Compass

Application Tutorial

Library Search Tutorial

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Version 1.4 (June 2008)

Bruker Daltonics

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Document History

Library Search Tutorial, Version 1.4 (June 2008)
Printed in Germany

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Preliminary remarks

Related software:
Compass 1.3 for esquire / HCT incl. esquireControl 6.2 and DataAnalysis 4.0
HyStar 3.2
BioTools 3.1
ProteinScape 1.3
Mascot 1.9
MetaboliteTools 1.1

This document describes how to generate custom libraries (MS and MS/MS) with the Compass 1.0 software and search unknown components against the libraries. However, as mass spectra obtained using API techniques typically produces only pseudo-molecular ions, it is recommended to build MS/MS libraries.

This document is divided into two parts.

1st generate the library and

2nd using the library for identification.

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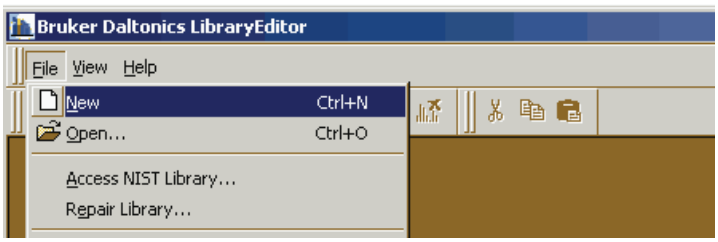
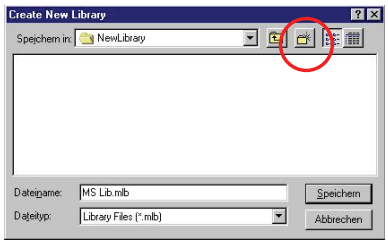
1 Generating a Library

1.1 Create new Library

The program is accessible from the start button in the task bar:
Start / Programs / Bruker Daltonics / LibraryEditor
or via main menu *Compass / LibraryEditor* of **Bruker Daltonics DataAnalysis**.

The program **Bruker Daltonics LibraryEditor** has been used for generation of a new library.
Every library must be stored in a separate directory. The function "*New Folder*" is used in the **file new dialog / create new folder**. As the second step the library itself is saved in the new folder. Therefore you have to choose a name for the new library.





1.2 Open Data File in DataAnalysis

The data set for extracting spectra is **SULF0024.d** which contains the following compounds:

Compound	**MW**	**est. m/z** (MSMS)
Sulfamethizole	270	156, 108
Sulfamethazine	278	204, 186, 156, 124
Sulfachloropyridazine	284	156, 108
Sulfadimethoxime	310	245, 218, 156, 108

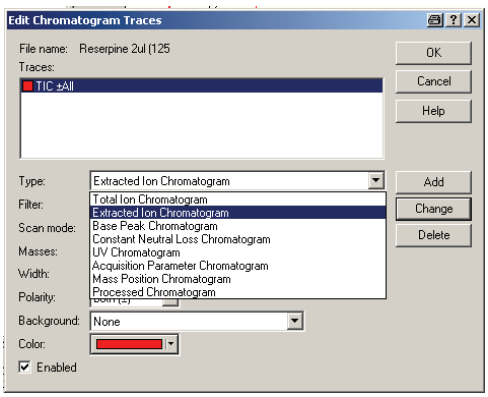
In the region of 3, 4 and 7 min. are peaks visible. These trace are used for creating a peak list with function **Find / Compounds – Chromatogram**.

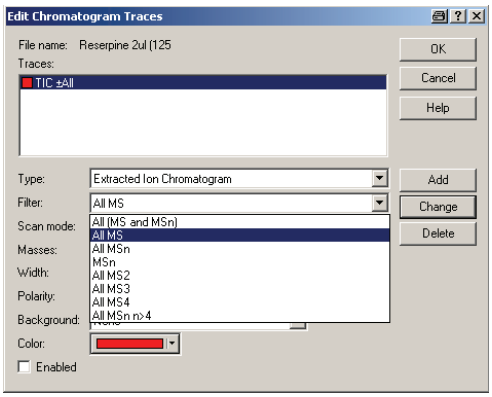
The demo data can be found in the following directory:
"D:\Data\Demo\Esquire, HCT\Library Search\CARB0016.d"
"D:\Data\Demo\Esquire, HCT\Library Search\SULF0024.d"

1.2.1 Create Chromatogram Traces

1.2.1.1 Known Compounds

If your compound of interest and the polarity of the acquisition are known, it is easy to display the respective mass traces of the pseudomolecular ions. In most cases, the **Extracted Ion Chromatogram** (EIC) is chosen for display.





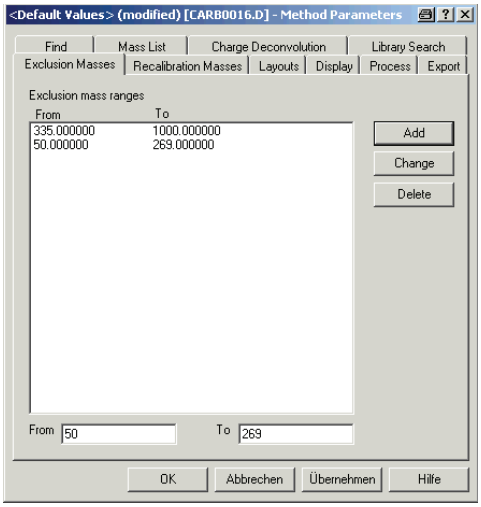
→ Select **Edit | Chromatograms (F7)**: the **Edit Chromatogram Traces** dialog box is opened.
→ Select the **Type** and the **Filter** "All(MS and MSn)" .
Single mass traces or mass ranges can be selected in **Masses (**Not available for the **Type:** Total Ion Chromatogram**)**.
Extract the mass lines: 271, 279, 285, 311 m/z or type in a hyphen "-" to cover the whole mass range.
→ Select **Width** "± 0.5" and **Polarity** to "both".
→ Click on the **Add** button and confirm with **OK**.

1.2.1.2 Unknown Compounds

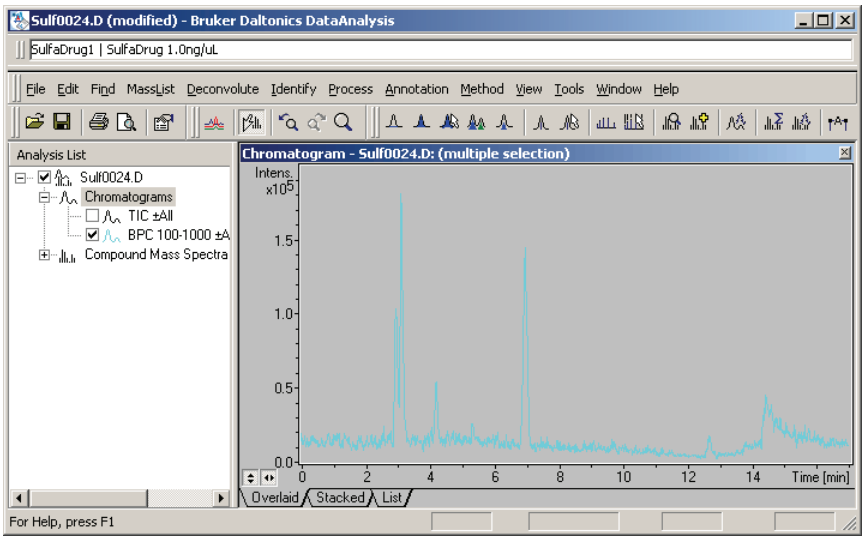
When searching unknown compounds, the crucial step to find characteristic mass spectra is to determine the background signal (chemical noise). It is possible to exclude masses from further processing in a base peak chromatogram as well as in the TIC.

In the example file **Sulf0024.d**
(D:\Data\Demo\Esquire, HCT\Library Search\SULF0024.d), the TIC exhibits a high background level with four little peaks.

Create the trace “Base Peak Chromatogram”.
Select **Method | Parameters...** (**alt+F2**) the method parameter dialog opens.
Here the tab **Exclusion Masses** is used.



Type in the mass range “50 to 269”, “Add” and “335 to 3000” “Add” and press OK.



1.3 Export of Spectra from DataAnalysis

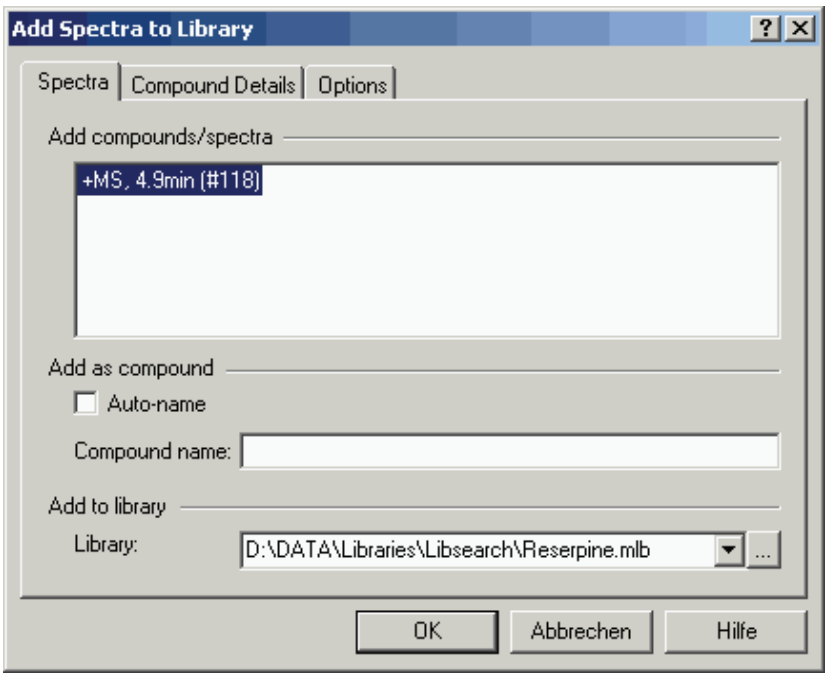
The Bruker library is able to hold a double-dimensional data set. For one compound are several MS spectra allowed (e.g. MS stages, polarity, settings).

1.3.1 Directly Export of Spectra

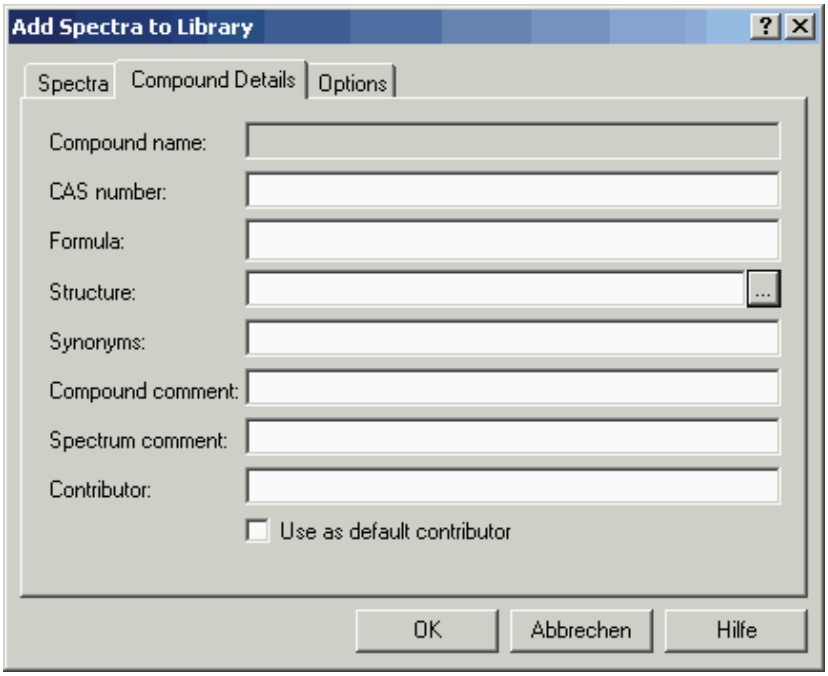
The first way to fill a library is to export spectra directly to the target library. It's done with the context function **Add to Library.... .** With the function **File / Export / Mass Spectrum** any spectrum can be saved to disk for further using.

Activate a spectrum by pressing control and clicking into the **chromatogram** window. A spectrum should be shown in the **Spectrum View** window. A right mouse click into this window offers the command *Copy to Compound Spectra*.

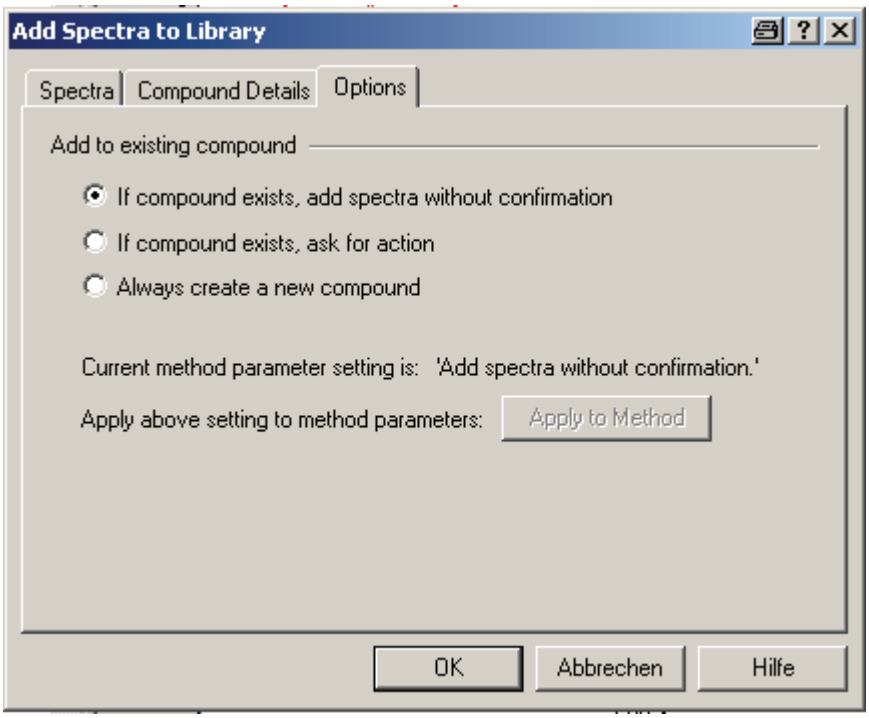
From the **Compound Spectra** window the context menu offers the function **Add to Library.** The user must type in the name of this spectrum or activate the function Auto-name. In this case the spectrum gets the filename.



Additionally, compound information can be typed in on the page "Compound Details".



If the compound already is in the library, the user can decide how to handle the new entry, on the page “Options”.



All other information is sent directly to the selected library.

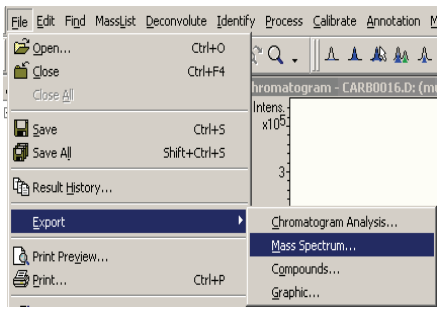
When a peak list is generated on this chromatogram the **Compound Spectra**

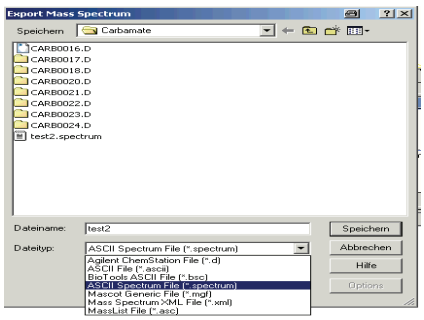
window shows all peaks. Activate a spectrum, choose the context menu of this spectrum and use **Add to Library** directly. When in the processing method a library is selected, this target library is suggested. Otherwise select a library.

In case to add a spectrum from profile data only detected peaks are exported (That means all peaks of the **MassList (Spectrum Data)** are exported. Which peaks are in the MassList depends on the settings of **MassList / Parameters…**). In case of line spectra all mass lines are exported to library.

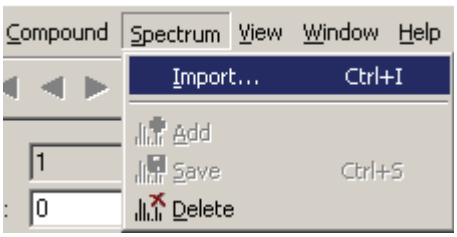
1.3.2 Export via saved files

The function **File / Export / Mass Spectrum** offers different file types to the user. Select *ASCII MS Spectrum File (*.spectrum)* to saved spectra at file. For this export function the user must type in a name.





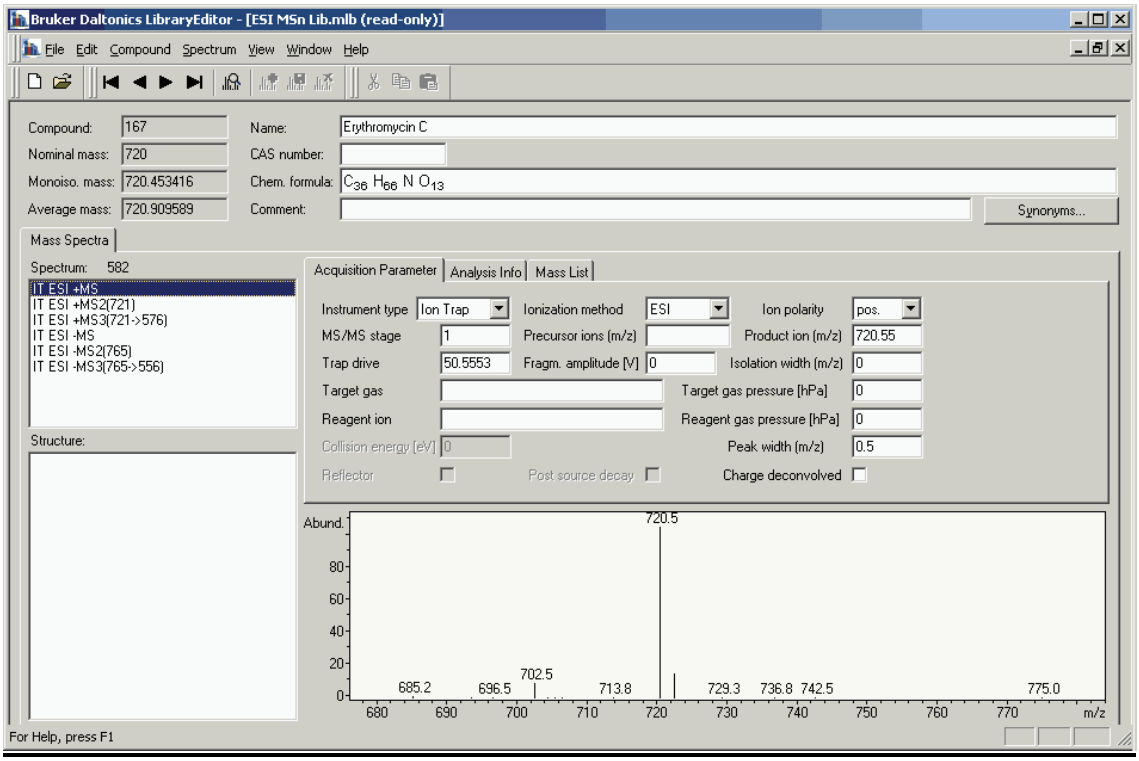
To import the spectra in the library use the function **Spectrum / Import…** of the Library Editor.



1.4 Modify Library

When the library holds multiple MS spectra, the LibraryEditor sorts all entries automatically.

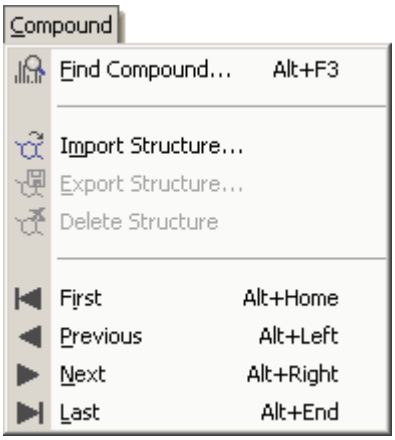
The **LibraryEditor** allows to hold additional parameters / values for any compound.



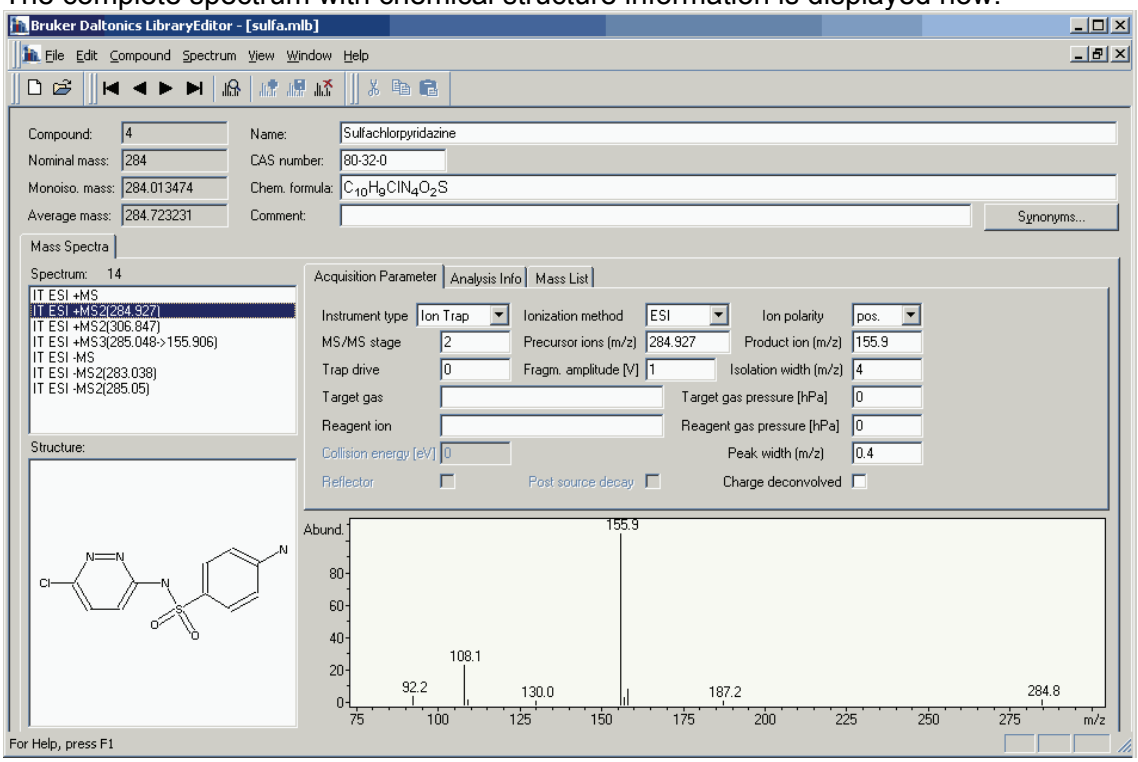
Repeat the selection of peaks or averaged spectra and the export for the other compounds of interest assigning representative names (with the function **Add to Library**). When the library is opened in the **LibraryEditor** use the *File / Refresh* function to read the new spectra.

1.5 Import of MOL-Files

For any compound in the library a structure (Mol-file) can be stored. To import the structure of a selected compound use the function **Compound / Import Structure**.



Chemical structures can be drawn in several external programs (e.g. ACD ChemSketch, MDL ISIS Draw).
The complete spectrum with chemical structure information is displayed now.

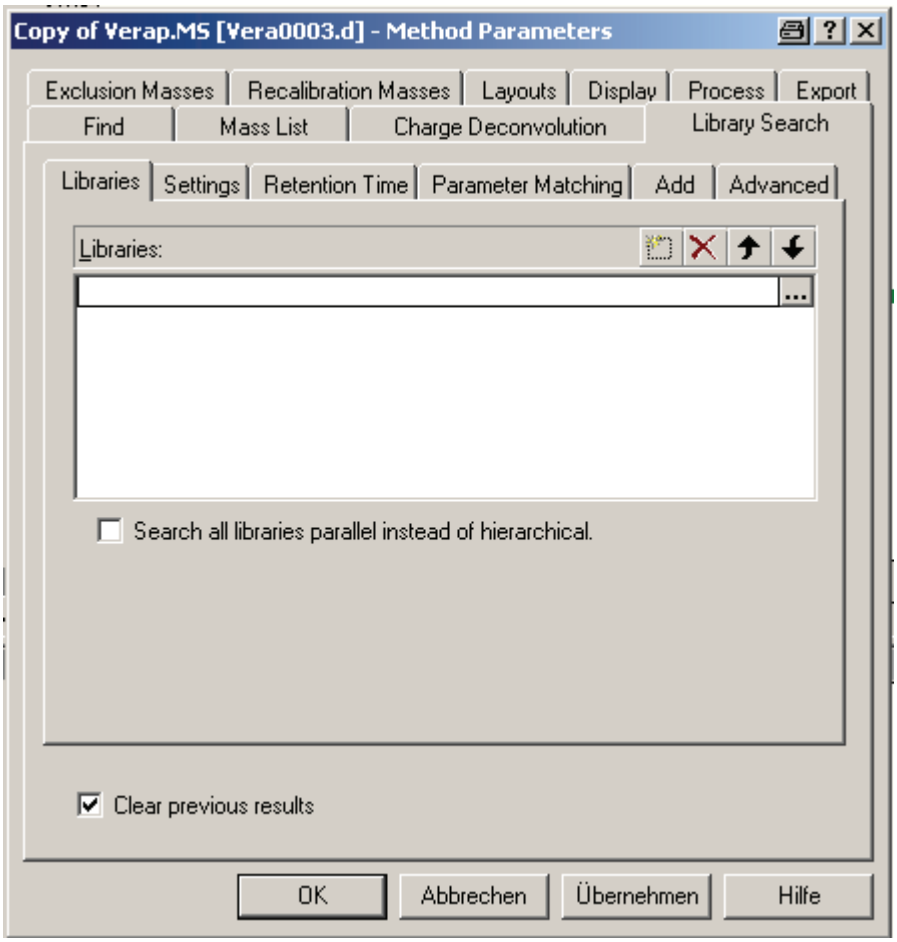


2 Identification with Library

2.1 Link the Library to Method

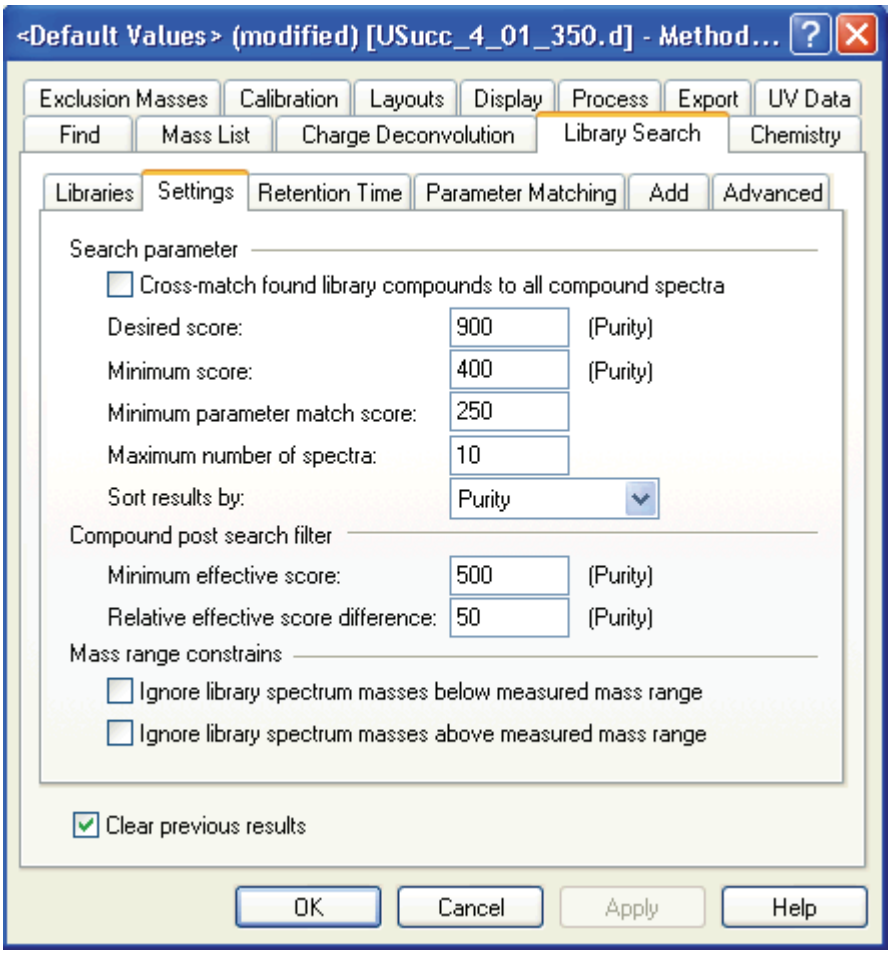
Link the just created library to the method used. Every data file hold a processing method.

As default the method **DEFAULT.ms** is used. In the dialog **Method / Parameters...** the actual setting is displayed. On the tab **Library Search** we'll now define our new generated library.



Method Parameters dialog – Library Search tab – Libraries

Use the Library Search – Settings tab to define the search parameters and mass range constrains to be used in library search.

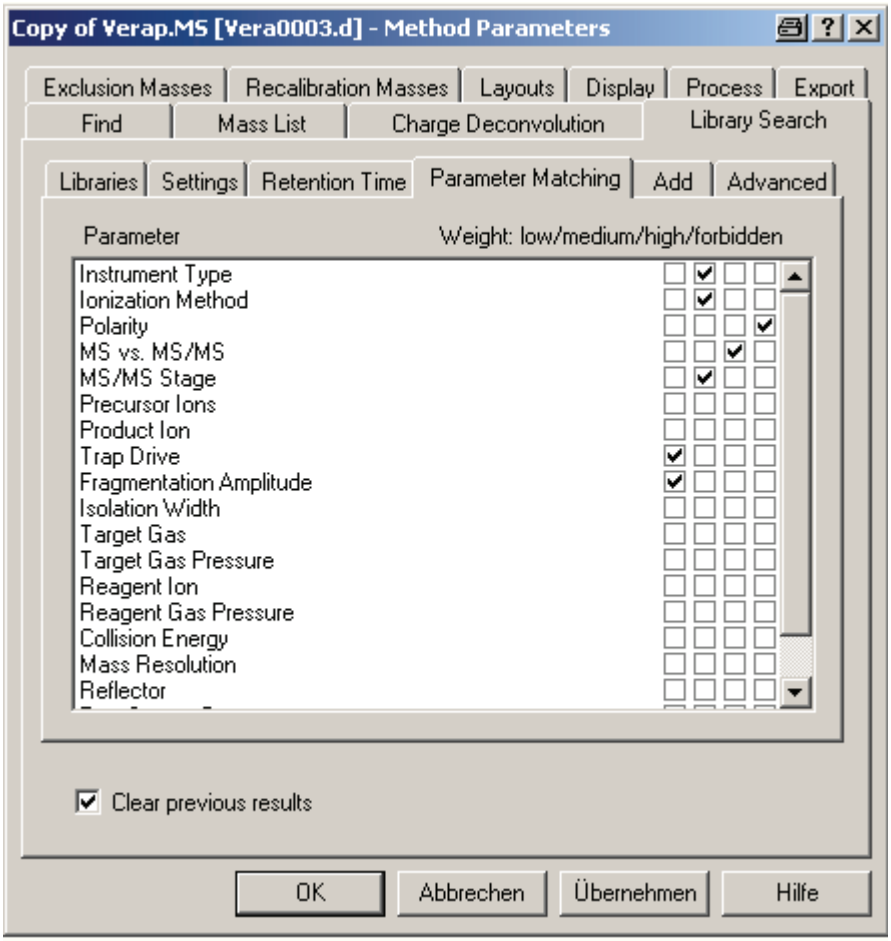


Method Parameters dialog – Library Search tab – Settings

To define the quality of your search you can select a **Minimum Purity score**. The **Purity** score indicates how well the masses and intensities of the library spectrum and the acquired spectrum agree. The maximum Purity is 1000 (acquired spectra and library spectra are identical).

The **Desired** score is only active if you use several libraries in a hierarchical order (using only one library the desired score is **not** used!).

To get an idea how well the data acquisition parameters used for the acquired spectrum and the library spectrum meet, the **Parameter Match** score is calculated. If both spectra are acquired with the same parameters, the Parameter Match score is 1000. Define the settings for calculating the **Parameter Match** score in the **Library Search - Parameter Matching** tab.



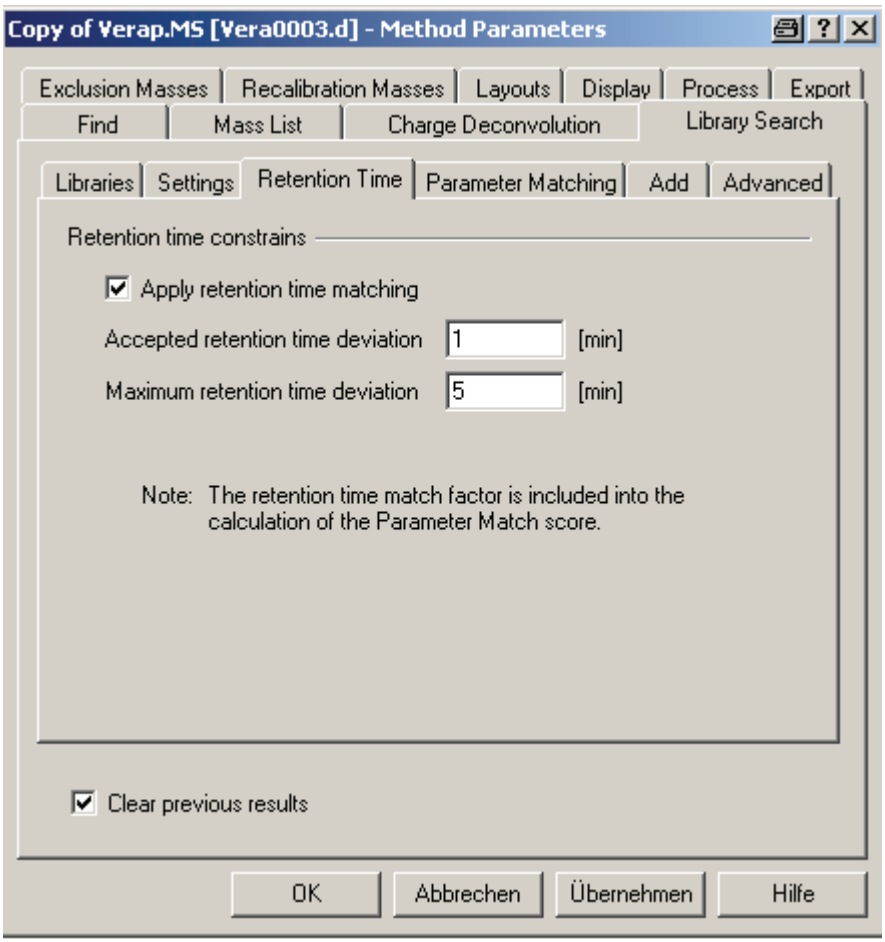
Method Parameters dialog – Library Search tab – Parameter Matching

Here you can define which instrumental parameters should be used for calculating the Parameter Match score and how they should be weighted.

Also you can define which instrumental parameter has to be equal (if not it is 'no hit'), therefore you define this parameter as forbidden. By default the Polarity and MS vs. MS/MS parameters are set to forbidden. This will prevent of getting any hits of spectra with different polarity or matching a precursor (parent) with a product (daughter) spectrum.

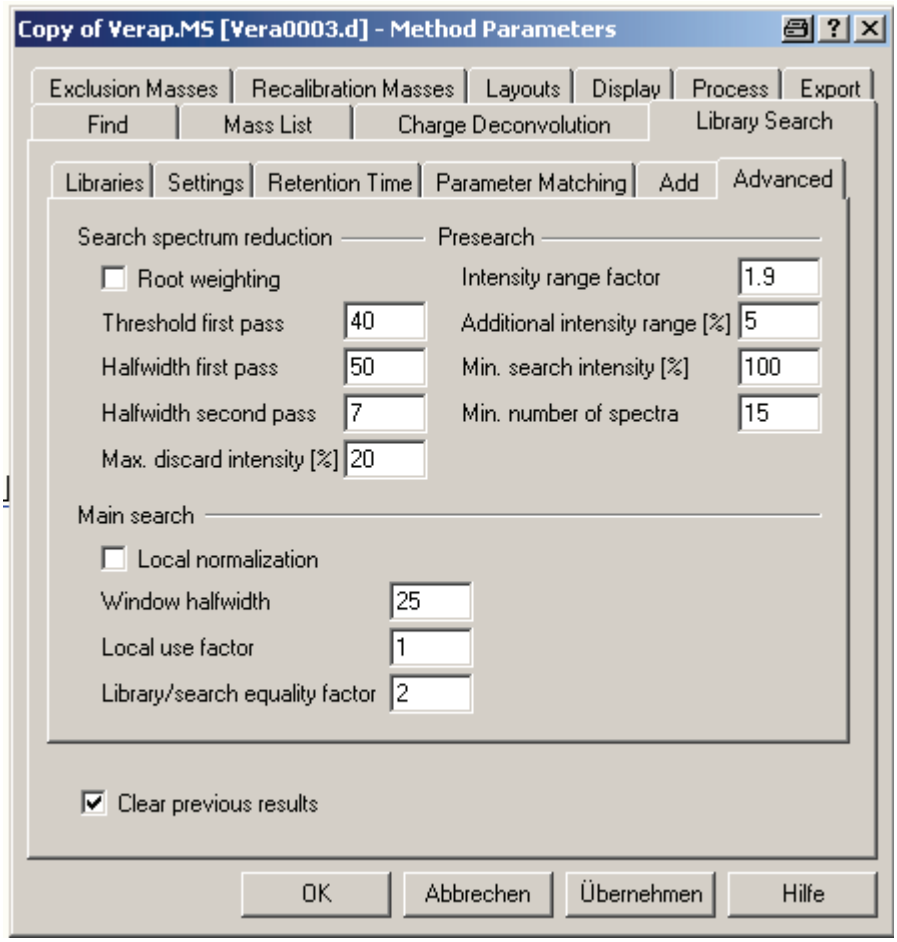
Additionally a Retention Time Match factor can be calculated. He is included into the calculation of the Parameter Match score.

Note: Retention time matching should only be applied when operating under the same LC conditions including the column.



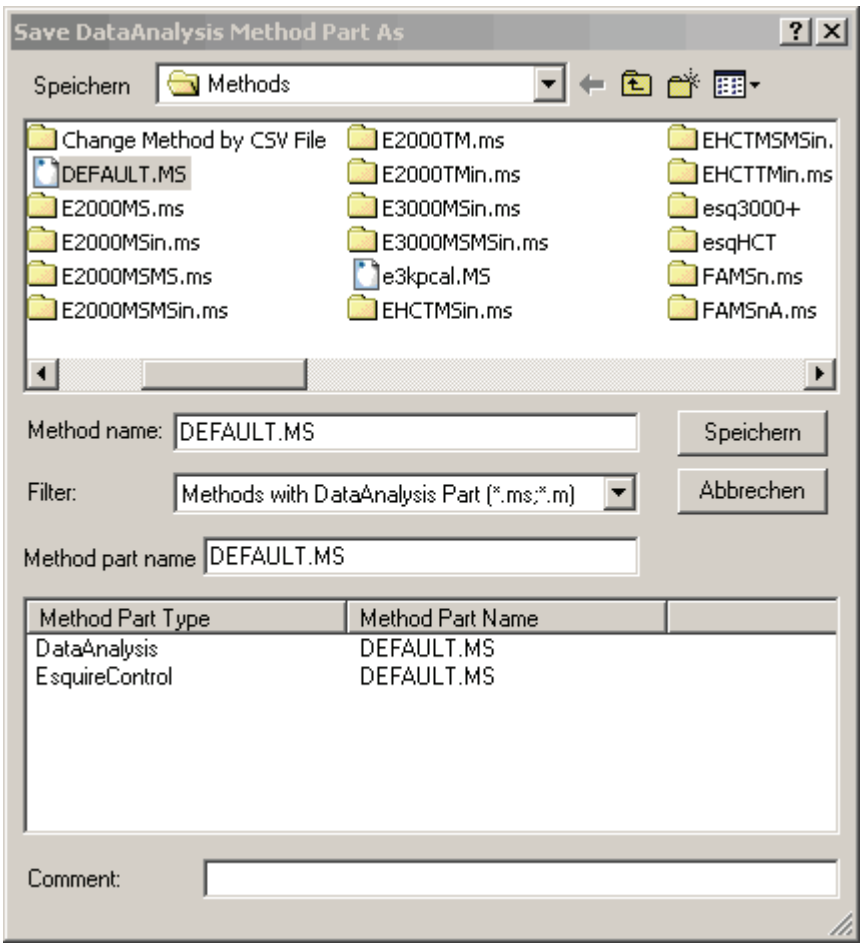
Method Parameters dialog – Library Search tab – Retention Time

The last tab **Advanced** parameter settings should be altered only by advanced users. For all parameters on this tab approved default values are set.



Method Parameters dialog – Library Search tab – Advanced

At the end the modified processing method is saved under a new name. Select **Method / Save As…** and save the method (Method name) and the method part (Method part name) with a new name.

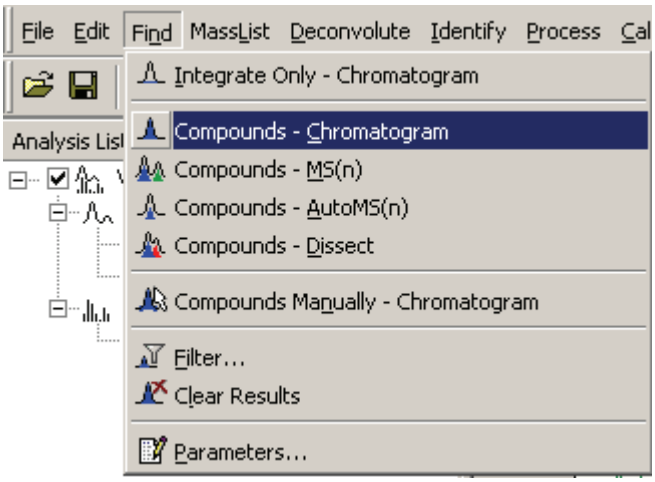


Method Save As dialog

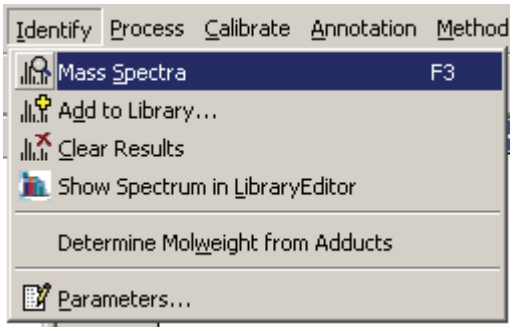
2.2 Identify Compound MS Spectra in DataAnalysis

The identification of mass spectra from a chromatogram is done in two steps. 1st generate a peak list and 2nd identify mass spectra. Delete any existing results with the function **Find / Clear Results**. Clear an existing identification with **Identify / Clear Results.**

Open the chromatogram with **File / Open...** dialog (here SULF0024.d), create Chromatogram Traces (see 1.2.1) and generate the peak list with function **Find / Compounds Chromatogram.**

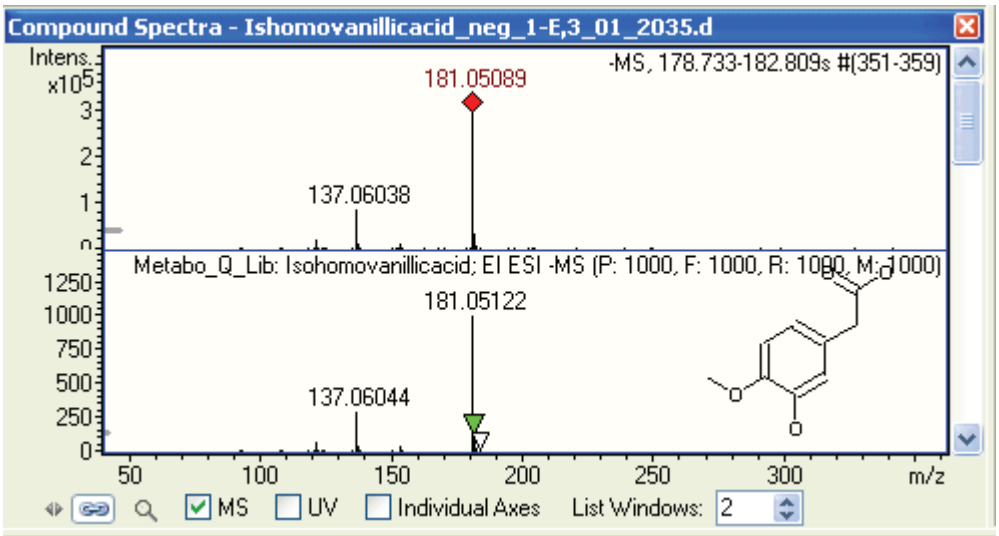


When a peak list is generated the next function **Identify / Mass Spectra** is used.



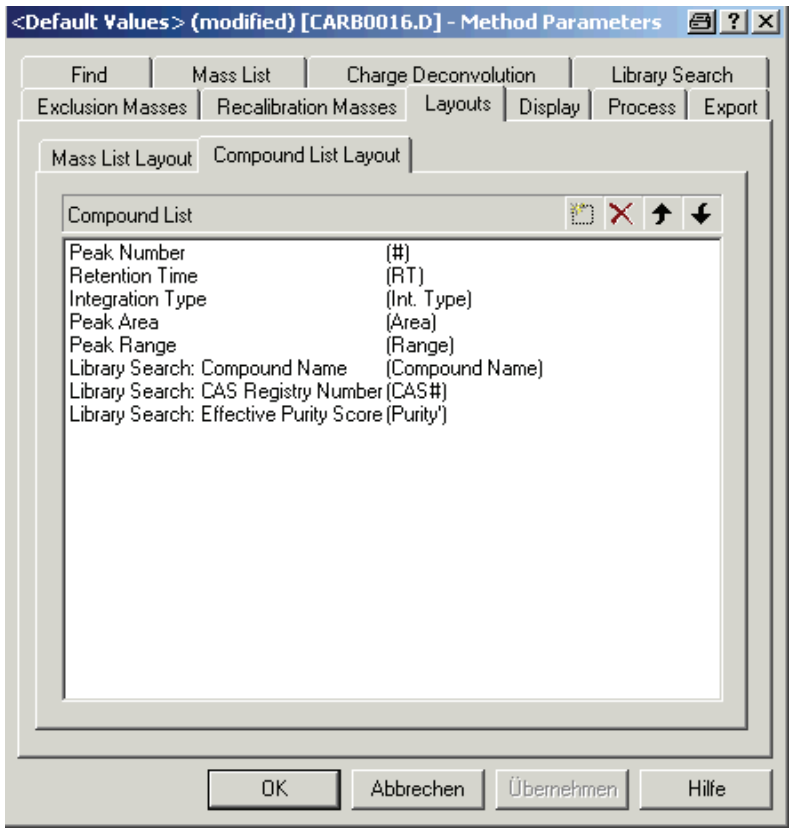
When the dialog “No library defined” comes up select the **Method / Parameters...** dialog. On the tab **Library Search** select NEW first and then the library itself. Close dialog with **OK** and use **Identify / Mass Spectra** again.

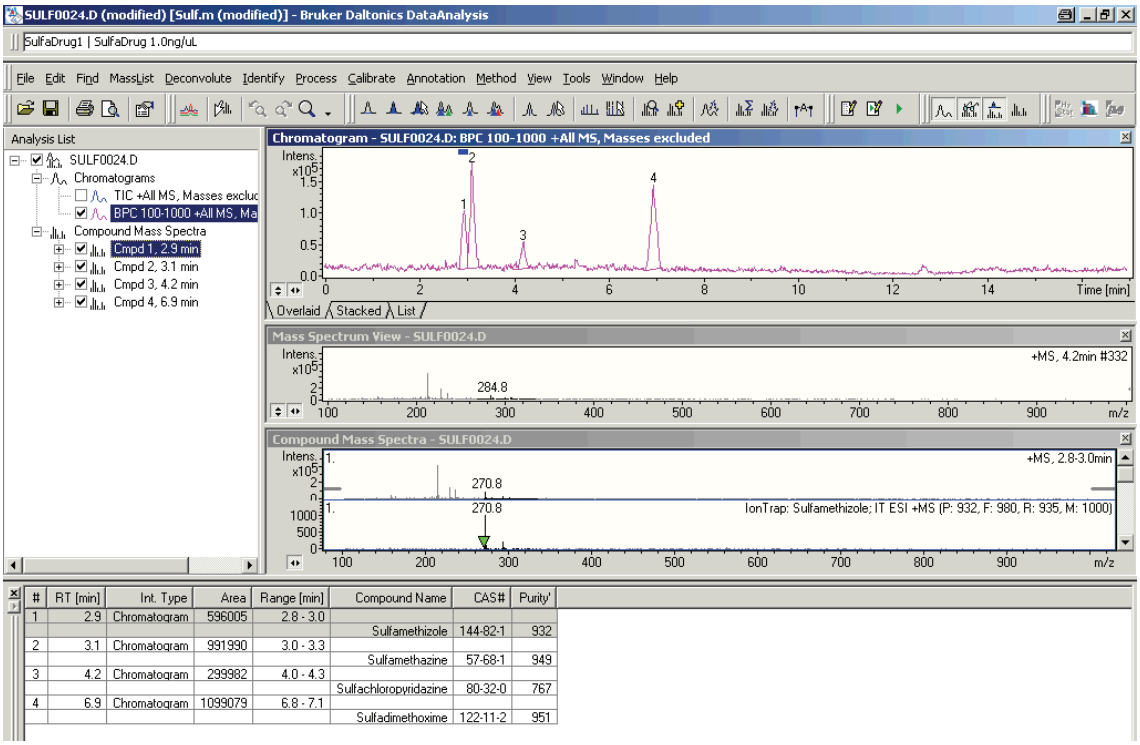
The results are displayed in the **Compound Spectra window**. Select List windows of this window and set it to “2”. This second window here keeps the information of the identification: library name, compound name and the scoring information.



Now the identification is ready.

With the tab **Layouts** under the function **Method / Parameters** you can design your own compound list layout.

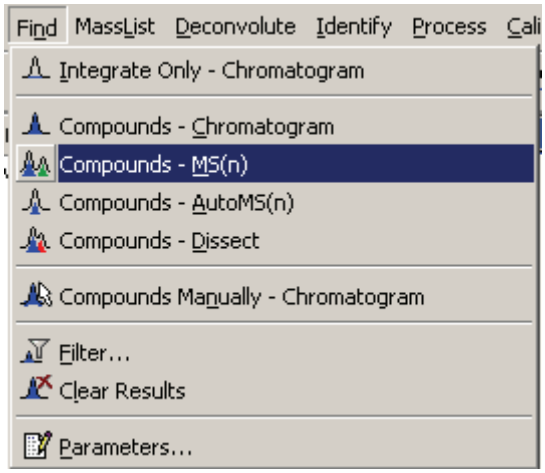




The result should look like this example.

2.3 Identify Compound MS/MS Spectra in DataAnalysis

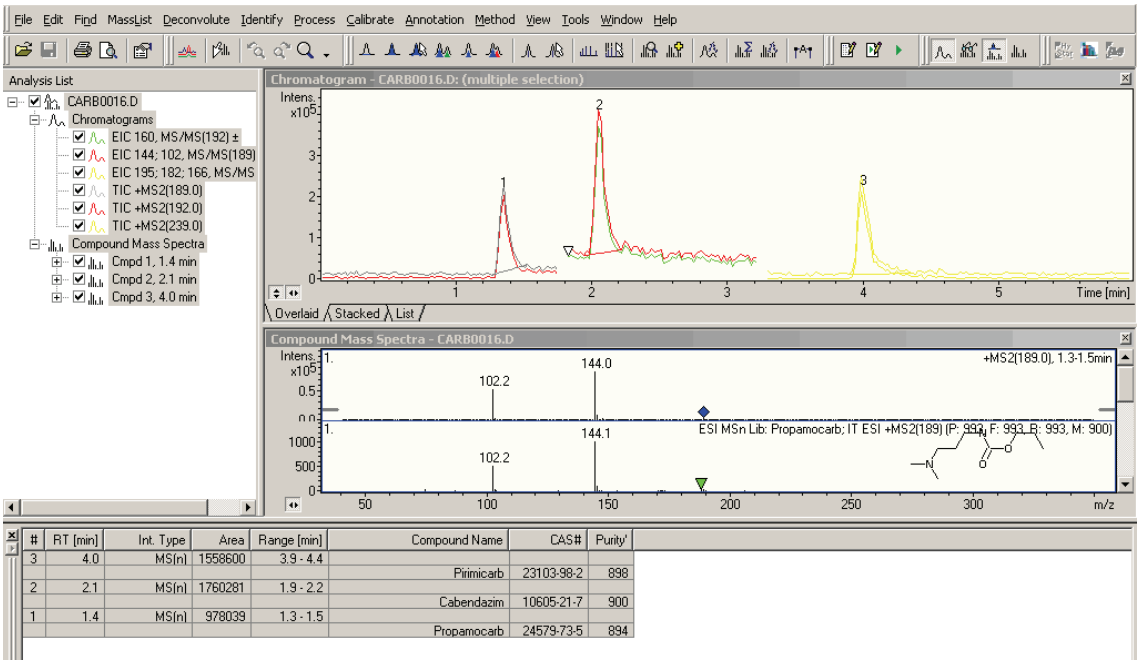
Load the data file **CARB0016.d (**D:\Data\Demo\Esquire, HCT\Library Search\CARB0016.d). Clear all results with the function **Find... / Clear Results** and create Chromatogram Traces (see chapter 1.2.1).



Then use the function **Find... / Compounds MS(n)** to generate the peaks. The demonstration library **ESI MSn Lib** is located in the directory **D:\Data\Libraries**. Connect this library to the actual used processing method (**Identify / Parameters**). The function **Identify Mass Spectra F3** should work now. In the Compound Spectra Window the result is displayed (mass spectra of the peak and the library spectra with the scoring information).

The result should look like this example.

To sort your results you can use the function **Find... / Filter…** Now you can decide which results you want to look at. Fore this example only the results which are identified by MS/MS spectra were displayed (because in the table below only the entry "**Identified by MS/MS**" is checked).



Filtering results in enabling only those compounds and mass spectra that match the specified criteria. All compounds and spectra not matching these criteria will be disabled in the Compound Spectra folder and hidden from being displayed in the Compound List. In the Chromatogram window the corresponding peak labels will be displayed in light grey. Thus, filtering may be a helpful tool in case of large Compound Lists as e.g. from AutoMSn runs.