Comprehensive Test Mix for MassHunter Forensics and Toxicology Personal Compound Database and Library

Method Setup Guide

NOTE The Comprehensive Forensics and Toxicology Test Mix is included with the G6855AA/G3876AA Forensics and Toxicology 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 Forensics and Toxicology 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*.

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Step 1. Set up the LC part of the method

1 Prepare the standards.

The concentration of the test mix stock solution is 100 µg/mL (100 ppm).

a Dilute 10 µL of the stock solution to 1.0 mL with acetonitrile.

For more accurate results, and if conservation of sample is not a concern, dilute 100 µL of the stock solution to 10.0 mL of solvent instead.

NOTE Submix 9 consists of 4 vials. Submix 10 consists of 3 vials. When you dilute either of these submixes, combine 10 µL from each vial, and then dilute to 1.0 mL with acetonitrile.

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

The final solution is a 1 µg/mL (1 ppm) working solution.

2 Set up the mobile phases.

This step is identical for all LC modules.

- Solvent A: 5 mM ammonium formate/0.01% formic acid in water
- Solvent B: 0.01% formic acid in methanol

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 Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm ZORBAX LC column (p/n 959758-902), included in the G6855AA/G3876AA Forensics and Toxicology PCD/PCDL 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	---	---
10.00	5.00	95.00	---	---
10.10	95.00	5.00	---	---

Stop time is 12 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
1.50	95.00	5.00	---	---
2.00	70.00	30.00	---	---
8.50	40.00	60.00	---	---
11.00	5.00	95.00	---	---
12.00	5.00	95.00	---	---
12.10	95.00	5.00	---	---

Stop time is 14 minutes with a post time of 2 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 tab.

  For a multi-component method, the ion source parameters shown in the next tables 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.

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)	35
VCap	3500 (Pos), 3000 (Neg)
Fragmentor	150 (Pos), 120 (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	150 (Pos), 120 (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)	250	120
Drying Gas (L/min)	6	15
Nebulizer (psig)	35	35
Sheath Gas Temp (°C)	375	375
Sheath Gas Flow (L/min)	11	12
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.

Step 3. Set up a worklist to run the submixes

- Set up the worklist as shown in Figure 1. 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	ForTox_ComprehensiveTestMix.m	todelete.d	Sample
2	✓	SubMix_01	P1-A1	ForTox_ComprehensiveTestMix.m	Submix_1.d	Sample
3	✓	SubMix_02	P1-A2	ForTox_ComprehensiveTestMix.m	Submix_2.d	Sample
4	✓	SubMix_03	P1-A3	ForTox_ComprehensiveTestMix.m	Submix_3.d	Sample
5	✓	SubMix_04	P1-A4	ForTox_ComprehensiveTestMix.m	Submix_4.d	Sample
6	✓	SubMix_05	P1-A5	ForTox_ComprehensiveTestMix.m	Submix_5.d	Sample
7	✓	SubMix_06	P1-A6	ForTox_ComprehensiveTestMix.m	Submix_6.d	Sample
8	✓	SubMix_07	P1-A7	ForTox_ComprehensiveTestMix.m	Submix_7.d	Sample
9	✓	SubMix_08	P1-A8	ForTox_ComprehensiveTestMix.m	Submix_8.d	Sample
10	✓	SubMix_09	P1-A9	ForTox_ComprehensiveTestMix.m	Submix_9.d	Sample
11	✓	SubMix_10	P1-A10	ForTox_ComprehensiveTestMix.m	Submix_10.d	Sample

Figure 1 Worklist

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.

Revision A, January 2015

G3876-90009

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