



Comprehensive Test Mix for MassHunter Veterinary Drug Personal Compound Database and Library

Method Setup Guide

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NOTE

The Comprehensive Veterinary Drug Test Mix is included with the G6856AA/G3879AA Veterinary Drug PCD/PCDL Application Kits.

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 Q-TOF methods for the test mix.

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 *Quick Start Guide* for this database, and the *MassHunter Data Acquisition for 6500 Series Quadrupole TOF LC/MS Familiarization Guide* and *online Help*.



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 G6856AA/G3879AA Veterinary Drug PCD/PCDL Application Kit.

Time [min]	Δ	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.

Step 1. Set up the LC part of the method

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.

Table 21 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 22 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

Step 2. Set up LC/MS ion source parameters

Table 23 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 that is used. The fragmentor voltage on the non-iFunnel configuration also depends on the molecule size. The masses in the Comprehensive Test Mix range from 200 dalton to 1200 dalton.

Step 3. Set up a worklist to run the submixes

Step 3. 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
1	✓	SubMix_01	P1-A1	Vetdrugs_ComprehensiveTestMix.m	todelete.d ...	Sample
2	✓	SubMix_01	P1-A1	Vetdrugs_ComprehensiveTestMix.m	Submix_1.d	Sample
3	✓	SubMix_02	P1-A2	Vetdrugs_ComprehensiveTestMix.m	Submix_2.d	Sample
4	✓	SubMix_03a	P1-A3	Vetdrugs_ComprehensiveTestMix.m	Submix_3a.d	Sample
5	✓	SubMix_03b	P1-A4	Vetdrugs_ComprehensiveTestMix.m	Submix_3b.d	Sample
6	✓	SubMix_04	P1-A5	Vetdrugs_ComprehensiveTestMix.m	Submix_4.d	Sample
7	✓	SubMix_05	P1-A6	Vetdrugs_ComprehensiveTestMix.m	Submix_5.d	Sample
8	✓	SubMix_06	P1-A7	Vetdrugs_ComprehensiveTestMix.m	Submix_6.d	Sample
9	✓	SubMix_07	P1-A8	Vetdrugs_ComprehensiveTestMix.m	Submix_7.d	Sample
10	✓	SubMix_08	P1-A9	Vetdrugs_ComprehensiveTestMix.m	Submix_8.d	Sample
11	✓	SubMix_09	P1-A10	Vetdrugs_ComprehensiveTestMix.m	Submix_9.d	Sample
12	✓	SubMix_10	P1-A11	Vetdrugs_ComprehensiveTestMix.m	Submix_10.d	Sample
13	✓	SubMix_11	P1-A12	Vetdrugs_ComprehensiveTestMix.m	Submix_11.d	Sample

For more information about Q-TOF methods, refer to the *Quick Start Guide* for this database, 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 set up. The methods are used for the Comprehensive Test Mix.

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