MassHunter Veterinary Drug Personal Compound Database and Library

Method Setup Guide

Use this guide to create Q-TOF methods to use with your Personal Compound Database and Library (PCDL).

Before you begin, make sure that your system meets the installation requirements that are described in the *MassHunter Veterinary Drug Personal Compound Database and Library Quick Start Guide*.

For more detailed instructions, see the *MassHunter PCDL for Qualitative Analysis Familiarization Guide*, and the MassHunter Data Acquisition for 6500 Series Quadrupole TOF LC/MS *Familiarization Guide* and *online Help*.

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Step 1. Set up the LC part of the method

1 Prepare the standards.

   a Dilute your standards to a final solution of 1 µg/mL (1 ppm) with the appropriate solvent:

   For standards that dissolve in acetonitrile, use acetonitrile.

   For standards that dissolve in 90% acetonitrile, use 90% acetonitrile.

   For standards that dissolve in DMSO, or for a mixture of standards that contain multiple solvents, use 50:50 acetonitrile:methanol (v/v).

   b Transfer 1 mL of the final sample solution to a standard 2-mL sample vial for analysis.

2 Set up the mobile phases.

   This step is identical for all LC modules.

   You can use the same mobile phase to detect most compounds in the PCDL, but sensitivity would not be optimized equally. To optimize sensitivity, create separate methods with different mobile phases, depending on the compound class.

   Prepare **Mobile Phase Set 1** for compounds that tend to form protonated adducts:

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

   Prepare **Mobile Phase Set 2** for compounds that tend to form ammonium adducts (such as Avermectins):

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

   Use the Agilent Poroshell 120 EC-C18, 2.1 mm × 150 mm, 2.7 µm column (p/n 693775-902) for both Mobile Phase Sets.

3 Check that the method is set up to make a 2-µL injection.

4 Set up the gradient.

   The gradient setup is dependent upon the LC configuration. The parameters that follow are examples.

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

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), sold separately.

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 18 minutes with a post time of 2 minutes.

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 18 minutes with a post time of 2 minutes.

Step 2. Set up LC/MS ion source parameters

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

  For a multicomponent method, the ion source parameters shown in the next tables are used to achieve the best overall sensitivity for most Veterinary Drug compounds. You can adjust the method to optimize for individual compounds.

Table 1 ESI Ion Source

ESI Ion Source Parameters	6520/6530/6540 Q-TOF LC/MS
Gas Temp (°C)	350
Drying Gas (L/min)	12
Nebulizer (psig)	40
VCap	3500 (Pos), 3000 (Neg)
Fragmentor	140 (Pos), 140 (Neg)
Skimmer	65
OCT 1 RF Vpp	750

Table 2 Dual ESI Ion Source

Dual ESI Ion Source Parameters	6520/6530/6540 Q-TOF LC/MS
Gas Temp (°C)	350
Drying Gas (L/min)	12
Nebulizer (psig)	35
VCap	3500 (Pos), 3000 (Neg)
Fragmentor	140 (Pos), 140 (Neg)
Skimmer	65
OCT 1 RF Vpp	750

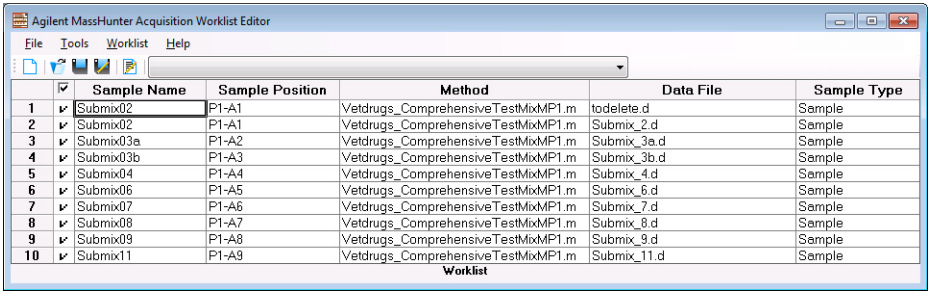
Table 3 Agilent Jet Stream Ion Source

Agilent Jet Stream Ion Source Parameters	6520/6530/6540 Q-TOF LC/MS	6550/6560 Q-TOF LC/MS
Gas Temp (°C)	200	130
Drying Gas (L/min)	7	16
Nebulizer (psig)	35	35
Sheath Gas Temp (°C)	375	375
Sheath Gas Flow (L/min)	11	11
Capillary (V)	3500 (Pos), 3000 (Neg)	3500 (Pos), 3000 (Neg)
Nozzle Voltage (V)	300 (Pos), 0 (Neg)	300 (Pos), 0 (Neg)
High Pressure RF (V)	N/A	150 (Pos), 90 (Neg)
Low Pressure RF (V)	N/A	60 (Pos), 60 (Neg)
Fragmentor	140	380
Skimmer	65	N/A
OCT 1 RF Vpp	750	750

Nebulizer pressure depends to a large extent on the flow rate that is used. The fragmentor voltage on the non-iFunnel configuration also depends on the molecule size. The masses for Veterinary Drugs typically range from 100 dalton to 1200 dalton.

Step 3. Set up worklists to run samples

1 To analyze compounds that tend to form protonated adducts, set up **worklist 1** as shown in Figure 1. Inject the first standard twice to allow the system to come to equilibrium.

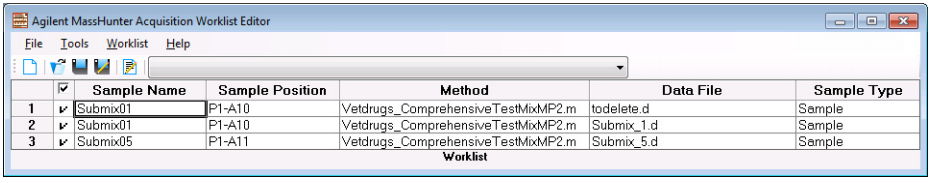


		Sample Name	Sample Position	Method	Data File	Sample Type
1	✓	Submix02	P1-A1	Vetdrugs_ComprehensiveTestMixMP1.m	todelete.d	Sample
2	✓	Submix02	P1-A1	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_2.d	Sample
3	✓	Submix03a	P1-A2	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_3a.d	Sample
4	✓	Submix03b	P1-A3	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_3b.d	Sample
5	✓	Submix04	P1-A4	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_4.d	Sample
6	✓	Submix06	P1-A5	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_6.d	Sample
7	✓	Submix07	P1-A6	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_7.d	Sample
8	✓	Submix08	P1-A7	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_8.d	Sample
9	✓	Submix09	P1-A8	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_9.d	Sample
10	✓	Submix11	P1-A9	Vetdrugs_ComprehensiveTestMixMP1.m	Submix_11.d	Sample

Figure 1 Worklist 1

2 To analyze compounds that tend to form ammonium adducts, change the mobile phase to Mobile Phase Set 2, then:

- **a** Purge the LC system (A:B 50:50 at 5 mL/min) for 10 minutes. Refer to the LC instrument guide for instructions.
- **b** Allow the column to equilibrate (A:B 50:50 at 0.4 mL/min) with Mobile Phase Set 2 for 10 minutes.
- **c** Set up **worklist 2** 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
1	✓	Submix01	P1-A10	Vetdrugs_ComprehensiveTestMixMP2.m	todelete.d	Sample
2	✓	Submix01	P1-A10	Vetdrugs_ComprehensiveTestMixMP2.m	Submix_1.d	Sample
3	✓	Submix05	P1-A11	Vetdrugs_ComprehensiveTestMixMP2.m	Submix_5.d	Sample

Figure 2 Worklist 2

For more information about Q-TOF methods, refer to the *MassHunter PCDL for Qualitative Analysis Familiarization Guide*, or the MassHunter Data Acquisition for 6500 Series Quadrupole TOF LC/MS *Familiarization Guide* or *online Help*.

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In this Book

The *Method Setup Guide* describes how to create methods for your specific LC/MS setup.

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