Comprehensive Test Mix for MassHunter Veterinary Drug Triggered MRM Database and Library

Method Setup Guide

NOTE The Comprehensive Veterinary Drug Test Mix is included with the G1735AA Veterinary Drug 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_VetDrugs.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 Veterinary Drug 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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The Comprehensive Veterinary Drug Test Mix is composed of 146 compounds, provided in 12 submixes. The methods described in this guide are suitable for the analysis of 134 of these compounds. The remaining 12 compounds require dedicated methods and so are not covered in the general screening method included in this kit. These 12 compounds are:

- Cefalonium
- Cefquinome
- Cefalexin
- Cefoperazone
- Flavophospholipol
- Clorsulon
- Ibuprofen
- Vedaprofen
- Dipyrone (Metamizolum)
- Oxytetracycline
- Chlortetracycline
- Doxycycline

Step 1. Set up the LC part of the method

1 Set up the solvent.

This step is identical for all LC configurations.

You can use one LC method to detect most of the compounds in the Comprehensive Test Submixes, but sensitivity would not be optimized equally. To optimize sensitivity, create separate methods with different solvent sets, depending on the Submix to run.

Method 1 (for **Submixes 2 through 4** and **Submixes 6 through 11**):

- Solvent A: 0.1% formic acid in water
- Solvent B: 0.1% formic acid in acetonitrile

Method 2 (for **Submix 1** and **Submix 5**):

- Solvent A: 5 mM ammonium formate/0.1% formic acid in water
- Solvent B: 5 mM ammonium formate/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 Poroshell 120 EC-C18, 2.1 mm × 150 mm, 2.7 µm column (p/n 693775-902), included in the G1735AA Veterinary Drug 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
2.00	95.00	5.00	---	---
5.00	60.00	40.00	---	---
13.00	5.00	95.00	---	---
14.00	0.00	100.00	---	---
16.00	0.00	100.00	---	---
16.10	95.00	5.00	---	---

Stop time is 16:10 minutes with a post time of 3 minutes.

Make sure that the **Column** temperature is set to 40°C.

1260 Infinity LC system

The 1260 Infinity LC system can have a lower backpressure limit (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
2.00	95.00	5.00	---	---
5.00	60.00	40.00	---	---
13.00	5.00	95.00	---	---
14.00	0.00	100.00	---	---
16.00	0.00	100.00	---	---
16.10	95.00	5.00	---	---

Stop time is 16:10 minutes with a post time of 3 minutes.

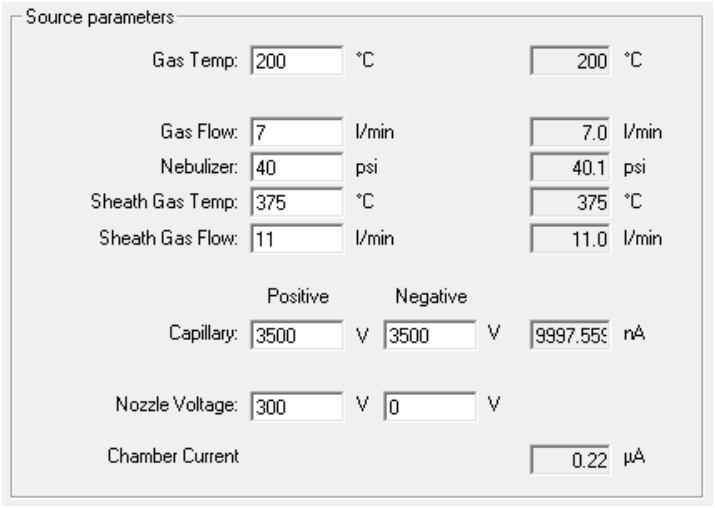
Make sure that the **Column** temperature is set to 40°C.

Step 2. Set up LC/MS ion source parameters

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

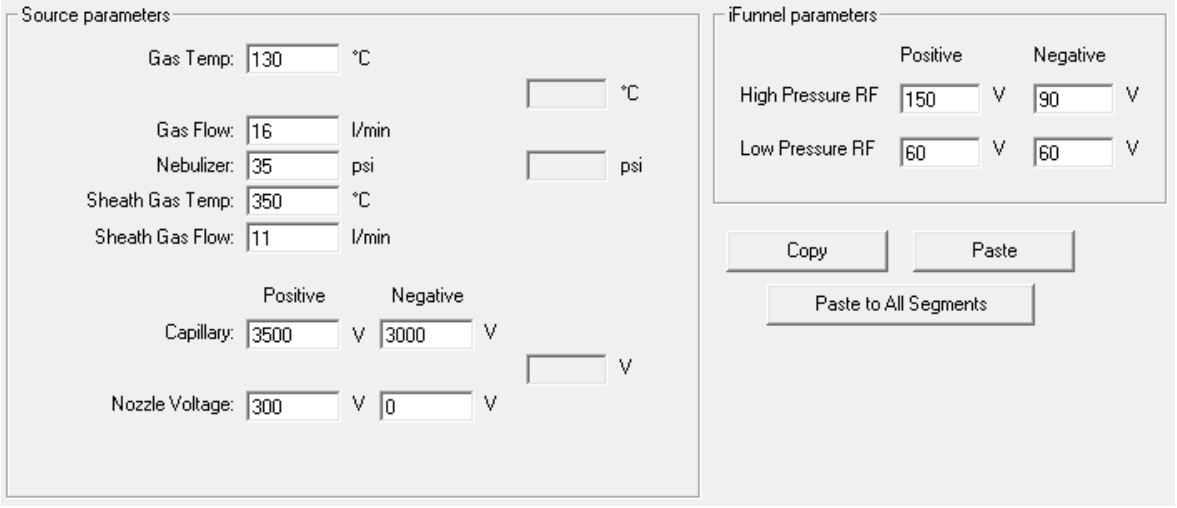
  For a multi-component method, the ion source parameters shown in the next figure are used to achieve the best overall sensitivity for most of the compounds in the Comprehensive Test Mix. You can make adjustments to optimize for individual compounds or submixes.

6460 LC/MS Ion source parameters for the 6460 LC/MS instruments.



Under **Time segments**, make sure that **Delta EMV (+)** and **Delta EMV (-)** are both set to **200**.

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



Under **Time segments**, make sure that **Delta EMV (+)** and **Delta EMV (-)** are both set to **200**.

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 online Help.

Step 3. Set up the MRM method

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

This spreadsheet file contains several tabs, one for each of the standard mixes in the Comprehensive Test Mix.

The Veterinary Drug Triggered MRM database contains both positive and negative polarity transitions for some compounds. The better polarity to use for a particular compound is often the one that gives the most selective transitions, not the most sensitive or abundant transitions.

Selectivity depends on the sample matrix and to a lesser degree the mobile phase composition. The **MRM_Methods_VetDrugs.xlsx** spreadsheet contains the most generally selective polarity and transitions to use in your dMRM and tMRM methods.

NOTE

During method development, the inclusion of both polarities for one compound is often desirable. Qualifier and quantifier ions must have the same precursor, so one compound cannot contain both negative and positive polarity transitions. The compounds that contain both polarities in the Triggered MRM database must be renamed in the method to "*compoundname*_pos" and "*compoundname*_neg"

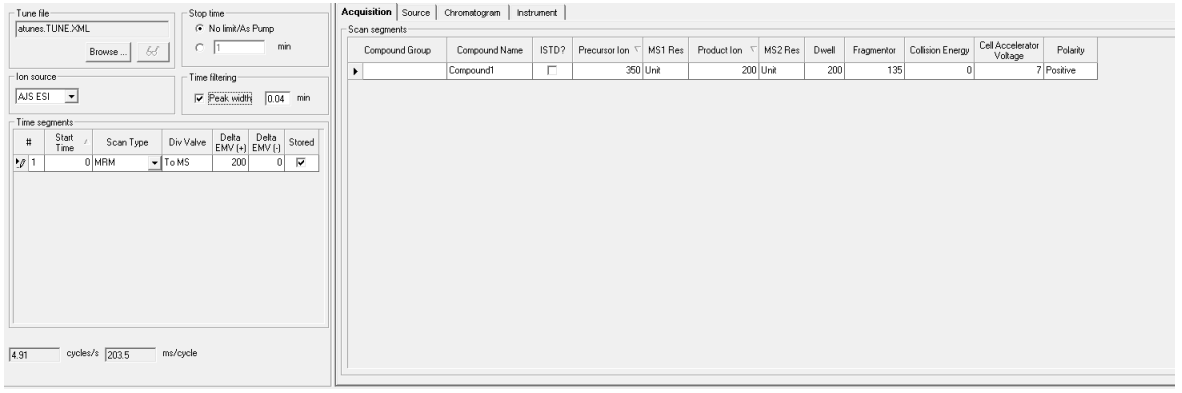
When the best polarity and transitions are found for a compound, remove from the method all other transitions for the compound. Then remove "_pos" or "_neg" from the remaining compound name.

	A	B	C	D	E	F	G	H	I	J	K	L
1	MRM											
2	Compoun	Compound Name	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Dwell	Fragmenti	Collision E	Cell Accel	Polarity
3		Amino-Mebendazole	FALSE	238.1	Unit	77.1	Unit	10	176	40	3	Positive
4		Amino-Mebendazole	FALSE	238.1	Unit	105.1	Unit	10	176	28	3	Positive
5		Avermectin B1a (Abamectin B1a)	FALSE	890.5	Unit	305.1	Unit	10	155	8	2	Positive
6		Avermectin B1a (Abamectin B1a)	FALSE	890.5	Unit	567.4	Unit	10	155	0	2	Positive
7		Doramectin	FALSE	921.5	Unit	183.1	Unit	10	320	60	3	Positive
8		Doramectin	FALSE	921.5	Unit	777.4	Unit	10	320	48	3	Positive
9		Emamectin B1a	FALSE	886.5	Unit	82.1	Unit	10	190	60	3	Positive
10		Emamectin B1a	FALSE	886.5	Unit	158	Unit	10	190	40	3	Positive
11		Eprinomectin B1a	FALSE	936.5	Unit	352.1	Unit	10	305	60	3	Positive
12		Eprinomectin B1a	FALSE	936.5	Unit	490.2	Unit	10	305	56	3	Positive
13		Febantel	FALSE	447.1	Unit	383.1	Unit	10	128	16	3	Positive
14		Febantel	FALSE	447.1	Unit	415.1	Unit	10	128	10	3	Positive
15		Fenbendazole	FALSE	300.1	Unit	159	Unit	10	156	36	3	Positive
16		Fenbendazole	FALSE	300.1	Unit	268.1	Unit	10	156	20	3	Positive
17		Flubendazole	FALSE	314.1	Unit	123	Unit	10	141	0	4	Positive
18		Flubendazole	FALSE	314.1	Unit	282.1	Unit	10	141	0	4	Positive
19		Ivermectin B1a	FALSE	892.5	Unit	307.3	Unit	10	80	24	2	Positive
20		Ivermectin B1a	FALSE	892.5	Unit	551.4	Unit	10	80	16	2	Positive
21		Levamisole	FALSE	205.1	Unit	91.1	Unit	10	141	44	3	Positive
22		Levamisole	FALSE	205.1	Unit	178.1	Unit	10	141	20	3	Positive
23		Mebendazole	FALSE	296.1	Unit	77	Unit	10	151	48	3	Positive
24		Mebendazole	FALSE	296.1	Unit	264.1	Unit	10	151	20	3	Positive
25		Mebendazole-hydroxy	FALSE	298.1	Unit	79.1	Unit	10	156	44	3	Positive
26		Mebendazole-hydroxy	FALSE	298.1	Unit	266.1	Unit	10	156	20	3	Positive
27		Moxidectin	FALSE	662.4	Unit	369.1	Unit	10	320	32	3	Positive
28		Moxidectin	FALSE	662.4	Unit	383.1	Unit	10	320	40	3	Positive
29		Oxfendazole	FALSE	316.1	Unit	159	Unit	10	166	32	3	Positive
30		Oxfendazole	FALSE	316.1	Unit	191.1	Unit	10	166	16	3	Positive

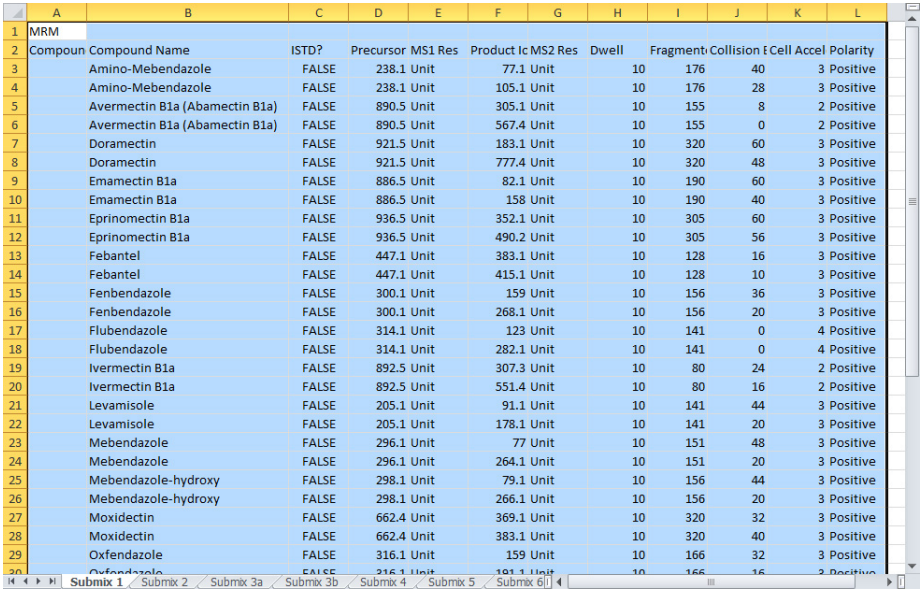
Submix 1 | Submix 2 | Submix 3a | Submix 3b | Submix 4 | Submix 5 | Submix 6

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!*

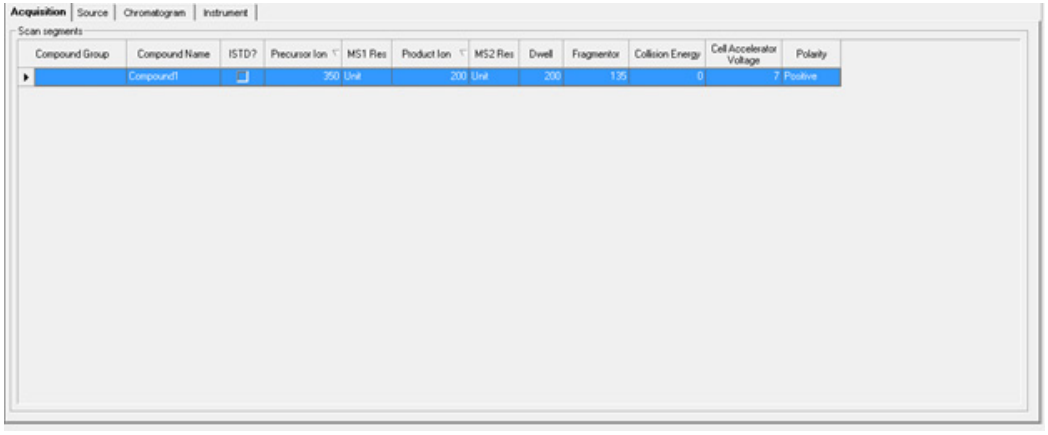


	A	B	C	D	E	F	G	H	I	J	K	L
1	MRM											
2	Compoun	Compound Name	ISTD?	Precursor	MS1 Res	Product Ic	MS2 Res	Dwell	Fragment	Collision E	Cell Accel	Polarity
3		Amino-Mebendazole	FALSE	238.1	Unit	77.1	Unit	10	176	40	3	Positive
4		Amino-Mebendazole	FALSE	238.1	Unit	105.1	Unit	10	176	28	3	Positive
5		Avermectin B1a (Abamectin B1a)	FALSE	890.5	Unit	305.1	Unit	10	155	8	2	Positive
6		Avermectin B1a (Abamectin B1a)	FALSE	890.5	Unit	567.4	Unit	10	155	0	2	Positive
7		Doramectin	FALSE	921.5	Unit	183.1	Unit	10	320	60	3	Positive
8		Doramectin	FALSE	921.5	Unit	777.4	Unit	10	320	48	3	Positive
9		Emamectin B1a	FALSE	886.5	Unit	82.1	Unit	10	190	60	3	Positive
10		Emamectin B1a	FALSE	886.5	Unit	158	Unit	10	190	40	3	Positive
11		Eprinomectin B1a	FALSE	936.5	Unit	352.1	Unit	10	305	60	3	Positive
12		Eprinomectin B1a	FALSE	936.5	Unit	490.2	Unit	10	305	56	3	Positive
13		Febantel	FALSE	447.1	Unit	383.1	Unit	10	128	16	3	Positive
14		Febantel	FALSE	447.1	Unit	415.1	Unit	10	128	10	3	Positive
15		Fenbendazole	FALSE	300.1	Unit	159	Unit	10	156	36	3	Positive
16		Fenbendazole	FALSE	300.1	Unit	268.1	Unit	10	156	20	3	Positive
17		Flubendazole	FALSE	314.1	Unit	123	Unit	10	141	0	4	Positive
18		Flubendazole	FALSE	314.1	Unit	282.1	Unit	10	141	0	4	Positive
19		Ivermectin B1a	FALSE	892.5	Unit	307.3	Unit	10	80	24	2	Positive
20		Ivermectin B1a	FALSE	892.5	Unit	551.4	Unit	10	80	16	2	Positive
21		Levamisole	FALSE	205.1	Unit	91.1	Unit	10	141	44	3	Positive
22		Levamisole	FALSE	205.1	Unit	178.1	Unit	10	141	20	3	Positive
23		Mebendazole	FALSE	296.1	Unit	77	Unit	10	151	48	3	Positive
24		Mebendazole	FALSE	296.1	Unit	264.1	Unit	10	151	20	3	Positive
25		Mebendazole-hydroxy	FALSE	298.1	Unit	79.1	Unit	10	156	44	3	Positive
26		Mebendazole-hydroxy	FALSE	298.1	Unit	266.1	Unit	10	156	20	3	Positive
27		Moxidectin	FALSE	662.4	Unit	369.1	Unit	10	320	32	3	Positive
28		Moxidectin	FALSE	662.4	Unit	383.1	Unit	10	320	40	3	Positive
29		Oxfendazole	FALSE	316.1	Unit	159	Unit	10	166	32	3	Positive

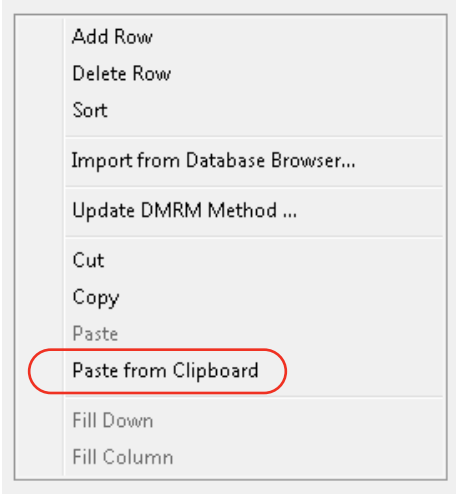
Submix 1 | Submix 2 | Submix 3a | Submix 3b | Submix 4 | Submix 5 | Submix 6

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	4	Positive
	Amino-Mebendazole	☐	238.1	Unit	77.1	Unit	10	176	40	3	Positive
	Amino-Mebendazole	☐	238.1	Unit	105.1	Unit	10	176	28	3	Positive
	Avermectin B1a (Abamectin B1a)	☐	890.5	Unit	305.1	Unit	10	155	8	2	Positive
	Avermectin B1a (Abamectin B1a)	☐	890.5	Unit	567.4	Unit	10	155	0	2	Positive
	Doramectin	☐	921.5	Unit	183.1	Unit	10	320	60	3	Positive
	Doramectin	☐	921.5	Unit	777.4	Unit	10	320	48	3	Positive
	Emamectin B1a	☐	886.5	Unit	82.1	Unit	10	190	60	3	Positive
	Emamectin B1a	☐	886.5	Unit	158	Unit	10	190	40	3	Positive
	Eprinomectin B1a	☐	936.5	Unit	352.1	Unit	10	305	60	3	Positive
	Eprinomectin B1a	☐	936.5	Unit	490.2	Unit	10	305	56	3	Positive
	Febantel	☐	447.1	Unit	383.1	Unit	10	128	16	3	Positive
	Febantel	☐	447.1	Unit	415.1	Unit	10	128	10	3	Positive
	Fenbendazole	☐	300.1	Unit	159	Unit	10	156	36	3	Positive
	Fenbendazole	☐	300.1	Unit	268.1	Unit	10	156	20	3	Positive
	Flubendazole	☐	314.1	Unit	123	Unit	10	141	0	4	Positive
	Flubendazole	☐	314.1	Unit	282.1	Unit	10	141	0	4	Positive
	Ivermectin B1a	☐	892.5	Unit	307.3	Unit	10	80	24	2	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

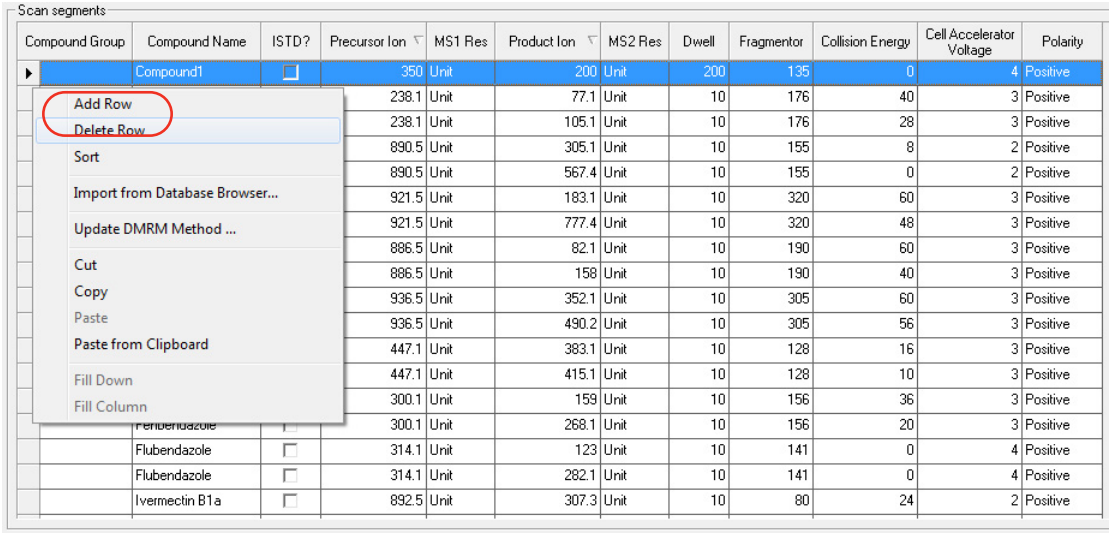
Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Compound1	☐	350	Unit	200	Unit	200	380	0	5	Positive
	Amino-Mebendazole	☐	238.1	Unit	77.1	Unit	10	380	40	3	Positive
	Amino-Mebendazole	☐	238.1	Unit	105.1	Unit	10	380	28	3	Positive
	Avermectin B1a (Aba	☐	890.5	Unit	305.1	Unit	10	380	8	2	Positive
	Avermectin B1a (Aba	☐	890.5	Unit	567.4	Unit	10	380	0	2	Positive
	Doramectin	☐	921.5	Unit	183.1	Unit	10	380	60	3	Positive
	Doramectin	☐	921.5	Unit	777.4	Unit	10	380	48	3	Positive
	Emamectin B1a	☐	886.5	Unit	82.1	Unit	10	380	60	3	Positive
	Emamectin B1a	☐	886.5	Unit	158	Unit	10	380	40	3	Positive
	Eprinomectin B1a	☐	936.5	Unit	352.1	Unit	10	380	60	3	Positive
	Eprinomectin B1a	☐	936.5	Unit	490.2	Unit	10	380	56	3	Positive
	Febantel	☐	447.1	Unit	383.1	Unit	10	380	16	3	Positive
	Febantel	☐	447.1	Unit	415.1	Unit	10	380	10	3	Positive
	Fenbendazole	☐	300.1	Unit	159	Unit	10	380	36	3	Positive
	Fenbendazole	☐	300.1	Unit	268.1	Unit	10	380	20	3	Positive
	Flubendazole	☐	314.1	Unit	123	Unit	10	380	0	4	Positive
	Flubendazole	☐	314.1	Unit	282.1	Unit	10	380	0	4	Positive
	Ivermectin B1a	☐	892.5	Unit	307.3	Unit	10	380	24	2	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.

Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Amino-Mebendazole	☐	238.1	Unit	105.1	Unit	10	176	28	3	Positive
	Amino-Mebendazole	☐	238.1	Unit	77.1	Unit	10	176	40	3	Positive
	Avermectin B1a (Aba	☐	890.5	Unit	567.4	Unit	10	155	0	2	Positive
	Avermectin B1a (Aba	☐	890.5	Unit	305.1	Unit	10	155	8	2	Positive
	Doramectin	☐	921.5	Unit	777.4	Unit	10	320	48	3	Positive
	Doramectin	☐	921.5	Unit	183.1	Unit	10	320	60	3	Positive
	Emamectin B1a	☐	886.5	Unit	158	Unit	10	190	40	3	Positive
	Emamectin B1a	☐	886.5	Unit	82.1	Unit	10	190	60	3	Positive
	Eprinomectin B1a	☐	936.5	Unit	490.2	Unit	10	305	56	3	Positive
	Eprinomectin B1a	☐	936.5	Unit	352.1	Unit	10	305	60	3	Positive
	Febantel	☐	447.1	Unit	415.1	Unit	10	128	10	3	Positive
	Febantel	☐	447.1	Unit	383.1	Unit	10	128	16	3	Positive
	Fenbendazole	☐	300.1	Unit	268.1	Unit	10	156	20	3	Positive
	Fenbendazole	☐	300.1	Unit	159	Unit	10	156	36	3	Positive
	Flubendazole	☐	314.1	Unit	282.1	Unit	10	141	0	4	Positive
	Flubendazole	☐	314.1	Unit	123	Unit	10	141	0	4	Positive
	Ivermectin B1a	☐	892.5	Unit	551.4	Unit	10	80	16	2	Positive
	Ivermectin B1a	☐	892.5	Unit	307.3	Unit	10	80	24	2	Positive

9 Click **Method > Save As** (or click  in the Method Editor toolbar) and save the method as **VetDrugs_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 **VetDrugs_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	VetDrugs_MRM_Mix1.m	todelete.d	Sample	
2	✓	SubMix_01	P1-A1	VetDrugs_MRM_Mix1.m	SubMix_1.d	Calibration	1
3	✓	SubMix_02	P1-A2	VetDrugs_MRM_Mix2.m	SubMix_2.d	Calibration	1
4	✓	SubMix_03a	P1-A3	VetDrugs_MRM_Mix3a.m	SubMix_3a.d	Calibration	1
5	✓	SubMix_03b	P1-A4	VetDrugs_MRM_Mix3b.m	SubMix_3b.d	Calibration	1
6	✓	SubMix_04	P1-A5	VetDrugs_MRM_Mix4.m	SubMix_4.d	Calibration	1
7	✓	SubMix_05	P1-A6	VetDrugs_MRM_Mix5.m	SubMix_5.d	Calibration	1
8	✓	SubMix_06	P1-A7	VetDrugs_MRM_Mix6.m	SubMix_6.d	Calibration	1
9	✓	SubMix_07	P1-A8	VetDrugs_MRM_Mix7.m	SubMix_7.d	Calibration	1
10	✓	SubMix_08	P1-A9	VetDrugs_MRM_Mix8.m	SubMix_8.d	Calibration	1
11	✓	SubMix_09	P1-A10	VetDrugs_MRM_Mix9.m	SubMix_9.d	Calibration	1
12	✓	SubMix_10	P1-A11	VetDrugs_MRM_Mix10.m	SubMix_10.d	Calibration	1
13	✓	SubMix_11	P1-A12	VetDrugs_MRM_Mix11.m	SubMix_11.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.

Revision B, January 2014

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Agilent Technologies