Agilent RapidFire 300 High-throughput Mass Spectrometry System

Data Analysis Guide

What is Agilent RapidFire Integrator?

The Agilent RapidFire Integrator data analysis software can be used to integrate all of the data points collected in a single Agilent G9213AA RapidFire 300 High-throughput Mass Spectrometry System run.

Data can be acquired from LC/MS instruments controlled by the Agilent MassHunter Data Acquisition, AB Sciex Analyst, or Thermo Fisher Xcalibur software.

Each sequence of a RapidFire-MS batch is processed individually. There is no limit on the number of plates.

Agilent Technologies

Installation

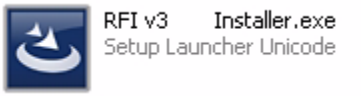
To install Agilent RapidFire Integrator software

The RapidFire Integrator data analysis software can be installed on any personal computer.

Before you begin

If a previous version of the RapidFire Integrator software is already present on the system, use the **Add/remove programs** utility in the Windows Control Panel to remove it.

1 Double-click the **Installer.exe** file supplied by Agilent Technologies.



2 Follow the prompts displayed by the installation wizard.

The software is installed in **C:\ Program Files\ Agilent\ RapidFire Integrator**.

Preparing Data Collected in Plates Mode for RapidFire Integrator

A run in Plates mode is defined as the data that is collected when you click the **Play** button. Unlike Sequences mode, data acquired in Plates mode are named and saved in folders with *different* names on the RapidFire and MS computers.

If the data that you collect in Plates mode is in MassHunter, Analyst, or Xcalibur format, you need to prepare the data before you can analyze it in RapidFire Integrator.

To prepare Agilent MassHunter data

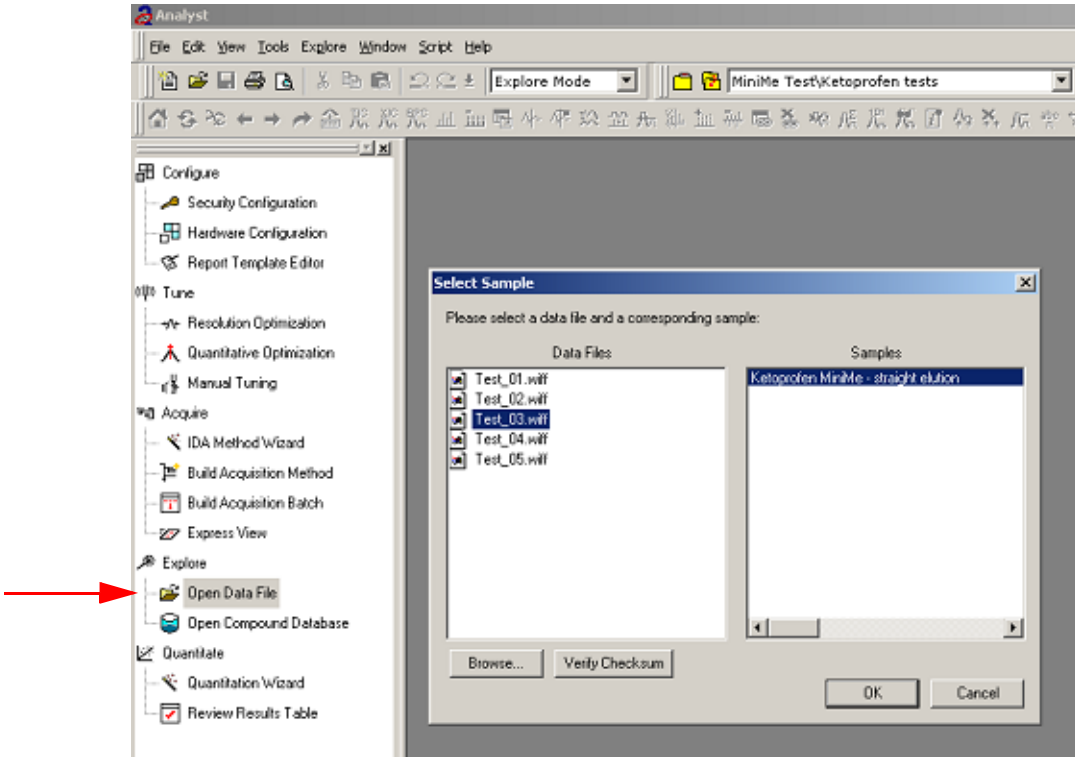
Repeat this process for each RapidFire-MS run.

1. Rename the MS data files to **sequence1.d**, **sequence2.d**, **sequence3.d**, and so on.
2. Copy the following files to a unique folder, such as **\data sequenceX**:
   - **sequenceX.d**
   - **batch.rftime** (from the RapidFire computer, see "RapidFire data" on page 18)
   - mass spectrometry files for each compound in the reaction, such as **xic-MSprod.txt** and **xic-MSis.txt**.

To prepare AB Sciex Analyst data

1 Open the Analyst program.

a Click the **Explore > Open Data File**.



b Select the appropriate **.WIFF** file from the list.

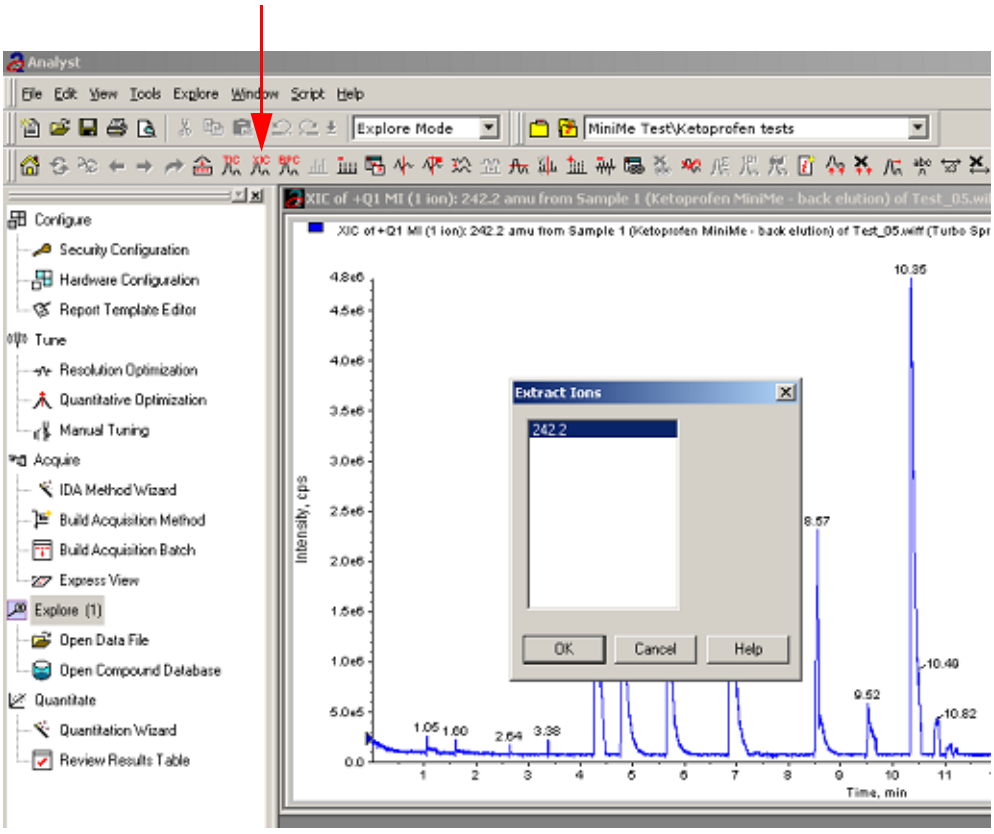
c Click to select the sample of interest, then click **OK**.

The MS chromatogram traces for the selected sample appear in the main window.

2 Create extracted ion chromatograms (XIC) as follows:

a Click on the **XIC** button on the Analyst toolbar.

b Click to select the masses of interest, which are the individual MS chromatograms for each of the MRM channels that were monitored during the experiment.



3 Save *each* XIC as a text file as follows:

   a Right-click on the panel for the XIC of interest and select **Save to Text File**.

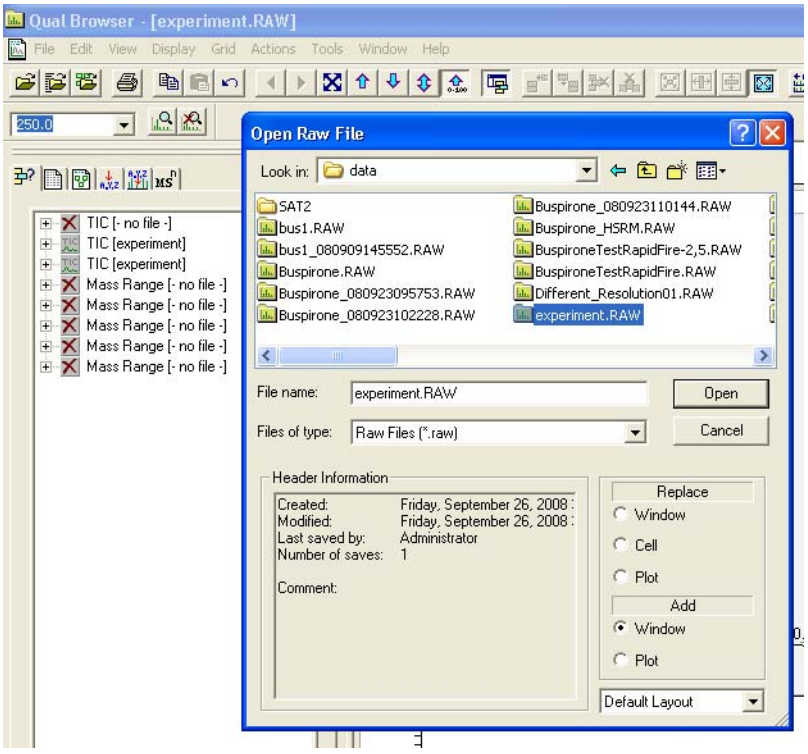
   b Select the folder where you want to save the file. Use the same directory where the RapidFire **batch.rftime** data file is already saved.

   c Name the MS data files **xic-*XXX*.txt**, where ***XXX*** is any unique string of alphanumerical characters, such as **xic-MSprod.txt**.

To prepare Thermo Fisher Xcalibur data

1 Open the Xcalibur program.

a Click **File > Open**.



b Select the appropriate **.RAW** file from the list.

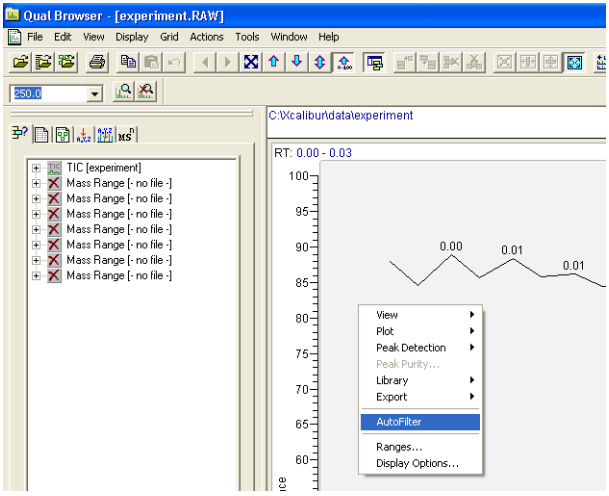
c Click **Open**.

The MS chromatogram traces for the selected sample appear in the main window.

2 Create extracted ion chromatograms (XIC) as follows:

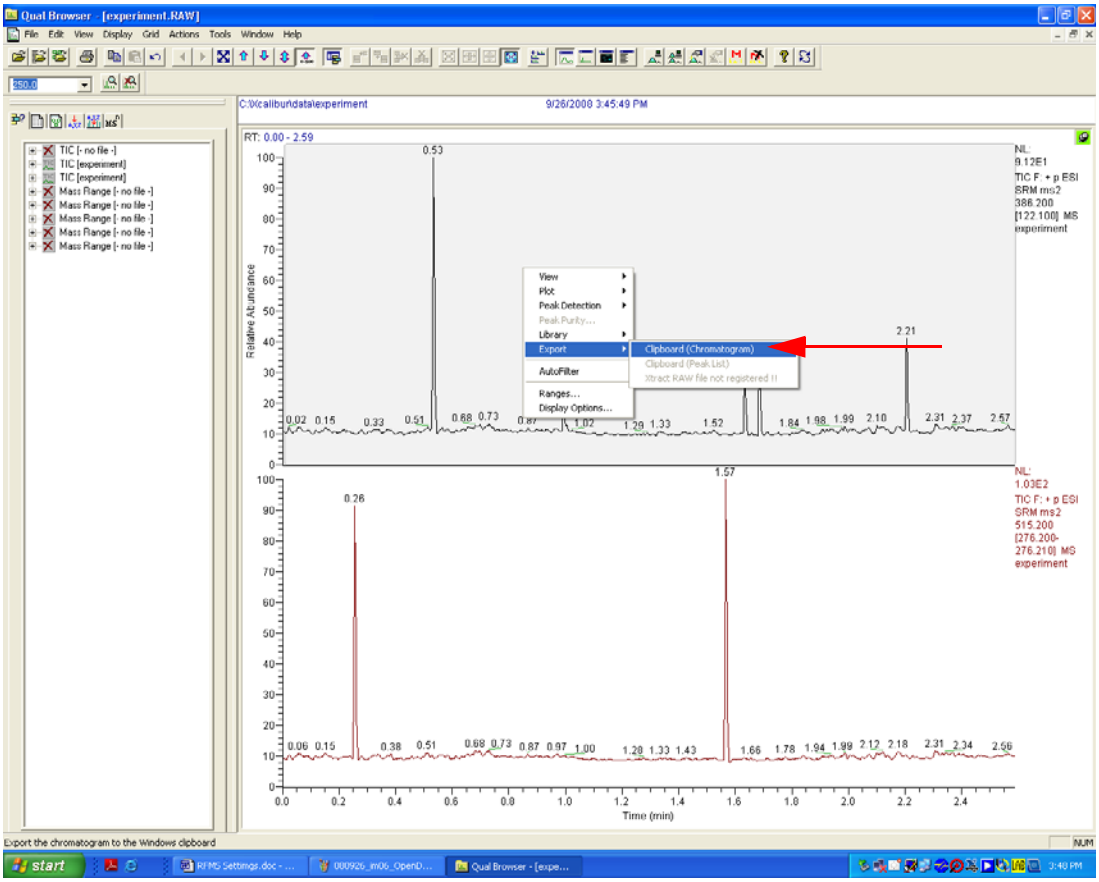
a Right-click right on the total ion chromatogram (TIC) and select **AutoFilter**.

b Delete the TIC panel at the top of the window.



3 Save *each* XIC as a text file as follows:

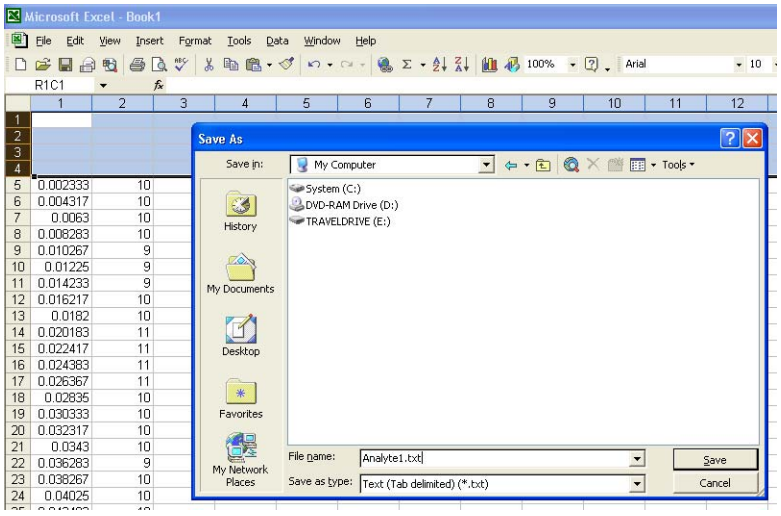
a Right-click on the panel for the XIC of interest and select **Export to Clipboard (Chromatograms)**.



b Paste the selection into Excel.

c Save it as a text file (**.TXT**) in the same directory where the RapidFire **batch.rftime** data file is already saved.

d Name the MS data files **xic-*XXX*.txt**, where ***XXX*** is any unique string of alphanumerical characters, such as **xic-MSprod.txt**.



	1	2
5	0.002333	10
6	0.004317	10
7	0.0063	10
8	0.008283	10
9	0.010267	9
10	0.01225	9
11	0.014233	9
12	0.016217	10
13	0.0182	10
14	0.020183	11
15	0.022417	11
16	0.024383	11
17	0.026367	11
18	0.02835	10
19	0.030333	10
20	0.032317	10
21	0.0343	10
22	0.036283	9
23	0.038267	10
24	0.04025	10

Analyzing Data in RapidFire Integrator

To access data acquired in Sequences mode

This procedure applies to Agilent MassHunter and AB Sciex Analyst data. RapidFire is not fully integrated with Thermo Fisher Xcalibur at this time.

1 Open the configuration file in which the variable **BASE_DATA_DIR** is set:

- **C:\Program Files\ Agilent\ RapidFire\ cfgs\ RFMassHunterS.cfg** (for Agilent MassHunter)
- **C:\Program Files\ Agilent\ RapidFire\ cfgs\ RFAAOS.cfg** (for AB Sciex Analyst)

2 Locate the variable **BASE_DATA_DIR**.

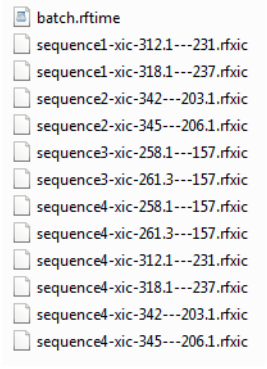
Typical values for **BASE_DATA_DIR** are:

- **D:\ MassHunter\ Data\ RapidFire** (for Agilent MassHunter)
- **D:\ Analyst Data\ RapidFire** (for AB Sciex Analyst)

3 Make note of the path name that is assigned to **BASE_DATA_DIR**.

Tip The following files are automatically moved to a unique folder, such as **D:\ MassHunter\ Data\RapidFire\2011\April\5\1** (for the first run on April 5, 2011):

- **batch.rftime** (automatically transferred from the RapidFire computer to the MS computer)
- **sequenceX.d** (for Agilent MassHunter)
- **sequenceX-xic-YZ.rfxic** (for AB Sciex Analyst)



To analyze data in RapidFire Integrator

1 Double-click the desktop shortcut to start the Agilent RapidFire Integrator software.

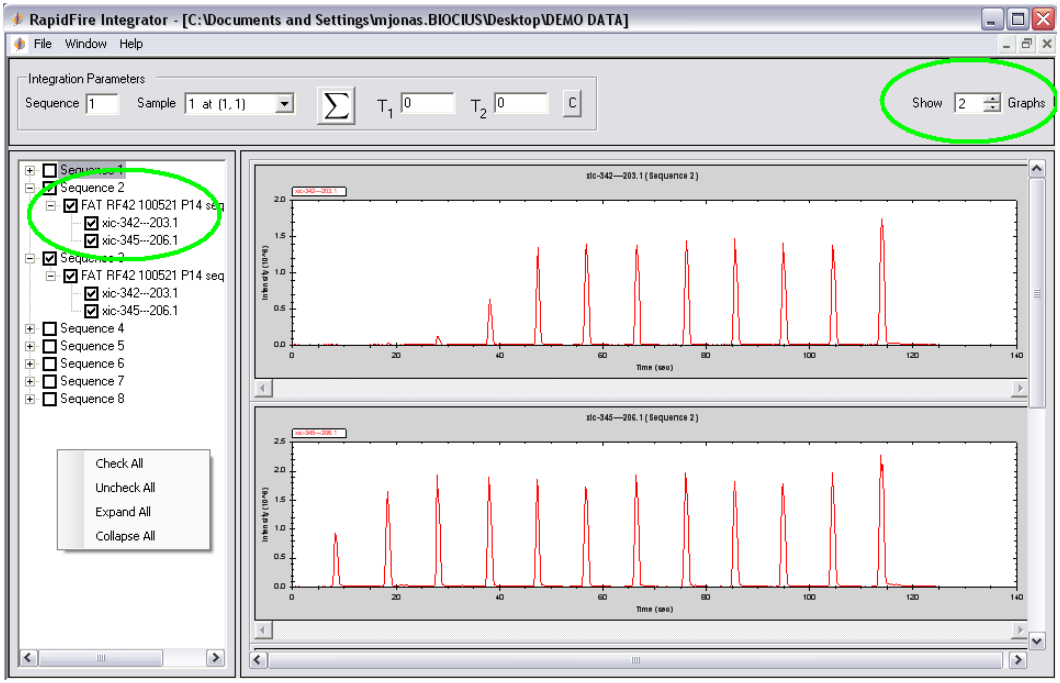
2 Click **File > Load RF300 Data Set**.

3 When the Browse For Folder window appears:

a Select the folder that contains the **.RFTIME** and **.RFXIC** or **.D** files.

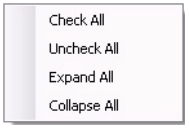
b Click **OK**.

4 Click to select the sequence of interest in the left pane, as shown for Sequence 2 in the following example.



5 Review the data using the following features:

- Click the + sign next to the sequence to display more information about the sequence, such as plate name, and *m/z* chromatograms.
- Right-click in the left pane to display the following menu options:



- Click to select the number of graphs to display in the upper right area of the window.
- Drag the mouse to select an area of the graph to expand. Repeat to expand the area further.
- Right-click in the graph and select **Un-zoom** to zoom out each successive level.

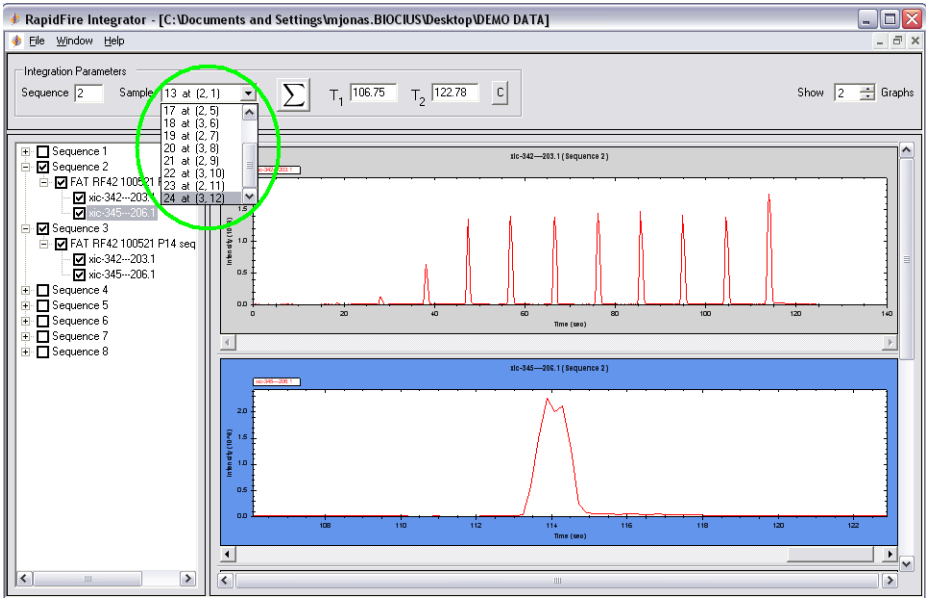
6 Integrate peaks as follows:

a Zoom in on a known landmark peak within the sequence.

- Any peak within the experiment can be selected as a landmark.
- Data analysis can be based on any MRM transition monitored by the MS (either product or internal standard, for example).
- Monitored analytes are identified by their Q1 *m/z* ---Q3 *m/z* (title of the plot).

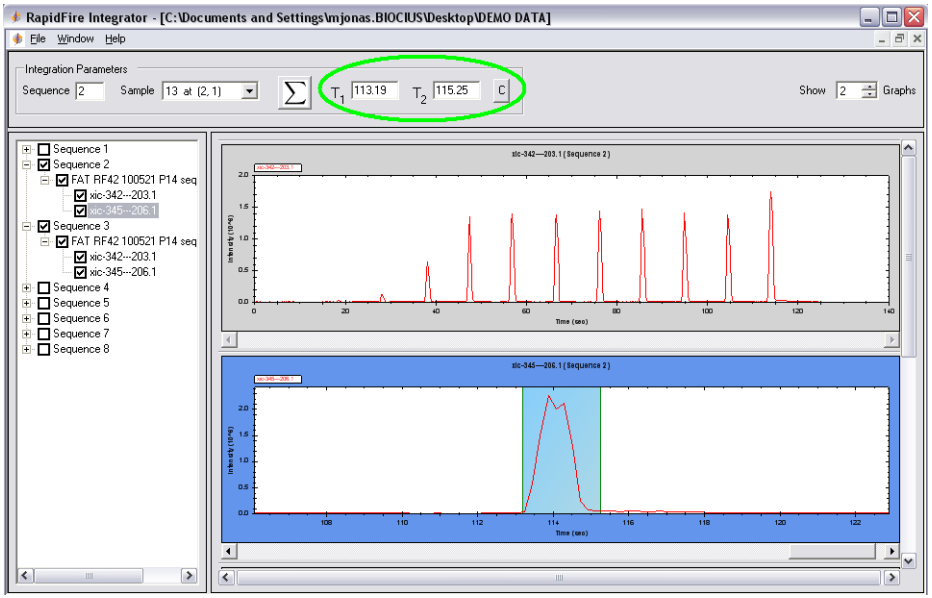
  Use the scroll bar on the right side of the window to navigate from one MRM to another.

b Select the identity of the peak from the **Sample** list.



c Drag the mouse in a thin slab rectangle from the front edge of the peak to its tail end to set the peak width for integration for area-under-the-curve (AUC) calculations.

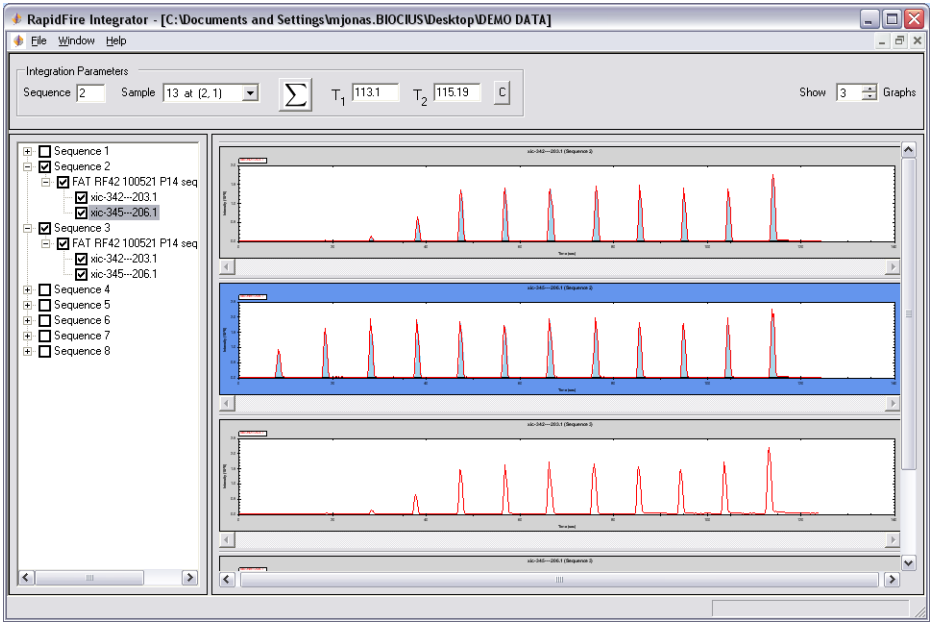
The defined time range is displayed in the $\mathbf{T_1}$ and $\mathbf{T_2}$ boxes above the graph, and area also emphasized by teal coloring as shown in the following example:



d Click the summation button to process the data for the current sequence.

All MRMs of the sequence are processed together.

AUCs of injections turn blue, but background signals remain red.



e To save partial progress in data analysis before all of the sequences of the run are analyzed, click **File >Save As**.

- The results are saved in the specified **.RFD** file.
- Partially processed experiment files can be reopened by clicking **File > Open**.

7 Export data.

a When processing is complete for all the sequences of a run, click **File > Export Peak Data** to export the data.

A **.RFPKS** text file is created, which contains a list of injections.

Only the masses of interest are reported for each well.

You can analyze this file with a spreadsheet program such as Microsoft Excel.

	A	B	C	D	E	F
1	Sequence 1					
2	PlateName	Row	Column	xic-342---203.1	xic-345---206.1	Injection Time (sec)
3	Plate1	1	1	18800	3346420	8.42
4	Plate1	1	2	67890	6215320	18.42
5	Plate1	1	3	352140	6442985	28.23
6	Plate1	1	4	1707265	5712430	37.58
7	Plate1	1	5	4602310	6090260	47.2
8	Plate1	1	6	5283070	7169040	56.89
9	Plate1	1	7	4763010	6029000	66.08
10	Plate1	1	8	5072060	6568350	75.56
11	Plate1	1	9	4681235	6430260	84.95
12	Plate1	1	10	4934730	6827290	94.59
13	Plate1	1	11	23465	49370	103.96
14	Plate1	1	12	10939460	14423820	113.34
15	Sequence 2					
16	PlateName	Row	Column	xic-342---203.1	xic-345---206.1	Injection Time (sec)
17	Plate1	2	1	18230	3743510	7.72
18	Plate1	3	2	81380	6190690	17.83
19	Plate1	2	3	410120	7120610	27.55
20	Plate1	3	4	2192220	7106175	37.47

b You can also export data using **File > Export Peak Data Plate-wise**. Keep in mind the following caveats:

- All data pertaining to MAT# and WASH# injections is not displayed.
- This option is only offered if each sample well within a plate is not visited more than once.

c You can analyze this file with a spreadsheet program, as shown in the following example in Microsoft Excel.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	XIC = xic-342---203.1												
2		1	2	3	4	5	6	7	8	9	10	11	12
3	A	17060	69240	351195	1675510	4485350	5145550	4675030	5006445	4630220	4912200	5900	10379525
4	B	22620	101930	409910	2519030	5382330	6074400	5543170	6310075	5374720	5889960	5441500	9831590
5	C	15530	82915	487280	2191820	5966590	5331980	6205310	5569820	6738300	5162370	6531670	7544630
6	D	35660	116230	635105	2999890	7014370	6974840	7239930	7294740	6773310	6273410	6765560	12194250
7	E	28270	123070	619530	3241380	7240720	7195915	7231920	6897140	6673390	6537440	6863870	14057730
8	F	16730	113440	648320	3263800	3135335	7091210	6938345	5974780	6590740	6263830	6233490	13339040
9	G	9220	138810	613570	3266685	7388100	6034660	7144950	6912480	5846570	5996250	6499790	7195990
10	H	5000	126890	702220	3093510	7312870	7475130	5911630	5634800	6832050	6414380	6990580	9278240
11													
12	XIC = xic-345---206.1												
13		1	2	3	4	5	6	7	8	9	10	11	12
14	A	3225680	6069120	6334215	5616810	5918680	6971700	5893470	6458745	6351660	6765690	13070	13552035
15	B	3762920	7471300	7134560	8075850	7261610	7952300	7361700	8446565	7231980	8255770	7480010	12304715
16	C	4614840	6195005	7830980	7109350	7752745	7066970	8158290	7669750	9088970	7261570	8688110	10037330
17	D	5137720	8598850	9067560	9328840	9354920	9053750	9416945	9146200	9264970	8556380	9120250	15759350
18	E	5040400	8489620	8636100	9187590	9358630	9060130	9115380	9003460	9253780	9332390	9280810	18907170
19	F	4970310	8287770	9052850	8977820	4413770	8956190	8779035	8012300	8917535	8807140	8532365	16720920
20	G	5260530	8750170	8687950	9301155	9386290	7878040	9091835	8854535	8031880	8598980	8799570	9218770
21	H	5259840	8857620	9088550	8940810	9437290	9680240	7971770	7499840	9280300	9121100	9257000	11785570

Data Analysis Reference

The following reference material applies to RapidFire Integrator data analysis software.

Data files

The following file types make up a data set for analysis with the RapidFire Integrator software when used for data acquired with the RapidFire-MS System. *These file types must be present in the same folder.*

- **batch.rftime** file from the RapidFire computer
- **sequenceX.d** mass spectrometer (MS) data files in Agilent MassHunter
- **sequenceX-xic-YZ.rfxic** in AB Sciex Analyst or Thermo Fisher Xcalibur

If the data is acquired within a single experiment in Plates mode, then multiple 96-well or 384-well plates are analyzed within a single folder.

RapidFire data

RapidFire data is stored in a folder within the operating system software. A new data folder is created each day with the date as the folder name.

A new folder is created each time a new experiment (in Plates mode) or a new batch (in Sequences mode) is started within the RapidFire acquisition software. A data file named **batch.rftime** is saved in that folder.

Example The first run performed on April 5, 2011 is saved in the following folder on the RapidFire computer:

C:\ Program Files\ Agilent\ RapidFire\ data\ 2011\ April\ 5\ 1

Data columns

The following columns of data are saved in the data file:

- **Plate identity**
- **Injection number**
- **Sequence number**
- **Row**
- **Column**
- **Time stamp** (actuation of valve 2 from inject to load position: start of elution)
- **Sip sensor value** = The displayed value is **1** if the optical sip sensor detected the presence of liquid, or **0** if it did not.

Example

	A	B	C	D	E	F	G	H
1	plate	sip	seq	row	col	siptime	sip sensor	
2	----------							
3	DEMO PLATE	1	1	2001	2	3.304	0	WASH STATION
4	DEMO PLATE	2	1	1	1	12.529	1	
5	DEMO PLATE	3	1	1	3	21.341	1	
6	DEMO PLATE	4	1	1	5	30.238	1	
7	DEMO PLATE	5	1	2001	2	38.486	1	
8	DEMO PLATE	6	1	2	1	47.582	1	B1
9	DEMO PLATE	7	1	2	3	56.322	1	B3
10	DEMO PLATE	8	1	2	5	65.164	1	B5
11	DEMO PLATE	9	1	2001	2	73.49	1	
12	DEMO PLATE	10	1	3	1	82.726	1	
13	DEMO PLATE	11	1	3	3	91.705	1	
14	DEMO PLATE	12	1	3	5	100.552	1	
15	DEMO PLATE	13	1	2001	2	108.996	1	
16	DEMO PLATE	14	1	4	1	118.225	1	
17	DEMO PLATE	15	1	4	3	127.228	1	
18	DEMO PLATE	16	1	4	5	136.047	1	

Mass spectrometry (MS) data

The path where the MS data is automatically stored on the MS computer is specified by the variable **BASE_DATA_DIR**, which is defined in:

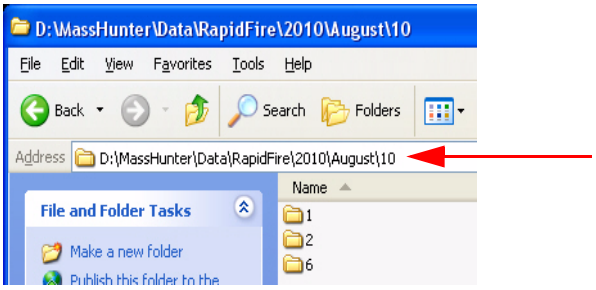
- **C:\Program Files\ Agilent\ RapidFire\ cfgs\ RFMassHunterS.cfg** (for Agilent MassHunter)
- **C:\Program Files\ Agilent\ RapidFire\ cfgs\ RFAAOS.cfg** (for AB Sciex Analyst)

Typical values for **BASE_DATA_DIR** are:

- **D:\ MassHunter\ Data\ RapidFire** (for Agilent MassHunter)
- **D:\ Analyst Data\ RapidFire** (for AB Sciex Analyst)

In Sequences mode

When data is acquired in Sequences mode, the MS data is automatically named and saved in folders with the same names on the RapidFire and MS computers.



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In This Guide

This guide has instructions for installing and using the Agilent RapidFire Integrator.

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Agilent Technologies