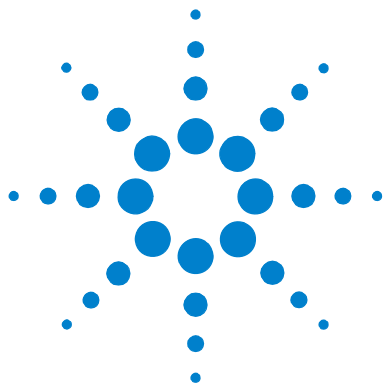




# Agilent Mass Hunter Software

## Quick Start Guide

Use this guide to get started with the Mass Hunter software.

### What is Mass Hunter Software?

Mass Hunter is an integral part of Agilent TOF software (version A.02.00). Mass Hunter operates on chromatographic and electrophoretic mass spectral data to extract information to reduce data complexity, eliminate potential interferences, and generate a list of molecular features.

A feature is a discrete molecular entity defined by the combination of retention time and mass.

### Working with the extracted information

You can use the feature information from Mass Hunter to perform many different tasks. Here are a few examples:

- Compare the original (raw data) total ion chromatogram (TIC) with the processed TIC
- Compare the original and processed mass spectra at a specific retention time (RT), or averaged over an RT range, on the TIC
- Show extracted ion chromatograms based on a mass spectral range.
- View the species clusters (isotopic, dimers, adducts) for each feature

For a complete list of tasks, see the Mass Hunter online help.



**Agilent Technologies**

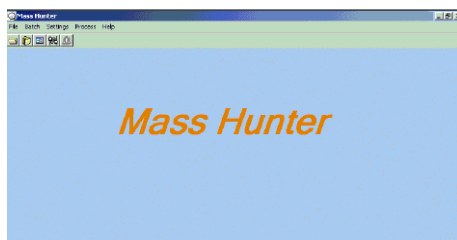
## Getting started with the Mass Hunter software

### Start the Mass Hunter software

This section tells you how to start the Mass Hunter software.

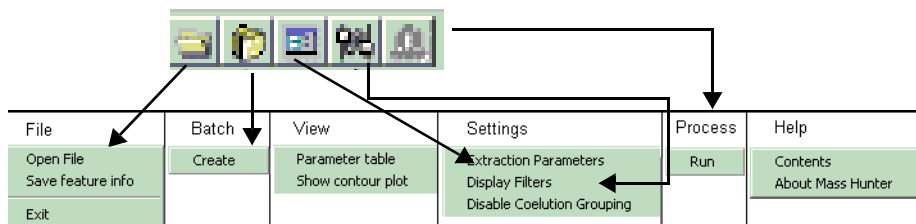
- Either do this:
  - a** Double-click the Agilent TOF Software folder icon on the desktop, or  
Select **Start > Programs > Agilent > TOF Software** from the desktop.
  - b** In the Agilent TOF Software folder, double-click the **Mass Hunter** icon .
- Or do this:  
Select **Start > Programs > Agilent > TOF Software > Mass Hunter** from the desktop.

The system displays the Mass Hunter main window.



### Learn how to access Mass Hunter functions

You can use the toolbar or the menus to perform many of the Mass Hunter tasks:



#### HINT

To access other functions, double-click or **Ctrl**-click a plot or table row number. You may use the middle mouse button click for **Ctrl**-click in the Mass Hunter software

## Learn how to use Mass Hunter

Try these exercises to familiarize yourself with the Mass Hunter application. Try the **Steps** on the left in the exercises on the next pages without the **Detailed Instructions**. If you need more help, follow the detailed instructions.

| If you want to do this:                                                                | Refer to this section or exercise:                                                                |
|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Extract feature information                                                            | <a href="#">“Exercises: Extracting feature information”</a> on page 4                             |
| Extract feature information for a single file                                          | <a href="#">“Exercise 1—Extract feature information for a single file”</a> on page 4              |
| Extract feature information for a batch                                                | <a href="#">“Exercise 2—Extract feature information for a batch”</a> on page 9                    |
| Reprocess a file with different parameters                                             | <a href="#">“Exercise 3—Reprocess files with different parameters”</a> on page 11                 |
| Display specific feature information or export and save information                    | <a href="#">“Exercises: Reviewing and saving feature information”</a> on page 14                  |
| Show species clusters, mass spectra and extracted ion chromatograms (EIC) for features | <a href="#">“Exercise 4—Show species clusters, EIC and mass spectra for features”</a> on page 14. |
| Show possible feature compositions                                                     | <a href="#">“Exercise 5—Show possible feature compositions”</a> on page 17                        |
| Export and save feature information                                                    | <a href="#">“Exercise 6—Export and save feature information”</a> on page 19                       |
| Work with chromatograms, mass spectra and contour plots                                | <a href="#">“Exercises: Working with plots”</a> on page 22                                        |
| Show mass spectra from chromatograms and show and hide other plots                     | <a href="#">“Exercise 7—Working with processed chromatograms (TICs or EICs)”</a> on page 22       |
| Show EICs from mass spectra                                                            | <a href="#">“Exercise 8—Working with mass spectra”</a> on page 24                                 |
| Learn to use contour plots                                                             | <a href="#">“Exercise 9—Working with contour plots”</a> on page 25                                |

# Exercises: Extracting feature information

## Exercise 1—Extract feature information for a single file

This exercise guides you through the process to extract feature information from a TOF .wiff file.

### CAUTION

To process Agilent TOF data files (.wiff files) with the Mass Hunter software, the .wiff files must be local to the software. That is, the files must reside on the computer where the Mass Hunter software is running. Also, the files must not be read-only. Use Windows Explorer to check and change the file attributes.

| Steps                                                                                                                                                                                          | Detailed Instructions                                                                                                                                                                                                                                                                                                     | Comments                                                                                                                                                                                                                                                                                                                                                                           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Open <b>UrineNeg 1027_9_1A.wiff</b> . <ul style="list-style-type: none"><li>Copy the example Mass Hunter files that come with the TOF software to a folder that only you will use.</li></ul> | <p><b>a</b> Select <b>File &gt; Open File</b>.</p> <p><b>b</b> Go to your folder that contains the Mass Hunter example TOF data files.</p> <p><b>c</b> Select <b>UrineNeg 1027_9_1A.wiff</b>.</p> <p><b>d</b> Click <b>Open</b>.</p> <p>The data file appears in the Raw Data Window. (See <a href="#">Figure 1</a>.)</p> | <ul style="list-style-type: none"><li>This example file contains a single sample time segment and scan data. When you open a data file that contains multiple data sets, the Select Data Set dialog box (<a href="#">Figure 2</a>) appears. You must then select one combination of sample, time segment and scan segment to extract before the Raw Data Window appears.</li></ul> |

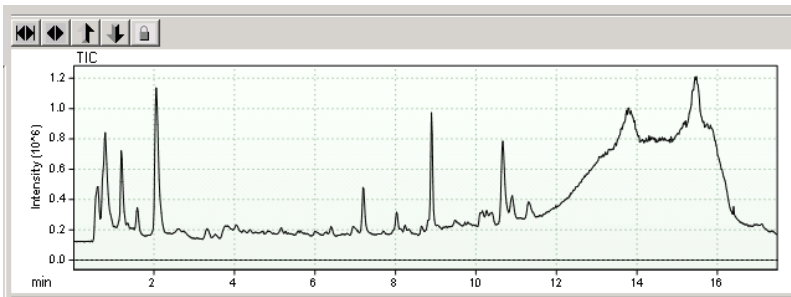
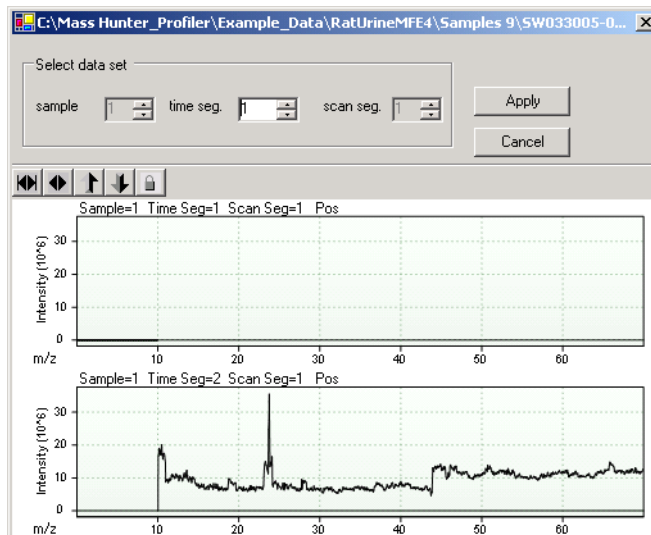
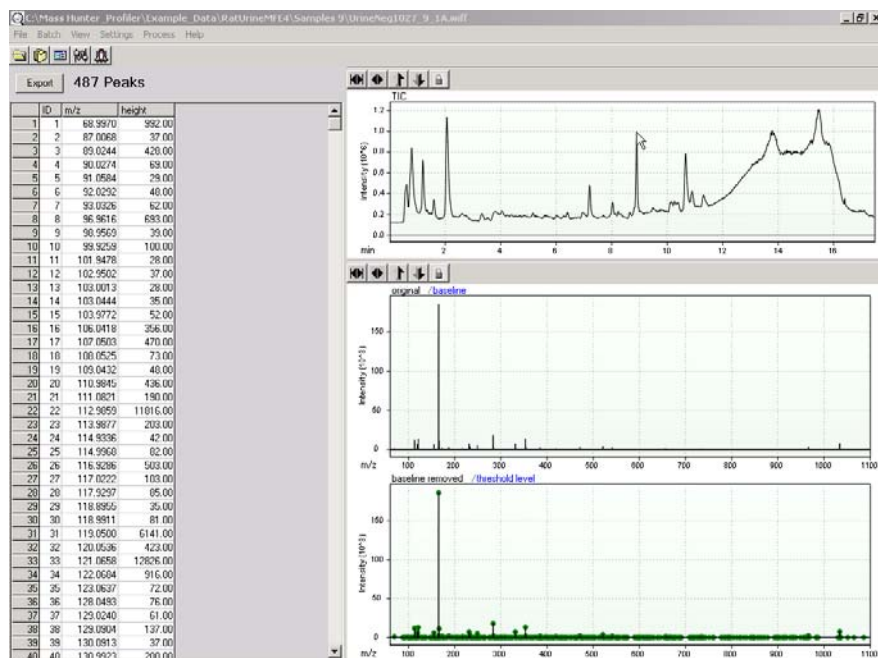


Figure 1 Raw Data Window

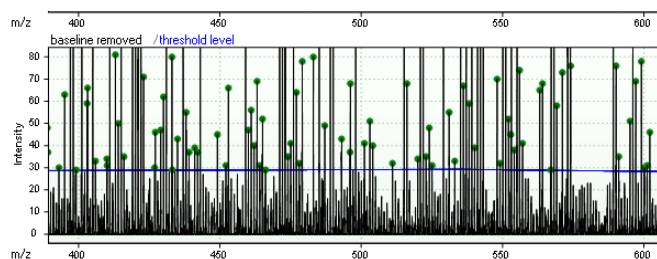


**Figure 2** Select a data set dialog box

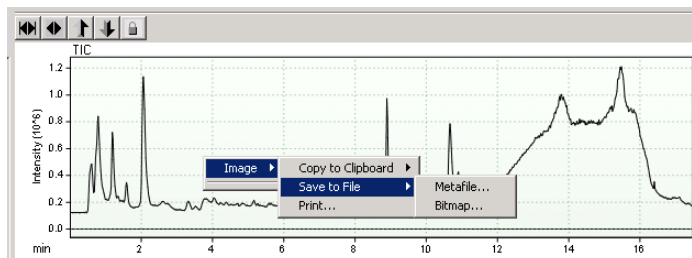
| Steps                                                                                      | Detailed Instructions                                                                                                                                                                                                                     | Comments                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2 View the mass spectra and peak information of the raw data at retention time of 8.9 min. | <ul style="list-style-type: none"> <li>Double-click the TIC at 8.9 minutes. Two mass spectra for that RT appear below the TIC, and the peak information for the mass spectrum appears in the Raw Data Peak Viewer on the left.</li> </ul> | <ul style="list-style-type: none"> <li>One mass spectrum shows the baseline, and the other shows the threshold level, which can be changed by the user (see <a href="#">“Exercise 3—Reprocess files with different parameters”</a> on page 11).</li> </ul> |



| Steps | Detailed Instructions                                                                                                                                                                                                                                                                                                       | Comments |
|-------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| 3     | <p>View the threshold level for the mass spectrum between 400 and 600 m/z.</p> <p><b>a</b> In the lower spectra viewer, hold the mouse button down and draw a rectangle at the baseline between 400 and 600 m/z.</p> <p><b>b</b> Release the mouse button.</p> <p><b>c</b> Repeat the zoom until you see the blue line.</p> |          |



- |                                        |                                                                                                                                                                                                                                             |
|----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>4 Save the TIC image to a file.</p> | <p><b>a</b> Right-click the TIC.</p> <p><b>b</b> Select <b>Image &gt; Save to File &gt; Bitmap</b>.</p> <p><b>c</b> Go to the folder into which you want to place the file, and enter the file name.</p> <p><b>d</b> Click <b>Save</b>.</p> |
|----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|



| Steps | Detailed Instructions | Comments |
|-------|-----------------------|----------|
|-------|-----------------------|----------|

- |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>5 Process the file.</p> <ul style="list-style-type: none"> <li>An .mhd file is created upon processing. This file contains the extraction result. Although you can open this file directly, you should open the original .wiff file so that you access more information.</li> </ul> | <ul style="list-style-type: none"> <li>Select <b>Process &gt; Run</b>, or click the Process button on the toolbar. The processed total ion chromatogram appears under the original TIC in the Processed Data Window (Figure 3), and the Feature Summary Table appears in the Features Viewer.</li> </ul> | <ul style="list-style-type: none"> <li>An Excel file is automatically created containing attributes for each feature. The Excel file takes the name of the .wiff file processed. In this case, the file is named UrineNeg1027_9_1A.xls. (Figure 4).</li> </ul> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Note: if you attempt to process the \*.wiff file with the same set of extraction parameters (see “Exercise 3—Reprocess files with different parameters” on page 11) as the ones stored in the \*.mhd file, the program simply loads the result from the \*.mhd file.

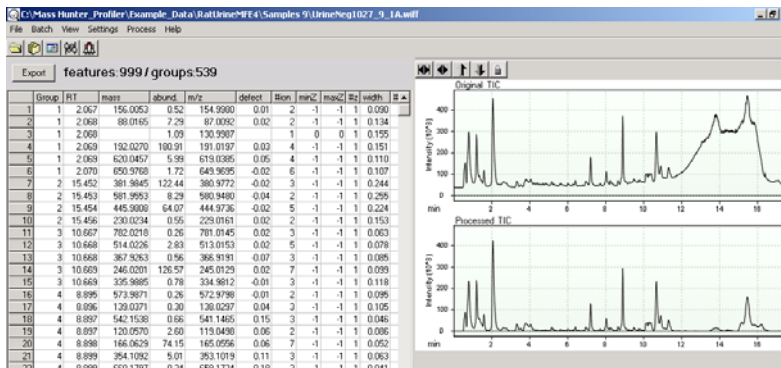


Figure 3 Processed Data Window

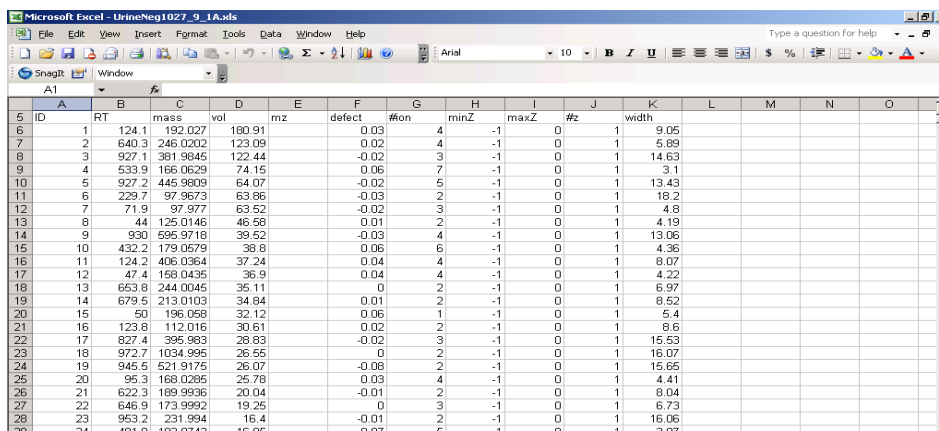


Figure 4 Excel spreadsheet with feature information automatically saved after processing



## Exercise 2—Extract feature information for a batch

This exercise shows you how to automatically extract feature information for a batch of TOF data files for downstream use or later review.

### CAUTION

To process Agilent TOF data files (.wiff files) with the Mass Hunter software, the .wiff files must be local to the software. That is, the files must reside on the computer where the Mass Hunter software is running. Also, the files must not be read-only. Use Windows Explorer to check and change the file attributes.

| Steps                                                                                                                                                                                                                                                                                                                                        | Detailed Instructions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Create a batch of files to extract. Use the following files: <ul style="list-style-type: none"><li>All three <b>sample 9</b> .wiff files: <b>UrineNeg1027_0_3A.wiff</b>, <b>UrineNeg1027_9_2A.wiff</b>, and <b>UrineNeg1027_9_1A.wiff</b>.</li><li>The first file in the <b>sample 10</b> folder: <b>UrineNeg1027_10_2A.wiff</b></li></ul> | <p><b>a</b> Select <b>Batch &gt; Create</b>.<br/>Or, click the <b>Create Batch</b> button in the toolbar.</p> <p><b>b</b> Hold the Shift key as you select all three files in the <b>sample 9</b> folder, and click <b>Open</b>.<br/>The Batch Process Window (Figure 5) appears with the .wiff files from sample 9 listed.</p> <p><b>c</b> Click the <b>Create Batch</b> button in the toolbar again.</p> <p><b>d</b> Select the first file in the sample 10 folder.</p> <p><b>e</b> Click <b>Open</b>.<br/>The Batch Process Window now contains all four files.</p> | <ul style="list-style-type: none"><li>If any of the .wiff files contains more than one data set, the Batch Data Set Options dialog box appears. You then select if you want to use all the data sets in all the files or one spectra set per file.</li><li>If you click One spectra set per file, the Select Data Set dialog box appears.</li><li>For more details about using these dialog boxes, see the Mass Hunter online help.</li></ul> |

| Files                                                                                | Sample | Time seg. | Scan seg. | Status  |
|--------------------------------------------------------------------------------------|--------|-----------|-----------|---------|
| C:\Mass Hunter_Profiler\Example_Data\RatUrineMFE4\Samples 9\UrineNeg1027_9_3A.wiff   | 1      | 1         | 1         | Pending |
| C:\Mass Hunter_Profiler\Example_Data\RatUrineMFE4\Samples 9\UrineNeg1027_9_2A.wiff   | 1      | 1         | 1         | Pending |
| C:\Mass Hunter_Profiler\Example_Data\RatUrineMFE4\Samples 9\UrineNeg1027_9_1A.wiff   | 1      | 1         | 1         | Pending |
| C:\Mass Hunter_Profiler\Example_Data\RatUrineMFE4\Samples 10\UrineNeg1027_10_2A.wiff | 1      | 1         | 1         | Pending |

**Figure 5** Batch Process Window

| Steps                | Detailed Instructions                                                                                                                            | Comments                                                                                                                                                                                                                                                                                                                                                                                   |
|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2 Process the files. | <ul style="list-style-type: none"> <li>• Select <b>Process &gt; Execute Batch</b>, or click the <b>Process</b> button in the toolbar.</li> </ul> | <ul style="list-style-type: none"> <li>• Notice the Progress bar on the left. When the runs are complete for all data files, you see a message saying that the batch run is complete.</li> <li>• To see the features, groups and mass spectra for each of the processed .mhd files in the batch, you must open each individually and work with it in the Processed Data Window.</li> </ul> |

## Exercise 3—Reprocess files with different parameters

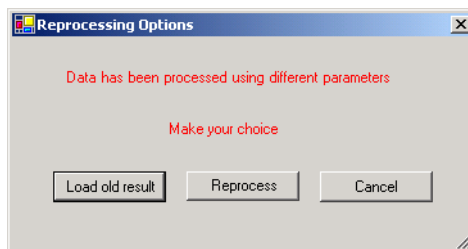
You can reprocess original .wiff files or already processed .mhd files with new extraction parameters.

| Steps                                                                                                                                                                                        | Detailed Instructions                                                                                                         | Comments |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|----------|
| 1 Open the file <b>UrineNeg 1027_9_1A.wiff</b> .                                                                                                                                             | <b>a</b> Select <b>File &gt; Open File</b> .<br><b>b</b> Select <b>UrineNeg 1027_9_1A.wiff</b> , and click <b>Open</b> .      |          |
| 2 Change the extraction parameters: <ul style="list-style-type: none"><li>• RT range of 6 to 12 minutes</li><li>• m/z range of 300 to 800</li><li>• 250 x 1000 mass spectral peaks</li></ul> | <b>a</b> Select <b>Settings &gt; Extraction Parameters</b> , or click the <b>Extraction Parameters</b> button in the toolbar. |          |

The screenshot shows the 'Extraction Parameters' dialog box with the following settings:

- Data Ranges:**
  - ☐ Use all the available data
  - RT: Min 0.00, Max 20000.00 min
  - M/Z: 0, 1000000 Da
- Spectral Peak Detection:**
  - S/N threshold: 5
- Feature Detection:**
  - Max spectral peaks to use: 500 X 1000
  - ☐ Peptidic isotope distribution
  - ☐ Single charge only
- Adducts:**
  - ☐ Salt dominated
  - Positive ions:**
    - Input field: [ ] Add
    - Selected: ☒ K, ☒ Na
    - Delete
  - Negative ions:**
    - Input field: [ ] Add
    - Selected: [ ]
    - Delete
- Buttons: OK, Cancel

| Steps                                                                                                                                                                                                                                                                                                 | Detailed Instructions                                                                                                                                                                                                                                                                                     | Comments                                                                                                                                                                                                                                                                                                                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                                                                                                                                       | <p><b>b</b> To set the RT range, enter 6 as the <b>Min RT</b> and 12 as the <b>Max RT</b>.</p> <p><b>c</b> To set the m/z range, enter 300 as the <b>Min M/Z</b> and 800 as the <b>Max M/Z</b>.</p> <p><b>d</b> For the <b>Max spectral peaks to use</b>, enter 250.</p> <p><b>e</b> Click <b>OK</b>.</p> |                                                                                                                                                                                                                                                                                                                             |
| <p><b>3</b> Reprocess the file.</p> <ul style="list-style-type: none"> <li>Because the open .wiff file was previously processed and produced an .mhd file, the system displays the Reprocessing Options dialog box.</li> <li>Make sure to reprocess the file and not load the old results.</li> </ul> | <p><b>a</b> Select <b>Process &gt; Run</b>, or click the <b>Process</b> button in the toolbar.</p> <p><b>b</b> Click <b>Reprocess</b> in the Reprocessing Options dialog box.</p>                                                                                                                         | <ul style="list-style-type: none"> <li>This dialog box appears only when you try to process a newly opened *.wiff file which has been processed previously. If you try to reprocess a file whose feature information is displayed now, the software assumes that is your intention and will not show the dialog.</li> </ul> |



| Steps                                                           | Detailed Instructions                                                                        | Comments                                                                                                                                              |
|-----------------------------------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| 4 Review the extraction parameters used for the current result. | <b>a</b> Select <b>View &gt; Parameter Table</b> .<br><b>b</b> Close the table after review. | <ul style="list-style-type: none"> <li>You may want to occasionally review the parameters used to display the currently displayed results.</li> </ul> |

| Parameter Table                            |           |
|--------------------------------------------|-----------|
| Parameters used for UrineNeg1027_9_1A.wiff |           |
| Export                                     |           |
| Parameter                                  | Value     |
| 1 Data Ranges                              |           |
| 2 Min RT                                   | 6.00 min  |
| 3 Max RT                                   | 12.00 min |
| 4 Min M/Z                                  | 300.00 Da |
| 5 Max M/Z                                  | 800.00 Da |
| 6                                          |           |
| 7 Spectral Peak Detection                  |           |
| 8 S/N                                      | 5.0       |
| 9                                          |           |
| 10 Feature Detection                       |           |
| 11 Max spectral peaks used                 | 250 x1000 |
| 12 Peptidic isotope distribution           | no        |
| 13 Single charge only                      | no        |
| 14                                         |           |
| 15 Adducts                                 |           |
| 16 Salt Dominated                          | no        |
| 17 Positive Adducts                        | K         |
| 18                                         | Na        |
| 19 Negative Adducts                        |           |

# Exercises: Reviewing and saving feature information

## Exercise 4—Show species clusters, EIC and mass spectra for features

The Feature Summary Table presents a list of features and their attributes. When coelution grouping is enabled (default), the list presents group features that coelute at almost equivalent retention times. This exercise shows you how to bring up the Feature Details Table, which lists the features and their species clusters for the coeluting group. When coelution grouping is disabled, the two tables list only individual features.

Steps

Detailed Instructions

Comments

- 1 Display the species clusters for the features that coelute together in a group.
  - Display group #2 in the .wiff file.

- a Double-click row 25 of the Feature Summary Table.  
Double-clicking the row header of any feature of the group brings up the Feature Details Table for the group.

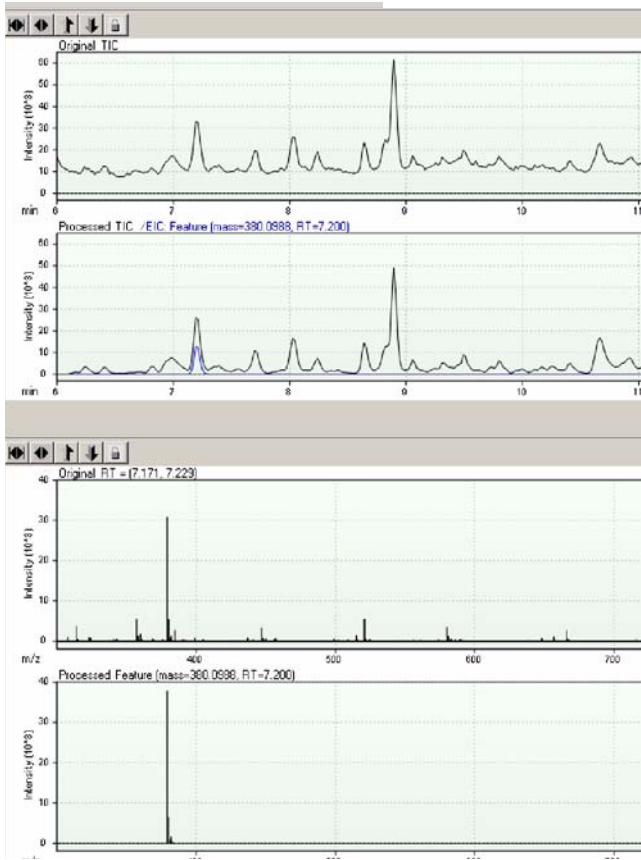
- The Feature Details Table contains the species clusters for the features in the group.
- The Plot Viewer displays the mass spectra for the group of features ,

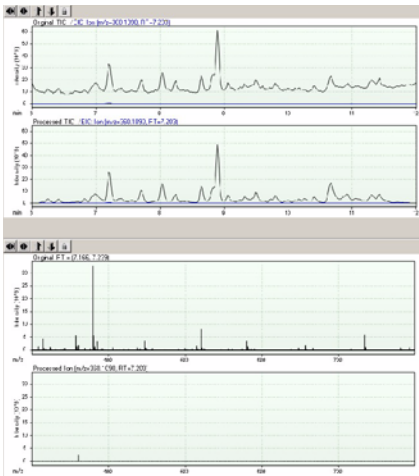
The screenshot displays the Agilent Mass Hunter software interface. The top window shows the 'features: 1298 / groups: 501' table. The bottom window shows the 'Group #2 (RT=7.200) --- 10 Features' table. To the right, three mass spectra plots are shown: 'Original TIC', 'Processed TIC - EIC, Group #2 (RT=7.200) - 10 Features', and 'Original RT = 7.163, 7.230'.

| Group | RT    | mass     | abund    | is/7     | detect   | Blon  | ms/2 | ms/2 | ms/2 | ms/2  | ms/2  | ms/2 |
|-------|-------|----------|----------|----------|----------|-------|------|------|------|-------|-------|------|
| 1     | 0.897 | 730.1901 | 0.13     | 729.1903 | 0.30     | 2     | -1   | -1   | 1    | 0.040 |       |      |
| 2     | 1     | 0.897    | 542.1530 | 0.66     | 541.1405 | 0.15  | 3    | -1   | -1   | 1     | 0.046 |      |
| 3     | 1     | 0.897    | 573.9071 | 0.21     | 572.9798 | -0.01 | 2    | -1   | -1   | 1     | 0.073 |      |
| 4     | 1     | 0.898    | 552.1007 | 0.11     | 551.1044 | 0.11  | 3    | -1   | -1   | 1     | 0.032 |      |
| 5     | 1     | 0.898    | 602      | 723.1701 |          |       | 1    | 0    | 0    | 1     | 0.036 |      |
| 6     | 1     | 0.898    | 602      | 625.0629 |          |       | 1    | 0    | 0    | 1     | 0.090 |      |
| 7     | 1     | 0.898    | 619      | 411.0999 | 0.07     | 3     | -1   | -1   | 1    | 0.069 |       |      |
| 8     | 1     | 0.898    | 604      | 590.1053 |          |       | 1    | 0    | 0    | 1     | 0.041 |      |
| 9     | 1     | 0.898    | 590.1406 | 0.10     | 589.1533 | 0.16  | 2    | -1   | -1   | 1     | 0.036 |      |
| 10    | 1     | 0.898    | 376.0902 | 0.23     | 375.0833 | 0.09  | 2    | -1   | -1   | 1     | 0.091 |      |
| 11    | 1     | 0.898    | 660.1797 | 0.24     | 659.1724 | 0.18  | 2    | -1   | -1   | 1     | 0.040 |      |
| 12    | 1     | 0.898    | 354.1092 | 4.72     | 353.1019 | 0.11  | 3    | -1   | -1   | 1     | 0.057 |      |
| 13    | 1     | 0.898    | 568.1012 | 0.10     | 567.1140 | 0.10  | 3    | -1   | -1   | 1     | 0.042 |      |
| 14    | 1     | 0.898    | 532.1262 | 2.25     | 531.1193 | 0.13  | 3    | -1   | -1   | 1     | 0.046 |      |
| 15    | 1     | 0.898    | 601      | 762.1290 |          |       | 1    | 0    | 0    | 1     | 0.030 |      |
| 16    | 1     | 0.898    | 606      | 431.0450 |          |       | 1    | 0    | 0    | 1     | 0.061 |      |
| 17    | 1     | 0.898    | 472.1259 | 0.08     | 471.1286 | 0.14  | 2    | -1   | -1   | 1     | 0.045 |      |
| 18    | 1     | 0.898    | 607      | 476.0413 |          |       | 1    | 0    | 0    | 1     | 0.054 |      |
| 19    | 1     | 0.898    | 603      | 596.0954 |          |       | 1    | 0    | 0    | 1     | 0.042 |      |
| 20    | 1     | 0.898    | 738.1508 | 0.04     | 738.1433 | 0.15  | 2    | -1   | -1   | 1     | 0.037 |      |
| 21    | 1     | 0.900    | 603      | 609.1287 |          |       | 1    | 0    | 0    | 1     | 0.044 |      |
| 22    | 1     | 0.900    | 610      | 437.0067 |          |       | 1    | 0    | 0    | 1     | 0.079 |      |
| 23    | 1     | 0.900    | 612      | 658.0797 |          |       | 1    | 0    | 0    | 1     | 0.036 |      |
| 24    | 2     | 7.199    | 607      | 343.1040 |          |       | 1    | 0    | 0    | 1     | 0.073 |      |
| 25    | 2     | 7.199    | 610      | 367.0145 |          |       | 1    | 0    | 0    | 1     | 0.127 |      |
| 26    | 2     | 7.199    | 612      | 762.1009 | 0.12     | 0.041 |      |      |      |       |       |      |

| Species  | RT    | mass     | abund    | width |       |  |
|----------|-------|----------|----------|-------|-------|--|
| 1 M      | 7.200 | 380.0980 | 4.72     | 0.063 |       |  |
| 2 M+1    | 7.202 | 379.0915 | 390.0989 | 3.78  | 0.057 |  |
| 3 M+1    | 7.203 | 380.0955 | 0.05     | 0.056 |       |  |
| 4 M+2    | 7.197 | 381.0955 | 0.10     | 0.060 |       |  |
| 5 M+3    | 7.204 | 382.0994 | 0.16     | 0.064 |       |  |
| 6 M+4    | 7.154 | 383.0960 | 0.03     | 0.061 |       |  |
| 7        |       |          |          |       |       |  |
| 8 M      | 7.199 | 361.1162 | 0.29     | 0.076 |       |  |
| 9 M+1    | 7.203 | 360.1090 | 361.1162 | 0.24  | 0.072 |  |
| 10 M+1   | 7.196 | 361.1095 | 0.05     | 0.091 |       |  |
| 11       |       |          |          |       |       |  |
| 12 M     | 7.201 | 516.0737 | 0.25     | 0.067 |       |  |
| 13 M+1   | 7.206 | 515.0665 | 516.0737 | 0.20  | 0.069 |  |
| 14 M+1   | 7.195 | 516.0710 | 0.04     | 0.066 |       |  |
| 15       |       |          |          |       |       |  |
| 16 M     | 7.199 |          | 0.20     | 0.127 |       |  |
| 17 M + 7 | 7.199 | 362.0145 | 0.20     | 0.127 |       |  |
| 18       |       |          |          |       |       |  |
| 19 M     | 7.200 | 762.1009 | 0.12     | 0.041 |       |  |

| Steps                                                                       | Detailed Instructions                                                                                         | Comments                                                                                             |
|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| 2 Show the feature mass spectra and EIC for the first feature in the group. | <ul style="list-style-type: none"> <li>Double-click the row number 1 in the Feature Details Table.</li> </ul> | <ul style="list-style-type: none"> <li>The mass spectra and EICs appear for that feature.</li> </ul> |

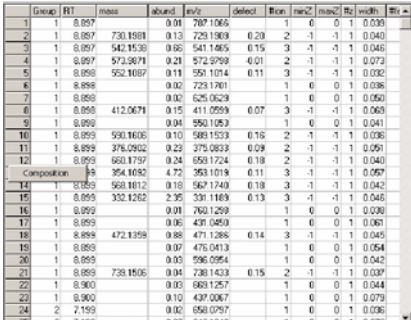
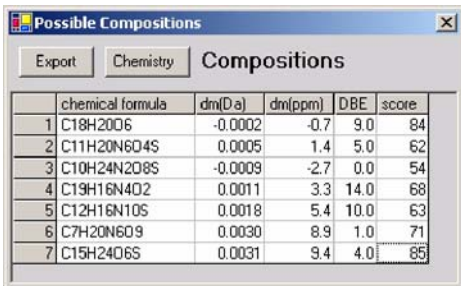


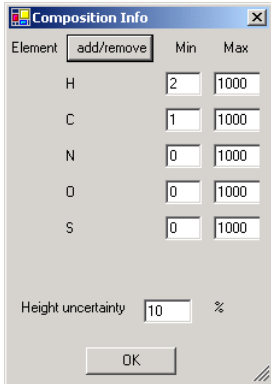
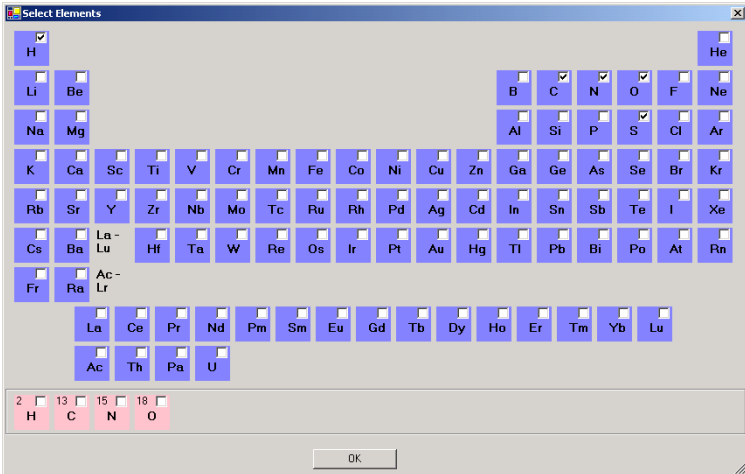
| Steps                                                                                | Detailed Instructions                                                                                                                                                                                                                                                                                                                                                | Comments                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3 Show ion mass spectra and EICs for the M+H ion of the second feature in the group. | <ul style="list-style-type: none"> <li>Double-click the row #9 in the Feature Details table.</li> </ul>                                                                                                                                                                                                                                                              | <ul style="list-style-type: none"> <li>The Plot Viewer displays the mass spectrum and EIC for the original data, and a mass spectrum and EIC for the processed ion specified in row 9.</li> </ul>          |
|     |                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                            |
| 4 Show feature mass spectra for a feature when coelution grouping is disabled.       | <ol style="list-style-type: none"> <li>Select <b>Settings &gt; Disable Coelution Grouping</b>.</li> <li>Click the <b>Process</b> icon to bring up the Feature Summary Table with a listing of features and no groups.</li> <li>Double-click the row #2.<br/>You now see the Feature Details Table with species cluster information for only that feature.</li> </ol> | <ul style="list-style-type: none"> <li>The mass spectra for the selected feature appear in the Plot Viewer, and the species clusters for that feature only appear in the Feature Details Table.</li> </ul> |
| 5 Re-enable Coelution Grouping.                                                      | <ol style="list-style-type: none"> <li>Select <b>Settings &gt; Enable Coelution Grouping</b>.</li> <li>Click the <b>Process</b> icon.</li> </ol>                                                                                                                                                                                                                     |                                                                                                                                                                                                            |



## Exercise 5—Show possible feature compositions

The Mass Hunter software calculates the possible compositions for any feature you select either from the Feature Summary Table or the Feature Details Table. This exercise shows you how to display these compositions and set up the “rules” for calculating the composition.

| Steps                                                      | Detailed Instructions                                                                                                                                                                                                                                                                  | Comments                                                                                                                                                                                                                                                                                                                             |
|------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Display the possible compositions for feature at row 15. | <p>a In the Feature Summary table, right-click row #15 at the row header.</p>  <p>b Click <b>Composition</b>.</p>  | <ul style="list-style-type: none"> <li>When you click Composition, the Possible Compositions dialog box appears with the possible compositions listed.</li> <li>If the MW is greater than 800 amu, a warning message appears to let you know that the calculation may take awhile and to ask you if you want to continue.</li> </ul> |

| Steps                                                                                          | Detailed Instructions                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Comments                                                                                                                                                                                                                     |
|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2 Add isotope N15 to the list of elements used in the calculation.                             | <p><b>a</b> In the Possible Compositions dialog box, click <b>Chemistry</b>.</p>  <p><b>b</b> In the Composition Info dialog box, click <b>Add/Remove</b>.</p>  <p><b>c</b> In the Select Elements dialog box, right-click <b>N</b> and select isotope <b>15N</b>.</p> <p><b>d</b> Click <b>OK</b>.</p> | <ul style="list-style-type: none"> <li>After you follow these instructions, Mass Hunter adds the N15 isotope to the element list. The isotope appears at the bottom of the Periodic Table with a marked checkbox.</li> </ul> |
| 3 Set up the calculation so that the software uses no fewer than zero and no more than 10 N15. | <p><b>a</b> Enter the <b>Min</b> number of atoms for N15 of 0 and <b>Max</b> number of 10.</p> <p><b>b</b> Click <b>OK</b>.</p>                                                                                                                                                                                                                                                                                                                                             | <ul style="list-style-type: none"> <li>When you click OK, the software automatically recalculates the possible compositions.</li> </ul>                                                                                      |

## Exercise 6—Export and save feature information

You can export the information in each Mass Hunter table to an Excel file:

- Feature Summary Table
- Feature Details Table
- Possible Compositions Table
- Parameter View

You can also save molecular ion peak information for all the features.

| Steps                                                                                                                                                                                                                                                  | Detailed Instructions                                                                                                                                                                                                                                                                                                                                                       | Comments                                                                                                                                                                                                                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1</b> Export the Feature Composition table to a Microsoft Excel file. <ul style="list-style-type: none"><li>• If you are already in the Possible Compositions dialog box after Exercise 5, skip to step b.</li><li>• View the Excel file.</li></ul> | <b>a</b> Right-click any feature in the Feature Summary Table, and click <b>Composition</b> .<br><b>b</b> Click <b>Export</b> .<br><b>c</b> Specify a destination folder.<br><b>d</b> Specify a file name.<br><b>e</b> Click <b>OK</b> .<br><b>f</b> Close the Possible Compositions dialog box.<br><b>g</b> Go to the folder containing the Excel file, and open the file. | <ul style="list-style-type: none"><li>• If you are in the Mass Hunter main window, open the file UrineNeg1027_9_1A_1_1_1.mhd. Then follow the instructions in this step.</li></ul>                                                                                                 |
| <b>2</b> Export the Feature Summary table to a Microsoft Excel file. <ul style="list-style-type: none"><li>• View the Excel file.</li></ul>                                                                                                            | <b>a</b> At the top of the Feature Summary Table, click <b>Export</b> .<br><b>b</b> Specify a destination folder.<br><b>c</b> Specify a file name.<br><b>d</b> Click <b>OK</b> .<br><b>e</b> Go to the folder containing the Excel file, and open the file.                                                                                                                 | <ul style="list-style-type: none"><li>• See <a href="#">Figure 6</a>, “Feature Summary Table in Excel,” on page 20.</li></ul>                                                                                                                                                      |
| <b>3</b> Export the Feature Details table. <ul style="list-style-type: none"><li>• View the Excel file.</li></ul>                                                                                                                                      | <b>f</b> At the top of the Feature Details Table, click <b>Export</b> .<br><b>g</b> Specify a destination folder.<br><b>h</b> Specify a file name.<br><b>i</b> Click <b>OK</b> .<br><b>j</b> Go to the folder containing the Excel file, and open the file.                                                                                                                 | <ul style="list-style-type: none"><li>• See <a href="#">Figure 7</a>, “Feature Details Table in Excel,” on page 21.</li><li>• If the Feature Details Table is not present, double-click the row number of the group or feature of interest in the Feature Summary Table.</li></ul> |

| Steps                                          | Detailed Instructions                                                                                                                                                                                 | Comments                                                                                                                   |
|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| 4 Save and view the molecular ion information. | <b>a</b> Select <b>File &gt; Save Feature Info.</b><br><b>b</b> Specify a destination folder.<br><b>c</b> Click <b>OK.</b><br><b>d</b> Go to the folder containing the Excel file, and open the file. | <ul style="list-style-type: none"> <li>See <a href="#">Figure 8</a>, “Feature Info Table in Excel,” on page 21.</li> </ul> |

|    | A          | B         | C                    | D      | E   | F      | G   | H    | I    | J  | K     | L         | M |
|----|------------|-----------|----------------------|--------|-----|--------|-----|------|------|----|-------|-----------|---|
|    | Group      | RT        | mass                 | abund. | m/z | defect | #on | minZ | maxZ | #z | width | #features |   |
| 1  | MassHunter | (1.0.0.0) | 9/23/2005 2:12:38 PM |        |     |        |     |      |      |    |       |           |   |
| 2  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 3  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 4  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 5  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 6  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 7  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 8  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 9  |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 10 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 11 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 12 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 13 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 14 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 15 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 16 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 17 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 18 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 19 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 20 |            |           |                      |        |     |        |     |      |      |    |       |           |   |
| 21 |            |           |                      |        |     |        |     |      |      |    |       |           |   |

**Figure 6** Feature Summary Table in Excel

Microsoft Excel - detailTable.xls

File Edit View Insert Format Tools Data Work Items Window

MassHunter(1.0.0.0) 9/23/2005 2:14:56 PM

| A  | B                                   | C                    | D        | E    | F      |
|----|-------------------------------------|----------------------|----------|------|--------|
| 1  | MassHunter(1.0.0.0)                 | 9/23/2005 2:14:56 PM |          |      |        |
| 2  |                                     |                      |          |      |        |
| 3  | Group #2 (RT=7.200) --- 10 Features |                      |          |      |        |
| 4  |                                     |                      |          |      |        |
| 5  | species                             | RT                   | m/z      | mass | abund. |
| 6  |                                     |                      |          |      |        |
| 7  | M                                   | 7.2                  | 380.0988 | 4.72 | 0.063  |
| 8  | M-H                                 | 7.202                | 379.0915 | 3.78 | 0.057  |
| 9  | M-H+1                               | 7.203                | 380.0966 | 0.65 | 0.056  |
| 10 | M-H+2                               | 7.197                | 381.0955 | 0.1  | 0.06   |
| 11 | M-H+3                               | 7.204                | 382.0894 | 0.16 | 0.064  |
| 12 | M-H+4                               | 7.194                | 383.096  | 0.03 | 0.081  |
| 13 |                                     |                      |          |      |        |
| 14 | M                                   | 7.199                | 361.1162 | 0.29 | 0.076  |
| 15 | M-H                                 | 7.203                | 360.109  | 0.24 | 0.072  |
| 16 | M-H+1                               | 7.196                | 361.1095 | 0.05 | 0.081  |
| 17 |                                     |                      |          |      |        |
| 18 | M                                   | 7.201                | 516.0737 | 0.25 | 0.067  |
| 19 | M-H                                 | 7.206                | 515.0665 | 0.2  | 0.069  |
| 20 | M-H+1                               | 7.195                | 516.071  | 0.04 | 0.086  |
| 21 |                                     |                      |          |      |        |

detailTable

**Figure 7** Feature Details Table in Excel

Microsoft Excel - info.xls

File Edit View Insert Format Tools Data Work Items Window Help

feature ID

| A  | B          | C            | D            | E      | F         | G    | H |
|----|------------|--------------|--------------|--------|-----------|------|---|
| 1  | feature ID | multimer m/z | mono peak rt | m/z    | abundance |      |   |
| 2  | 1          | 1            | -1 M-H       | 8.899  | 363.1019  | 4.03 |   |
| 3  | 2          | 1            | -1 M-H       | 7.2    | 379.0915  | 3.78 |   |
| 4  | 3          | 1            | -1 M-H       | 8.645  | 429.0836  | 2.91 |   |
| 5  | 4          | 1            | -1 M-H       | 7.707  | 341.0676  | 2.68 |   |
| 6  | 5          | 1            | -1 M-H       | 10.668 | 513.0153  | 2.14 |   |
| 7  | 6          | 1            | -1 M-H       | 8.899  | 331.1189  | 1.97 |   |
| 8  | 7          | 1            | -1 M-H       | 8.839  | 308.1175  | 1.73 |   |
| 9  | 8          | 1            | -1 M-H       | 11.112 | 344.98    | 1.81 |   |
| 10 | 8          | 2            | -1 2M-H      | 11.112 | 690.9686  | 0.02 |   |
| 11 | 9          | 1            | -1 M-H       | 9.502  | 417.1195  | 1.42 |   |
| 12 | 10         | 1            | -1 M-H       | 8.031  | 408.1221  | 1.26 |   |
| 13 | 11         | 1            | -1 M-H       | 11.428 | 315.2536  | 1.18 |   |
| 14 | 12         | 1            | -1 M-H       | 9.061  | 325.0836  | 1.17 |   |
| 15 | 13         | 1            | -1 M-H       | 11.435 | 305.2079  | 1.06 |   |
| 16 | 14         | 1            | -1 M-H       | 6.985  | 325.0898  | 0.92 |   |
| 17 | 15         | 1            | -1 M-H       | 9.322  | 445.0779  | 0.79 |   |
| 18 | 16         | 1            | -1 M-H       | 6.931  | 307.0443  | 0.83 |   |
| 19 | 16         | 2            | -1 2M-H      | 6.931  | 615.0897  | 0.02 |   |
| 20 | 17         | 1            | -1 M-H       | 6.817  | 363.0831  | 0.78 |   |
| 21 | 18         | 1            | -1 M-H       | 8.034  | 305.1407  | 0.79 |   |

info

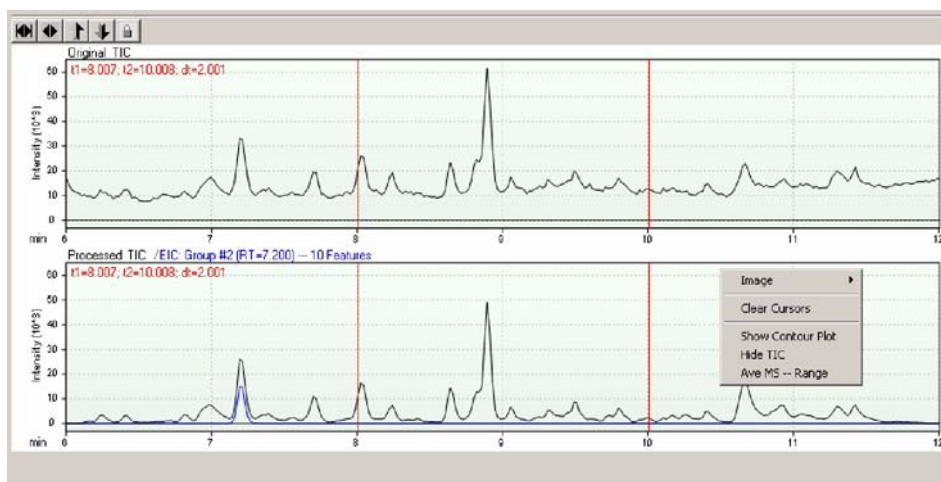
**Figure 8** Feature Info Table in Excel

## Exercises: Working with plots

### Exercise 7—Working with processed chromatograms (TICs or EICs)

You can perform the chromatogram operations on total ion chromatograms (TICs) and extracted ion chromatograms (EICs) and on the original and processed chromatograms, either together or separately.

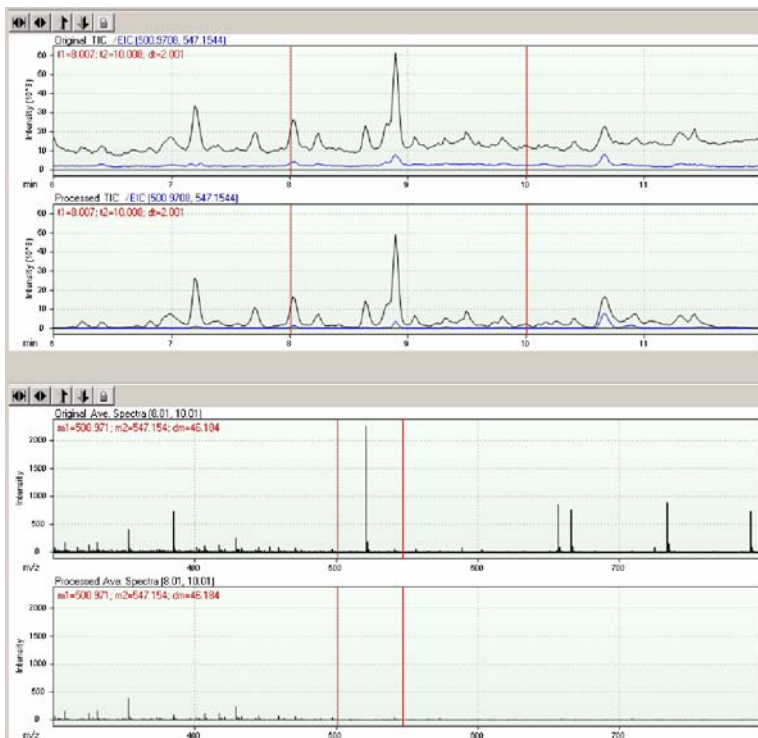
| Steps                                                                                                                                                                                                              | Detailed Instructions                                                                                                                                                                                                                                                                                                                                                                 | Comments                                                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1</b> Do the following: <ul style="list-style-type: none"> <li>Zoom in on the chromatograms between 8 to 10 minutes to include the entire peak around 9 minutes.</li> <li>Zoom out the chromatogram.</li> </ul> | <b>a</b> Hold the mouse button down as you draw a rectangle around the specified time window.<br><b>b</b> Release the mouse button.<br><b>c</b> Click the <b>Full Zoom Out</b> button.                                                                                                               | <ul style="list-style-type: none"> <li>When the zoom function is locked (default), the zoom works on both chromatograms simultaneously.</li> <li>If you click the Lock button to unlock the zoom function, you can zoom in or out of each chromatogram separately.</li> </ul>                             |
| <b>2</b> View the mass spectra (original and processed) at around RT 9 minutes.                                                                                                                                    | <ul style="list-style-type: none"> <li>Double-click the RT at around 9 minutes in the chromatogram.</li> </ul>                                                                                                                                                                                                                                                                        | <ul style="list-style-type: none"> <li>The system displays the mass spectrum at RT 9 min. for both the original and processed chromatograms.</li> </ul>                                                                                                                                                   |
| <b>3</b> View the average mass spectra over the range of RT 8 to 10 minutes.                                                                                                                                       | <b>a</b> <b>CTRL</b> -click the left mouse button (or click the middle mouse button) to place a line on the plot to set the low end of the range over which the average is calculated.<br><b>b</b> <b>CTRL</b> -click the left mouse button again to place a line on the plot to set the high end of the range.<br><b>c</b> Right-click the image, and select <b>Ave MS – Range</b> . | <ul style="list-style-type: none"> <li>See <a href="#">Figure 9</a>, “Chromatographic range context menu,” on page 23.</li> <li>The average mass spectra calculated over the selected range appears below the chromatogram plots for the original chromatogram and the processed chromatogram.</li> </ul> |
| <b>4</b> Hide the TIC.                                                                                                                                                                                             | <b>a</b> Right-click the image, and select <b>Hide EIC</b> from the context menu (see <a href="#">Figure 9</a> ).                                                                                                                                                                                                                                                                     | <ul style="list-style-type: none"> <li>When an EIC is superimposed on a TIC, you can hide the TIC.</li> </ul>                                                                                                                                                                                             |
| <b>5</b> Re-show the TIC.                                                                                                                                                                                          | <b>a</b> Right-click the image, and select <b>Show EIC</b> from the context menu.                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                           |



**Figure 9** Chromatographic range context menu

## Exercise 8—Working with mass spectra

| Steps                                                                                   | Detailed Instructions                                                                                                                                                                                                                                                                                                                                                                                | Comments                                                                                                                                                |
|-----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Show an extracted ion chromatogram at about 500 m/z (highest peak)                    | 1 Double-click the highest peak in the mass spectrum.                                                                                                                                                                                                                                                                                                                                                | <ul style="list-style-type: none"> <li>The Plot Viewer displays the EIC for this RT in both the original and processed chromatogram windows.</li> </ul> |
| 2 Show an extracted ion chromatogram for the m/z range of 500 to 550 m/z (approximate). | <p>a CTRL-click the left mouse button (or click the middle mouse button) to place a line on the mass spectrum to set the low end of the range over which the chromatogram is extracted.</p> <p>b CTRL-click the left mouse button again to place a line on the mass spectrum to set the high end of the range.</p> <p>c Right-click the image, and select <b>Show EIC</b> from the context menu.</p> | <ul style="list-style-type: none"> <li>The EICs appear superimposed on the TICs.</li> </ul>                                                             |

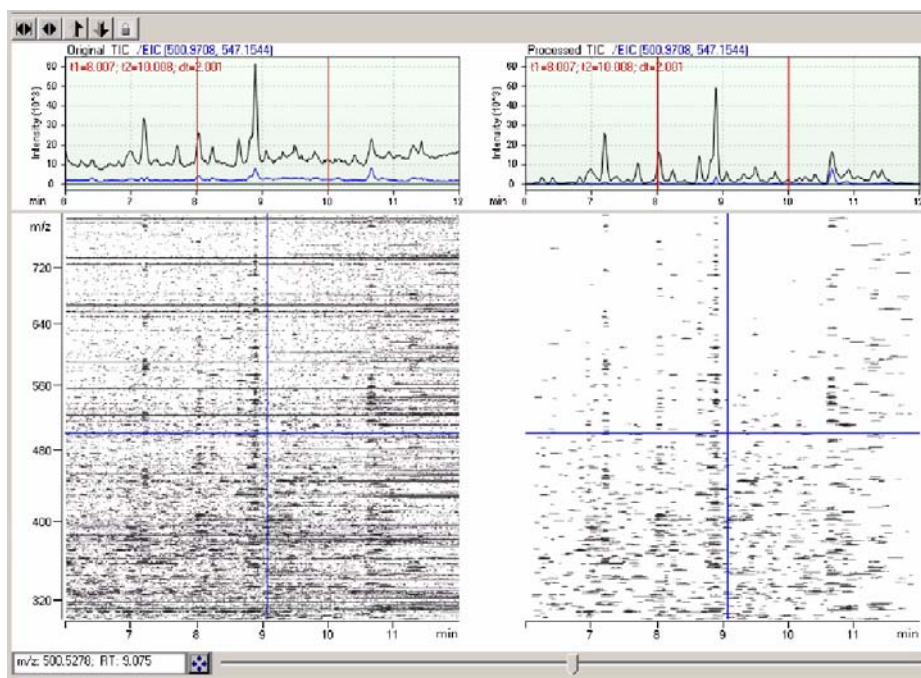




## Exercise 9—Working with contour plots

The contour plot is a two-dimensional representation of 3D data, with  $m/z$  as the x-axis, RT as the y-axis and intensity as the z-axis (the darkness or lightness of the image on the plot). Two plots are shown, one for the original data and the other, for the processed data.

| Steps                                             | Detailed Instructions                                                                                                                                                                                                                                                                                                                          | Comments                                                                                                                                                                                                              |
|---------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Show the contour plots.                         | <p><b>a</b> Right-click any one of the chromatograms, and select <b>Show Contour Plot</b>.</p>                                                                                                                                                                                                                                                 | <ul style="list-style-type: none"> <li>• See <a href="#">Figure 9</a>, “Chromatographic range context menu,” on page 23.</li> <li>• The contour plots appear for the original and processed chromatograms.</li> </ul> |
| 2 Zoom into and out of the contour plot.          | <p><b>a</b> Hold down the mouse button and draw a rectangle around the area of interest.</p> <p><b>b</b> Release the mouse button.</p> <p><b>c</b> To zoom out, click the <b>Zoom Out</b> button next to the coordinates text field.</p>                      |                                                                                                                                                                                                                       |
| 3 Find the position of $m/z = 500$ and $RT = 9$ . | <p><b>a</b> CTRL-click the left mouse button (or click the middle mouse button). Two perpendicular blue lines (cross-hair cursor) appear in the plot.</p> <p><b>b</b> With the mouse button held down, move the cross-hair cursor to the point specified in step 3.</p> <p><b>c</b> Double-click the plot to remove the cross-hair cursor.</p> | <ul style="list-style-type: none"> <li>• You can see the coordinates of the position in the box to the left and below the left contour plot. (<a href="#">Figure 10</a>)</li> </ul>                                   |
| 4 Hide the contour plot.                          | <p><b>a</b> Right-click the plot, and select <b>Hide Counter Plot</b>.</p>                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                       |



**Figure 10** Contour Plots with cross-hair cursor



**www.agilent.com**

## **In this guide**

This Quick Start Guide includes an overview of the Mass Hunter software, quick reference information to get started using the software, and a set of tutorials to learn how to use the software.

© Agilent Technologies, Inc. 2005

Printed in USA  
First edition, September 2005



G3297-90000



**Agilent Technologies**