Comprehensive Test Mix for MassHunter Forensics and Toxicology Triggered MRM Database and Library

Method Setup Guide

NOTE The Comprehensive Forensics and Toxicology Test Mix is included with the G1734BA Forensics and Toxicology 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_ForTox.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 Forensics and Toxicology 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 0.01% formic acid in water
- Solvent B: 0.01% 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 × 100 mm, 1.8 µm ZORBAX LC column (p/n 959758-902), included in the G1734BA Forensics and Toxicology 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	---	---
1.50	70.00	30.00	---	---
6.50	40.00	60.00	---	---
9.00	5.00	95.00	---	---

Stop time is 9 minutes with a post time of 3.5 minutes.

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
1.50	95.00	5.00	---	---
2.00	70.00	30.00	---	---
8.50	40.00	60.00	---	---
11.00	5.00	95.00	---	---

Stop time is 11 minutes with a post time of 3.5 minutes.

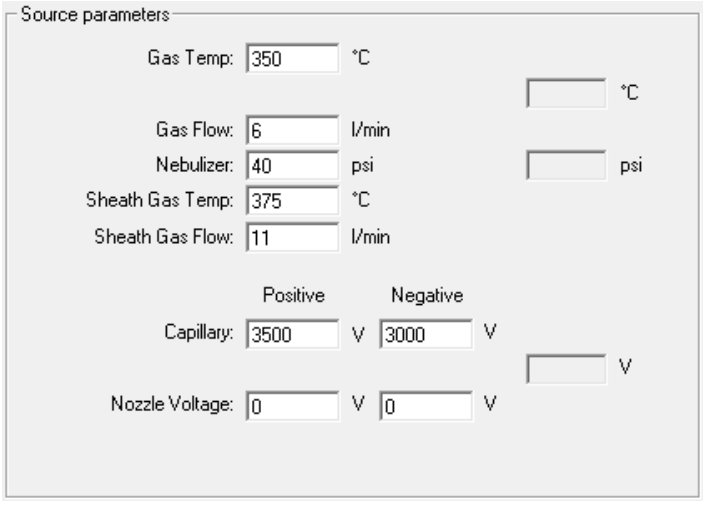
These settings are optimized over the whole Comprehensive Test Mix. For best sensitivity of SubMix 5, use pure water and methanol in negative mode.

Step 2. Set up LC/MS ion source parameters

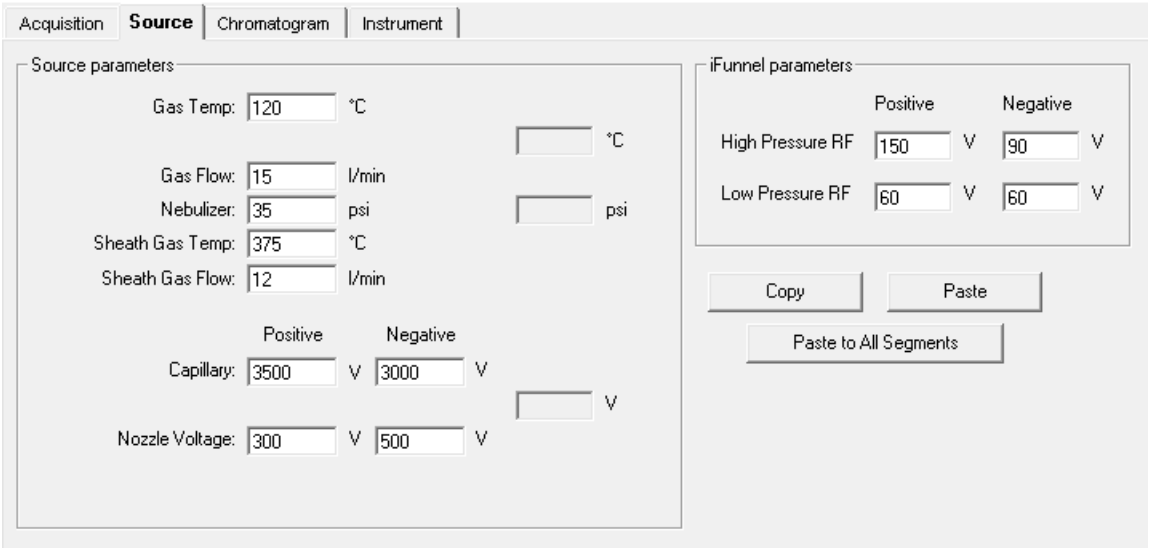
- Set up the ion source parameters in the MS part of the method.

  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:

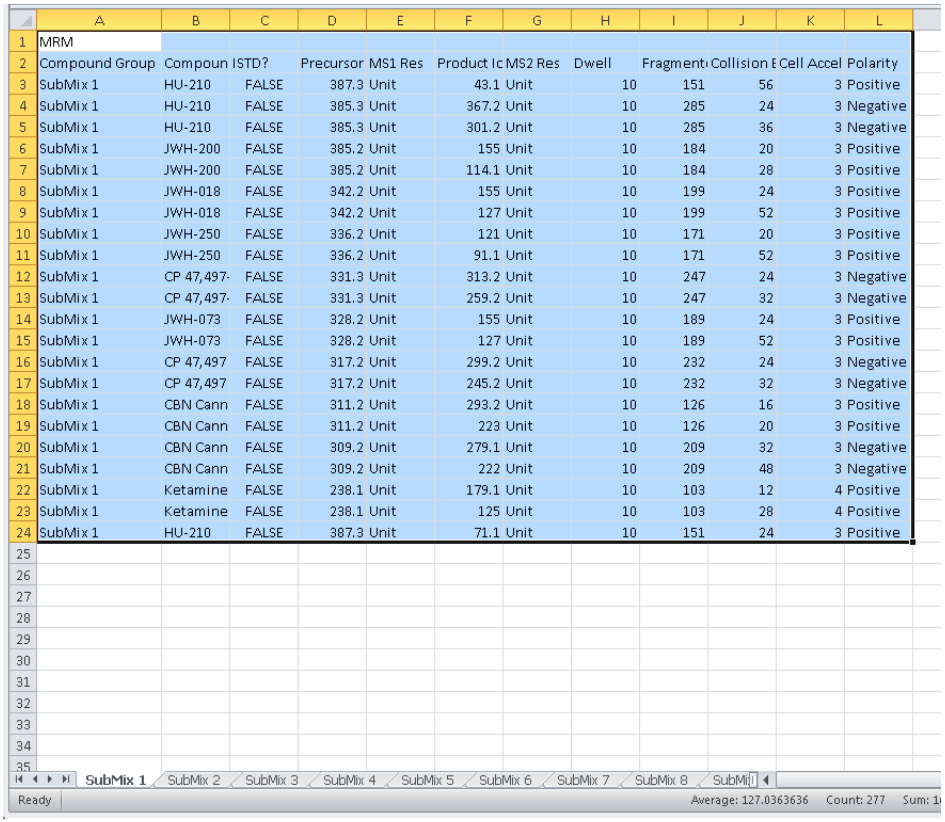


The ion source parameters shown for the 6490 LC/MS also include the iFunnel parameters. These iFunnel parameters ensure the best overall sensitivity for all of the compounds in the Comprehensive Test Mix. You can use the Source and iFunnel Optimizer program to optimize for individual compounds or submixes. 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_ForTox.xlsx**.

This spreadsheet file contains ten tabs, **SubMix 1** through **SubMix 10**, one for each of the ten standard mixes in the Comprehensive Test Mix.



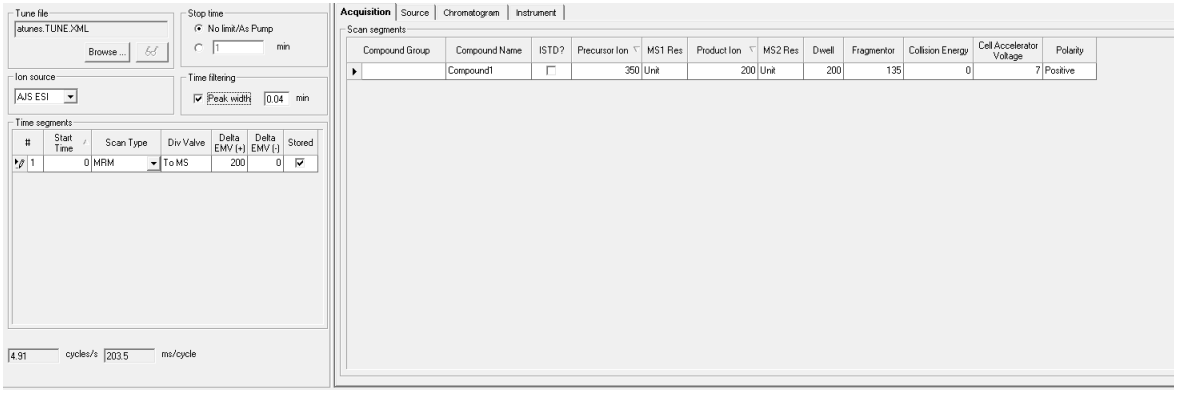
	A	B	C	D	E	F	G	H	I	J	K	L
1	MRM											
2	Compound Group	Compoun	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Dwell	Fragment	Collision E	Cell Accel	Polarity
3	SubMix 1	HU-210	FALSE	387.3	Unit	43.1	Unit	10	151	56	3	Positive
4	SubMix 1	HU-210	FALSE	385.3	Unit	367.2	Unit	10	285	24	3	Negative
5	SubMix 1	HU-210	FALSE	385.3	Unit	301.2	Unit	10	285	36	3	Negative
6	SubMix 1	JWH-200	FALSE	385.2	Unit	155	Unit	10	184	20	3	Positive
7	SubMix 1	JWH-200	FALSE	385.2	Unit	114.1	Unit	10	184	28	3	Positive
8	SubMix 1	JWH-018	FALSE	342.2	Unit	155	Unit	10	199	24	3	Positive
9	SubMix 1	JWH-018	FALSE	342.2	Unit	127	Unit	10	199	52	3	Positive
10	SubMix 1	JWH-250	FALSE	336.2	Unit	121	Unit	10	171	20	3	Positive
11	SubMix 1	JWH-250	FALSE	336.2	Unit	91.1	Unit	10	171	52	3	Positive
12	SubMix 1	CP 47,497-	FALSE	331.3	Unit	313.2	Unit	10	247	24	3	Negative
13	SubMix 1	CP 47,497-	FALSE	331.3	Unit	259.2	Unit	10	247	32	3	Negative
14	SubMix 1	JWH-073	FALSE	328.2	Unit	155	Unit	10	189	24	3	Positive
15	SubMix 1	JWH-073	FALSE	328.2	Unit	127	Unit	10	189	52	3	Positive
16	SubMix 1	CP 47,497	FALSE	317.2	Unit	299.2	Unit	10	232	24	3	Negative
17	SubMix 1	CP 47,497	FALSE	317.2	Unit	245.2	Unit	10	232	32	3	Negative
18	SubMix 1	CBN Cann	FALSE	311.2	Unit	293.2	Unit	10	126	16	3	Positive
19	SubMix 1	CBN Cann	FALSE	311.2	Unit	223	Unit	10	126	20	3	Positive
20	SubMix 1	CBN Cann	FALSE	309.2	Unit	279.1	Unit	10	209	32	3	Negative
21	SubMix 1	CBN Cann	FALSE	309.2	Unit	222	Unit	10	209	48	3	Negative
22	SubMix 1	Ketamine	FALSE	238.1	Unit	179.1	Unit	10	103	12	4	Positive
23	SubMix 1	Ketamine	FALSE	238.1	Unit	125	Unit	10	103	28	4	Positive
24	SubMix 1	HU-210	FALSE	387.3	Unit	71.1	Unit	10	151	24	3	Positive
25												
26												
27												
28												
29												
30												
31												
32												
33												
34												
35												

SubMix 1 | SubMix 2 | SubMix 3 | SubMix 4 | SubMix 5 | SubMix 6 | SubMix 7 | SubMix 8 | SubMi

Ready | Average: 127.0363636 | Count: 277 | Sum:

2 Open the MassHunter Data Acquisition program.

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

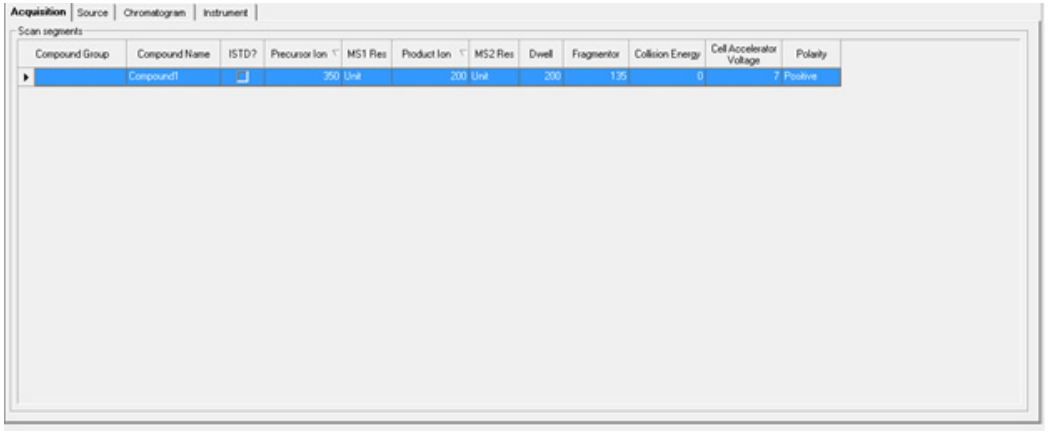


4 In the spreadsheet file, in the **SubMix 1** tab, select all of the cells that contain MRM information. Make sure that you select the two header rows. *Do not select the entire table!*

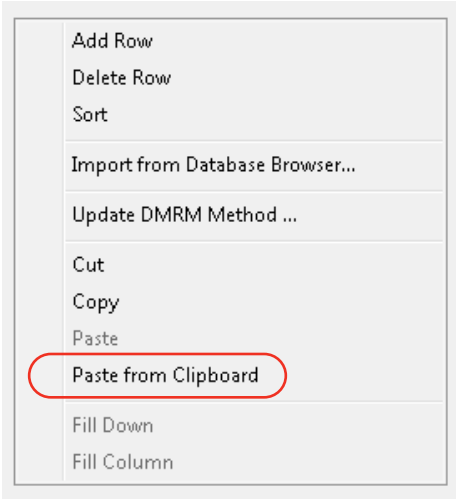
	A	B	C	D	E	F	G	H	I	J	K	L
1	MRM											
2	Compound Group	Compoun	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Dwell	Fragment	Collision E	Cell Accel	Polarity
3	SubMix 1	HU-210	FALSE	387.3	Unit	43.1	Unit	10	151	56	3	Positive
4	SubMix 1	HU-210	FALSE	385.3	Unit	367.2	Unit	10	285	24	3	Negative
5	SubMix 1	HU-210	FALSE	385.3	Unit	301.2	Unit	10	285	36	3	Negative
6	SubMix 1	JWH-200	FALSE	385.2	Unit	155	Unit	10	184	20	3	Positive
7	SubMix 1	JWH-200	FALSE	385.2	Unit	114.1	Unit	10	184	28	3	Positive
8	SubMix 1	JWH-018	FALSE	342.2	Unit	155	Unit	10	199	24	3	Positive
9	SubMix 1	JWH-018	FALSE	342.2	Unit	127	Unit	10	199	52	3	Positive
10	SubMix 1	JWH-250	FALSE	336.2	Unit	121	Unit	10	171	20	3	Positive
11	SubMix 1	JWH-250	FALSE	336.2	Unit	91.1	Unit	10	171	52	3	Positive
12	SubMix 1	CP 47,497-	FALSE	331.3	Unit	313.2	Unit	10	247	24	3	Negative
13	SubMix 1	CP 47,497-	FALSE	331.3	Unit	259.2	Unit	10	247	32	3	Negative
14	SubMix 1	JWH-073	FALSE	328.2	Unit	155	Unit	10	189	24	3	Positive
15	SubMix 1	JWH-073	FALSE	328.2	Unit	127	Unit	10	189	52	3	Positive
16	SubMix 1	CP 47,497	FALSE	317.2	Unit	299.2	Unit	10	232	24	3	Negative
17	SubMix 1	CP 47,497	FALSE	317.2	Unit	245.2	Unit	10	232	32	3	Negative
18	SubMix 1	CBN Cann	FALSE	311.2	Unit	293.2	Unit	10	126	16	3	Positive
19	SubMix 1	CBN Cann	FALSE	311.2	Unit	223	Unit	10	126	20	3	Positive
20	SubMix 1	CBN Cann	FALSE	309.2	Unit	279.1	Unit	10	209	32	3	Negative
21	SubMix 1	CBN Cann	FALSE	309.2	Unit	222	Unit	10	209	48	3	Negative
22	SubMix 1	Ketamine	FALSE	238.1	Unit	179.1	Unit	10	103	12	4	Positive
23	SubMix 1	Ketamine	FALSE	238.1	Unit	125	Unit	10	103	28	4	Positive
24	SubMix 1	HU-210	FALSE	387.3	Unit	71.1	Unit	10	151	24	3	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	HU-210	☐	387.3	Unit	43.1	Unit	10	151	56	3	Positive
SubMix 1	HU-210	☐	385.3	Unit	367.2	Unit	10	285	24	3	Negative
SubMix 1	HU-210	☐	385.3	Unit	301.2	Unit	10	285	36	3	Negative
SubMix 1	JWH-200	☐	385.2	Unit	155	Unit	10	184	20	3	Positive
SubMix 1	JWH-200	☐	385.2	Unit	114.1	Unit	10	184	28	3	Positive
SubMix 1	JWH-018	☐	342.2	Unit	155	Unit	10	199	24	3	Positive
SubMix 1	JWH-018	☐	342.2	Unit	127	Unit	10	199	52	3	Positive
SubMix 1	JWH-250	☐	336.2	Unit	121	Unit	10	171	20	3	Positive
SubMix 1	JWH-250	☐	336.2	Unit	91.1	Unit	10	171	52	3	Positive
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	313.2	Unit	10	247	24	3	Negative
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	259.2	Unit	10	247	32	3	Negative
SubMix 1	JWH-073	☐	328.2	Unit	155	Unit	10	189	24	3	Positive
SubMix 1	JWH-073	☐	328.2	Unit	127	Unit	10	189	52	3	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

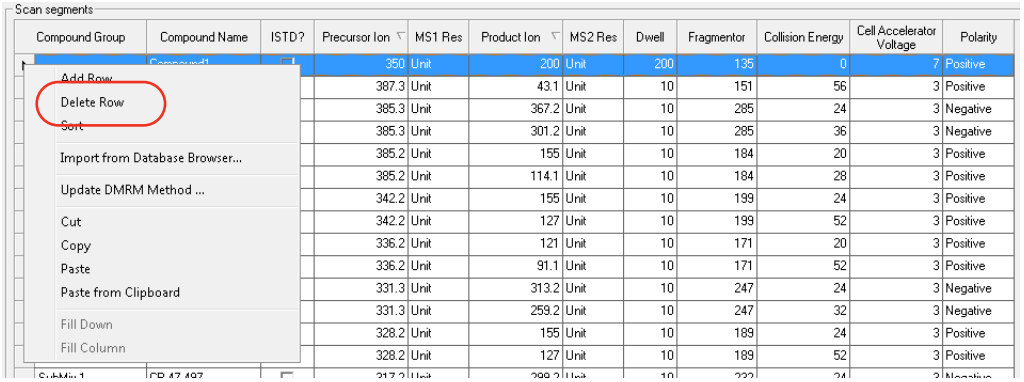
Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
SubMix 1	JWH-200	☐	385.2	Unit	114.1	Unit	10	380	28	3	Positive
SubMix 1	JWH-018	☐	342.2	Unit	155	Unit	10	380	24	3	Positive
SubMix 1	JWH-018	☐	342.2	Unit	127	Unit	10	380	52	3	Positive
SubMix 1	JWH-250	☐	336.2	Unit	121	Unit	10	380	20	3	Positive
SubMix 1	JWH-250	☐	336.2	Unit	91.1	Unit	10	380	52	3	Positive
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	313.2	Unit	10	380	24	3	Negative
SubMix 1	CP 47,497-C8 homolc	☐	331.3	Unit	259.2	Unit	10	380	32	3	Negative
SubMix 1	JWH-073	☐	328.2	Unit	155	Unit	10	380	24	3	Positive
SubMix 1	JWH-073	☐	328.2	Unit	127	Unit	10	380	52	3	Positive
SubMix 1	CP 47,497	☐	317.2	Unit	299.2	Unit	10	380	24	3	Negative
SubMix 1	CP 47,497	☐	317.2	Unit	245.2	Unit	10	380	32	3	Negative
SubMix 1	CBN Cannabinol	☐	311.2	Unit	293.2	Unit	10	380	16	3	Positive
SubMix 1	CBN Cannabinol	☐	311.2	Unit	223	Unit	10	380	20	3	Positive
SubMix 1	CBN Cannabinol	☐	309.2	Unit	279.1	Unit	10	380	32	3	Negative
SubMix 1	CBN Cannabinol	☐	309.2	Unit	222	Unit	10	380	48	3	Negative
SubMix 1	Ketamine	☐	238.1	Unit	179.1	Unit	10	380	12	4	Positive
SubMix 1	Ketamine	☐	238.1	Unit	125	Unit	10	380	28	4	Positive
SubMix 1	HU-210	☐	387.3	Unit	71.1	Unit	10	380	24	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.



Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
			350	Unit	200	Unit	200	135	0	7	Positive
			387.3	Unit	43.1	Unit	10	151	56	3	Positive
			385.3	Unit	367.2	Unit	10	285	24	3	Negative
			385.3	Unit	301.2	Unit	10	285	36	3	Negative
			385.2	Unit	155	Unit	10	184	20	3	Positive
			385.2	Unit	114.1	Unit	10	184	28	3	Positive
			342.2	Unit	155	Unit	10	199	24	3	Positive
			342.2	Unit	127	Unit	10	199	52	3	Positive
			336.2	Unit	121	Unit	10	171	20	3	Positive
			336.2	Unit	91.1	Unit	10	171	52	3	Positive
			331.3	Unit	313.2	Unit	10	247	24	3	Negative
			331.3	Unit	259.2	Unit	10	247	32	3	Negative
			328.2	Unit	155	Unit	10	189	24	3	Positive
			328.2	Unit	127	Unit	10	189	52	3	Positive

The final method now looks like 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
SubMix 1	HU-210		387.3	Unit	43.1	Unit	10	151	56	3	Positive
SubMix 1	HU-210		385.3	Unit	367.2	Unit	10	285	24	3	Negative
SubMix 1	HU-210		385.3	Unit	301.2	Unit	10	285	36	3	Negative
SubMix 1	JWH-200		385.2	Unit	155	Unit	10	184	20	3	Positive
SubMix 1	JWH-200		385.2	Unit	114.1	Unit	10	184	28	3	Positive
SubMix 1	JWH-018		342.2	Unit	155	Unit	10	199	24	3	Positive
SubMix 1	JWH-018		342.2	Unit	127	Unit	10	199	52	3	Positive
SubMix 1	JWH-250		336.2	Unit	121	Unit	10	171	20	3	Positive
SubMix 1	JWH-250		336.2	Unit	91.1	Unit	10	171	52	3	Positive
SubMix 1	CP 47,497-C8 homolc		331.3	Unit	313.2	Unit	10	247	24	3	Negative
SubMix 1	CP 47,497-C8 homolc		331.3	Unit	259.2	Unit	10	247	32	3	Negative
SubMix 1	JWH-073		328.2	Unit	155	Unit	10	189	24	3	Positive
SubMix 1	JWH-073		328.2	Unit	127	Unit	10	189	52	3	Positive

9 Click **Method > Save As** (or click  in the Method Editor toolbar) and save the method as **ForTox_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 **ForTox_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. Include all submixes. 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	ForTox_MRM_Mix_1.m	to_delete.d	Calibration	1
2	✓	SubMix_01	P1-A1	ForTox_MRM_Mix_1.m	SubMix_01.d	Calibration	1
3	✓	SubMix_02	P1-A2	ForTox_MRM_Mix_2.m	SubMix_02.d	Calibration	1
4	✓	SubMix_03	P1-A3	ForTox_MRM_Mix_3.m	SubMix_03.d	Calibration	1
5	✓	SubMix_04	P1-A4	ForTox_MRM_Mix_4.m	SubMix_04.d	Calibration	1
6	✓	SubMix_05	P1-A5	ForTox_MRM_Mix_5.m	SubMix_05.d	Calibration	1
7	✓	SubMix_06	P1-A6	ForTox_MRM_Mix_6.m	SubMix_06.d	Calibration	1
8	✓	SubMix_07	P1-A7	ForTox_MRM_Mix_7.m	SubMix_07.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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