to be processed.<br>• 4: Use when extreme deconvolutions are required. This value is only relevant for good signal-to-noise data where there is only a very small change in peak width across the region to be processed. The designed model must also be a good fit to the peak profiles.<br>You can use non-integer values. Negative values indicate an absolute number of iterations to perform.<br>Decreasing the value for this parameter reduces the computation time. Starting at 0, the computation time roughly doubles for each integer increase. |
| Noise Compensation | Determines whether the ReSpect algorithm improves signal detection where the noise level varies across the data.<br>• (Default) Selected: Improves signal detection where the noise level varies across the data.<br>• Unselected: Does not improve signal detection where the noise level varies across the data. |

**Table 17.** Parameters page parameters for ReSpect deconvolution (Sheet 7 of 7)

| Parameter | Description |
|---|---|
| Minimum Adjacent Charges | Specifies the minimum number of peaks in a row that must appear at the lower and the upper end of the spectrum if the ReSpect algorithm is to recognize a component as real. The ReSpect algorithm rejects potential components with fewer than this number of adjacent peaks.<br><br>Minimum values: 1 and 1<br><br>Maximum values: None<br><br>Default values: 6 and 10 |
| **Peak Model Parameters** | Displays parameters that place restrictions on the width and shape that a peak must have to be associated with a compound. |
| Number of Peak Models | Controls the resolution of the peak modeling process by dividing the observed *m/z* range into a uniformly spaced set of regions equal to this number. The Protein Deconvolution application generates a single peak model for each of these regions on the basis of the observed *m/z* value and instrument resolution at the midpoint of each region.<br><br>Default: 1 |
| Resolution @ 400 | Defines the resolution at mass 400. This parameter is read-only. |
| Left/Right Peak Shape | Defines the sharpness of a peak.<br><br>Default for both the left and the right peak shape: 2 |

# Selecting the Spectrum to Deconvolve

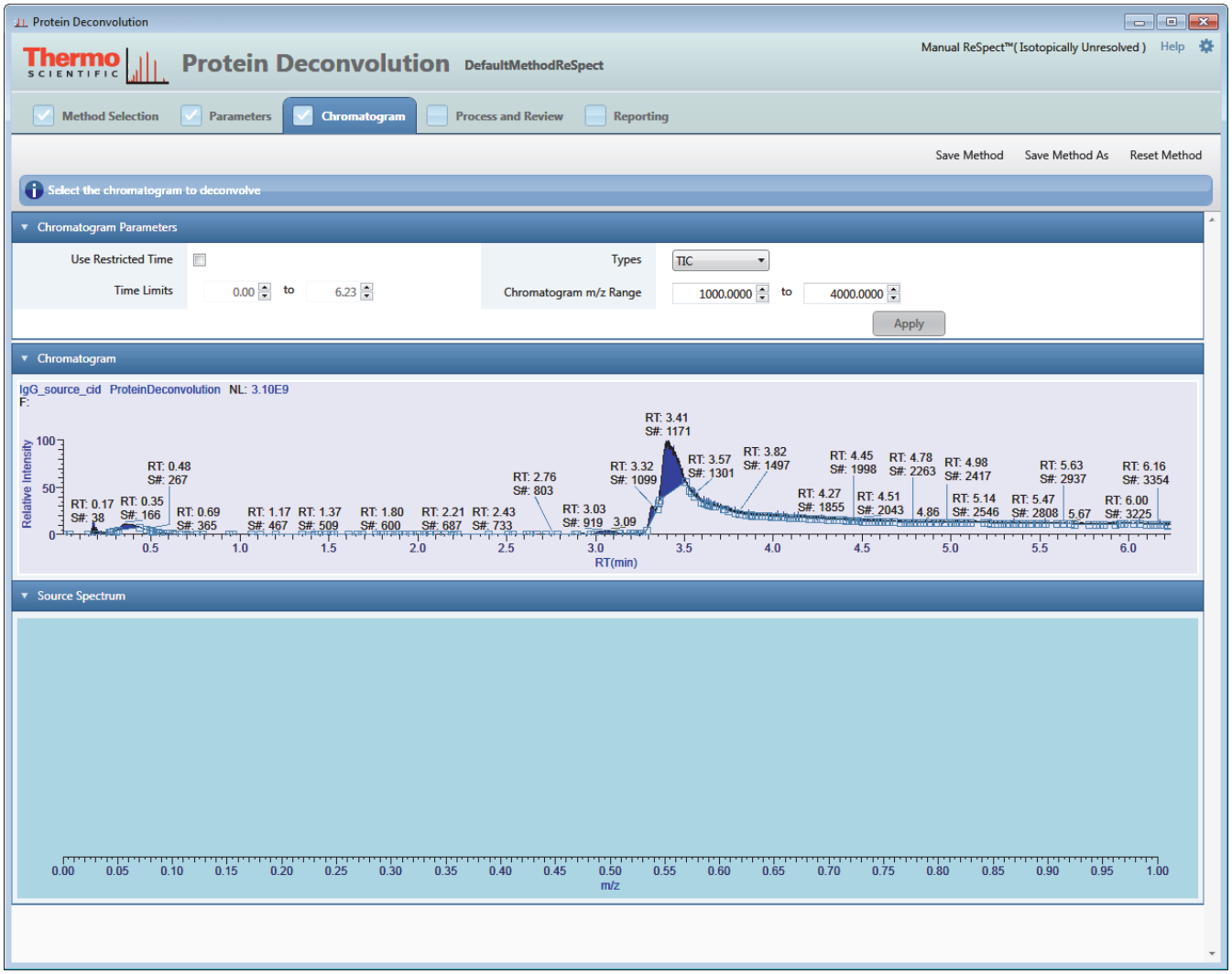
When you click Save Method or Save Method As after you have set the parameters on the Parameters page or when you load an existing parameter set and click Load Method in the Method Selection page, the Protein Deconvolution application automatically transfers you to the Chromatogram page, shown in Figure 41. Use the Chromatogram page to select the best possible spectrum for the target protein for deconvolution.

### ❖ To select the spectrum to deconvolve

1. Click the **Chromatogram** tab, if it is not already selected.

   Figure 41 shows the Chromatogram page.

**Figure 41.** Chromatogram page for ReSpect deconvolution




The Chromatogram page displays three panes:

- Chromatogram Parameters: Contains parameters that you can use to adjust the view in the Chromatogram pane.
- Chromatogram: Displays a chromatogram of the data in the RAW file. A chromatogram view shows the intensities of one or more masses as a function of time. By default, the Chromatogram pane displays a TIC chromatogram, as shown in Figure 41. The chromatogram is fully magnified. You can use the zooming and averaging functions in this pane to generate a spectrum (for instructions, see step 4).
- Source Spectrum: Displays the spectrum to deconvolve, either single-scan or averaged, that you selected in the Chromatogram pane. The mass spectrum in this pane is empty until you select a region in the chromatogram.

2. (Optional) Use the parameters in the Chromatogram Parameters pane to adjust the chromatogram displayed in the Chromatogram pane.

   - Use Restricted Time: Determines whether Protein Deconvolution application zooms the part of the chromatogram that you designate with the Time Limits parameters.
     - Selected: Zooms the designated part of the chromatogram.
     - (Default) Unselected: Displays the entire chromatogram.
   - Time Limits: Specifies the beginning and the end of the chromatogram that you want to zoom.

     The default is 0.00 for both limits.

     This parameter is only available when you select the Use Restricted Time check box.
   - Types: Specifies the type of chromatogram to display in the Chromatogram pane:
     - (Default) TIC: Displays a total ion current chromatogram.
     - BPC: Displays a base peak chromatogram. The base peak is the largest peak in a spectrum.

     For information on these types of chromatograms, see Table 18 on page 73.
   - Chromatogram m/z Range: Specifies the range of mass-to-charge (*m/z*) values used as input to the chromatogram. The ReSpect algorithm ignores the portions of the spectrum outside this range.

     The default is 0.0000 for both limits.

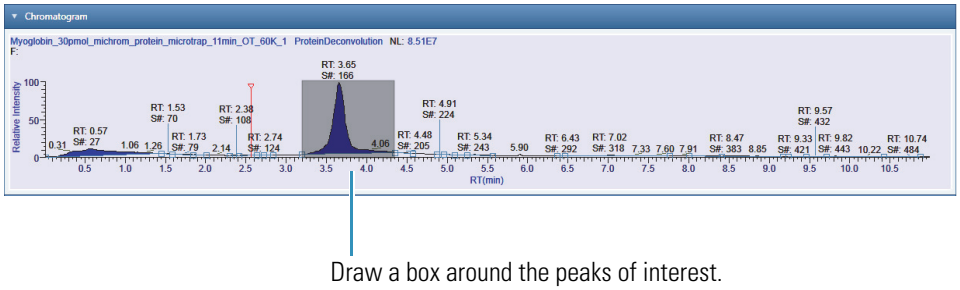
3. If you want to return the parameters in the Chromatogram Parameters pane to their original default settings, click **Reset Method**; otherwise, click **Apply**.

   If you change any parameters in this pane but do not click Apply and then click another tab, the message box shown in Figure 39 on page 57 appears. Click **Yes** to apply the parameter changes or **No** to restore the parameter defaults.

4. (Optional) Adjust the view in the Chromatogram pane.

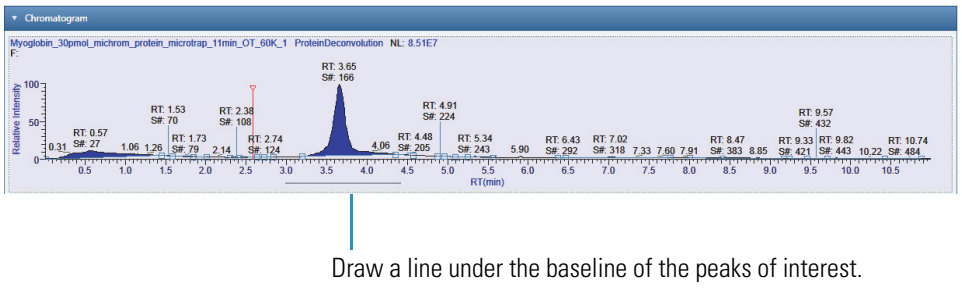
   - To enlarge the view to see more detail, do one of the following:
     - Right-click and choose **Mode > Auto Zooming** from the shortcut menu if it is not already selected, and drag the red cross-shaped cursor over the peak or peaks of interest to form a box, as shown in Figure 42.

**Figure 42.** Enlarging an area by drawing a box around the peaks of interest




– Keeping the left mouse button pressed, draw a line beneath the baseline of the peaks of interest, as shown in Figure 43.

**Figure 43.** Enlarging an area by drawing a line beneath the baseline of the peaks of interest




– Right-click and choose **Zoom In** from the shortcut menu to zoom the entire chromatogram.

If there is no obvious chromatographic peak, change the limits of the m/z Range parameter in the Parameters pane to find it.

- To shrink the view of the entire spectrum, right-click and choose **Zoom Out**.
- To reset the view to the original spectrum, right-click and choose **Reset Scale**.

5. Create a spectrum in the Source Spectrum pane by doing one of the following:

- In the Chromatogram pane, place the red cross-shaped cursor on the chromatogram to select a single scan and to display the associated mass spectrum at that time point, as shown in Figure 16 on page 25. You can use the left and right arrow keys to move to the previous or next time point in the chromatogram. The spectrum window automatically updates.

–or–

- Select a region of the chromatogram to display an averaged spectrum for all the scans in the selected region in the Source Spectrum pane:

  i. Right-click and choose **Mode > Averaging**.

  ii. Drag the red cross-shaped cursor across the area of interest, as shown in Figure 44.

The horizontal line of this cursor aids in assessing peak height. The Protein Deconvolution application calculates an average spectrum for this interval.

The averaging method is better suited to complex data than the single-scan method. It is recommended for ReSpect deconvolution.

**Tip** (Optional) You can perform step 1 through step 5 in Qual Browser in the Xcalibur data system, and then right-click and choose **Export > Write to RAW File** so that you can import the file into the Protein Deconvolution application.

The spectrum appears in the Source Spectrum pane of the Chromatogram page, as shown in Figure 44.

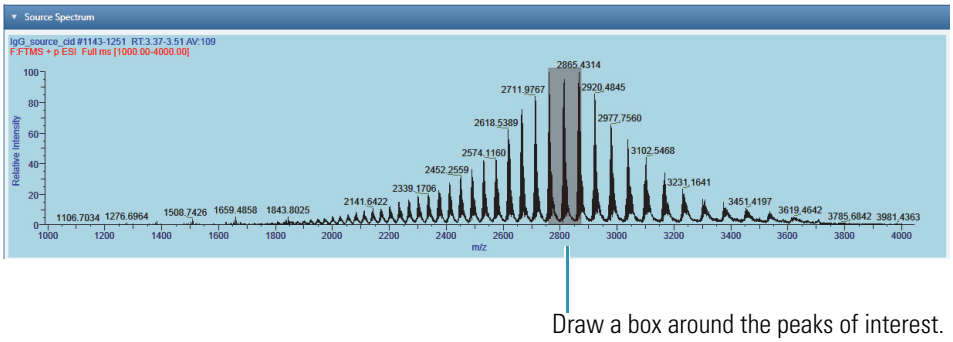
**Figure 44.** Spectrum in the Source Spectrum pane of the Chromatogram page for ReSpect deconvolution

The Source Spectrum pane shows the actual spectrum, either single-scan or averaged, to be deconvolved. This spectrum also appears in the Process and Review pane for deconvolution. The ReSpect algorithm can deconvolve only profile spectra.

- Centroid data represents mass spectral peaks in terms of two parameters: the centroid (the weighted center of the mass) and the intensity (the normalized area of the peak). The data is displayed as a bar graph.
- Profile data represents the entire spectrum as a succession of points in *m/z* and intensity. The data is displayed as a line.

6. (Optional) Adjust the view in the Source Spectrum pane if necessary.
   - To enlarge the view to see more detail, do one of the following:
     – Drag the red cross-shaped cursor over the peak or peaks of interest to form a box, as shown in Figure 45.

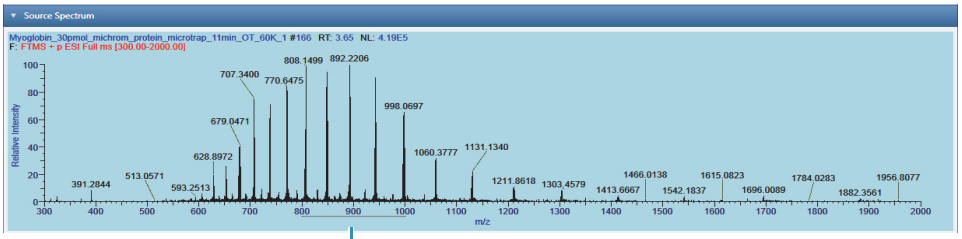
**Figure 45.** Enlarging an area by drawing a box around the peaks of interest




Draw a box around the peaks of interest.

     – Keeping the left mouse button pressed, draw a line beneath the baseline of the peaks of interest, as shown in Figure 46.

**Figure 46.** Enlarging an area by drawing a line beneath the baseline of the peaks of interest

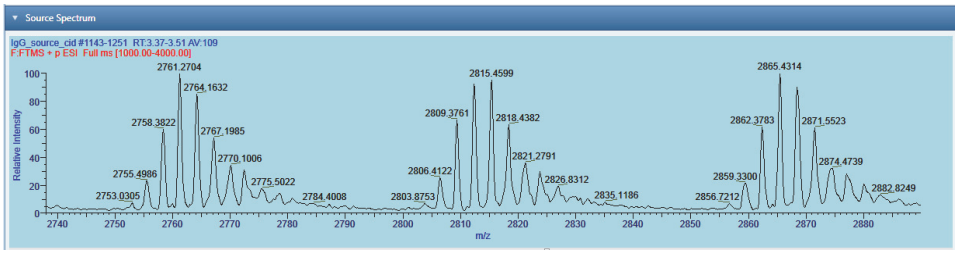



Draw a line under the baseline of the peaks of interest.

     – Right-click and choose **Zoom In** to enlarge the view of the entire spectrum.

Figure 47 gives an example of the enlarged peaks.

**Figure 47.** Enlarged peaks in the Source Spectrum for ReSpect deconvolution




- To shrink the view of the entire spectrum, right-click and choose **Zoom Out**.
- To reset the view to the original spectrum, right-click and choose **Reset Scale**.

Unlike adjustments in the Chromatogram pane, which you use to select a spectrum for processing, adjustments in the Source Spectrum pane do not affect the spectrum that the Protein Deconvolution application deconvolves. In particular, they do not change the *m/z* range that the deconvolution algorithm uses.

7. When the spectrum is suitable for ReSpect processing, click the **Process and Review** tab, and follow the instructions in "Deconvolving the Spectrum" on page 76.

## Obtaining the Best Results with the ReSpect Algorithm

Low, outlying peaks in the source spectrum are less accurate than high peaks and fade into noise. Follow these suggestions to increase the stringency of the deconvolution, decrease noise, and produce better results:

- Use the most abundant peaks in the distribution for deconvolution.
- Narrow the mass-to-charge (*m/z*) range as much as possible.
- In the Main Parameters (ReSpect) pane of the Process and Review page or the Parameters page, adjust the values of the Output Mass Range parameter. Harmonics (overtones) are an artifact of FTMS detectors. They are normal in a distribution, but you can avoid them by narrowing the range to the area around the target mass.
- In the Main Parameters (ReSpect) pane of the Process and Review page or the Parameters page, set the Mass Tolerance parameter to a lower value, in daltons, to make the output results cleaner. When you decrease this value, the delta mass value for each charge state also drops.
- In the Advanced Parameters (ReSpect) pane of the Parameters page, raise the values of the Minimum Adjacent Charges parameter.

## Chromatogram Page Parameters

Table 18 lists the parameters that are available on the Chromatogram page for ReSpect deconvolutions.

**Table 18.** Chromatogram page parameters for ReSpect deconvolution (Sheet 1 of 3)

| Parameter | Description |
|---|---|
| **Chromatogram Parameters pane** | Displays the parameters that govern the appearance of the chromatogram in the Chromatogram pane. |
| Use Restricted Time | Determines whether the Protein Deconvolution application zooms the part of the chromatogram that you define with the Time Limits parameters.<br>• Selected: Zooms the specified part of the chromatogram.<br>• (Default) Unselected: Displays the entire chromatogram. |
| Time Limits | Specifies the beginning and the end of the chromatogram that you want to zoom.<br>The default values for both limits depend on the data in the RAW file.<br>This parameter is only available when you select the Use Restricted Time parameter. |

**Table 18.** Chromatogram page parameters for ReSpect deconvolution (Sheet 2 of 3)

| Parameter | Description |
|---|---|
| Types | Determines the type of chromatogram displayed in the Chromatogram pane:<br>• (Default) TIC: Displays a total ion current chromatogram, which shows the summed intensity across the entire range of masses being detected at every point in the analysis. The range is typically several hundred mass-to-charge units or more. In complex samples, the TIC chromatogram often provides limited information as multiple analytes elute simultaneously, obscuring individual species.<br>A TIC in combination with a narrow *m/z* range is effectively an extracted ion chromatogram (XIC).<br>• BPC: Displays a base peak chromatogram, which shows only the most intense peak in each spectrum. This means that the base peak chromatogram represents the intensity of the most intense peak at every point in the analysis. Base peak chromatograms often have a cleaner look and are therefore more informative than TIC chromatograms because the background is reduced by focusing on a single analyte at every point.<br>For intact protein spectra, the TIC often looks better. The BPC is usually best for smaller molecules where all of the signal exists in a single charge state. |
| Chromatogram m/z Range | Specifies the range of mass-to-charge (*m/z*) values used as input to the chromatogram. The ReSpect algorithm ignores the portions of the spectrum outside this range.<br>The default values for both limits depend on the data in the RAW file. |
| Apply | Implements the parameter settings that you selected. This button is only available if you have changed any parameter settings in the Chromatogram Parameters pane. |
| **Chromatogram pane** | Displays the chromatogram contained in the RAW file. |
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| RT (min) (*x* axis) | Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column. |

**Table 18.** Chromatogram page parameters for ReSpect deconvolution (Sheet 3 of 3)

| Parameter | Description |
|---|---|
| **Source Spectrum pane** | Displays the spectrum that you selected. |
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| m/z (*x* axis) | Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion. |

## Chromatogram Pane Shortcut Menu

When you right-click in the Chromatogram pane, a shortcut menu appears that contains the commands listed in Table 19.

**Table 19.** Chromatogram page shortcut menu

| Parameter | Description |
|---|---|
| Mode | Determines whether dragging the cursor zooms or selects a range of scans to average.<br>• Averaging: Averages the spectra for all the scans in the region that you drag the cursor over in the Chromatogram pane and displays them in the Source Spectrum pane.<br>• (Default) Auto Zooming: Enlarges the area that you drag the cursor over in the Chromatogram pane without changing the view displayed in the Source Spectrum pane. |
| Scale | Restores the original chromatogram that first appeared in the Chromatogram pane. |
| Copy | Copies the view in the Chromatogram pane. |
| Zoom Out | Shrinks the view in the Chromatogram pane two times. |
| Zoom In | Enlarges the view in the Chromatogram pane two times. |

## Chromatogram Pane Header

The header in the Chromatogram pane displays the following information. The example values come from Figure 44 on page 70.

- The name of the RAW file, for example, IgG_source_cid
- The name of the product, Protein Deconvolution

- NL: The the intensity of the most abundant peak in the entire LC/MS run, for example, 3.10E9
- F: The scan filter used during the LC/MS run. The scan filter indicates the type of mass analyzer used to acquire the data in the raw data file and the ionization technique used. If this field is blank, no scan filter was used.

## Source Spectrum Pane Shortcut Menu

When you right-click in the Source Spectrum pane, a shortcut menu appears that contains all of the commands in Table 19 except Mode, but they apply to the Source Spectrum pane rather than the Chromatogram pane. For information on these commands, see Table 19 on page 75.

## Source Spectrum Pane Header

The header in the Source Spectrum pane displays the following information. The example values come from Figure 44 on page 70.

- Name of the RAW file, for example, IgG_source_cid
- Scan number or range of scan numbers, for example, #1138 –1290
- RT: Retention time, which is the time in the mass chromatogram when any particular precursor ion is observed, for example, 3.37 – 3.52
- AV: The number of samples, for example, 123. AV does not appear when you use the default Auto Zooming mode in the ReSpect algorithm.
- F: The scan filter used during the LC/MS run, for example, FTMS + p ESI Full ms [1000.00–4000.00]. The scan filter indicates the type of mass analyzer used to acquire the data in the raw data file and the ionization technique used. If this field is blank, no scan filter was used.

# Deconvolving the Spectrum

When you arrive at the Process and Review page, shown in Figure 48, you have already selected the chromatogram and source spectrum on the Chromatogram page. You can zoom in and out of these views, but you cannot change them on the Process and Review page. You must manually navigate back to the Chromatogram pane to change these views.

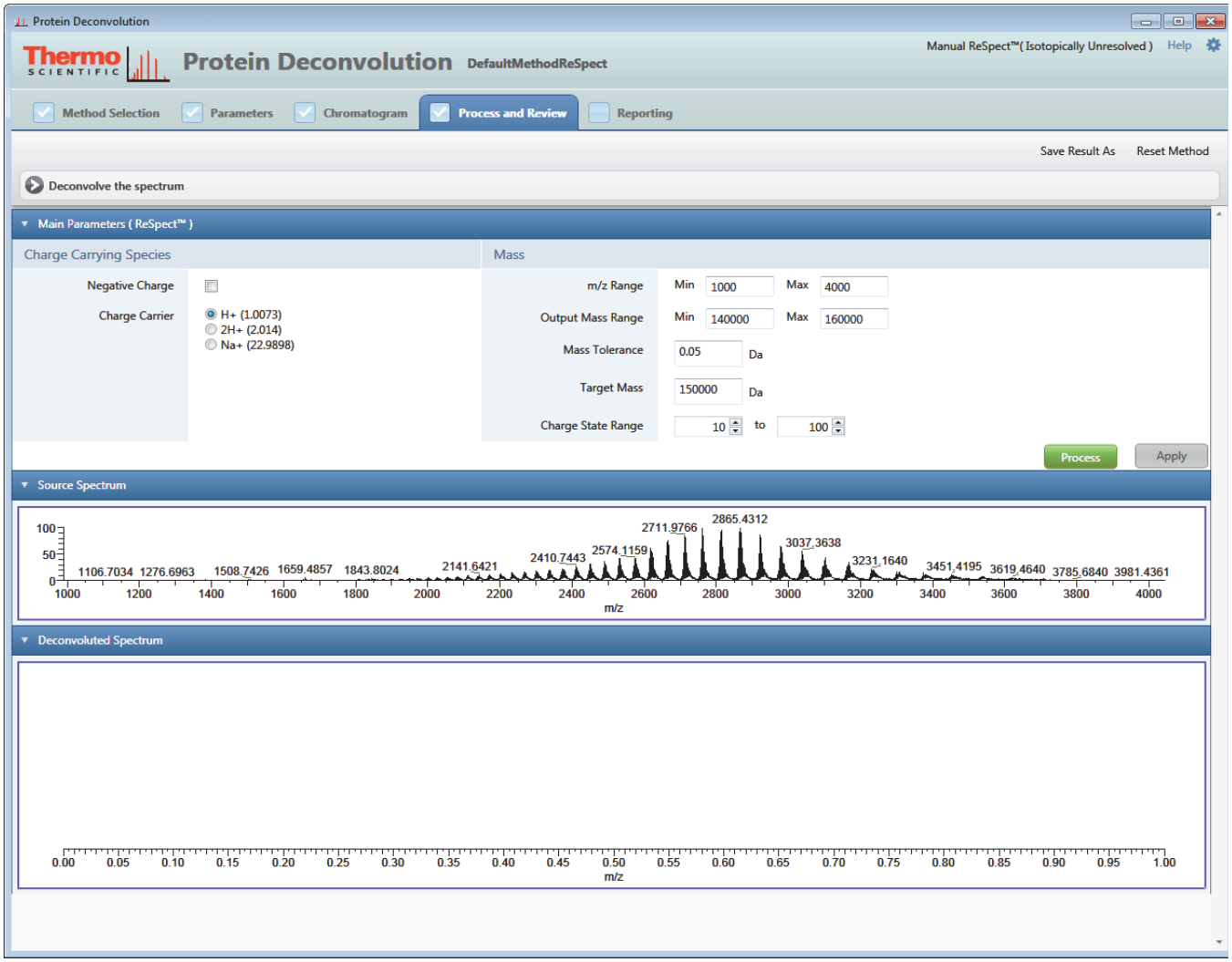
Use the Process and Review page to deconvolve the selected spectrum and to view the resulting data to ensure that the results make sense. You can also export the data into an Excel file for use in other applications.

### ❖ To deconvolve the spectrum

1. If you are not already on the Process and Review page, click the **Process and Review** tab.

   Figure 48 shows the initial Process and Review page.

**Figure 48.** Initial Process and Review page for ReSpect deconvolution




The initial Process and Review page displays three panes:

- Main Parameters (ReSpect): Displays the same parameters as those in the Main Parameters (ReSpect) pane on the Parameters page so that you can adjust them.
- Source Spectrum: Displays the spectrum that you selected in the Source Spectrum pane of the Chromatogram page.
- Deconvolved Spectrum: Displays the deconvolved spectrum resulting from applying the ReSpect algorithm.

2. (Optional) Adjust any parameters in the Main Parameters (ReSpect) pane. For information on these parameters, see Table 17 on page 60.

3. Click **Process** in the Main Parameters (ReSpect) pane.

   The ReSpect algorithm finishes processing, producing a deconvolved spectrum and a list of the components that it detected.

The Protein Deconvolution application displays the output spectrum in the Deconvolved Spectrum pane as a profile in mass and intensity along with a set of peak labels. It displays the component list in the Results pane as a table of masses, intensities, charge state information, and mass shifts. You can expand each entry in this table to display detailed information about the individual charge states that the entry contains.

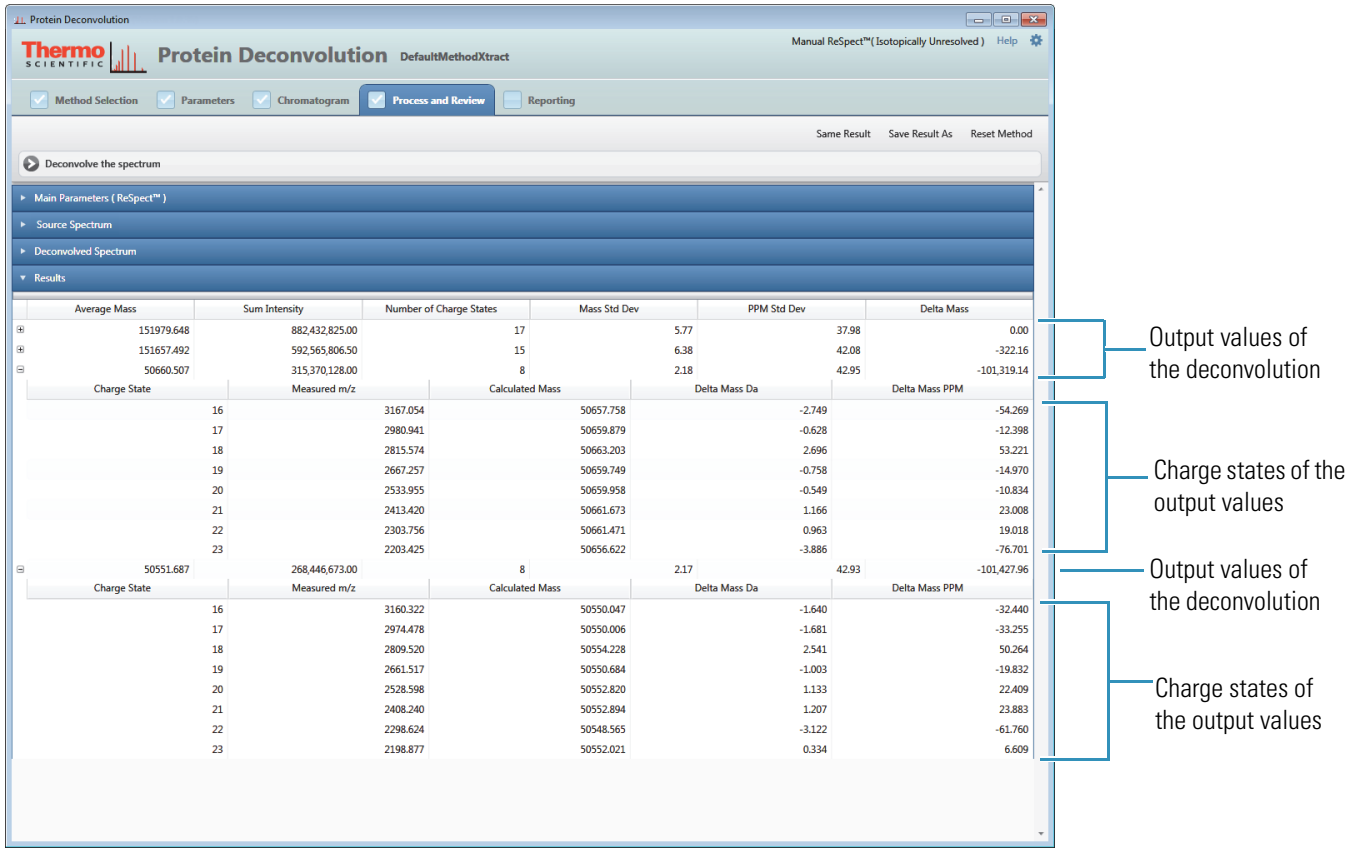
As shown in Figure 49, the values in the Average Mass, Sum Intensity, Number of Charge States, Mass Std Dev, PPM Std Dev, and Delta Mass columns represent the outputs of the deconvolution. Each peak in the Results table is composed of charge-state peaks. Each charge-state peak in the original spectrum provides evidence for the peak in the deconvolved spectrum.

**Figure 49.** Deconvolved spectrum on the Process and Review page for a ReSpect deconvolution

You can sort the data in each column of the peak table from lowest to highest or highest to lowest by clicking the column header. For example, click the Number of Charge States column header. The Protein Deconvolution application, which initially displays the number of charge states in this column in order from lowest to highest, now displays the number of charge states from highest to lowest. Click again to display the numbers from lowest to highest.

Click the plus sign (+) to the far left of a row in the peak table. As shown in Figure 50, five new columns appear: Charge State, Measured m/z, Calculated Mass, Delta Mass Da, and Delta Mass PPM. These values represent the charge-state peaks that constitute the peaks shown in the five output columns.

**Figure 50.** Hierarchical table in Results pane for a ReSpect deconvolution

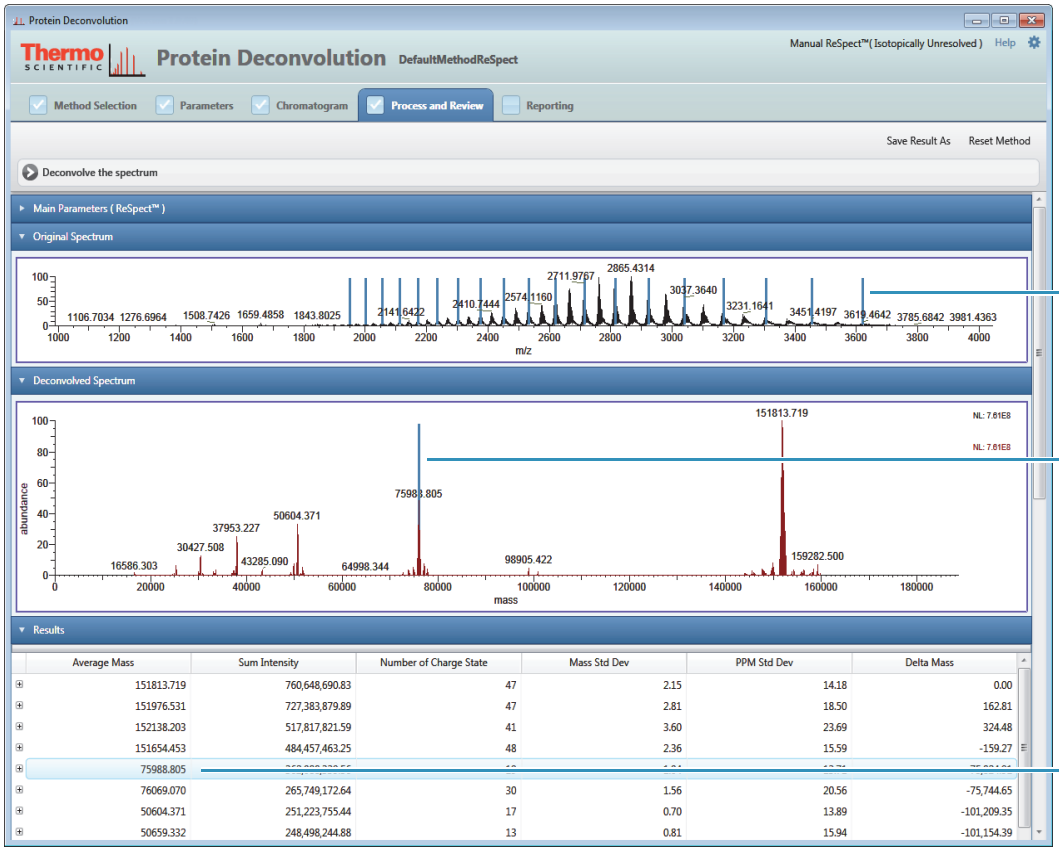



In addition, the peak table includes the following features:

- The Charge State column lists the individual charge states.
- The Average Mass column displays the calculated mass of a molecule based on the average atomic weight of each element. When you click on a value in this column, the application automatically highlights the corresponding mass in the Deconvolved Spectrum pane with a blue line, as shown in Figure 51. You might have to zoom in to see the highlighted line; for instructions, see "Selecting the Spectrum to Deconvolve" on page 21.

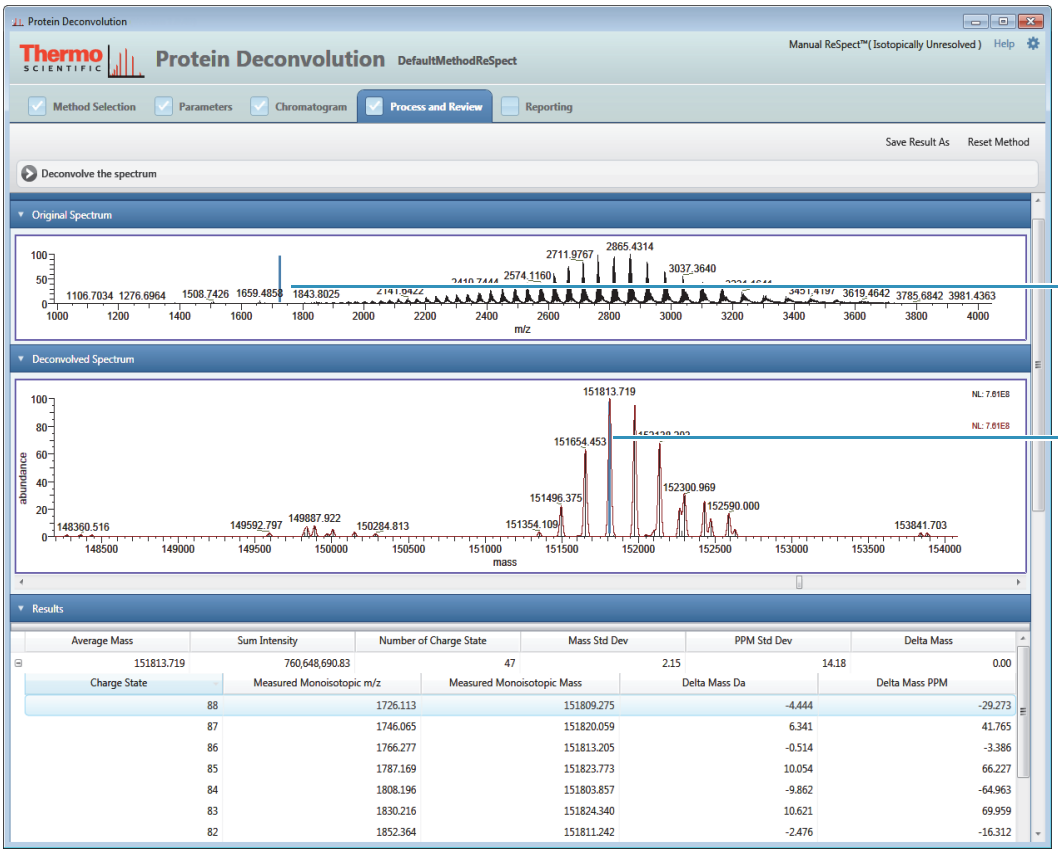
In the Source Spectrum pane, the application highlights all the charge states corresponding to the selected average mass, as shown in Figure 51.

**Figure 51.** Highlighted average mass and corresponding charge states




- When you click on one of the charge states in the Charge State column, the application highlights the corresponding calculated mass in the Deconvolved Spectrum pane, as shown in Figure 52. In the Source Spectrum pane, it highlights the corresponding value of the Measured m/z column.

**Figure 52.** Highlighted values corresponding to individual charge state




Line highlighting the measured *m/z* corresponding to a single charge state

Line highlighting the calculated mass corresponding to a single charge state

For information on the columns in the Results table, see Table 20 on page 83.

### ❖ To adjust the ReSpect deconvolution results

1. If you are not satisfied with the deconvolution results, do the following:

   - Adjust the parameters in the Main Parameters (Xtract) pane on either the Process and Review page or the Parameters page, and click **Apply**.

   –or–

   - Return to the Parameters page, adjust the parameters in the Advanced Parameters (Xtract) pane, and click **Apply**.

2. When you finish adjusting the parameters, click **Process** on the Process and Review page again.

   "Obtaining the Best Results with the ReSpect Algorithm" on page 72 offers some suggestions for obtaining better results when you use the ReSpect algorithm.

If you are satisfied with the results, you can save them by using the following procedure.

### ❖ To save the results of the deconvolution

1. Click **Save Result As**.

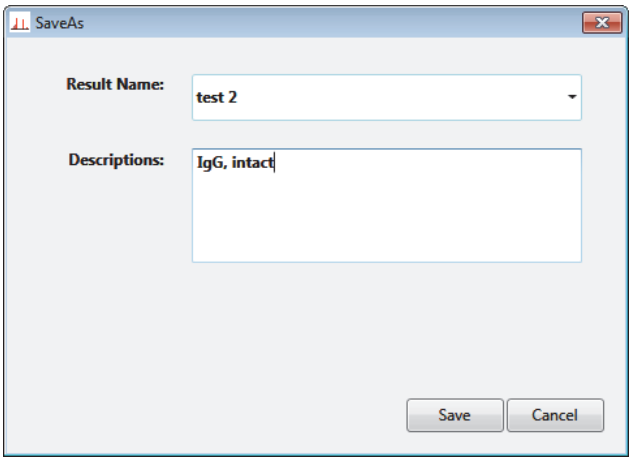
1. In the SaveAs dialog box, do the following:

   a. In the Result Name box, type the name of the results file.

   b. In the Description box, type a brief description of the results.

   The dialog box should resemble that shown in Figure 53.

   **Figure 53.** SaveAs dialog box

   c. Click **Save**.

   The Protein Deconvolution application saves the results of the deconvolution in a file with an .sqlite suffix in the same directory where you stored the RAW files.

   You can also copy and paste any one of the views in this window to a PowerPoint presentation file.

2. If you want to analyze another averaged spectrum from the same LC/MS data file, navigate back to the Chromatogram pane and follow the instructions in "Selecting the Spectrum to Deconvolve" on page 21.

### ❖ To export the results of the deconvolution

1. Double-right-click anywhere in the Results pane.

2. Choose **Export**.

3. In the Save As dialog box, browse to or type the name of the file to store the results in.

4. Click **Save**.

The Protein Deconvolution application stores the data in the Results pane in an Excel file with an .xls suffix.

## Process and Review Page Parameters for the ReSpect Algorithm

The Process and Review page displays parameters that you can set for the protein deconvolution, the source spectrum, the deconvolved spectrum, and a table showing the results of the deconvolution.

Table 20 describes the types of information available on the Process and Review page for ReSpect deconvolutions.

**Table 20.** Process and Review page information for ReSpect deconvolution (Sheet 1 of 2)

| Parameter | Description |
|---|---|
| **Main Parameters (ReSpect) pane** | The parameters in the Main Parameters (ReSpect) pane are the same as those in the Main Parameters (ReSpect) pane of the Parameters page. For information on the parameters on this page, see Table 17 on page 60. |
| Process | Deconvolves the spectrum with the specified algorithm. |
| Apply | Implements the parameter settings that you selected. |
| **Source Spectrum pane** | Displays the selected spectrum before deconvolution. |
| m/z (*x* axis) | Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion. |
| **Deconvolved Spectrum pane** | Displays the deconvolved spectrum. |
| Abundance (*y* axis) | Displays the peak height. |
| Mass (*x* axis) | Displays the actual mass of an ion in atomic mass units. |
| **Results pane** | Displays the masses and intensities of the peaks that the ReSpect algorithm detected during the deconvolution, along with their quality scores. |
| Average Mass | Displays the calculated mass of a molecule based on the average atomic weight of each element. |
| Sum Intensity | Displays the sum of the intensities of the peaks for a charge state. |
| Number of Charge States | Displays the number of component in the list. |
| Mass Std Dev | Displays the standard deviation, in daltons, of the delta masses for all the charge states of a component (for example, the standard deviation of Delta Mass Da). |
| PPM Std Dev | Displays the standard deviation, in parts per million, of the delta masses for all the charge states of a component (for example, the standard deviation of Delta Mass PPM). |

**Table 20.** Process and Review page information for ReSpect deconvolution (Sheet 2 of 2)

| Parameter | Description |
|---|---|
| Delta Mass | Displays the difference between the mass of a specific compound and the mass of the highest-intensity compound. |
| Charge State | Displays the imbalance between the number of protons (in the nuclei of the atoms) and the number of electrons that a molecular species (or adduct ion) possesses. If the species possesses more protons than electrons, its charge state is positive. If it possesses more electrons than protons, its charge state is negative. |
| Measured m/z | Displays the mass-to-charge ratio of the peak in the source spectrum. |
| Calculated Mass | Displays the mass calculated from the measured mass-to-charge ratio and the charge state. It represents the deconvolved mass for a particular charge state. |
| Delta Mass Da | Displays the difference between the average mass for a component and the calculated mass for that charge state, in daltons. |
| Delta Mass PPM | Displays the difference between the average mass for a component and the calculated mass for that charge state, in parts per million. |

# Displaying a Deconvolution Report

When you click Process on the Process and Review page, the Protein Deconvolution application generates a report displaying several aspects of the deconvolution so that you can track the progression of the data. You can view this report on the Reporting page. You can also save this report as a PDF file.

### ❖ To display a report

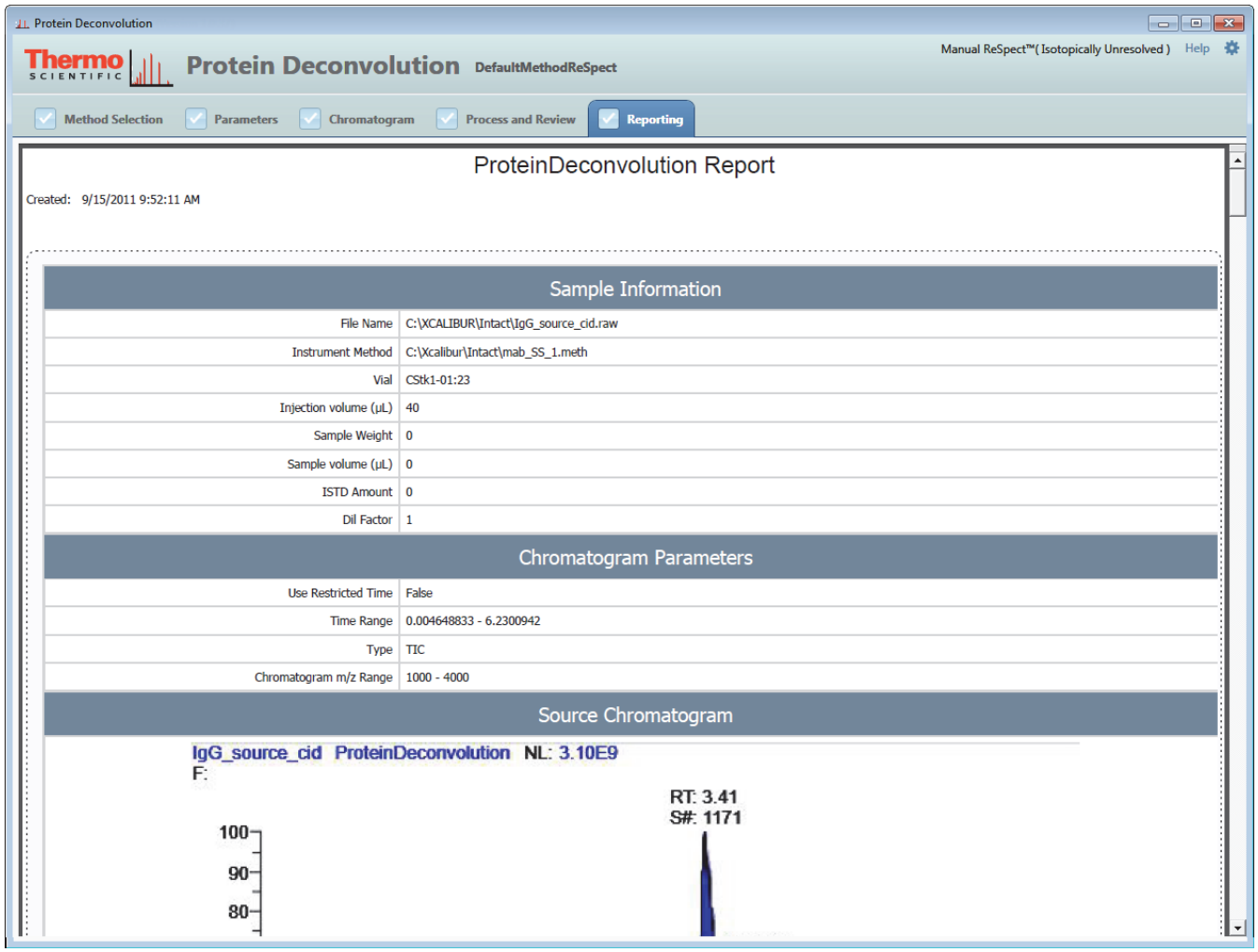
- Click the **Reporting** tab when you have finished analyzing the data.

  The Reporting page, partially shown in Figure 54, displays a summary of all results for a given data file. It contains the following sections:

  - Sample Information Section
  - Chromatogram Parameters Section
  - Source Chromatogram Section
  - Source Spectrum Section
  - Main Parameters (ReSpect) Section

- Advanced Parameters (ReSpect) Section
- Deconvolved Spectrum Section
- ReSpect Masses Table Section
- Source Spectrum Evidence Section

**Figure 54.** Partial view of the Reporting page for ReSpect deconvolution




### ❖ To save the report in a PDF file

1. Move the cursor near the bottom of the screen.

   The Reporting page toolbar shown in Figure 27 on page 42 appears.

2. Click the **Show Acrobat** icon,  .

   The Adobe Acrobat toolbar appears at the top of the screen.

3. On the Adobe toolbar, click the **Save File** icon,  .

   The Save a Copy dialog box opens.

4. Specify the path and name of a PDF file to store the reports in, and click **Save**.

❖ **To print a report**

1. Hover the cursor near the bottom of the screen.
2. Click the **Print File** icon,  , on the Reporting page toolbar shown in Figure 27 on page 42.
3. In the Print dialog box, set the appropriate printing parameters, and click **OK**.

## Reporting Page Toolbar

For information about the icons on the Reporting page toolbar, see "Reporting Page Toolbar" on page 42.

## Sample Information Section

The Sample Information section of the report, shown in Figure 55, displays information about the sample that the spectrum was taken from.

**Figure 55.** Sample Information section for ReSpect deconvolution

| Sample Information | |
|---|---|
| File Name | C:\XCALIBUR\Intact\IgG_source_cid.raw |
| Instrument Method | C:\Xcalibur\Intact\mab_SS_1.meth |
| Vial | CStk1-01:23 |
| Injection volume (µL) | 40 |
| Sample Weight | 0 |
| Sample volume (µL) | 0 |
| ISTD Amount | 0 |
| Dil Factor | 1 |

Table 21 lists the parameters in the Sample Information section. All the parameters in this section are read-only.

**Table 21.** Sample Information section parameters for ReSpect deconvolution (Sheet 1 of 2)

| Parameter | Description |
|---|---|
| File Name | Displays the name of the RAW file. |
| Instrument Method | Displays the name of the instrument method file. |
| Vial | Displays the position number of the sample in the autosampler. |
| Injection Volume (µL) | Displays the injection volume of the sample to be injected, in microliters. |

**Table 21.** Sample Information section parameters for ReSpect deconvolution (Sheet 2 of 2)

| Parameter | Description |
|---|---|
| Sample Weight | Displays the amount of a component in the sample. |
| Sample Volume (µL) | Displays the volume of a component in the sample. |
| ISTD Amount | Specifies the correction for the internal standard amount. If the value in this box is not 0.000, the value is used in an algorithm to correct for a case when any internal standard amounts specified in the active instrument method are correct, but when the amount of internal standard actually in one or more samples is different than the amount specified in the instrument method.<br><br>This correction eliminates the necessity of remaking any samples to the internal standard concentrations or amounts specified in the instrument method and re-running the samples. |
| Dial Factor | Specifies the dilution factor that was used to prepare the sample. |

## Chromatogram Parameters Section

The Chromatogram Parameters section, shown in Figure 56, displays the settings that you chose in the Chromatogram Parameters pane of the Chromatogram page. For more information on these parameters, see Table 18 on page 73.

**Figure 56.** Chromatogram Parameters section for ReSpect deconvolution

| Chromatogram Parameters | |
|---|---|
| Use Restricted Time | False |
| Time Range | 0.004648833 - 6.2300942 |
| Type | TIC |
| Chromatogram m/z Range | 1000 - 4000 |

## Source Chromatogram Section

The Source Chromatogram section, shown in Figure 57, displays the chromatogram contained in the RAW file. It is the same chromatogram that appears in the Chromatogram pane of the Chromatogram page.

**Figure 57.** Source Chromatogram section for ReSpect deconvolution

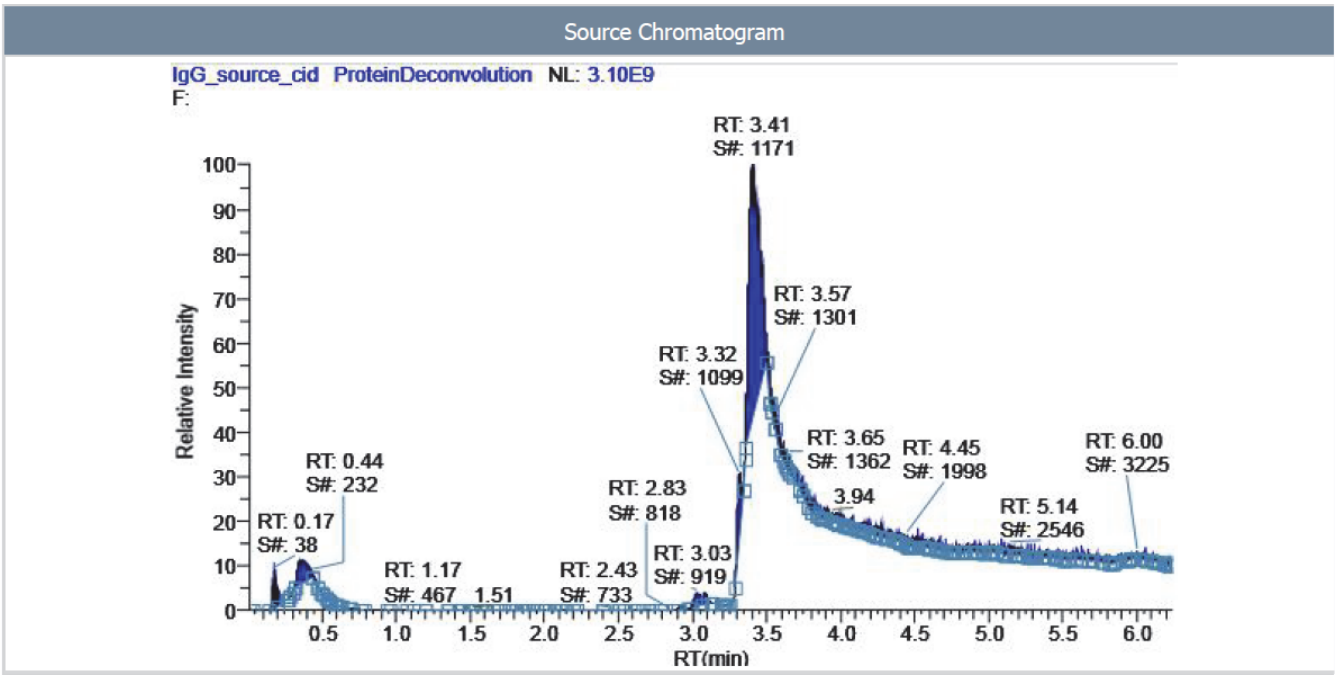



Table 22 lists the parameters in the Source Chromatogram section.

**Table 22.** Source Chromatogram section parameters for ReSpect deconvolution

| Parameter | Description |
|---|---|
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| RT (min) (*x* axis) | Displays the retention time of the spectrum, which is the time after injection at which a compound elutes. Retention time can also refer to the total time that the compound is retained on the chromatograph column. |

## Source Spectrum Section

The Source Spectrum section, shown in Figure 58, displays the spectrum that you selected in the Source Spectrum pane of the Chromatogram page.

**Figure 58.** Source Spectrum section for ReSpect deconvolution

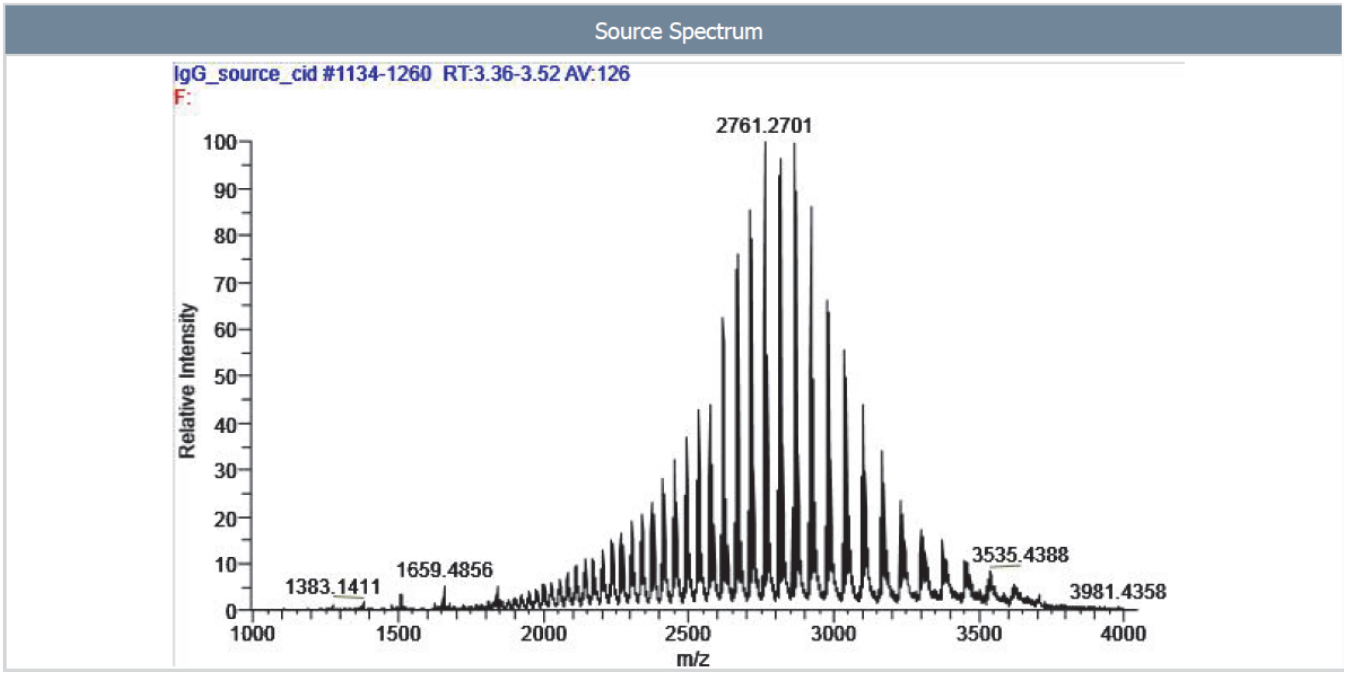



Table 23 lists the parameters in the Source Spectrum section.

**Table 23.** Source Spectrum section parameters for ReSpect deconvolution

| Parameter | Description |
|---|---|
| Relative Intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| m/z (*x* axis) | Displays the mass-to-charge ratio of ions formed from molecules. This ratio is the quantity formed by dividing the mass of an ion, in daltons, by the number of charges carried by the ion. |

## Main Parameters (ReSpect) Section

The Main Parameters (ReSpect) section, shown in Figure 59, displays the parameter settings that you selected in the Main Parameters (ReSpect) pane of the Parameters page for the deconvolution. For information on these parameters, see Table 17 on page 60.

**Figure 59.** Main Parameters (ReSpect) section for ReSpect deconvolution

| Main Parameters ( ReSpect™ ) | |
|---|---|
| Negative Charge | False |
| Charge Carrier | H |
| m/z Range | 1000 - 4000 |
| Output Mass Range | 10000 - 160000 |
| Mass Tolerance | 0.05 |
| Target Mass | 150000 |
| Charge State Range | 10 - 100 |

## Advanced Parameters (ReSpect) Section

The Advanced Parameters (ReSpect) section, shown in Figure 59, displays the parameter settings that you selected in the Advanced Parameters (ReSpect) pane of the Parameters page for the deconvolution. For information on these parameters, see Table 17 on page 60.

**Figure 60.** Main Parameters (ReSpect) section for ReSpect deconvolution

| Advanced Parameters ( ReSpect ™) | |
|---|---|
| Minimum Peak Significance | 1 |
| Noise Rejection | 95% Confidence |
| Use Relative Intensities | True |
| Peak Width | 0 |
| Feature Width | 0 |
| Degree of Fit | 0 |
| Number of Iterations | 3 |
| Noise Compensation | True |
| Minimum #. Adjacent Charges | 6 - 10 |
| Number of Peak Models | 10 |
| Resolution @ 400 | 12374 |
| Left/Right Peak Shape | 1.5:1.5 |

## Deconvolved Spectrum Section

The Deconvolved Spectrum section, shown in Figure 61, displays the same information that appears in the Deconvolved Spectrum pane of the Process and Review page.

**Figure 61.** Deconvolved Spectrum section for ReSpect deconvolution

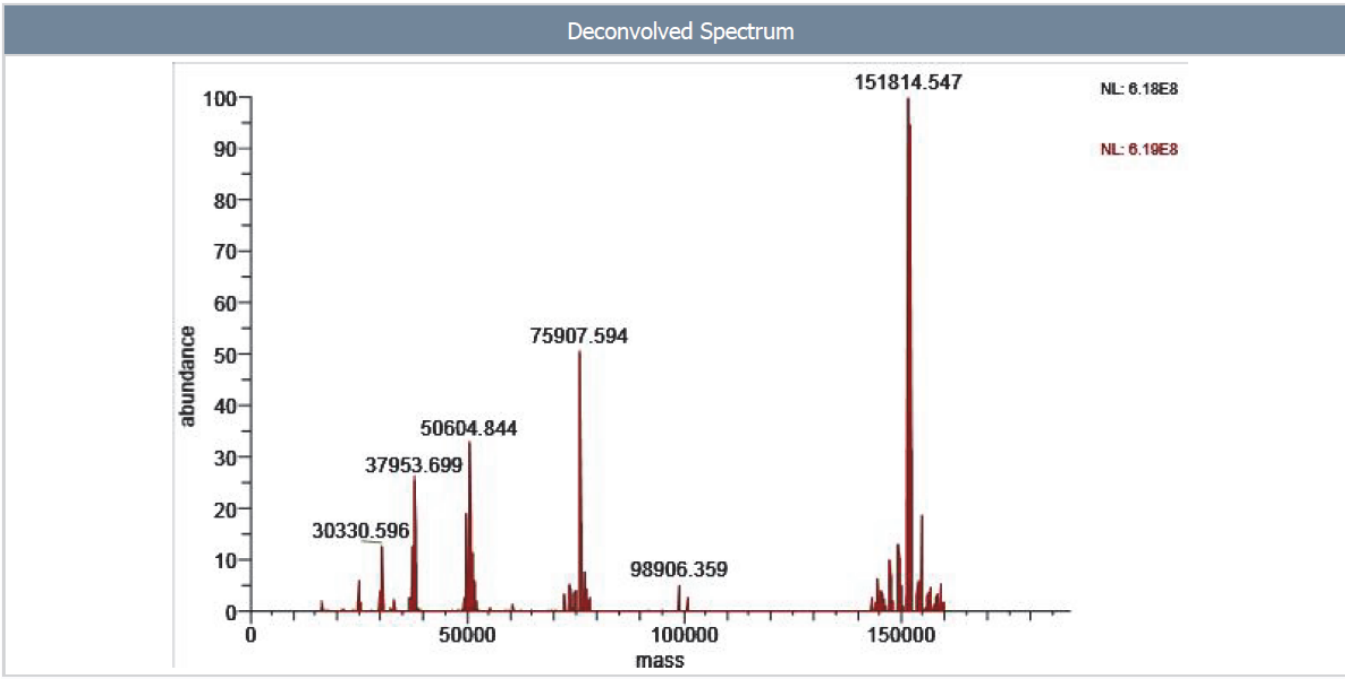



Table 24 lists the parameters in the Deconvolved Spectrum section.

**Table 24.** Deconvolved Spectrum section parameters for ReSpect deconvolution

| Parameter | Description |
|---|---|
| Abundance (*y* axis) | Displays the peak height. |
| Mass (*x* axis) | Displays the actual mass of an ion in atomic mass units. |

## ReSpect Masses Table Section

The ReSpect Masses Table section, shown in Figure 62, displays the results of the deconvolution. It contains the same columns as those on the Results pane on the Process and Review page. For information on the columns in this table, see Table 20 on page 83.

**Figure 62.** ReSpect Masses Table section for ReSpect deconvolution

| ReSpect Masses Table | | | | | |
|---|---|---|---|---|---|
| Average Mass | Sum Intensity | Number of Charge States | Mass Std Dev | PPM Std Dev | Delta Mass |
| 151813.438 | 649078553.70 | 47 | 1.95 | 12.85 | 0.00 |
| 151976.641 | 623899549.77 | 46 | 2.48 | 16.34 | 163.20 |
| 152138.453 | 456451240.16 | 52 | 3.20 | 21.05 | 325.02 |
| 151654.313 | 417716261.19 | 43 | 1.98 | 13.08 | -159.13 |
| 75907.242 | 328918554.69 | 29 | 0.98 | 12.97 | -75906.20 |
| 75988.516 | 311666151.06 | 20 | 0.82 | 10.77 | -75824.92 |
| 76069.406 | 229705768.72 | 30 | 1.42 | 18.71 | -75744.03 |
| 50604.398 | 214522927.28 | 17 | 0.61 | 12.05 | -101209.04 |
| 50658.773 | 213614767.36 | 19 | 0.93 | 18.30 | -101154.66 |
| 75827.117 | 210266759.47 | 24 | 1.26 | 16.59 | -75986.32 |
| 152302.531 | 202820202.38 | 26 | 4.24 | 27.86 | 489.09 |
| 152430.828 | 159574155.75 | 25 | 3.92 | 25.75 | 617.39 |
| 37994.117 | 159375169.88 | 10 | 0.65 | 17.01 | -113819.32 |
| 152268.609 | 152164554.25 | 24 | 4.26 | 27.94 | 455.17 |
| 50712.938 | 149225282.45 | 16 | 1.07 | 21.03 | -101100.50 |
| 151496.797 | 143887122.94 | 26 | 3.58 | 23.63 | -316.64 |
| 152588.000 | 119998704.56 | 23 | 6.47 | 42.41 | 774.56 |
| 152469.016 | 100891944.38 | 20 | 6.37 | 41.78 | 655.58 |
| 37913.480 | 100307771.50 | 8 | 0.64 | 16.81 | -113899.96 |
| 76133.164 | 83301385.13 | 17 | 2.67 | 35.03 | -75680.27 |
| 30330.582 | 79337710.28 | 8 | 0.46 | 15.05 | -121482.86 |
| 76213.711 | 74840094.94 | 15 | 2.41 | 31.68 | -75599.73 |
| 75748.586 | 73572457.56 | 15 | 2.07 | 27.28 | -76064.85 |

## Source Spectrum Evidence Section

The Source Spectrum Evidence section, shown in Figure 63, displays a table and an accompanying graph for every scan in the sample. The table shows all the charge states that the Protein Deconvolution application detected. It displays the same parameters as those displayed in the Results pane on the Process and Review page. For information on these parameters, see Table 20 on page 83. The graph shows the peaks in the scan that are associated with a particular component.

The table in Figure 63 shows only a partial list of values.

**Figure 63.** Source Spectrum Evidence section for ReSpect deconvolution

| Average Mass:151813.4375 | | | | |
|---|---|---|---|---|
| Charge | Measured m/z | Calculated Mass | Delta Mass Da | Delta Mass PPM |
| 42 | 3615.632 | 151814.221 | 0.784 | 5.163 |
| 43 | 3531.824 | 151825.139 | 11.702 | 77.078 |
| 44 | 3451.433 | 151818.715 | 5.277 | 34.763 |
| 45 | 3374.662 | 151814.467 | 1.030 | 6.784 |
| 46 | 3301.375 | 151816.938 | 3.500 | 23.056 |
| 47 | 3231.144 | 151816.417 | 2.979 | 19.623 |
| 48 | 3163.862 | 151817.041 | 3.604 | 23.739 |
| 49 | 3099.340 | 151818.308 | 4.870 | 32.081 |
| 50 | 3037.324 | 151815.823 | 2.385 | 15.711 |
| 51 | 2977.776 | 151815.211 | 1.774 | 11.683 |
| 52 | 2920.538 | 151815.577 | 2.139 | 14.091 |
| 53 | 2865.456 | 151815.759 | 2.322 | 15.294 |
| 54 | 2812.402 | 151815.294 | 1.857 | 12.229 |
| 55 | 2761.287 | 151815.364 | 1.926 | 12.690 |
| 56 | 2711.979 | 151814.389 | 0.952 | 6.270 |
| 57 | 2664.419 | 151814.493 | 1.055 | 6.952 |
| 58 | 2618.514 | 151815.385 | 1.948 | 12.829 |
| 59 | 2574.135 | 151814.551 | 1.113 | 7.332 |
| 60 | 2531.271 | 151815.823 | 2.386 | 15.714 |
| 61 | 2489.797 | 151816.166 | 2.728 | 17.969 |
| 62 | 2449.640 | 151815.237 | 1.800 | 11.856 |
| 63 | 2410.742 | 151813.299 | -0.138 | -0.910 |
| 64 | 2373.122 | 151815.331 | 1.894 | 12.474 |
| 65 | 2336.593 | 151813.073 | -0.364 | -2.400 |

Source Spectrum Evidence

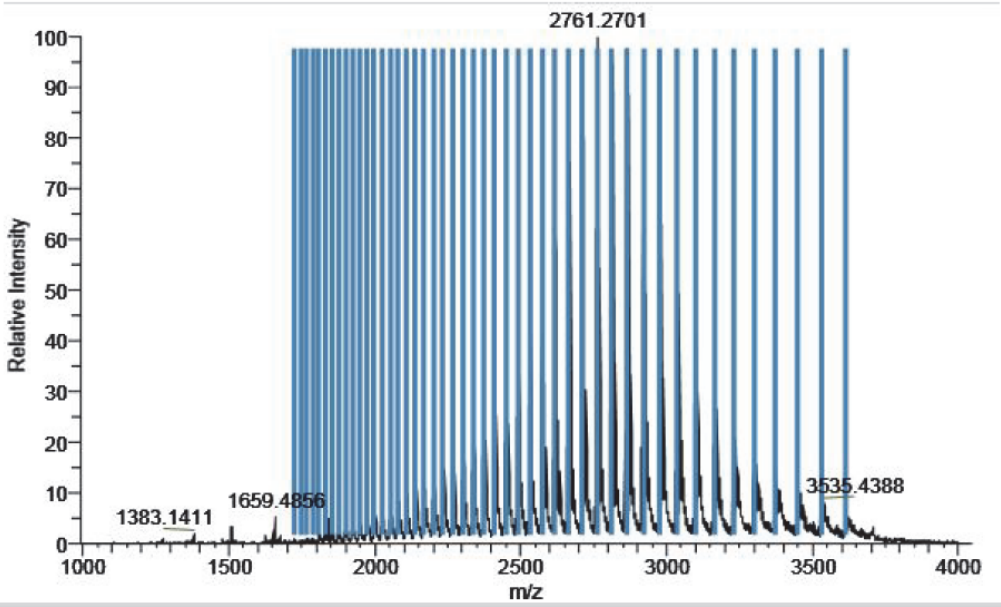


Table 25 lists the parameters in the Source Spectrum Evidence section.

**Table 25.** Source Spectrum Evidence section parameters for ReSpect deconvolution

| Parameter | Description |
|---|---|
| Relative intensity (*y* axis) | Displays the ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity. |
| m/z (*x* axis) | Displays the mass-to-charge ratio. |

# Loading Saved Results

If you saved the results of a deconvolution, you can reload them at a later time.

### ❖ To load saved results

1. Click the **Method Selection** tab.
2. In the Experiment Types pane, click **Load Previous Results**.
3. In the Raw Data Directory of the Load Result File pane, type the path and name of the file containing the saved results or click the **Browse** button (...) to browse to the location of the file.
4. In the Select Result Files box, select the name of the .sqlite file containing the results, and click **Load**.

# Index


## A

About Protein Deconvolution dialog box vi
Abundance *y* axis label 38, 47, 83, 91
adduct ions 13–14, 18–19, 55, 60
Adobe Acrobat toolbar 41–42, 85
Advanced Parameters (ReSpect) pane
  parameters on 57, 62
  purpose 54
Advanced Parameters (ReSpect) report section 90
Advanced Parameters (Xtract) pane
  parameters on 15, 20
  purpose 12
Advanced Parameters (Xtract) report section 46
Auto Zooming mode 24, 68
Average Charge column 32, 39
Average Mass column 78–79, 83
averagine isotope distribution 15, 20, 39
Averaging mode 25, 69

## B

base peak chromatogram 23, 28, 68, 74
baseline correction 64
baseline subtraction 3

## C

Calculated Mass column 79, 84
Calculated Monoisotopic m/z column 33, 39
centroid data 26, 71
Charge Carrier parameter 14, 19, 55, 60
Charge column 33–34, 39
charge deconvolution 3
Charge Normalized Intensity column 33, 39
Charge Range parameter 16, 21
Charge State column 79–80, 84
Charge State Range parameter 56, 61
Chromatogram m/z Range parameter 23, 28, 68, 74
Chromatogram page
  Chromatogram pane 22, 67
  Chromatogram Parameters pane 22, 67
  parameters on 27, 73
  selecting spectrum to deconvolve 21, 66
  Source Spectrum pane 22, 67
Chromatogram pane
  adjusting the view 24, 68
  creating spectrum in Source Spectrum pane 24
  header 30, 75
  parameters on 28, 74
  purpose 22, 67
  shortcut menu 29, 75
Chromatogram Parameters pane
  parameters on 23, 27, 68, 73
  purpose 22, 67
Chromatogram Parameters report section 44, 87
Consider Overlaps parameter 15, 20
contacting us viii
Copy command 29

## D

deconvolution
  algorithms in Protein Deconvolution application 1
  charge 3
  description 1
  peak 3
  results of 32, 78
Deconvolved Spectrum pane
  deconvolved spectrum in 32, 78
  highlighting calculated mass 80
  parameters on 38, 83
  purpose 32, 77
Deconvolved Spectrum report section 47, 91
Degree of Fit parameter 58, 64
deisotoping 2
Delta Mass column 32, 78, 84
Delta Mass Da column 79, 84
Delta Mass parameter 39
Delta Mass PPM column 79, 84

deprotonation 55, 60
deuterium 56, 60
Dil Factor column 43, 87
documentation survey ix

## E

electrospray ionization (ESI) 1
Exactive mass spectrometers 5, 14, 18
Excel files 37, 82
exiting Protein Deconvolution application 7
Expected Intensity Error parameter 16, 21
Experiment Types pane
  parameters on 11, 53
  purpose 9, 51

## F

Feature Width parameter 58, 64
FileName column 43, 86
Fit column 36
Fit Factor (%) parameter 15, 20
Fit% column 33, 39
Fit% Left column 33, 39
Fit% Right column 33, 39
FTMS mass spectrometers 5, 14, 18

## G

Global Settings dialog box 6
Global Settings icon 6

## H

hydrogen 14, 19, 55, 60

## I

Injection Volume (uL) column 43, 86
inputs to Protein Deconvolution application 5
Instrument Method column 43, 86
isotopic clusters 15, 20, 32, 38
isotopically resolved mass spectra 1–2, 9
isotopically unresolved mass spectra 1–2, 51
ISTD Amount column 43, 87

## L

Left/Right Peak Shape parameter 59, 66
License Activation dialog box vii
license for Protein Deconvolution application vi
Load Raw Data File pane
  parameters on 12, 53
  purpose 10, 52

## M

m/z Range parameter 14, 19, 56, 61
m/z *x* axis label 29, 46, 49, 75, 89, 94
Main Parameters (ReSpect) pane
  parameters on 55, 60, 83
  purpose 54, 77
Main Parameters (ReSpect) report section 90
Main Parameters (Xtract) pane
  parameters on 13, 18, 38
  purpose 12, 32
Main Parameters (Xtract) report section 46
Mass Std Dev column 78, 83
Mass Tolerance parameter 56, 61, 72
Mass *x* axis label 38, 47, 83, 91
Measured m/z column 79, 84
Method Selection page
  Experiment Types pane 9, 11, 51, 53
  Load Raw Data File pane 10, 12, 52–53
  Methods pane 10, 12, 52, 54
  parameters on 11, 53
  selecting algorithm, RAW file, and parameter set 9, 51
Methods pane
  parameters on 12, 54
  purpose 10, 52
Minimum Adjacent Charges parameter 58, 66, 72
Minimum Intensity parameter 16, 21
Minimum Peak Significance parameter 57, 62
Mode command 29, 75
monoisotopic mass 2, 13, 39
Monoisotopic Mass column 32
Monoisotopic Mass for This Charge column 33, 35, 39
Monoisotopic Mass parameter 38
monoisotopic MH+ mass 13
Mostabund m/z column 33–34, 39

## N

Negative Charge parameter 55, 60
Noise Compensation parameter 58, 65
Noise Rejection parameter 57, 62
Number of Charge States column 32, 38, 78, 83
Number of Iterations parameter 58, 65
Number of Peak Models parameter 59, 66

## O

Orbitrap mass spectrometers 5, 14, 18
Output Mass parameter 13, 18
Output Mass Range parameter 56, 61, 72
outputs of Protein Deconvolution algorithm 5

## P

parameter sets
  creating
    in ReSpect algorithm 53–54
    in Xtract algorithm 11, 13
  deleting 17, 60
  existing 11, 53
  loading 11, 53
  parameters available in Advanced Parameters (ReSpect) pane 57, 62
  parameters available in Advanced Parameters (Xtract) pane 15, 20
  parameters available in Main Parameters (ReSpect) pane 55, 60
  parameters available in Main Parameters (Xtract) pane 13, 18
  saving 16–17, 59–60
Parameters page
  Advanced Parameters (ReSpect) pane 54, 57, 62
  Advanced Parameters (Xtract) pane 12, 15, 20
  Main Parameters (ReSpect) pane 54–55, 60
  Main Parameters (Xtract) pane 12–13, 18
  setting parameters for ReSpect deconvolution 54
  setting parameters for Xtract deconvolution 12
PDF files 40–41, 84–85
peak deconvolution 3
Peak Width parameter 57, 64
potassium 14, 19
PPM Std Dev column 83
Print dialog box 42, 86
Print File icon 42, 86
Process and Review page
  Deconvolved Spectrum pane 32, 77
  deconvolving the spectrum 31, 77
  Main Parameters (ReSpect) pane 77
  Main Parameters (Xtract) pane 32
  parameters on 38, 83
  Source Spectrum pane 32, 77
Process button 38, 83
profile data 26, 71
Protein Deconvolution application
  activating license vi
  exiting 7
  features 1
  inputs 5
  outputs 5
  ReSpect algorithm 1–2
  specifying default RAW file directory 6
  starting 5
  workflow 3
  Xtract algorithm 1–2
Protein Deconvolution window 5
protonation 55, 60

## Q

Qual Browser 3, 25, 70

## R

RAW file directory 6
RAW files 9–10, 12, 52, 54
Relative Intensity *y* axis label
  Chromatogram pane 28, 74
  Source Chromatogram report section 45, 88
  Source Spectrum Evidence report section 49, 94
  Source Spectrum pane 29
Relative Intensity y axis label
  Source Spectrum pane 75
Relative Intensity *y* axis label
  Source Spectrum report section 46, 89
Remainder Threshold (%) parameter 15, 20
Reporting page
  Advanced Parameters (Xtract) section 46
  Chromatogram Parameters section 44, 87
  Deconvolved Spectrum section 47, 91
  displaying deconvolution report 40, 84
  displaying deconvolution results 40, 84
  Main Parameters (Xtract) section 46
  Sample Information section 43, 86
  sections in 40, 84
  Source Chromatogram section 44, 88
  Source Spectrum Evidence section 48, 92
  Source Spectrum section 45, 89
  toolbar 42
  Xtract Masses Table section 47, 92
resetting parameters to defaults 15, 56, 59
Resolution @ 400 parameter 59, 66
Resolution at 400 m/z parameter 14, 18
ReSpect algorithm
  adjusting deconvolution results 81
  creating a parameter set 54
  deconvolving the spectrum 77
  displaying reports 84
  exporting results of deconvolution 82
  loading saved deconvolution results 94
  obtaining best results 72
  profile spectra 71

purpose 1–2
results of deconvolution 78
saving results of deconvolution 82
selecting spectrum to deconvolve 66
setting up protein deconvolution 51
types of spectra to use 51
ReSpect Masses Table report section 92
Results pane
parameters on 38, 83
results of deconvolution in 32, 78
retention time 28, 74
RT (min) *x* axis label 28, 45, 74, 88


## S


S/N Threshold parameter 14, 19
Sample Information report section 43, 86
Sample Volume (uL) column 43, 87
Sample Weight column 43, 87
Save a Copy dialog box 41–42, 86
Save a Copy icon 42
Save dialog box 16, 59
Save File icon 41, 86
SaveAs dialog box 16, 37, 59, 82
Scale command 29, 75
Show Acrobat icon 41–42, 85
signal-to-noise threshold 14, 19, 63
sodium 14, 19, 56, 60
Source Chromatogram report section 44, 88
Source Spectrum Evidence report section 48, 92
Source Spectrum pane
adjusting the view 26, 71
creating spectrum in 24, 69
displaying selected spectrum 26, 71
header 30, 76
highlighting charge states 80
highlighting most abundant isotope 34
parameters on 29, 38, 75, 83
purpose 22, 32, 67, 77
shortcut menu 30, 76
Source Spectrum report section 45, 89
.sqlite files
deleting from database 17
loading previous results 50
location of saved parameter sets 17, 60
saving deconvolution results in 37
starting Protein Deconvolution application 5
Sum Intensity column 32, 38, 78, 83
survey link ix


## T


Target Mass parameter 56, 61
Time Limits parameter 23, 27, 68, 73
total ion current chromatogram 23, 28, 68, 74
Types parameter 23, 28, 68, 74


## U


Use Relative Intensities parameter 57, 63
Use Restricted Time parameter 23, 27, 68, 73


## V


Vial column 43, 86


## W


workflow for Protein Deconvolution algorithm 3


## X


Xcalibur data system 3, 25, 70
Xtract algorithm
adjusting deconvolution results 36
centroid spectra 26
creating a parameter set 13
deconvolving the spectrum 31
displaying reports 40
exporting results of deconvolution 37
loading saved deconvolution results 50
printing a report 42, 86
profile spectra 26
purpose 1–2
results of deconvolution 32
saving a report in PDF file 41, 85
saving results of deconvolution 37
selecting spectrum to deconvolve 21
setting up protein deconvolution 9
types of spectra to use 9
Xtract Masses Table report section 47


## Z


Zoom In command 29, 75
Zoom Out command 29, 75