Agilent RapidFire 360 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 G9214AA RapidFire 360 High-throughput Mass Spectrometry System run.

Data can be acquired from LC/MS instruments controlled by the Agilent MassHunter Data Acquisition software.

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

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.

RFI v3 Installer.exe
Setup Launcher Unicode

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.

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 13)
- **sequenceX.tofmap.txt**

Analyzing Data in RapidFire Integrator

To access data acquired in Sequences mode

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

- **C:\Program Files\ Agilent\ RapidFire\ cfgs\ RFMassHunterS.cfg**

2 Locate the variable **BASE_DATA_DIR**.

Typical value for **BASE_DATA_DIR** is:

- **D:\ MassHunter\ Data\ RapidFire**

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

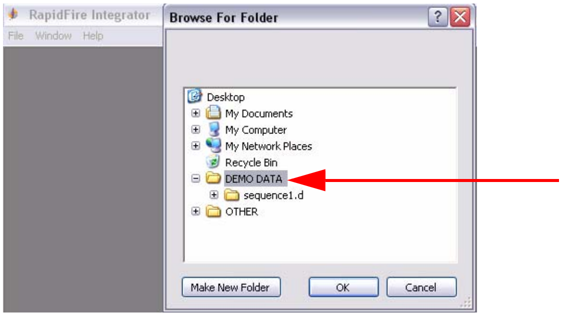
Tip The following files are automatically moved to a unique folder, such as **D:\ MassHunter\ Data\RapidFire\2011\April\5\1** (for the first run on April 5, 2011):

- **batch.rftime** (automatically transferred from the RapidFire computer to the MS computer)
- **sequenceX.d**
- **sequenceX.tofmap.txt**

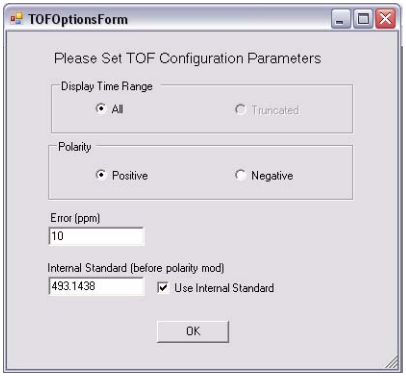
To analyze date in RapidFire Integrator

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

2. Click **File > Load TOF Data Set**.
3. When the Browse For Folder window appears:
   a. Select the folder that contains the **.D**, **.RFTIME** and **.TOFMAP.TXT** files.



   b. Click **OK**.
4. Click **Data > Extract TOF XICs**.
5. Set the following parameters on the TOF Options Form dialog box, then click **OK**:
   - **Display Time Range** (set to all by default)
   - **Polarity**
   - **Error (ppm)**, which determines the accuracy
   - Whether or not to **Use Internal Standard**, and if marked, then enter the mass of the internal standard



Please Set TOF Configuration Parameters

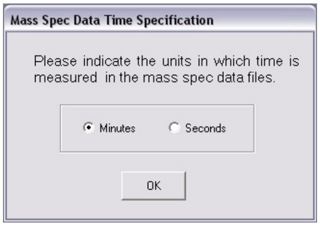
Display Time Range
● All ○ Truncated

Polarity
● Positive ○ Negative

Error (ppm): 10

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

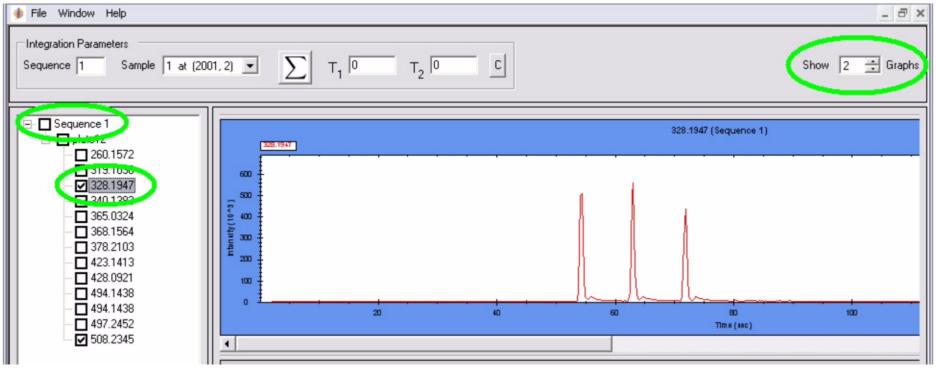
6 Select **Minutes** for the MS data files time units.



Please indicate the units in which time is measured in the mass spec data files.

● Minutes ○ Seconds

7 Click to select the sequence of interest in the left pane, as shown for Sequence 1 in the following example.



Sequence: 1 Sample: 1 at (2001, 2) T_1: 0 T_2: 0

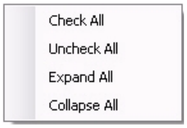
Show 2 Graphs

☐ Sequence 1
☐ 260.1572
☑ 328.1947
☐ 365.0324
☐ 368.1564
☐ 378.2103
☐ 423.1413
☐ 428.0921
☐ 494.1438
☐ 494.1438
☐ 497.2452
☑ 508.2345

8 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.

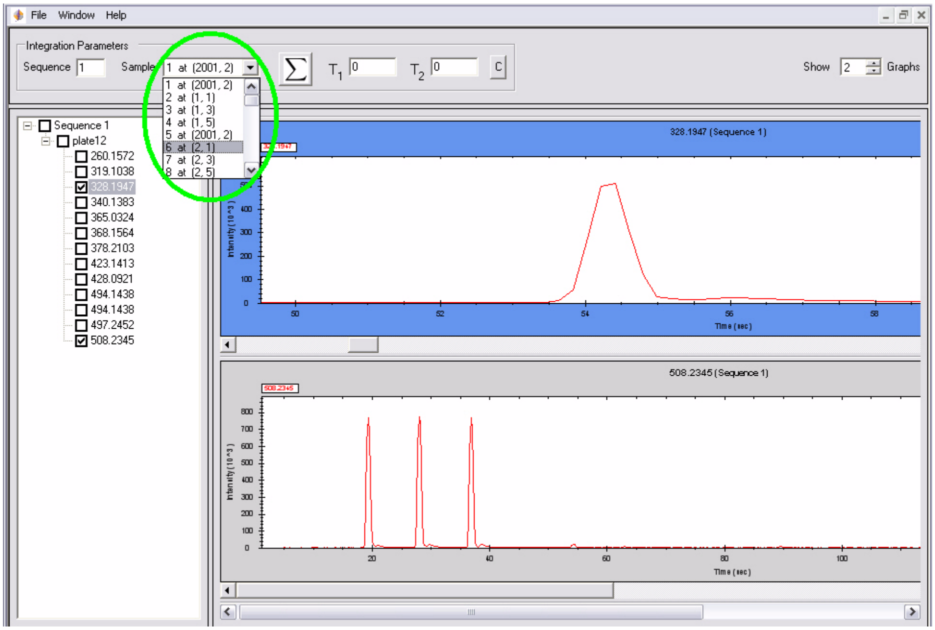
9 Integrate peaks as follows:

   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 extracted mass.
   - Monitored analytes are identified by their *m/z* (title of the plot).

   Use the scroll bar on the right side of the window to navigate from one *m/z* to another.

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



Sequence 1

Sample 1 at (2001, 2)

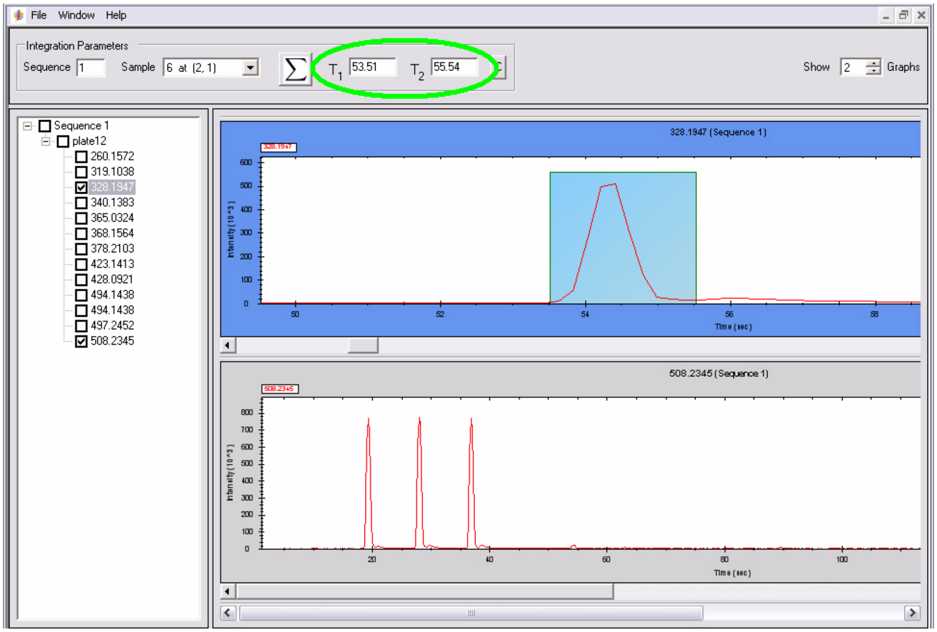
T1 0

T2 0

Show 2 Graphs

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 **T_1** and **T_2** boxes above the graph, and area also emphasized by teal coloring as shown in the following example:



Integration Parameters

Sequence 1 Sample 6 at (2, 1) ∑ T_1 53.51 T_2 55.54

Show 2 Graphs

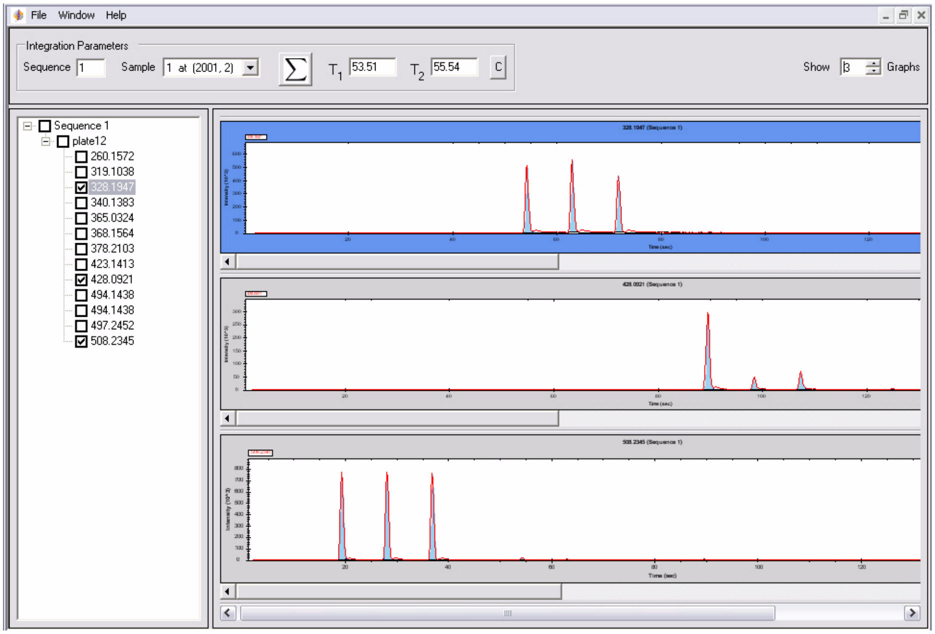
- ☐ Sequence 1
  - ☐ plate12
    - ☐ 260.1572
    - ☐ 319.1038
    - ☑ 328.1947
    - ☐ 340.1383
    - ☐ 365.0324
    - ☐ 368.1564
    - ☐ 378.2103
    - ☐ 423.1413
    - ☐ 428.0921
    - ☐ 494.1438
    - ☐ 494.1438
    - ☐ 497.2452
    - ☑ 508.2345

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

∑

All masses of the sequence are processed together.

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



Sequence 1

Sample 1 at (2001, 2)

T_1 53.51

T_2 55.54

Show 3 Graphs

- **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**.

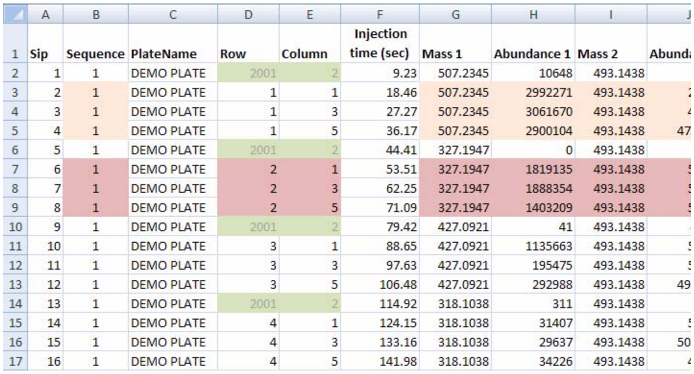
10 Export data.

- **a** When processing is complete for all the sequences of a run, click **File > Export Peak TOF Format** 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. An example file opened in Microsoft Excel is shown on the next page.



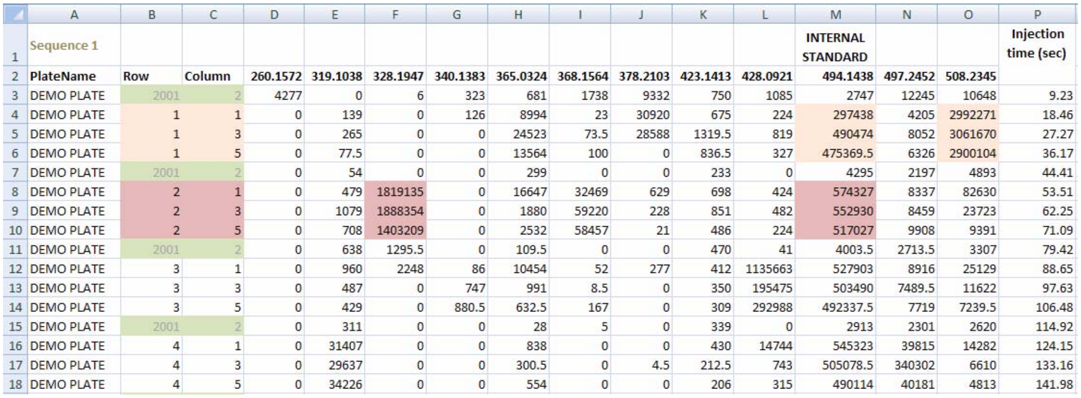
	A	B	C	D	E	F	G	H	I	J
1	Sip	Sequence	PlateName	Row	Column	Injection time (sec)	Mass 1	Abundance 1	Mass 2	Abund
2	1	1	DEMO PLATE	2001	2	9.23	507.2345	10648	493.1438	
3	2	1	DEMO PLATE	1	1	18.46	507.2345	2992271	493.1438	2
4	3	1	DEMO PLATE	1	3	27.27	507.2345	3061670	493.1438	4
5	4	1	DEMO PLATE	1	5	36.17	507.2345	2900104	493.1438	47
6	5	1	DEMO PLATE	2001	2	44.41	327.1947	0	493.1438	
7	6	1	DEMO PLATE	2	1	53.51	327.1947	1819135	493.1438	5
8	7	1	DEMO PLATE	2	3	62.25	327.1947	1888354	493.1438	5
9	8	1	DEMO PLATE	2	5	71.09	327.1947	1403209	493.1438	5
10	9	1	DEMO PLATE	2001	2	79.42	427.0921	41	493.1438	
11	10	1	DEMO PLATE	3	1	88.65	427.0921	1135663	493.1438	5
12	11	1	DEMO PLATE	3	3	97.63	427.0921	195475	493.1438	5
13	12	1	DEMO PLATE	3	5	106.48	427.0921	292988	493.1438	49
14	13	1	DEMO PLATE	2001	2	114.92	318.1038	311	493.1438	
15	14	1	DEMO PLATE	4	1	124.15	318.1038	31407	493.1438	5
16	15	1	DEMO PLATE	4	3	133.16	318.1038	29637	493.1438	50
17	16	1	DEMO PLATE	4	5	141.98	318.1038	34226	493.1438	4

b You can also export data using **File > Export Peak Data**,

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

All the masses of interest throughout the sequence are reported for every well.

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	N	O	P
1	Sequence 1												INTERNAL STANDARD			Injection time (sec)
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	
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

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. These are full scans acquired by the TOF and can be several gigabytes in size.
- A **.tofmap.txt** tab-delimited text file, which you create to indicate the platemap of the experiment. The platemap indicates the exact masses of interest that are expected for each well.

Here is an example folder that contains the three required files:

sequence1.d		File Folder
batch.rftime	2 KB	RFTIME File
Exact mass plate map.tofmap.txt	2 KB	Text Document

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:

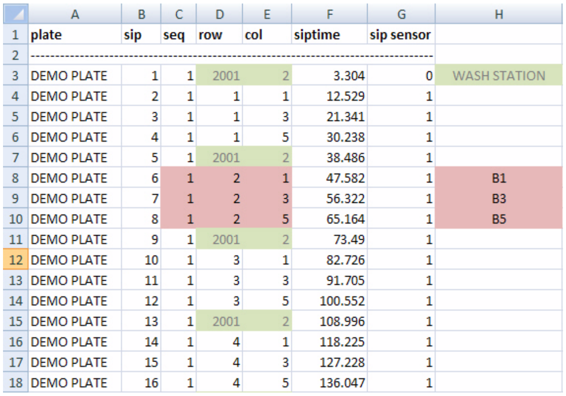
C:\ Program Files\ Agilent\ RapidFire\ data\ 2011\ April\ 5\ 1

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

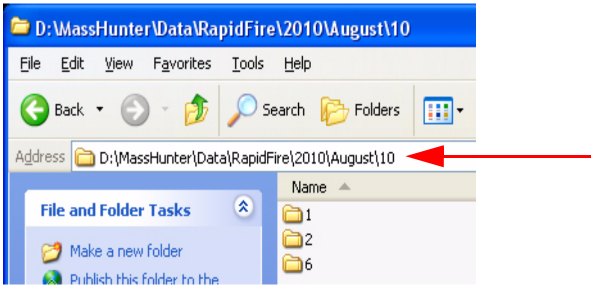
The path where the MS data is automatically stored on the MS computer is specified by the variable **BASE_DATA_DIR**, which is defined in:

- **C:\Program Files\ Agilent\ RapidFire\ cfgs\ RFMassHunterS.cfg**

Typical value for **BASE_DATA_DIR** is:

- **D:\ MassHunter\ Data\ RapidFire**

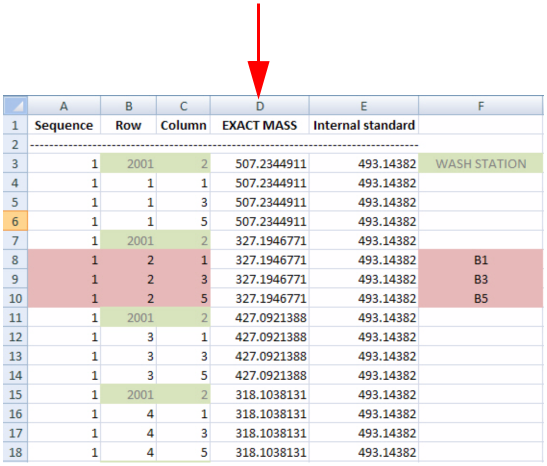
In Sequences mode When data is acquired in Sequences mode, the MS data is automatically named and saved in folders with the same names on the RapidFire and MS computers.



Exact mass platemap

The scientist must provide a **.tofmap.txt** file describing the platemap of the run. The platemap indicates the exact mass of the compounds for each well injected. The RapidFire Integrator uses these values to extract the appropriate ion chromatograms from the TOF data for each well.

Example



	A	B	C	D	E	F
1	Sequence	Row	Column	EXACT MASS	Internal standard	
2	--					
3	1	2001	2	507.2344911	493.14382	WASH STATION
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	B1
9	1	2	3	327.1946771	493.14382	B3
10	1	2	5	327.1946771	493.14382	B5
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	

Platemap file format

The columns in the **.tofmap.txt** must appear in the following order:

- sequence number
- row
- column
- exact mass 1
- exact mass 2, and so on

Additional columns can be appended as needed.

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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