Introduction

2D Chromatogram Creator for MassHunter provides an easy access to 2D-LC measurements for Agilent MassHunter users. Similar to the 2D-LC Viewer, it generates 2D chromatograms (abundance over time, usually the TIC) for cuts created in Multiple Heart-Cutting or High-Resolution Sampling 2D-LC for display and data analysis in MassHunter.

Comprehensive 2D-LC measurements can be displayed and analyzed with GC Image LCxLC Edition Software, which is described in the 2D-LC system manual, that can be found in folder documentation of the 2D-LC Software.

Figure 1  Splitting the raw data file into 2D chromatograms

The raw MS TIC signal continuously measures MS data of the 2D eluent. 2D Chromatogram Creator for MassHunter creates one 2D chromatogram per cut made in the first dimension of a multiple heart-cutting or high-resolution sampling measurement. These chromatograms can be used for further data analysis in MassHunter.
For running 2D-LC/MS measurements, this is a typical system:

The LC part is an LC system as described in the 2D-LC system manual. A typical system uses a UV detector in the first dimension and an optional UV detector in the second dimension. The 2D-LC instrument control and UV signal acquisition is done with OpenLAB CDS ChemStation (CS) Edition.

The MS detector, a TOF, Q-TOF or Triple Quadrupole, is used in the second dimension. It is controlled by MassHunter (MH) Acquisition Software, which acquires the MS signal.

For synchronizing signals of LC and MS, an APG remote cable connects both parts of the system, which communicates start and stop signals.
MassHunter has no knowledge about the size, order and position of cuts created by OpenLAB CDS ChemStation Edition. Therefore, 2D Chromatogram Creator for MassHunter is used for combining UV signals and meta data of cuts in OpenLAB CDS ChemStation Edition with the MS signal acquired by MassHunter.

As output, it creates following MassHunter chromatograms:

- A series of 2D chromatograms, one per cut including MS and optional UV signals
- If applicable, a 1D UV chromatogram (for a typical but optional 1D UV detector) in MassHunter data format including cut data
- An optional raw 2D UV signal and a
- 2D MS raw signal

**Compatibility**

MassHunter LC/MS Data Acquisition Version 10.0 requires 2D Chromatogram Creator rev. 1.1.20.

**NOTE**

2D Chromatogram Creator for MassHunter, OpenLAB CDS ChemStation Edition and MassHunter have been tested for co-execution (i.e. installed and running on same PC) with respect to parallel data acquisition in following configuration:

- 2D Chromatogram Creator 1.1.20
Installation

- OpenLAB CDS ChemStation Edition C.01.07 SR3 (Workstation)
- Agilent OpenLAB 2D-LC Software A.01.04
- MassHunter Q-TOF B.08.00
- MassHunter LC/QQQ B.08.02
- MassHunter Data Analysis B.08.00
- Operating system: Windows 10 Professional 64-Bit

Modules:
- 1290 Infinity II modules supported with LC Driver A.02.16
- QQQ or Q-TOF

PC Hardware:
- MassHunter bundle PC HP Z440 or equivalent

2D Chromatogram Creator for MassHunter has also been tested with OpenLAB CDS ChemStation Edition C.01.07 SR2, MassHunter Acquisition for TOF and QTOF B.06.01 SP1, MassHunter Acquisition for QQQ B.08.00 SP1, MassHunter Qualitative Software B.07.00 SP1 and SP2 and MassHunter Quantitative Software B.07.01. No co-execution of OpenLAB CDS ChemStation Edition and MassHunter was tested in this configuration.

MassHunter Qualitative B.07.00 is known to display misleading signal names.

Installation

Install the 2D-LC/MS system as outlined above. The 2D-LC system installation is described in the 2D-LC system manual. The installation of the MS instrument and its hydraulic and electronic connection to an LC system is described in the MS manual. Installations of OpenLAB CDS ChemStation Edition and MassHunter software packages are described in respective user manuals.

Install 2D Chromatogram Creator for MassHunter by double-clicking setup.exe in folder software of the 2D-LC Software DVD. For simple file handling, 2D Chromatogram Creator for MassHunter should preferably be installed on the PC with MassHunter data analysis software.
Example Data

Folder "example Data\2D Chromatogram" on the 2D-LC software CD contains two subfolders MassHunter and ChemStation with example data which can be used for trying out this software. It uses a sequence/worklist with 3 measurements of 3 compounds:

Table 1  Example Data

<table>
<thead>
<tr>
<th>#</th>
<th>Compound</th>
<th>m/z</th>
<th>1D retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Imipramine</td>
<td>280.407</td>
<td>1.33 min</td>
</tr>
<tr>
<td>2</td>
<td>Protriptyline</td>
<td>263.37</td>
<td>1.66 min</td>
</tr>
<tr>
<td>3a</td>
<td>Nortriptyline</td>
<td>263.38</td>
<td>1.84 min</td>
</tr>
<tr>
<td>3b</td>
<td>Verapramide</td>
<td>454.6</td>
<td>1.84 min</td>
</tr>
</tbody>
</table>

I.e. compounds 3a and 3b are co-eluting in 1D and are separated in 2D. Compounds 2 and 3a have the same mass but are separated in the 1D chromatogram. See also figures 1 and 13.

Workflow for a Single Measurement

For a 2D-LC/MS measurement, methods need to be set up in both OpenLAB CDS ChemStation Edition and MassHunter. Set up the 2D-LC method as described in the 2D-LC system manual. Set the stop time in the 2D pump. Then create a method in MassHunter for the MS.

For synchronizing run times (i.e. starting and stopping measurements simultaneously), please use “External start” as a run parameter:

In section “Additional information”, add parameter “run type” and choose option “external run”. Set stop time “no limit” in MassHunter.
Workflow for a Single Measurement

Then start the run in OpenLAB CDS ChemStation Edition, which will automatically start the MassHunter run. Both chromatographic data systems will create data files, which will be used for combined data analysis. Make OpenLAB CDS ChemStation Edition and MassHunter data files available through a network share or copy data to the PC with 2D Chromatogram Creator for MassHunter. Having both files on one PC is faster, more secure and recommended.

Start 2D Chromatogram Creator for MassHunter.

![User interface for single data files](image)

**Figure 4** User interface for single data files

Use mode “Single Run”.

Provide data files for OpenLAB CDS ChemStation Edition and MassHunter. A result folder is proposed and can be changed as needed.

If an optional UV detector is used in 2D, a delay time can be set between this detector and the MS detector. This value is used for aligning MS versus UV data by compensating the time offset.

Click the start button for starting the file processing. This can take some time depending on the file size.
Workflow for a Sequence/Worklist

Create methods in both OpenLAB CDS ChemStation Edition and MassHunter.

Set up a sequence in OpenLAB CDS ChemStation Edition.

![Sequence parameters](image)

**Figure 5** Using counters for data files in OpenLAB CDS ChemStation Edition

In sequence parameters, use an incremental counter for the data file names. This counter is the recommended way for later linking OpenLAB CDS ChemStation Edition data files to MassHunter data files.

Similarly set up a worklist in MassHunter and use an incremental counter. There are various possibilities for applying a counter, e.g. inserting multiple samples using a counter.
Workflow for a Sequence/Worklist

Figure 6  Using counters for data files in MassHunter Worklists

Set “External Start” as run type in “Worklist Run Parameters” for synchronizing runs.

Start 2D Chromatogram Creator for MassHunter with option “Split multiple data files”: 
Workflow for a Sequence/Worklist

In table “Data file mapping” OpenLAB CDS ChemStation Edition versus MassHunter data files are matched. Each line lists the OpenLAB CDS ChemStation Edition data file and the corresponding MassHunter data file. Please verify if the default linkage is correct, which sorts data files for both software editions by their creation date (same chronologic order is applied). However, this date may change when copying files. Sorting can be adjusted by right-clicking on the headers of the data file mapping table. Different sorting options are available for both OpenLAB CDS ChemStation Edition and MassHunter:
Extracting and Displaying Result Data

Sorting by file ending using the counter defined previously is the best user-defined way for linking data files.

Extracting and Displaying Result Data

Result files are stored to the result folder set above. Data for cuts can be imported as single files or by loading all data files at once. Each cut contains data for the 2D signals (MS, optional UV) and the 1D UV signal. By default, the 2D TIC is displayed:

![2D TIC raw signal](image)

Figure 9 Raw 2D MS signal (TIC)
Figure 10 shows an example for cut 1. 2D Chromatograms are named by their cut number, the first dimension retention time and the original MassHunter data file. This example uses a 2D gradient time of 3.5 minutes corresponding to the length of that 2D chromatogram.

In case of high-resolution sampling measurements, series of adjacent cuts can be defined. Then, cuts are numbered by the series and an incremental counter, i.e. cut 1-3 is the third cut of the first series.

The optional 2D UV chromatogram can be extracted by right-clicking the TIC scan and using function “Extract Chromatograms...”, see Figure 11.

Figure 11 Extracting chromatograms

Use Type Other Chromatograms and choose the 2D detector (in this example DAD2) and the signal with the wavelength of interest, see Figure 12.
Similarly, chromatograms can be extracted from the original 2D MS data file, which has been enriched by 2D-LC and UV data. Available signals are

- 1D UV signal
- 2D UV signal
- Cuts in 1D

Similar to the 2D-LC Software, these data files can be used for displaying the 1D measurement with cuts (here in red), see Figure 13.

All MS Chromatograms can then be processed further in MassHunter as usual, e.g. for extracting ion chromatograms, spectra and data analysis.
Figure 13  1D UV Chromatogram with cuts