Comprehensive Test Mix for MassHunter Pesticide Triggered MRM Database and Library

Method Setup Guide

NOTE The Comprehensive Pesticide Test Mix is included with the G1733 Pesticide Triggered MRM Application Kit.

Agilent does not provide the actual acquisition methods to use with the Comprehensive Test Mix, due to the large number of instrument configurations that are possible.

Instead, Agilent provides this guide to explain how to create MRM methods that are used to create dMRM and tMRM methods for the test mix.

You will copy the values from the file **MRM_Methods_Pesticide.xlsx**, found on the *Support Disc*, to set up your MRM methods.

Before you begin, make sure that your system meets the installation requirements that are described in the *MassHunter Pesticide Triggered MRM Database and Library Quick Start Guide*.

For more detailed instructions, see the *Quick Start Guide* for this database, and the MassHunter Data Acquisition for 6400 Series Triple Quadrupole LC/MS *Familiarization Guide* and *online Help*.

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Step 1. Set up the LC part of the method

1 Set up the solvent.

This step is identical for all LC configurations.

- Solvent A: 5 mM ammonium formate in 0.1% formic acid in water
- Solvent B: 5 mM ammonium formate in 0.1% formic acid in methanol

2 Set up the gradient.

The gradient setup is dependent upon the LC configuration. Some examples follow.

1290 Infinity LC system

1290 Infinity LC system with Agilent Eclipse Plus C18, 2.1 mm × 150 mm, 1.8 µm ZORBAX LC column (p/n 959759-902), included in the G1733BA Pesticide Triggered MRM Application Kit.

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	95.00	5.00	0.400	1200.00
0.50	95.00	5.00	0.400	1200.00
3.50	50.00	50.00	0.400	1200.00
17.00	0.00	100.00	0.400	1200.00
20.00	0.00	100.00	0.400	1200.00
20.10	95.00	5.00	0.400	1200.00

Stop time is 20:10.

1260 Infinity LC system

The 1260 Infinity LC system can have a lower backpressure (up to 600 bar) and a higher dead volume than the 1290 Infinity LC system.

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	95.00	5.00	0.400	600.00
3.50	50.00	50.00	0.400	600.00
17.00	0.00	100.00	0.400	600.00
20.00	0.00	100.00	0.400	600.00
20.10	95.00	5.00	0.400	600.00

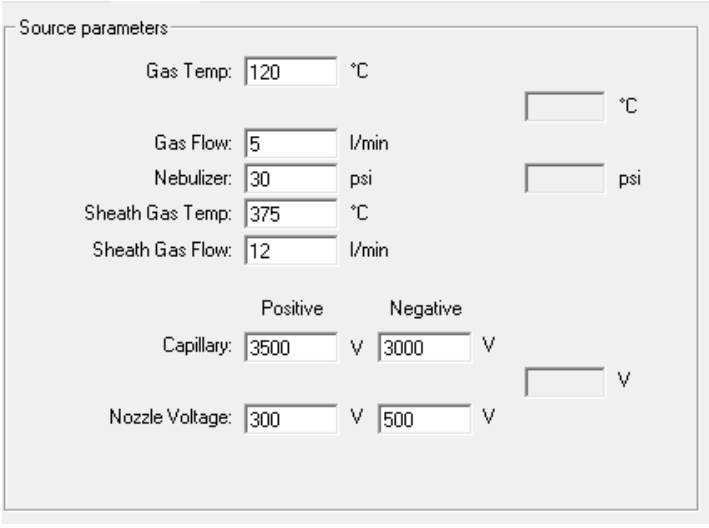
Stop time is 20:10.

Step 2. Set up LC/MS ion source parameters

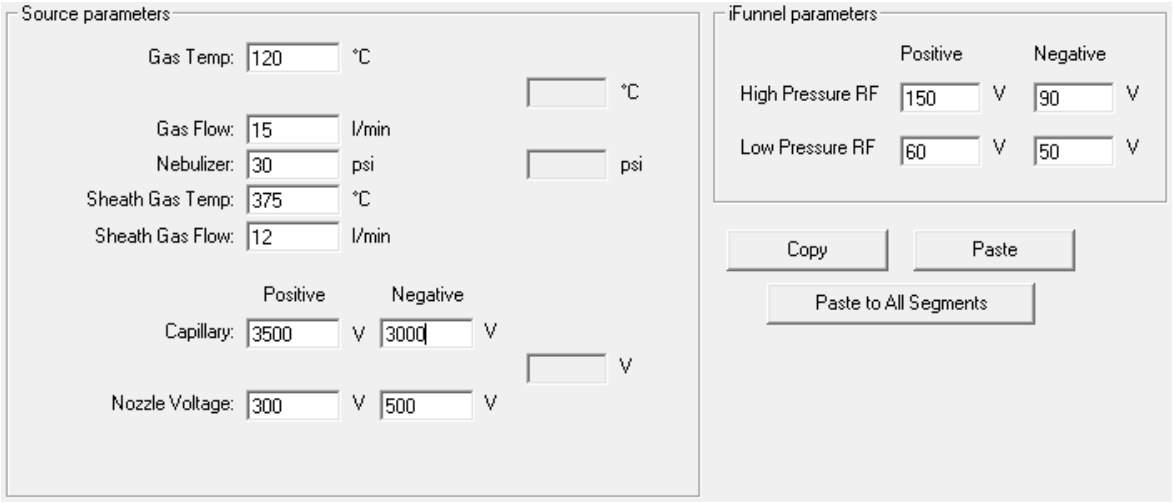
- Set up the ion source parameters in the MS part of the method.

  For a multi-component method, the ion source parameters shown in the next figure are used to achieve the best overall sensitivity for all of the compounds in the Comprehensive Test Mix. You can make adjustments to optimize for individual compounds or submixes.

6460 LC/MS Ion source parameters for the 6460 LC/MS instrument:



6490 LC/MS Ion source parameters for the 6490 LC/MS instrument:

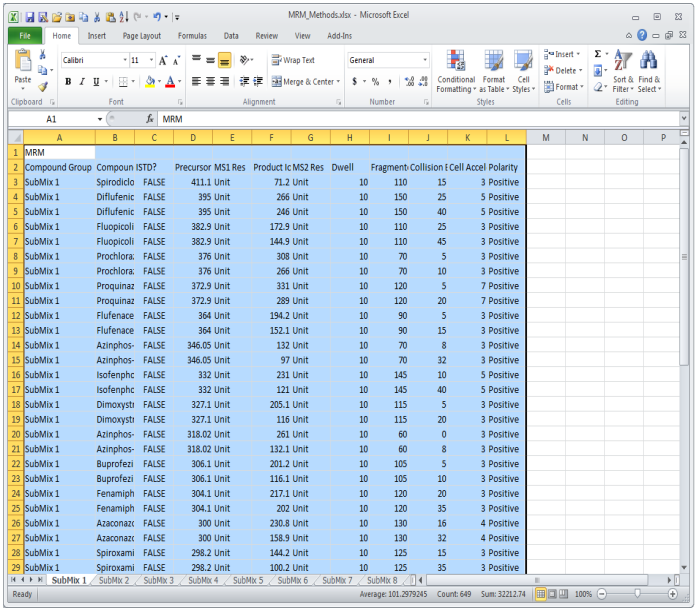


The ion source parameters shown for the 6490 LC/MS also include the iFunnel parameters. These iFunnel parameters ensure the best overall sensitivity for all of the compounds in the Comprehensive Test Mix. You can use the Source and iFunnel Optimizer program to optimize for individual compounds or submixes. Refer to the MassHunter Data Acquisition for 6400 Series Triple Quadrupole LC/MS *Familiarization Guide* and *online Help*.

Step 3. Set up the MRM method

1 From the *Support Disc*, open the file **MRM_Methods_Pesticide.xlsx**.

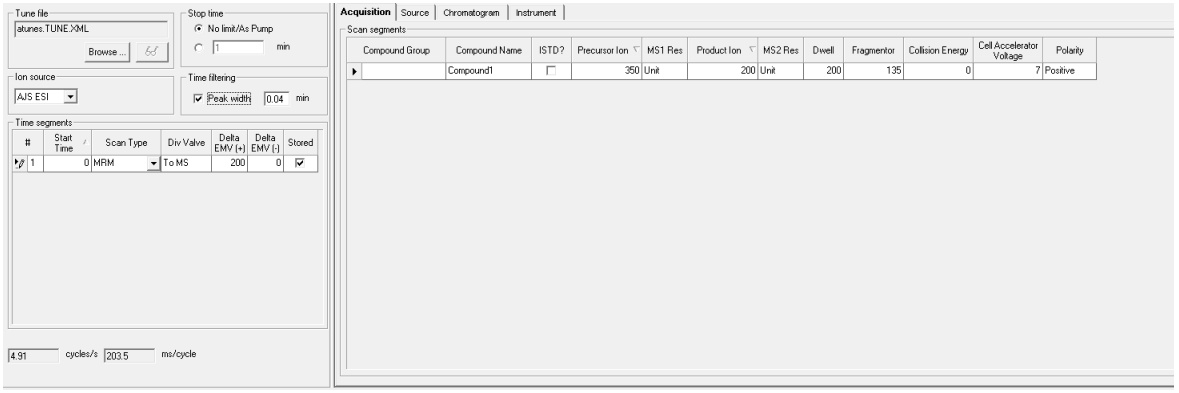
This spreadsheet file contains eight tabs, **SubMix 1** through **SubMix 8**, one for each of the eight standard mixes in the Comprehensive Test Mix.



	A	B	C	D	E	F	G	H	I	J	K	L
1	MRM											
2	Compound Group	Compoun	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Dwell	Fragment	Collision E	Cell Accel	Polarity
3	SubMix 1	Spirodiclo	FALSE	411.1	Unit	71.2	Unit	10	110	15	3	Positive
4	SubMix 1	Diflufenic	FALSE	395	Unit	266	Unit	10	150	25	5	Positive
5	SubMix 1	Diflufenic	FALSE	395	Unit	246	Unit	10	150	40	5	Positive
6	SubMix 1	Fluopicoli	FALSE	382.9	Unit	172.9	Unit	10	110	25	3	Positive
7	SubMix 1	Fluopicoli	FALSE	382.9	Unit	144.9	Unit	10	110	45	3	Positive
8	SubMix 1	Prochlora:	FALSE	376	Unit	308	Unit	10	70	5	3	Positive
9	SubMix 1	Prochlora:	FALSE	376	Unit	266	Unit	10	70	10	3	Positive
10	SubMix 1	Proquinaz	FALSE	372.9	Unit	331	Unit	10	120	5	7	Positive
11	SubMix 1	Proquinaz	FALSE	372.9	Unit	289	Unit	10	120	20	7	Positive
12	SubMix 1	Flufenace	FALSE	364	Unit	194.2	Unit	10	90	5	3	Positive
13	SubMix 1	Flufenace	FALSE	364	Unit	152.1	Unit	10	90	15	3	Positive
14	SubMix 1	Azinphos-	FALSE	346.05	Unit	132	Unit	10	70	8	3	Positive
15	SubMix 1	Azinphos-	FALSE	346.05	Unit	97	Unit	10	70	32	3	Positive
16	SubMix 1	Isofenphc	FALSE	332	Unit	231	Unit	10	145	10	5	Positive
17	SubMix 1	Isofenphc	FALSE	332	Unit	121	Unit	10	145	40	5	Positive
18	SubMix 1	Dimoxysti	FALSE	327.1	Unit	205.1	Unit	10	115	5	3	Positive
19	SubMix 1	Dimoxysti	FALSE	327.1	Unit	116	Unit	10	115	20	3	Positive
20	SubMix 1	Azinphos-	FALSE	318.02	Unit	261	Unit	10	60	0	3	Positive
21	SubMix 1	Azinphos-	FALSE	318.02	Unit	132.1	Unit	10	60	8	3	Positive
22	SubMix 1	Buprofezi	FALSE	306.1	Unit	201.2	Unit	10	105	5	3	Positive
23	SubMix 1	Buprofezi	FALSE	306.1	Unit	116.1	Unit	10	105	10	3	Positive
24	SubMix 1	Fenamiph	FALSE	304.1	Unit	217.1	Unit	10	120	20	3	Positive
25	SubMix 1	Fenamiph	FALSE	304.1	Unit	202	Unit	10	120	35	3	Positive
26	SubMix 1	Azaconaz	FALSE	300	Unit	230.8	Unit	10	130	16	4	Positive
27	SubMix 1	Azaconaz	FALSE	300	Unit	158.9	Unit	10	130	32	4	Positive
28	SubMix 1	Spiroxami	FALSE	298.2	Unit	144.2	Unit	10	125	15	3	Positive
29	SubMix 1	Spiroxami	FALSE	298.2	Unit	100.2	Unit	10	125	35	3	Positive

2 Open the MassHunter Data Acquisition program.

3 In the Method Editor window, click **QQQ > Acquisition**.

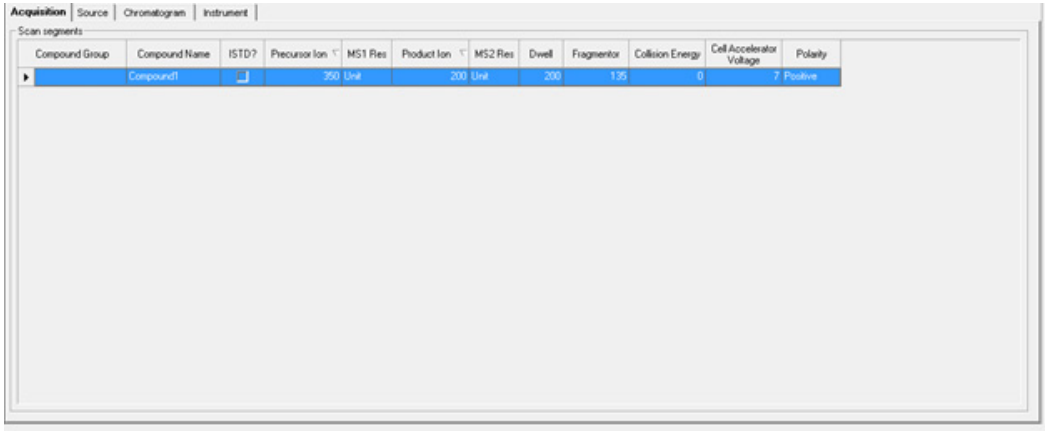


4 In the spreadsheet file, in the **SubMix 1** tab, select all of the cells that contain MRM information. Make sure that you select the two header rows. *Do not select the entire table!*

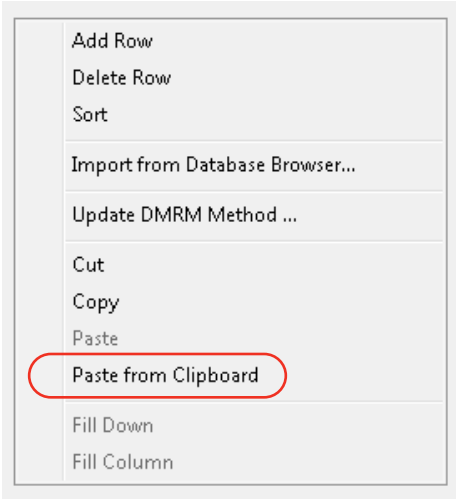
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	MRM												
2	Compound Group	Compoun	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Dwell	Fragment	Collision E	Cell Accel	Polarity	
3	SubMix 1	Spirodiclo	FALSE	411.1	Unit	71.2	Unit	10	110	15	3	Positive	
4	SubMix 1	Diflufenic	FALSE	395	Unit	266	Unit	10	150	25	5	Positive	
5	SubMix 1	Diflufenic	FALSE	395	Unit	246	Unit	10	150	40	5	Positive	
6	SubMix 1	Fluopicoli	FALSE	382.9	Unit	172.9	Unit	10	110	25	3	Positive	
7	SubMix 1	Fluopicoli	FALSE	382.9	Unit	144.9	Unit	10	110	45	3	Positive	
8	SubMix 1	Prochlora:	FALSE	376	Unit	308	Unit	10	70	5	3	Positive	
9	SubMix 1	Prochlora:	FALSE	376	Unit	266	Unit	10	70	10	3	Positive	
10	SubMix 1	Proquinaz	FALSE	372.9	Unit	331	Unit	10	120	5	7	Positive	
11	SubMix 1	Proquinaz	FALSE	372.9	Unit	289	Unit	10	120	20	7	Positive	
12	SubMix 1	Flufenace	FALSE	364	Unit	194.2	Unit	10	90	5	3	Positive	
13	SubMix 1	Flufenace	FALSE	364	Unit	152.1	Unit	10	90	15	3	Positive	
14	SubMix 1	Azinphos-	FALSE	346.05	Unit	132	Unit	10	70	8	3	Positive	
15	SubMix 1	Azinphos-	FALSE	346.05	Unit	97	Unit	10	70	32	3	Positive	
16	SubMix 1	Isofenphc	FALSE	332	Unit	231	Unit	10	145	10	5	Positive	
17	SubMix 1	Isofenphc	FALSE	332	Unit	121	Unit	10	145	40	5	Positive	
18	SubMix 1	Dimoxystr	FALSE	327.1	Unit	205.1	Unit	10	115	5	3	Positive	
19	SubMix 1	Dimoxystr	FALSE	327.1	Unit	116	Unit	10	115	20	3	Positive	
20	SubMix 1	Azinphos-	FALSE	318.02	Unit	261	Unit	10	60	0	3	Positive	
21	SubMix 1	Azinphos-	FALSE	318.02	Unit	132.1	Unit	10	60	8	3	Positive	
22	SubMix 1	Buprofezi	FALSE	306.1	Unit	201.2	Unit	10	105	5	3	Positive	
23	SubMix 1	Buprofezi	FALSE	306.1	Unit	116.1	Unit	10	105	10	3	Positive	
24	SubMix 1	Fenamiph	FALSE	304.1	Unit	217.1	Unit	10	120	20	3	Positive	
25	SubMix 1	Fenamiph	FALSE	304.1	Unit	202	Unit	10	120	35	3	Positive	
26	SubMix 1	Azaconazc	FALSE	300	Unit	230.8	Unit	10	130	16	4	Positive	
27	SubMix 1	Azaconazc	FALSE	300	Unit	158.9	Unit	10	130	32	4	Positive	
28	SubMix 1	Spiroxami	FALSE	298.2	Unit	144.2	Unit	10	125	15	3	Positive	
29	SubMix 1	Spiroxami	FALSE	298.2	Unit	100.2	Unit	10	125	35	3	Positive	
30	SubMix 1	Cyprocon:	FALSE	292.1	Unit	125.1	Unit	10	100	35	3	Positive	
31	SubMix 1	Cyprocon:	FALSE	292.1	Unit	70.1	Unit	10	100	15	7	Positive	
32	SubMix 1	Isoprothic	FALSE	291.1	Unit	231	Unit	10	80	8	4	Positive	
33	SubMix 1	Isoprothic	FALSE	291.1	Unit	188.8	Unit	10	80	20	4	Positive	
34	SubMix 1	Myclobut:	FALSE	289.1	Unit	125	Unit	10	110	35	3	Positive	
35	SubMix 1	Myclobut:	FALSE	289.1	Unit	70.1	Unit	10	110	15	7	Positive	
36	SubMix 1	Fosthiazat	FALSE	284	Unit	228.1	Unit	10	90	5	3	Positive	
37	SubMix 1	Fosthiazat	FALSE	284	Unit	104.1	Unit	10	90	20	3	Positive	
38	SubMix 1	Disulfotor	FALSE	275	Unit	89	Unit	10	130	5	5	Positive	

5 Copy the selected cells. (Press **Ctrl+C** or use the Copy command).

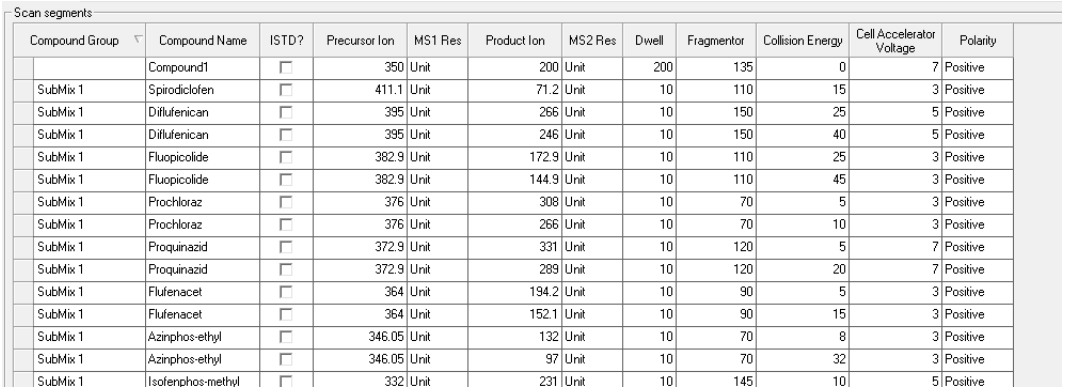
6 In the MassHunter Data Acquisition program, in the first line of the **Scan segments** table, click the leftmost column to select the first line.



7 Right-click and click **Paste from Clipboard**.



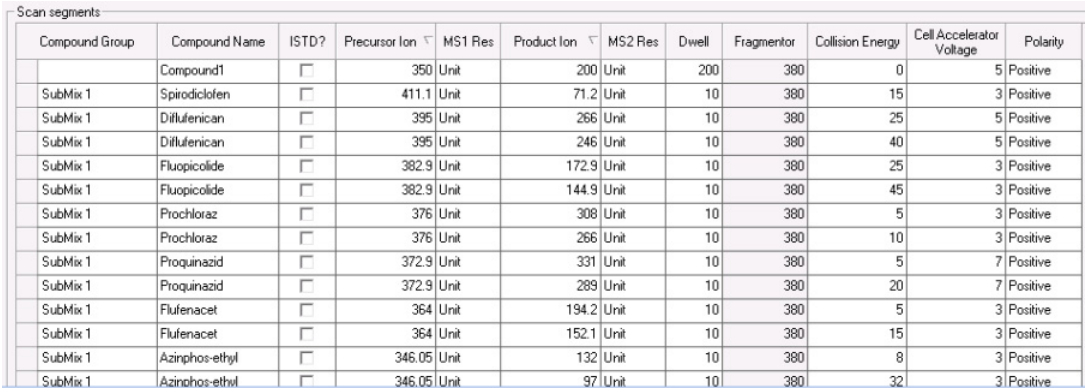
The Scan segments table for instruments that are not equipped with iFunnel technology looks similar to the next figure.



Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Compound1	☐	350	Unit	200	Unit	200	135	0	7	Positive
SubMix 1	Spirodiclofen	☐	411.1	Unit	71.2	Unit	10	110	15	3	Positive
SubMix 1	Diflufenican	☐	395	Unit	266	Unit	10	150	25	5	Positive
SubMix 1	Diflufenican	☐	395	Unit	246	Unit	10	150	40	5	Positive
SubMix 1	Fluopicolide	☐	382.9	Unit	172.9	Unit	10	110	25	3	Positive
SubMix 1	Fluopicolide	☐	382.9	Unit	144.9	Unit	10	110	45	3	Positive
SubMix 1	Prochloraz	☐	376	Unit	308	Unit	10	70	5	3	Positive
SubMix 1	Prochloraz	☐	376	Unit	266	Unit	10	70	10	3	Positive
SubMix 1	Proquinazid	☐	372.9	Unit	331	Unit	10	120	5	7	Positive
SubMix 1	Proquinazid	☐	372.9	Unit	289	Unit	10	120	20	7	Positive
SubMix 1	Flufenacet	☐	364	Unit	194.2	Unit	10	90	5	3	Positive
SubMix 1	Flufenacet	☐	364	Unit	152.1	Unit	10	90	15	3	Positive
SubMix 1	Azinphos-ethyl	☐	346.05	Unit	132	Unit	10	70	8	3	Positive
SubMix 1	Azinphos-ethyl	☐	346.05	Unit	97	Unit	10	70	32	3	Positive
SubMix 1	Isofenphos-methyl	☐	332	Unit	231	Unit	10	145	10	5	Positive

The Scan segments table for instruments that are equipped with iFunnel technology, such as the 6490, looks similar to the next figure.



Scan segments

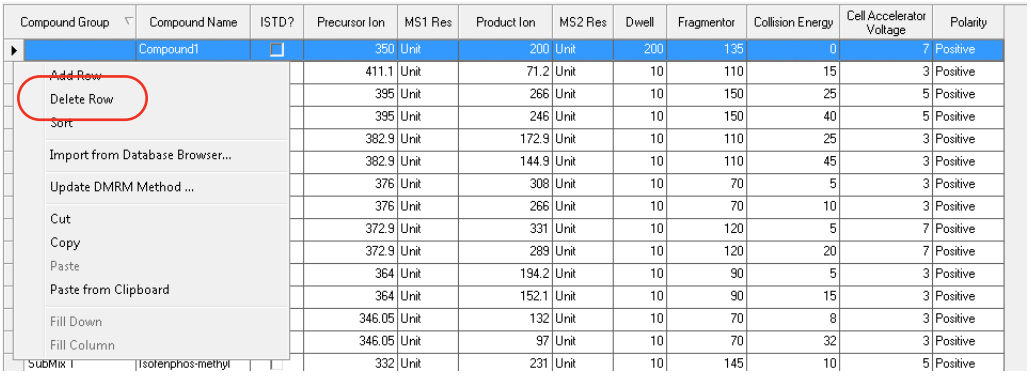
Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Compound1	☐	350	Unit	200	Unit	200	380	0	5	Positive
SubMix 1	Spirodiclofen	☐	411.1	Unit	71.2	Unit	10	380	15	3	Positive
SubMix 1	Diflufenican	☐	395	Unit	266	Unit	10	380	25	5	Positive
SubMix 1	Diflufenican	☐	395	Unit	246	Unit	10	380	40	5	Positive
SubMix 1	Fluopicolide	☐	382.9	Unit	172.9	Unit	10	380	25	3	Positive
SubMix 1	Fluopicolide	☐	382.9	Unit	144.9	Unit	10	380	45	3	Positive
SubMix 1	Prochloraz	☐	376	Unit	308	Unit	10	380	5	3	Positive
SubMix 1	Prochloraz	☐	376	Unit	266	Unit	10	380	10	3	Positive
SubMix 1	Proquinazid	☐	372.9	Unit	331	Unit	10	380	5	7	Positive
SubMix 1	Proquinazid	☐	372.9	Unit	289	Unit	10	380	20	7	Positive
SubMix 1	Flufenacet	☐	364	Unit	194.2	Unit	10	380	5	3	Positive
SubMix 1	Flufenacet	☐	364	Unit	152.1	Unit	10	380	15	3	Positive
SubMix 1	Azinphos-ethyl	☐	346.05	Unit	132	Unit	10	380	8	3	Positive
SubMix 1	Azinphos-ethyl	☐	346.05	Unit	97	Unit	10	380	32	3	Positive

Note that polarity switching is supported for MRM, but the transitions within each compound need to have the same polarity. Polarity switching (positive and negative transitions within a compound) is not supported.

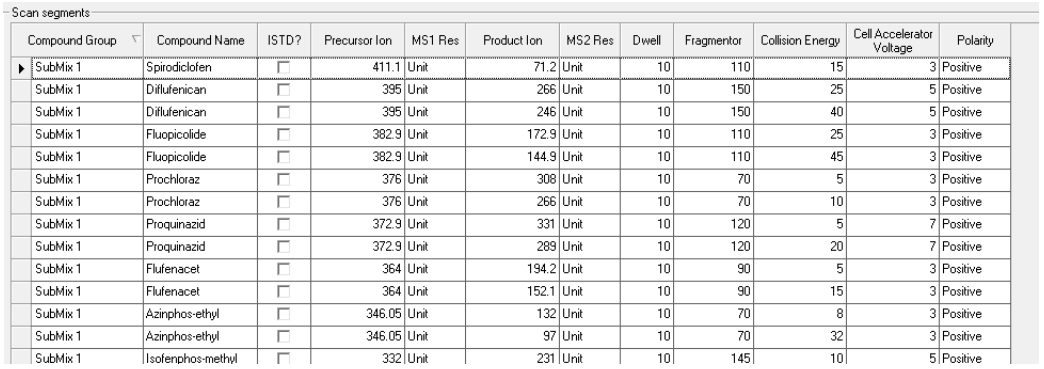
8 Remove the first compound from the Scan segments table:

a Select the first line. For the first method that you create, the line contains the compound **Compound1**. For the other methods, the line contains a compound from the previous submix.

b Right-click and click **Delete Row**. See the next figure.



The final method now looks like the next figure.



Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
SubMix 1	Spirodiclofen		411.1	Unit	71.2	Unit	10	110	15	3	Positive
SubMix 1	Diflufenican		395	Unit	266	Unit	10	150	25	5	Positive
SubMix 1	Diflufenican		395	Unit	246	Unit	10	150	40	5	Positive
SubMix 1	Fluopicolide		382.9	Unit	172.9	Unit	10	110	25	3	Positive
SubMix 1	Fluopicolide		382.9	Unit	144.9	Unit	10	110	45	3	Positive
SubMix 1	Prochloraz		376	Unit	308	Unit	10	70	5	3	Positive
SubMix 1	Prochloraz		376	Unit	266	Unit	10	70	10	3	Positive
SubMix 1	Proquinazid		372.9	Unit	331	Unit	10	120	5	7	Positive
SubMix 1	Proquinazid		372.9	Unit	289	Unit	10	120	20	7	Positive
SubMix 1	Flufenacet		364	Unit	194.2	Unit	10	90	5	3	Positive
SubMix 1	Flufenacet		364	Unit	152.1	Unit	10	90	15	3	Positive
SubMix 1	Azinphos-ethyl		346.05	Unit	132	Unit	10	70	8	3	Positive
SubMix 1	Azinphos-ethyl		346.05	Unit	97	Unit	10	70	32	3	Positive
SubMix 1	Isofenphos-methyl		332	Unit	231	Unit	10	145	10	5	Positive

9 Click **Method > Save As** (or click  in the Method Editor toolbar) and save the method as **Pesticide_MRM_Mix1.m**.

10 Delete all but one compound from the Scan segments table.

The Scan segments table cannot be empty. You need to leave one compound in the table.

11 Repeat step 4 through step 10 for each of the submixes.

When you save each method, use a name that reflects the submix name, such as **Pesticide_MRM_Mix2.m** for the values in the **SubMix 2** tab.

Step 4. Set up a worklist to run the submixes

- Set up the worklist as shown in the next figure. Inject the first standard twice to allow the system to come to equilibrium.

		Sample Name	Sample Position	Method	Data File	Sample Type	Level Name
1	✓	SubMix_01	P1-A1	Pesticide_MRM_Mix1.m	todelete.d	Sample	
2	✓	SubMix_01	P1-A1	Pesticide_MRM_Mix1.m	Submix_1.d	Calibration	1
3	✓	SubMix_02	P1-A2	Pesticide_MRM_Mix2.m	Submix_2.d	Calibration	1
4	✓	SubMix_03	P1-A3	Pesticide_MRM_Mix3.m	Submix_3.d	Calibration	1
5	✓	SubMix_04	P1-A4	Pesticide_MRM_Mix4.m	Submix_4.d	Calibration	1
6	✓	SubMix_05	P1-A5	Pesticide_MRM_Mix5.m	Submix_5.d	Calibration	1
7	✓	SubMix_06	P1-A6	Pesticide_MRM_Mix6.m	Submix_6.d	Calibration	1
8	✓	SubMix_07	P1-A7	Pesticide_MRM_Mix7.m	Submix_7.d	Calibration	1
9	✓	SubMix_08	P1-A8	Pesticide_MRM_Mix8.m	Submix_8.d	Calibration	1

To create the dMRM and tMRM methods from the MRM methods that you just created, refer to the *Quick Start Guide* for this database, or the MassHunter Data Acquisition for 6400 Series Triple Quadrupole LC/MS *Familiarization Guide* or *online Help*.

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In this Book

The *Method Setup Guide* describes how to create MRM methods for your specific LC/MS set up. The MRM methods are used to create Dynamic MRM (dMRM) and Triggered MRM (tMRM) methods for the Comprehensive Test Mix.

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