

# Agilent RapidFire System

## Data Analysis Guide



# Notices

## Document Identification

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## Software Revision

This guide is valid for the 4.3 revision or higher of the RapidFire Integrator program and compatible RapidFire Integrator programs, until superseded.

## Software Manufacturing



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# 1

## Preparation of RapidFire data for MassHunter quantitative analysis

This section describes how to prepare RapidFire data for analysis by MassHunter. It contains the following topics:

About analyzing RapidFire data **2**

About data analysis with MassHunter quantitative analysis **2**

To convert data for use with MassHunter quantitative analysis **3**

## About analyzing RapidFire data

RapidFire data can be acquired from triple-quadrupole (TQ) or time-of-flight (TOF/Q-TOF) LC/MS instruments controlled by the Agilent MassHunter Data Acquisition program.

After data acquisition is completed (see the *RapidFire 400 System User Guide*), you can process the data using RapidFire Integrator as described in this guide.

- The Agilent RapidFire Integrator data analysis program can be used to integrate all of the data points collected in a single RapidFire run.
- Each sequence of a RapidFire batch is processed individually. There is no limit on the number of plates.

RapidFire data can also be processed using MassHunter Workstation Quantitative Analysis. For more information see:

- **“About data analysis with MassHunter quantitative analysis”** below, and
- *Agilent MassHunter Workstation Quantitative Analysis Familiarization Guide*.

## About data analysis with MassHunter quantitative analysis

During the RapidFire-MassHunter data acquisition, data files (.D) are generated for each sequence.

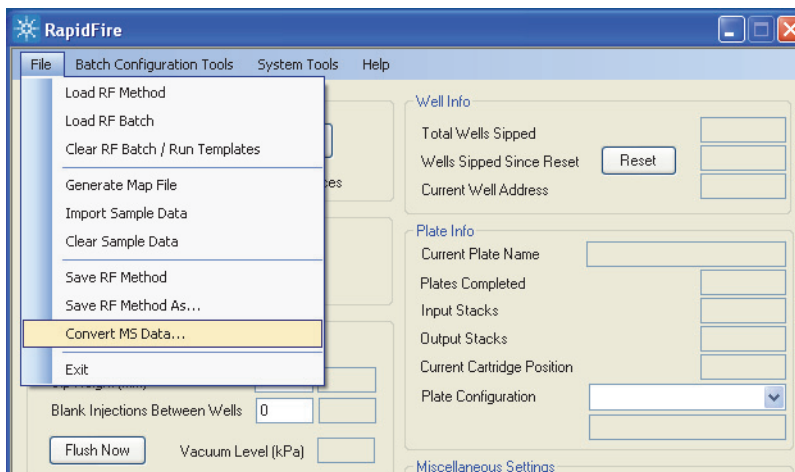
The total ion chromatogram (TIC) can be viewed in real time in MassHunter Data Acquisition, and can be inspected further in MassHunter Qualitative Analysis.

After a run is completed, the original data files (long **sequenceX.d** files that are generated by MassHunter Data Acquisition and **RFDataBase.xml** files that are generated by RapidFire software) must be split into individual injection files for the standard data analysis by MassHunter Quantitative Analysis program.

## To convert data for use with MassHunter quantitative analysis

### To convert data:

- 1 In the RapidFire software window, click **File > Convert MS Data**.



- 2 In the Convert MS Data dialog box:
  - a Select the whole-experiment data directory (**Data Path**) you want to convert.
  - b Select where to save the resulting single-injection data files (**Save To**).  
The data path of the latest run is displayed as a default selection.
  - c If needed, select options to indicate what you do not want to be converted.

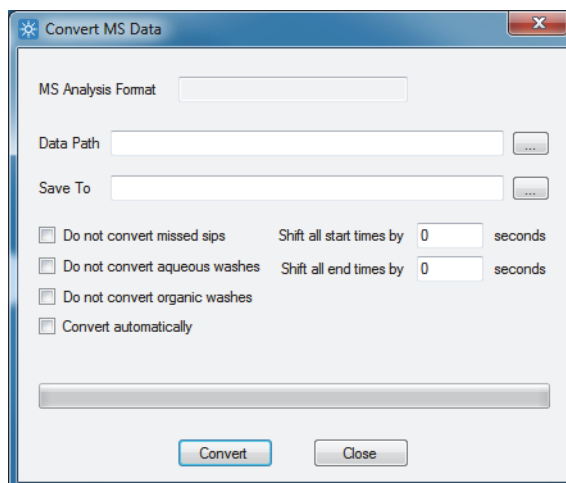
## Preparation of RapidFire data for MassHunter quantitative analysis

To convert data for use with MassHunter quantitative analysis

- d Select **Convert automatically** if you want data to be converted automatically.

While this option is selected and the dialog box opened, data is automatically converted. **Data Path** and **Save To** are automatically populated with the data folder that contains data most recently imported or generated by a run. Conversion begins on the data in that directory immediately. When you clear the check box, the automatic conversion in progress is canceled, and **Data Path** and **Save To** are no longer automatically populated.

- e Click **Convert**.

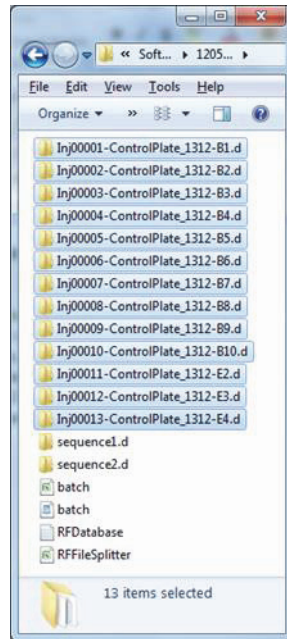




## Preparation of RapidFire data for MassHunter quantitative analysis

To convert data for use with MassHunter quantitative analysis

- View the list of converted files as shown in the following example. The single-peak data files are automatically named **InjXXXXX-barcode-well.d**, where:
  - XXXXXX is the injection order in the RapidFire batch.
  - barcode is the plate name.
  - well is the plate position of the sample on the RapidFire plate map.



- Proceed with viewing and processing the single-injection data files, as described in the MassHunter Quantitative Analysis online Help and User Guide.

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## 2

# Data analysis using RapidFire Integrator

This section describes how RapidFire 400 data is stored, prepared, and analyzed by RapidFire Integrator software.

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## Location, format, and preparation of data for RapidFire Integrator

### Location of data

RapidFire data is stored in the folder **D:\MassHunter\Data\RapidFire** on the MS controlling computer.

- A new data folder is created each day with the date as the folder name.
- A new folder is created when a new experiment (in Plates mode) or a new batch (in Sequences mode) is started in RapidFire Acquisition program.

#### Example

The first run performed on April 5, 2021 is saved in the following folder on the computer running the MS:

**D:\MassHunter\Data\RapidFire\2021\April\5\1**

#### NOTE

The data file folders that are created by the MS computer settings may be different than the scheme described above. Refer to the **BASE\_DATA\_DIR** variable in **RFMassHunterS.cfg** on your MS computer, which is located in **C:\Agilent\RapidFire Communicator\cfgs**.

## Data files

A data set for analysis with the RapidFire Integrator program includes the following types of files.

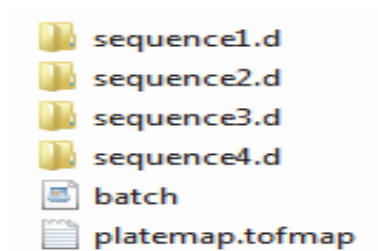
### NOTE

The following files must be present in the same folder.

- **batch.rftime**
- **sequenceX.d** mass spectrometer (MS) data files
- **platemap.tofmap.txt** tab-delimited text file (for TOF or Q-TOF LC/MS)

The original **platemap.tofmap.txt** file is empty. Change it as described on [page 11](#).

The following example data folder for a RapidFire Q-TOF system shows the three required file types:



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.

The following columns of data are saved in the **batch.rftime** text file:

- Plate identity
- Injection number
- Sequence number
- Row
- Column
- Sip Time

The Sip Time is the time stamp for the actuation of valve 2 from inject to load position, as well as the start of elution to MS.

- 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
1	plate	sip	seq	row	col	siptime	sipsensor
2	-----						
3	RF96-5	1	1	1	1	2.619	1
4	RF96-5	2	1	1	2	12.564	1
5	RF96-5	3	1	1	3	22.575	1
6	RF96-5	4	1	1	4	32.446	1
7	RF96-5	5	1	1	5	42.185	1
8	RF96-5	6	1	1	6	52.047	1
9	RF96-5	7	1	1001	5	62.187	1
10	RF96-5	8	1	1	7	71.874	1
11	RF96-5	9	1	1	8	81.752	1
12	RF96-5	10	1	1	9	91.54	1
13	RF96-5	11	1	1	10	101.031	1
14	RF96-5	12	1	1	11	110.671	1
15	RF96-5	13	1	1	12	120.131	1
16	RF96-5	14	1	2	1	130.12	1
17	RF96-5	15	1	2001	3	140.001	1
18	RF96-5	16	1	2001	2	149.968	1

### NOTE

- The aqueous and organic wash stations, WASH1 and WASH2, have (row, column) addresses of (2001, 2) and (2001, 3), respectively.
- The matrix vials MAT1, MAT2, MAT3, and MAT4 hve (row, column) addresses of (1001, 2), (1001, 3), (1001, 4), and (1001, 5), respectively.
- If data were acquired in Plates mode, make sure to name or rename the MS data files to **sequence1.d**.

## Exact mass plate map

For data that were acquired via RapidFire Q-TOF LC/MS in *MS/MS mode*, you do not need to modify the **platemap.tofmap.txt** file.

For RapidFire-TOF/Q-TOF experiments in *MS mode*, modify **platemap.tofmap.txt** to specify the exact masses of the compounds of interest for each well that was injected. RapidFire Integrator uses these values to extract the appropriate ion chromatograms from the TOF data of each well. For data that are acquired in *MS mode*, the columns of data in the **platemap.tofmap.txt** file are:

- Sequence number
- Row
- Column
- Exact mass 1 (or chemical formula 1)
- Exact mass 2 (or chemical formula 2), and so on

More columns can be appended as needed.

### Example

	A	B	C	D	E
1	seq	row	col	mass n	
2	-----				
3	1	2001	2	507.2344911	493.14382
4	1	1	1	507.2344911	493.14382
5	1	1	3	507.2344911	493.14382
6	1	1	5	507.2344911	493.14382
7	1	2001	2	327.1946771	493.14382
8	1	2	1	327.1946771	493.14382
9	1	2	3	327.1946771	493.14382
10	1	2	5	327.1946771	493.14382
11	1	2001	2	427.0921388	493.14382
12	1	3	1	427.0921388	493.14382
13	1	3	3	427.0921388	493.14382
14	1	3	5	427.0921388	493.14382
15	1	2001	2	318.1038131	493.14382
16	1	4	1	318.1038131	493.14382
17	1	4	3	318.1038131	493.14382
18	1	4	5	318.1038131	493.14382

## Loading RapidFire TQ LC/MS data in RapidFire Integrator

A run in Plates mode is defined as the data that are collected without setting up a batch. Unlike Sequences mode, data acquired in Plates mode are named and saved in folders possibly with different names and locations on the controlling computers.

If the data are collected in Plates mode, then prepare the data as described in this section before you analyze it with RapidFire Integrator.

If the data are collected in Sequences mode, proceed with **“To open RapidFire TQ LC/MS data”** on page 13.

### To prepare MassHunter data

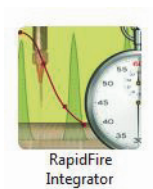
Repeat this process for each RapidFire-MS run:

- 1 Rename the MS data files to **sequence1.d**.
- 2 Copy the following files to a unique folder, such as **\data sequenceX\**
  - **sequence1.d**
  - **batch.rftime** (from the RapidFire computer, see **“Data files”** on page 9).

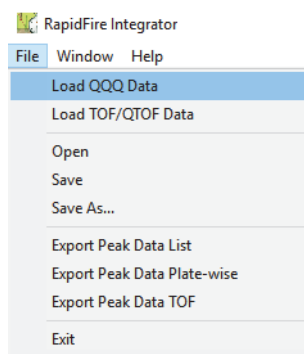


## To open RapidFire TQ LC/MS data

- 1 Start the Agilent RapidFire Integrator program by double-clicking the desktop icon.



- 2 Click **File > Load QQQ Data**.



- 3 When the Browse for folder window appears:
  - a Select the folder that contains the **.D** and **.RFTIME** files.
  - b Click **OK**.
- 4 Continue with **“Analyzing Data in RapidFire Integrator”** on page 17.

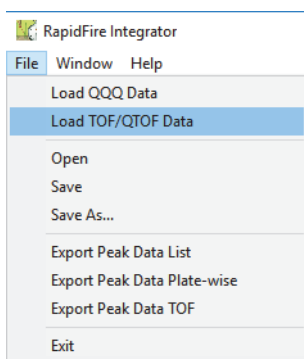
## Loading RapidFire TOF or Q-TOF LC/MS data in RapidFire Integrator

### To open RapidFire TOF or Q-TOF LC/MS data

- 1 Start the Agilent RapidFire Integrator program by double-clicking the desktop icon.



- 2 Click **File > Load TOF-QTOF Data**.



- 3 When the Browse For Folder window appears:
  - a Select the folder that contains the **.D**, **.RFTIME** and **platemap.TOFMAP.TXT** files.
  - b Click **OK**.
- 4 Click **Data > Extract TOF\_QTOF XICS**.
- 5 For data that were acquired with a TOF or Q-TOF LC/MS in *MS mode*:
  - a Set the following parameters in the **TOF Options** dialog box:

## Data analysis using RapidFire Integrator

To open RapidFire TOF or Q-TOF LC/MS data

- **Display Time Range** (set to all by default)
- **Polarity**
- **Error (ppm)**, which determines the accuracy
- **Internal Standard**

If **Use Internal Standard** is selected, enter the mass of the internal standard. To extract multiple internal standards in every well of the RapidFire-TOF run, use a comma to separate each mass.

TOF Options

Please Set TOF Configuration Parameters

Sequence: 1  Apply to all Sequences

Polarity:  Positive  Negative

Charge State: 1

Error (ppm): 10

Internal Standard (before polarity mod): 493.1438  Use Internal Standard

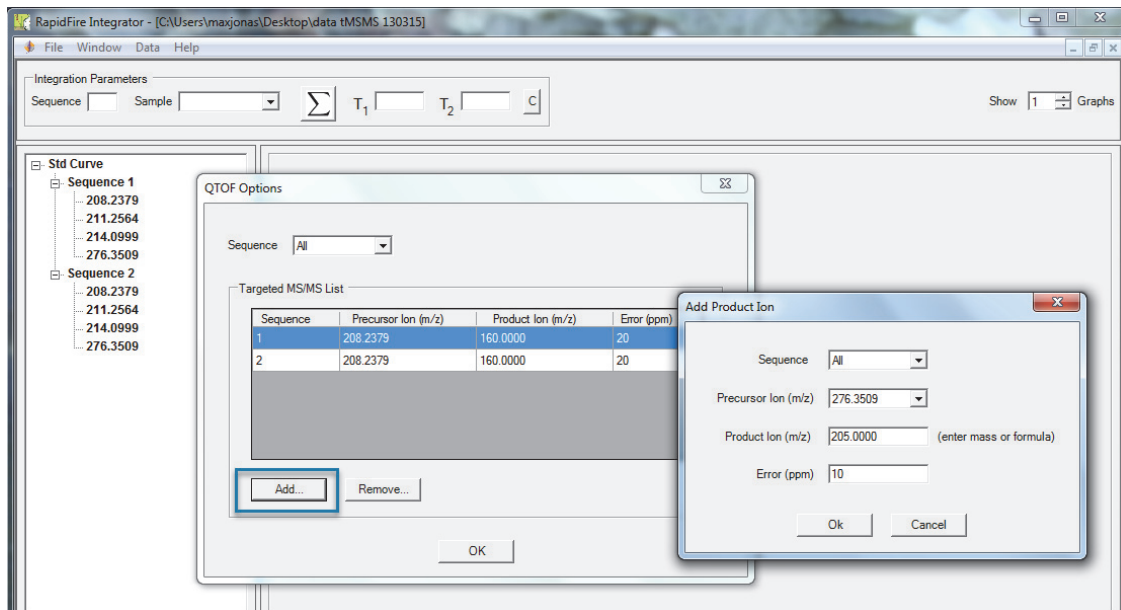
Metabolite Analysis

OK

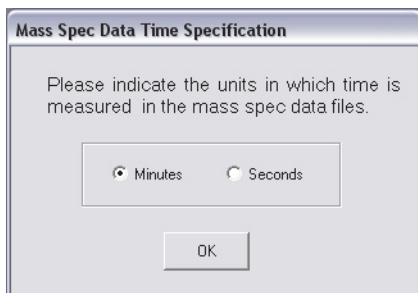
- b Click **OK** to close the dialog box.
- 6 For data that were acquired with a Q-TOF LC/MS in *MS/MS mode*:
  - a Click **Add** in the **QTOF Options** dialog box.
  - b In the **Add Product** dialog box:
    - Select **Sequence** and **Precursor Ion**.
    - Type the mass or chemical formula of one **Product Ion** of interest and the **Error** (ppm).
    - Click **OK**.
  - c Repeat for all Precursor Ions and all Product Ions of interest.

## Data analysis using RapidFire Integrator

To open RapidFire TOF or Q-TOF LC/MS data



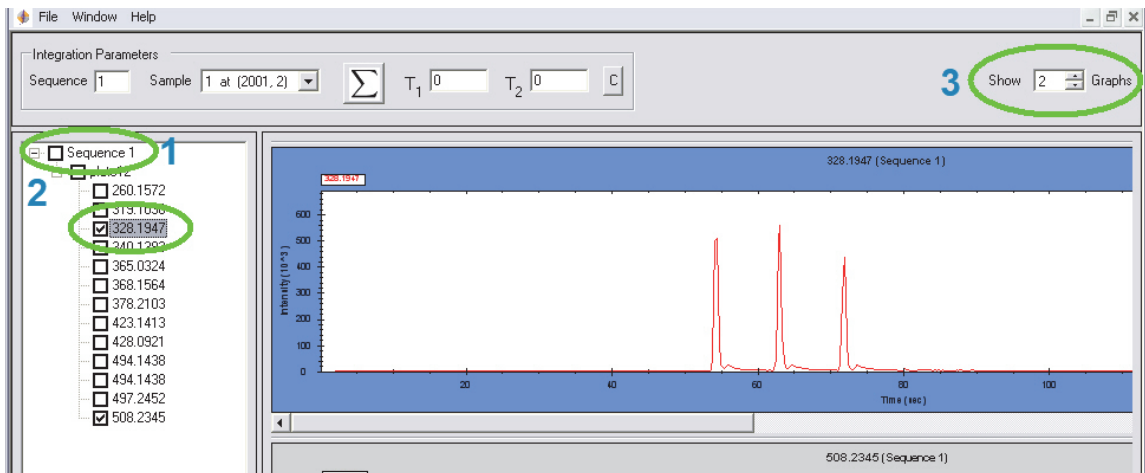
- d When you are finished, click **OK** to close the QTOF Options dialog box.
- 7 Select **Minutes** for the MS data files time units and click **OK**.



## Analyzing Data in RapidFire Integrator

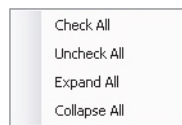
### To analyze the data in RapidFire Integrator:

- 1 Click the sequence of interest in the left pane (1), as shown for Sequence 1 in the following example.



- 2 Review the data using the following features:

- To display the list of  $m/z$ , click the + sign next to the sequence (2).
- To display the following menu options, right-click in the left pane (2).



- Select the number of graphs to display in the upper-right area of the window (3).
- To select an area of graph to expand, drag the mouse. Repeat to expand the area further.

To zoom out each successive level, right-click on the graph and select **Un-zoom**.

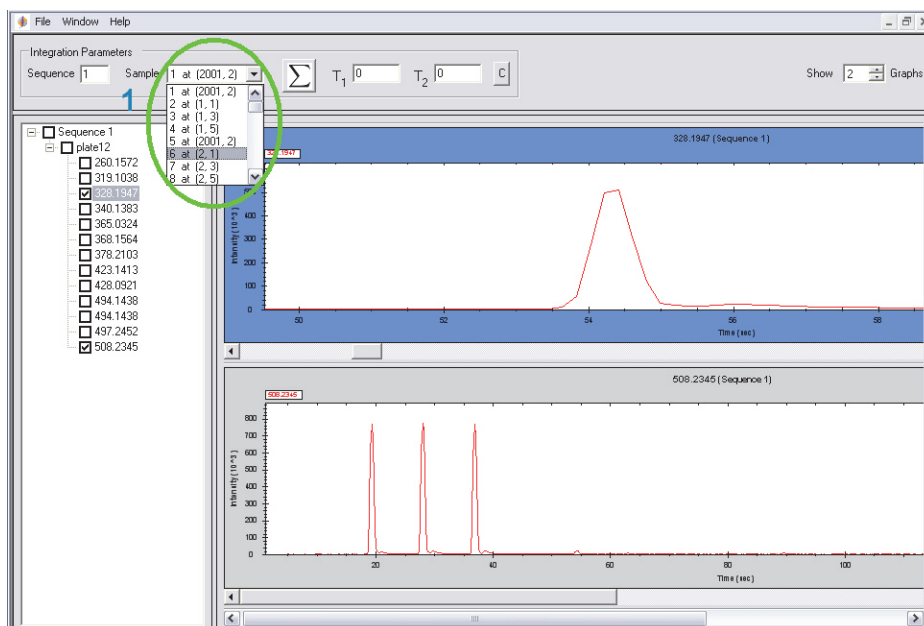
(1)

### 3 Integrate the 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 analyses can be based on any mass.
  - The title of the plot identifies monitored analytes by their  $m/z$ .

To navigate from one  $m/z$  to another, use the scroll bar on the right side of the window.

- b Select the identity of the peak from the **Sample** list (1).

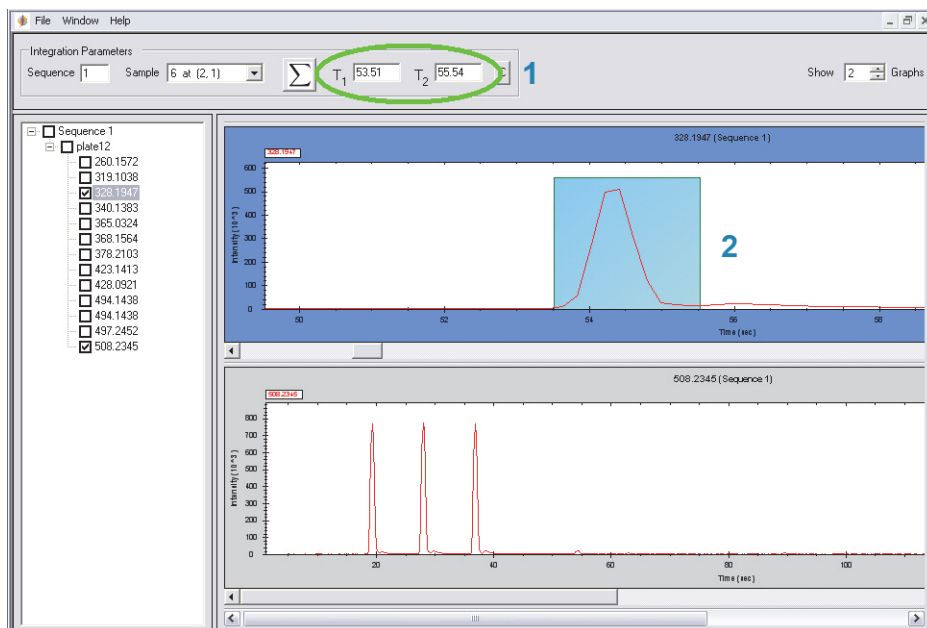


## Data analysis using RapidFire Integrator

### Analyzing Data in RapidFire Integrator

- c Define the peak to integrate for area-under-the-curve (AUC) calculations in either of the following ways:
  - Drag the mouse in a thin slab rectangle from the front edge of the peak to its tail end.
  - Use the keyboard to type in the  $T_1$  and  $T_2$  times (1).

The defined time range is displayed in the  $T_1$  and  $T_2$  boxes above the graph. The peak area is also emphasized by teal coloring as shown in the following example (2).

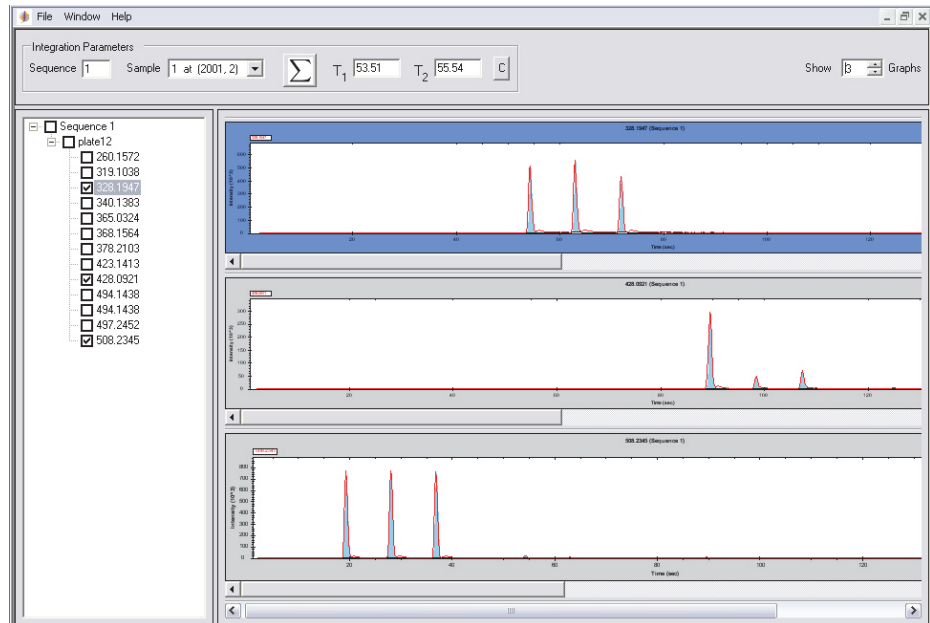


- d To process the data for the current sequence, click the summation button.



All masses within the same sequence are processed together. AUCs of injections turn blue, but background signals remain red.

- e Repeat **step a** through **step d** for each sequence of the run.



- 4 To save partial progress in data analysis before all of the sequences of the run are analyzed, click **File > Save As** and enter a name for the file.

The results are saved in the specified .RFD file.

- 5 Click **File > Open** to reopen experiment files that have been partially processed.
- 6 To export data that were acquired with a triple-quad LC/MS, click **File > Export Peak Data List**.

An **.rfpks** text file is created, which contains a list of injections.

You can analyze this file with a spreadsheet program. The following is an example file opened in Microsoft Excel.



## Data analysis using RapidFire Integrator

### Analyzing Data in RapidFire Integrator

	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

- 7 To export data that were acquired with a TOF or Q-TOF LC/MS in *MS mode*, click **File > Export Peak Data TOF**.

An **.rfpks** text file is created, which contains a list of injections.

Only the masses of interest are reported for each well.

You can further analyze the results with a spreadsheet program. The following is an example file opened in Microsoft Excel.

	A	B	C	D	E	F	G	H	I	J
1	Sip	Sequence	PlateName	Row	Column	Injection time (sec)	Mass 1	A1	Mass 2	A2
2	1	1	DEMO PLATE	2001	2	9.23	507.2345	10648	493.1438	2747
3	2	1	DEMO PLATE	1	1	18.46	507.2345	2992271	493.1438	297438
4	3	1	DEMO PLATE	1	3	27.27	507.2345	3061670	493.1438	490474
5	4	1	DEMO PLATE	1	5	36.17	507.2345	2900104	493.1438	475369.5
6	5	1	DEMO PLATE	2001	2	44.41	327.1947	0	493.1438	4295
7	6	1	DEMO PLATE	2	1	53.51	327.1947	1819135	493.1438	574327
8	7	1	DEMO PLATE	2	3	62.25	327.1947	1888354	493.1438	552930
9	8	1	DEMO PLATE	2	5	71.09	327.1947	1403209	493.1438	517027
10	9	1	DEMO PLATE	2001	2	79.42	427.0921	41	493.1438	4003.5
11	10	1	DEMO PLATE	3	1	88.65	427.0921	1135663	493.1438	527903
12	11	1	DEMO PLATE	3	3	97.63	427.0921	195475	493.1438	503490
13	12	1	DEMO PLATE	3	5	106.48	427.0921	292988	493.1438	492337.5
14	13	1	DEMO PLATE	2001	2	114.92	318.1038	311	493.1438	2913
15	14	1	DEMO PLATE	4	1	124.15	318.1038	31407	493.1438	545323
16	15	1	DEMO PLATE	4	3	133.16	318.1038	29637	493.1438	505078.5
17	16	1	DEMO PLATE	4	5	141.98	318.1038	34226	493.1438	490114

## Data analysis using RapidFire Integrator

### Analyzing Data in RapidFire Integrator

- 8 To export data that were acquired with a Q-TOF LC/MS in *MS/MS mode*, click **File > Export Peak Data List**.

An **.rfpks** text file is created, which contains a list of injections.

Only the precursor ion : product ion pairs of interest are reported for each sequence.

You can further analyze the results using a spreadsheet program. The following is an example file opened in Microsoft Excel.

	A	B	C	D	E	F	G
1	Sequence 1						
2	PlateName	Row	Column	208.2379 : 160.0000	211.2564 : 163.0000	276.3509 : 205.0000	Injection Time (sec)
3	Std Curve	1	3	27	7826	0	10.47
4	Std Curve	2	3	0	18471	202	27.1
5	Std Curve	3	3	395	16137	768	43.8
6	Std Curve	4	3	145	13475	406	60.38
7	Std Curve	5	3	0	19239	530	76.86
8	Std Curve	6	3	254	14069	1586	93.48
9	Std Curve	7	3	1019	20907	3064	109.94
10	Std Curve	8	3	1263	17357	5734	126.44
11	Std Curve	1	4	47	30059	106	142.87
12	Std Curve	2	4	85	34318	0	159.34
13	Std Curve	3	4	0	33395	0	176.12
14	Std Curve	4	4	120	37298	0	192.44
15	Sequence 2						
16	PlateName	Row	Column	208.2379 : 160.0000	211.2564 : 163.0000	276.3509 : 205.0000	Injection Time (sec)
17	Std Curve	1	3	68	8270	0	11.24
18	Std Curve	2	3	134	19820	257	27.8
19	Std Curve	3	3	77	15757	196	44.26
20	Std Curve	4	3	221	17702	632	60.83

- 9 To export RapidFire TQ LC/MS data by plates, click **File > Export Peak Data Plate-wise**.

#### NOTE

- Data for MAT# and WASH# injections are not displayed.
- This option is only offered if each sample well within a plate is visited only once.

You can analyze this file with a spreadsheet program, such as Microsoft Excel. An example is shown below.

	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

**10** To export RapidFire TOF/Q-TOF data acquired in *MS* mode, click **File > Export Peak Data TOF**.

An **.rfpks** text file is created, which contains a list of injections.

Only the masses of interest are reported for each well.

You can further analyze the results using a spreadsheet program, such as Microsoft Excel. An example is shown below.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Sequence 1															
2	PlateName	Row	Column	260.1572	319.1038	328.1947	340.1383	365.0324	368.1564	378.2103	423.1413	428.0921	494.1438	497.2452	508.2345	Injection time (sec)
3	DEMO PLATE	2001	2	4277	0	6	323	681	1738	9332	750	1085	2747	12245	10648	9.23
4	DEMO PLATE	1	1	0	139	0	126	8994	23	30920	675	224	297438	4205	2992271	18.46
5	DEMO PLATE	1	3	0	265	0	0	24523	73.5	28588	1319.5	819	490474	8052	3061670	27.27
6	DEMO PLATE	1	5	0	77.5	0	0	13564	100	0	836.5	327	475369.5	6326	2900104	36.17
7	DEMO PLATE	2001	2	0	54	0	0	299	0	0	233	0	4295	2197	4893	44.41
8	DEMO PLATE	2	1	0	479	1819135	0	16647	32469	629	698	424	574327	8337	82630	53.51
9	DEMO PLATE	2	3	0	1079	1888354	0	1880	59220	228	851	482	552930	8459	23723	62.25
10	DEMO PLATE	2	5	0	708	1403209	0	2532	58457	21	486	224	517027	9908	9391	71.09
11	DEMO PLATE	2001	2	0	638	1295.5	0	109.5	0	0	470	41	4003.5	2713.5	3307	79.42
12	DEMO PLATE	3	1	0	960	2248	86	10454	52	277	412	1135663	527903	8916	25129	88.65
13	DEMO PLATE	3	3	0	487	0	747	991	8.5	0	350	195475	503490	7489.5	11622	97.63
14	DEMO PLATE	3	5	0	429	0	880.5	632.5	167	0	309	292988	492337.5	7719	7239.5	106.48
15	DEMO PLATE	2001	2	0	311	0	0	28	5	0	339	0	2913	2301	2620	114.92
16	DEMO PLATE	4	1	0	31407	0	0	838	0	0	430	14744	545323	39815	14282	124.15
17	DEMO PLATE	4	3	0	29637	0	0	300.5	0	4.5	212.5	743	505078.5	340302	6610	133.16
18	DEMO PLATE	4	5	0	34226	0	0	554	0	0	206	315	490114	40181	4813	141.98

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## In This Guide

This guide has instructions on how to use the Agilent RapidFire Integrator program to analyze RapidFire data.

This guide applies to RapidFire Integrator 4.3 or higher.

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