LC/Q-TOF Applied Markets PCDL

User Guide

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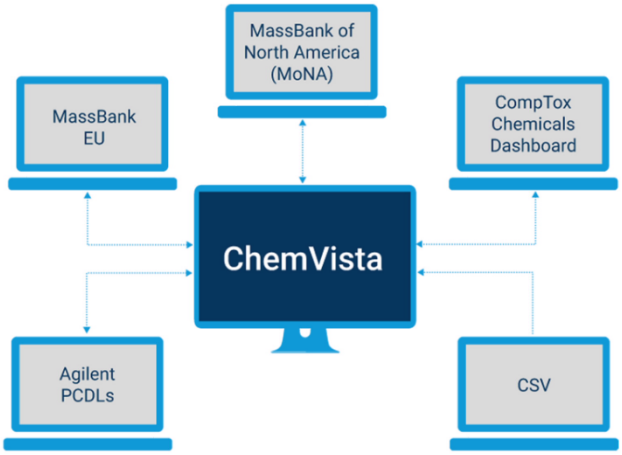

Notices

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Software Revision

This guide is valid for the C.01.00 revision or higher of the LC/Q-TOF Applied Markets PCDL program and compatible LC/Q-TOF Applied Markets PCDL programs, until superseded.

Software Manufacturing

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WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Book

This book describes the LC/Q-TOF Applied Markets PCDL. It explains the system configuration and/or method setup information pertaining to subset PCDLs (legacy PCDL products).

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Agilent Community

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Content

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1 Overview

This chapter provides an overview of the LC/Q-TOF Applied Markets PCDL.

What is the LC/Q-TOF Applied Markets PCDL?

The LC/Q-TOF Applied Markets Personal Compound Database and Library (PCDL) lets you screen a wide range of analytes in a single LC/MS analysis. Whether its analysis of pesticides in food products, environmental contaminants in wastewater, or forensic toxicological analysis of biological samples, confident identification remains a challenge. This PCDL contains over 14,500 compounds found in regulations, target lists of interest, and emerging areas of need such as designer drugs, enabling screening and identification throughout applied markets of interest.

The LC/Q-TOF Applied Markets PCDL includes:

- the complete content from the following legacy PCDL products: Extractables and Leachables PCDL, Mycotoxins PCDL, Pesticides PCDL, Veterinary Drugs PCDL, Water Screening PCDL, and Forensic and Toxicology PCDL
- designer drugs, human doping drugs, anabolic steroids, and hormones
- chemicals of concern present in public databases, such as pesticides, veterinary drugs, mycotoxins, cyanotoxins and industrial chemicals and their metabolites and transformation products
- contaminants of emerging concern, such as pharmaceuticals and personal care products (PPCPs) and per- and polyfluoroalkyl substances (PFAS)
- compounds from the EPA ToxCast Screening Library
- compounds that are present in regulations and region-specific lists

When used in tandem with Agilent ChemVista Library Manager, subsets are easily created using class tags (e.g. Estrogen, Designer Drug, Polyphenol), regulatory information, and legacy PCDL product tags (e.g., Veterinary Drug, Mycotoxin, Pesticide).

Regulatory lists include:

- US 40 CFR Part 355 Appendix A: Extremely Hazardous Chemicals
- US Environmental Protection Agency (EPA 521, EPA 539, EPA 1694, EPA 1698, and EPA Draft CCL 4)
- Chinese region specific lists and regulations (CN-NY-193, CN-NY-235, CN-NY-265, CN-NY-560, CN-EPA screening list, CN-antibiotics list and CN-CDC survey list)
- EU Water Framework Directive (EU WFD)

- Japanese region specific lists and regulations (Japanese Positive List System for Agricultural Chemical Residues in Foods (JPL) and Japan Drinking Water Quality Standard (JDWQS)

The LC/Q-TOF Applied Markets PCDL, together with an Agilent TOF or Q-TOF LC/MS, can be an appropriate supplement to single analyte or analyte-group detection methods at trace level.

Working with the PCDL

You can use the LC/Q-TOF Applied Markets PCDL as is to search for compounds. Or you can use the PCDL in Agilent ChemVista to build upon, subset, and manage the compound and spectral data contained in the PCDL to further your screening capabilities. Refer to the Agilent ChemVista Introduction Workbook, introductory videos, and online help to learn how to manage the compound and spectral data and:

- Add, remove and edit the compounds to meet the specific needs of your laboratory and your analyses.
- Add retention times generated experimentally based on standards and/or retention times for compounds you analyze.
- Add your own spectra. With MassHunter Qualitative Analysis B.07.00 and higher, you can:
  - Run a database search or use the Find by Formula algorithm to identify compounds and then send the MS/MS spectra to your custom PCDL. Import the updated PCDL into Agilent ChemVista to store new spectra all in one place.
  - Filter spectral noise and correct the product ions to their theoretical accurate mass.

The high mass accuracy of the Agilent time-of-flight (TOF or Q-TOF) LC/MS instrument provides the capability to screen all compounds in the library that are detected by their exact mass and retention time (if known). Searching the library can then identify the compounds found by comparison to their accurate product ion mass spectra.

Searching and managing the PCDL

The following are brief tips and ways to use the MassHunter Qualitative Analysis program to search the PCDL to identify compounds and spectrum peaks. For more information, see the *MassHunter PCDL for Qualitative Analysis Familiarization Guide*.

To run these algorithms, use the commands from the menu bar. To review the parameters for the algorithms, use the Method Editor window.

Table 1. Identifying Features

If you want to edit the method to...	Select this Method Editor section	Refer to Online Help topic
Find compounds using the Find by Formula algorithm restricted to formulas within a PCDL (with or without retention times)	Target/Suspect Screening > Find by Formula	Find compounds by formula
Search the database based on MS spectral information from compound features (with or without retention times)	Identification > Identification Workflow	Search database for a compound
Identify compounds from MS spectrum peaks (with or without retention times)	Identification > Database Search Settings	Search database from a spectrum
Search the spectral library based on MS/MS information from compound features	Identification > Identification Workflow	Search accurate mass library for compounds. Search unit mass library for compounds
Identify compounds from MS/MS spectra	Identification > Identification Workflow	Search accurate mass library for spectra Search unit mass library for spectra

Retention times as a search criterion

Use retention times with MS data as a search criterion:

- as not required (non-targeted screen)
- as optional providing a targeted and non-targeted screen
- required (targeted screen only)

Managing the PCDL content

Use Agilent ChemVista to manage the content of your PCDL:

- Import your PCDL into the standalone library manager to manage data in a compound-centric fashion. Alternatively, use the pre-load option during installation to have the PCDL content automatically loaded into ChemVista. When doing so, subset lists based on legacy PCDL content are automatically created. During pre-load, the classification feature in ChemVista is turned off. It may be desirable in certain circumstances to edit or turn off the classification feature prior to importing data in the case where multiple records have the same structure but should remain separate. See the ChemVista Online Help for more details.
- Create custom screening lists specific to your analysis by searching for compound class groups and regulation tags as well as searches using compound name, formula, mass, CAS, InChIKey, etc.
- Edit and add compounds, retention times, and MS/MS spectra.
- Search, browse, and store MS/MS centroid spectra acquired on a Q-TOF instrument.
- Merge compounds from your PCDL with compounds and spectra from MassBank, MassBank of North America (MoNA), and the EPA CompTox Chemicals Dashboard.
- For more information, see the *Agilent ChemVista Introduction Workbook*, introductory videos, and Online Help.
- Send spectra to your customized PCDL directly from the Qualitative Analysis program to create your own custom library. Choose from options to filter spectral noise and/or to correct the product ions to their theoretical accurate mass.
- Import the customized PCDL into Agilent ChemVista.

- Load spectra from either a .CEF file or by copy-and-pasting mass spectra from MassHunter Qualitative Analysis software into a PCDL using MassHunter PCDL Manager.
- For more information, see the *MassHunter Personal Compound Database and Library Manager Quick Start Guide*, PCDL Manager Online Help, and MassHunter Qualitative Analysis Help.

Product Content

Your PCDL product includes these parts:

- LC/Q-TOF Applied Markets PCDL (LC/Q-TOF Applied Markets PCDL.cdb)
- Legacy MassHunter PCDL Products, optionally installed (when installed, located under \MassHunter\LCQTOF Applied Markets PCDL\Legacy PCDLs)
- Checkout Mix PCDL (Checkout_TestMix_Std.cdb)
- Checkout Mix example data files

Where to find more information:

- The complete PCDL content listing is available on the installation media and is installed on your computer by default.
- *MassHunter Quant LC/Q-TOF Screener*: Use this guide to learn how to use PCDLs within the Quant Screener workflow. This guide, along with example data files, is available from Subscribenet (part number M6005-10006).

2 Configuration and Setup

This chapter explains the system configuration and/or method setup information pertaining to subset PCDLs (legacy PCDL products). These details can help you create Q-TOF methods to use with your PCDL and/or create chromatography methods to reproduce RT information and use RT information in screening and identification.

Extractables and Leachables PCDL

System Configuration

Retention times (RTs) for the E_n_L_AMRT_PCDL were estimated based on the conditions described in this document. These compounds can be identified by searching for "E&L Compound" as a class tag within Agilent ChemVista.

A number of factors can cause your retention times to differ from those determined by Agilent. These factors include different instrument delay volumes, dead volumes or configuration changes. Any deviation from the configuration described in this document can change the retention times. With the configuration described here, retention times are expected to be stable within a window of less than ± 0.5 minutes for the majority of compounds. To account for possible RT drifts during compound identification, adjust these parameters in MassHunter Qualitative Data Analysis.

- For Database Search or Find by Formula, in the Scoring tab set the Retention time expected data variation to ±1.
- For Find by Formula, in the Formula Matching tab set the Retention times match tolerance to ±1.25.

If expected compounds are not included with retention time information in the database, set the Values to Match to Mass and retention time (retention time optional) to see all possible mass matches. If retention times are expected to vary significantly compared with the retention time information in the database, set the Values to Match to Mass to see all possible mass matches over the entire chromatogram.

System configuration:

- Agilent 1290 Infinity I UHPLC system that includes:
  - Agilent 1290 Infinity Binary Pump (G4220A)
  - Agilent 1290 Infinity High Performance Autosampler (G4226A) equipped with a 40 µL injection loop and a low dispersion needle seat (G4226-87020, obsolete. Replaced by G4226-87030)
  - Agilent 1290 Infinity Thermostatted Column compartment (G1316C) equipped with 3 µL heat exchanger
- Agilent G6545A Quadrupole Time-of-Flight LC/MS System equipped with a Dual Spray Agilent Jet Stream electrospray ionization source.

Tubing

The individual modules were connected with the following tubing:

- Binary Pump to High Performance Autosampler (2-stack): Stainless Steel, 0.17 mm ID x 700 mm length (p/n G1312-87304)
- High Performance Autosampler to Heat Exchanger: Stainless Steel, 0.12 x 340 mm (p/n 5067-4647)
- Heat Exchanger to column: A-Line Quick Connect Fitting Assembly, Stainless Steel, 0.12 x 105 mm (p/n 5067-5957)
- Column to Q-TOF Inlet: PEEK Tubing, 0.12 mm ID, 1.6 mm OD, 370 mm length (p/n 0890-1915 or 5042-6461)

Agilent 1290 UHPLC parameters

- Column: Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 × 150 mm, 1.8 µm (p/n 959759-306)
- Mobile phase (A): Water, 4.5mM NH4Formate + 0.5mM NH4F + 0.1% formic acid
- Mobile phase (B): 80%MeOH + 20% IPA (v/v), 4.5mM NH4Formate + 0.5mM NH4F + 0.1% formic acid
- Column temperature: 45 °C
- Auto sampler temperature: 6 °C
- Needle wash: 10 s (80% MeOH /20% water)
- Flow rate: 0.4 mL/mins
- Injection volume: 2 µL
- LC gradient program

Time (min)	Mobile Phase B%
0	15
15	100
18	100

Time (min)	Mobile Phase B%
18.1	15
Stop Time 19 min; Post Time 3 min	

Extractable and leachable standards

Retention times were recorded by LC/MS analysis of mixed standard solutions of extractable and leachable compounds that purchased from AccuStandard (AccuStandard, Inc., New Haven, CT, USA) and Sigma Aldrich Inc. (Sigma-Aldrich Corp., St. Louis, MO, USA). Original stock solutions of E&L standards were prepared by dissolving standard compounds in tetrahydrofuran, isopropanol, or methanol depending on the physicochemical properties of E&L compounds. All standards were diluted by methanol for LC/MS analysis.

Forensic Toxicology PCDL

Use this Method Setup to create Q-TOF methods to use with your Personal Compound Database and Library (PCDL). These instructions are based on the 5190-0555 LC/MS Toxicology Comprehensive Test Mix (sold separately). The Comprehensive Test Mix is not required, but it can be a helpful tool to develop your own methods. To subset these compounds only, search for "Forensic and Toxicology Drug" as a class tag within Agilent ChemVista.

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)

- 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.
- 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.

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.

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.

Table 2. Mobile phase gradient with 1290 Infinity LC System

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	0.400	1200.00
1.50	70.00	30.00	0.400	1200.00
6.50	40.00	60.00	0.400	1200.00
9.00	5.00	95.00	0.400	1200.00
10.00	5.00	95.00	0.400	1200.00
10.10	95.00	5.00	0.400	1200.00

- Stop time is 12 minutes with a post time of 2 minutes.
- 1290 Infinity LC system with Agilent Eclipse Plus C18, 2.1 mm x 100 mm, 1.8um ZORBAX LC column (p/n 959758-902), sold separately.

Table 3. Mobile phase gradient with 1260 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	0.400	600.00
2.00	70.00	30.00	0.400	600.00
8.50	40.00	60.00	0.400	600.00
11.00	5.00	95.00	0.400	600.00
12.00	5.00	95.00	0.400	600.00
12.10	95.00	5.00	0.400	600.00

- Stop time is 14 minutes with a post time of 2 minutes.
- The 1260 Infinity LC system can have a lower backpressure limit (up to 600bar) and a higher dead volume than the 1290 Infinity LC system.

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 multicomponent method, the ion source parameters shown in the next tables are used to achieve the best overall sensitivity for all compounds in the Comprehensive Test Mix. You can adjust the method to optimize for individual compounds or submixes.

Table 4. 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 5. Dual 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 6. Agilent Jet Stream Ion Source

Agilent Jet Stream Ion Source Parameters	6520/6530/6540/6545/6546 Q-TOF LC/MS	6550/6560 Q-TOF LC/MS
Gas Temp (°C)	250	120
Drying Gas (L/min)	6	15

Table 6. Agilent Jet Stream Ion Source

Agilent Jet Stream Ion Source Parameters	6520/6530/6540/6545/6546 Q-TOF LC/MS	6550/6560 Q-TOF LC/MS
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

1 Set up the worklist as shown in the following figure. Include all submixes.

2 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 Familiarization Guide for this database, or the *LC/Q-TOF Data Acquisition for 6500 Series Quadrupole TOF LC/MS Familiarization Guide*, or Online Help.

Pesticide PCDL

Use this guide to create Q-TOF methods to use with your Personal Compound Database and Library (PCDL). The instructions in this guide are based on the 5190-0551 LC/MS Pesticide Comprehensive Test Mix (sold separately). The Comprehensive Test Mix is not required, but it can be a helpful tool to develop your own methods. To subset these compounds only, search for "Pesticide" as a class tag within Agilent ChemVista.

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)

- 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.
- 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: 5 mM ammonium formate/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 x 150 mm, 1.8um ZORBAX LC column (p/n 959759-902), sold separately.

Table 7. Mobile phase gradient with Infinity LC System

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	0.400	1200.00

Table 7. Mobile phase gradient with Infinity LC System

Time (min)	A (%)	B (%)	Flow (mL/min)	Max. Pressure Limit (bar)
3.50	50.00	50.00	0.400	1200.00
17.00	0.00	100.00	0.400	1200.00
20.00	0.00	100.00	0.400	1200.00
20.10	95.00	5.00	0.400	1200.00

- Stop time is 22 minutes with a post time of 2 minutes.
- 1290 Infinity LC system with Agilent Eclipse Plus C18, 2.1 mm x 150 mm, 1.8um ZORBAX LC column (p/n 959759-902), sold separately.

Table 8. Mobile phase gradient with 1260 Infinity LC system

Time (min)	A (%)	B (%)	Flow (mL/min)	Max. Pressure Limit (bar)
0.00	95.00	5.00	0.400	600.00
3.50	50.00	50.00	0.400	600.00
17.00	0.00	100.00	0.400	600.00
20.00	0.00	100.00	0.400	600.00
20.10	95.00	5.00	0.400	600.00

- Stop time is 22 minutes with a post time of 2 minutes.
- The 1260 Infinity LC system can have a lower backpressure limit (up to 600bar) and a higher dead volume than the 1290 Infinity LC system.

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 all compounds in the Comprehensive Test Mix. You can adjust the method to optimize for individual compounds or submixes.

Table 9. ESI Ion Source

ESI Ion Source Parameters	6520/6530/6540 Q-TOF LC/MS
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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 10. Dual 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 11. Agilent Jet Stream Ion Source

Agilent Jet Stream Ion Source Parameters	6520/6530/6540/6545/6546 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

Table 11. Agilent Jet Stream Ion Source

Agilent Jet Stream Ion Source Parameters	6520/6530/6540/6545/6546 Q-TOF LC/MS	6550/6560 Q-TOF LC/MS
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.
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Set up the worklist as shown in the following figure. Include all submixes. Inject the first standard twice to allow the system to come to equilibrium.

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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 2. Worklist

For more information about Q-TOF methods, refer to the Familiarization Guide for this database, or the *LC/Q-TOF Data Acquisition for 6500 Series Quadrupole TOF LC/MS Familiarization Guide*, or Online Help.

Veterinary Drug PCDL

Use this guide to create Q-TOF methods to use with your Personal Compound Database and Library (PCDL). To subset these compounds only, searching for "Veterinary Drug" as a class tag within Agilent ChemVista.

Step 1. Set up the LC part of the method.

1 Prepare the standards.

- 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).
- 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/0.01% formic acid in water
- Solvent B: 5 mM ammonium formate/0.01% 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.

Table 12. Mobile phase gradient with 1260 Infinity LC system

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	0.400	1200.00
5.00	60.00	40.00	0.400	1200.00
13.00	5.00	95.00	0.400	1200.00
14.00	0.00	100.00	0.400	1200.00
16.00	0.00	100.00	0.400	1200.00
16.10	95.00	5.00	0.400	1200.00

-.Stop time is 18 minutes with a post time of 2 minutes.
-.The 1260 Infinity LC system can have a lower backpressure limit (up to 600bar) and a higher dead volume than the 1290 Infinity LC system.

Table 13. Mobile phase gradient with 1260 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	0.400	600.00
5.00	60.00	40.00	0.400	600.00
13.00	5.00	95.00	0.400	600.00
14.00	0.00	100.00	0.400	600.00
16.00	0.00	100.00	0.400	600.00
16.10	95.00	5.00	0.400	600.00

- Stop time is 18 minutes with a post time of 2 minutes.
- The 1260 Infinity LC system can have a lower backpressure limit (up to 600bar) and a higher dead volume than the 1290 Infinity LC system.

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 all compounds in the Comprehensive Test Mix. You can adjust the method to optimize for individual compounds or submixes.

Table 14. 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 15. Dual 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

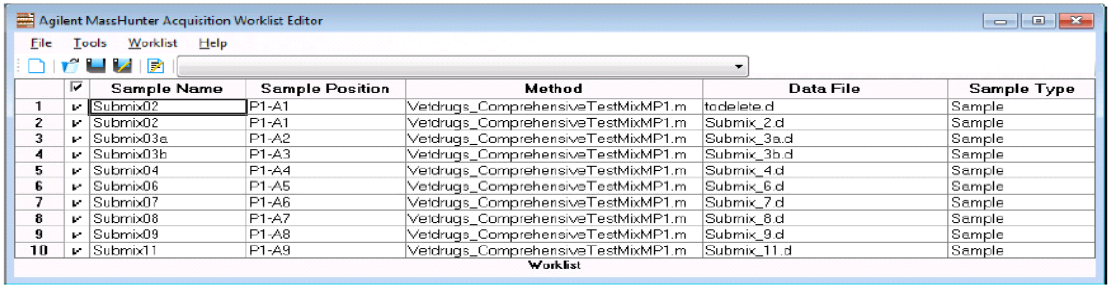
Table 16. Agilent Jet Stream Ion Source

Agilent Jet Stream Ion Source Parameters	6520/6530/6540/6545/6546 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.
The masses for Veterinary Drugs typically range from 100 Da to 1200 Da.

Step 3. Set up a worklist to run the samples

To analyze compounds that tend to form protonated adducts, set up worklist 1 as shown in the following figure. 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 3. Worklist

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

- Purge the LC system (A:B 50:50 at 5 mL/min) for 10 minutes. Refer to the LC instrument guide for instructions.
- Allow the column to equilibrate (A:B 50:50 at 0.4 mL/min) with Mobile Phase Set 2 for 10 minutes.
- Set up worklist 2 as shown in the next figure. Inject the first standard twice to allow the system to come to equilibrium.

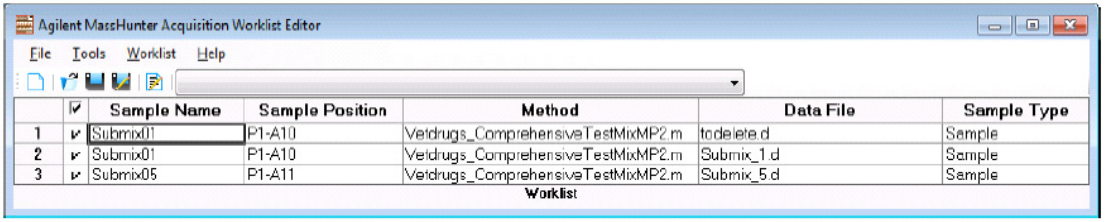


Figure 4. Worklist 2

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

Conditions for the Estimation of Retention Times for VetDrugs_AMRT_PCDL

Retention times for the VetDrugs_AMRT_PCDL and application note 5991-6651EN, "Analysis of 122 Veterinary Drugs in Meat Using All Ions MS/MS with an Agilent 1290 / 6545 UHPLC-Q-TOF System", were estimated based on the conditions described in this document.

A number of factors can cause your retention times to differ from those determined by Agilent. These factors include different instrument delay volumes, dead volumes or configuration changes. Any deviation from the configuration described in this document or the method described in application note 5991-6651EN can change the retention times significantly. With the configuration described here, retention times are expected to be stable within a

window of less than ± 0.5 minutes for the majority of compounds. To account for possible RT drifts during compound identification, adjust these parameters in MassHunter Qualitative Data Analysis.

- For Database Search or Find by Formula, in the Scoring tab set the Retention time expected data variation to ±1.
- For Find by Formula, in the Formula Matching tab set the Retention times match tolerance to ±1.25.
- For Find by Formula, in the Formula Matching tab set the Expected retention time to ±1.25.

If expected compounds are not included with retention time information in the database, set the Values to Match to Mass and retention time (retention time optional) to see all possible mass matches. If retention times are expected to vary significantly compared with the retention time information in the database, set the Values to Match to Mass to see all possible mass matches over the entire chromatogram.

System configuration

- Agilent 1290 Infinity I UHPLC system that includes:
  - Agilent 1290 Infinity Binary Pump (G4220A)
  - Agilent 1290 Infinity High Performance Autosampler (G4226A) equipped with a 40 μL injection loop and a 1.2 uL seat capillary
  - Agilent 1290 Infinity Thermostatted Column compartment (G1316C)
- Agilent G6545A Quadrupole Time-of-Flight LC/MS System equipped with a Dual Spray Agilent Jet Stream electrospray ionization source.

Tubing

The individual modules were connected with the following tubing:

- Binary Pump to High Performance Autosampler: Stainless Steel, 0.17 mm ID x 700 mm length (p/n G1312-87034)
- High Performance Autosampler to Heat Exchanger: Stainless Steel, 0.12 x 340 mm (p/n 5067-4647)

- Heat Exchanger to column: A-Line Quick Connect Fitting Assembly, Stainless Steel, 0.12 x 105 mm (p/n 5067-5961)
- Column to Q-TOF Inlet: PEEK Tubing, 0.12 mm ID, 1.6 mm OD, 500 mm length

Column and mobile phases

- Agilent ZORBAX RRHD Eclipse Plus C-8, 2.1 × 150 mm, 1.8 µm (p/n 959759-906) thermostatted to 30°C
- Guard column Zorbax Eclipse Plus C18 2.1x5 mm, 1.8 µm (p/n 821725-901)
- 0.1% formic acid (v/v) in water as mobile phase (A)
- 0.1% formic acid (v/v) in acetonitrile as mobile phase (B)

Standards

Retention times were estimated based on mixed standard solutions of veterinary drugs provided by Agilent collaborative research groups and purchased from Sigma Aldrich Inc.

Water Screening PCDL

Conditions for the Estimation of Retention Times for Water_AMRT_PCDL. To subset these compounds only, search for "Environmental contaminant" as a class tag within Agilent ChemVista.

System configuration

- Agilent 1290 Infinity I UHPLC system that includes:
  - Agilent 1290 Infinity Binary Pump (G4220A)
  - Agilent 1290 Infinity High Performance Autosampler (G4226A) equipped with a 20 μL injection loop and a 1.2 uL seat capillary connected to the large volume injection kit (p/n G4216-68711)
  - Agilent 1290 Infinity Thermostatted Column compartment (G1316C) equipped with a Low Dispersion Heat Exchanger double kit (p/n G1316-80022)
- Agilent G6550A iFunnel Quadrupole Time-of-Flight LC/MS System equipped with a Dual Spray Agilent Jet Stream electrospray ionization source.

Tubing

The individual modules were connected with the following capillaries:

- Binary Pump to High Performance Autosampler: Stainless Steel, 0.17 x 600 mm (p/n 5067-4670)
- High Performance Autosampler to Heat Exchanger: Stainless Steel, 0.12 x 340 mm (p/n 5067-4647)
- Heat Exchanger to column: A-Line Quick Connect Fitting Assembly, Stainless Steel, 0.12 x 105 mm (p/n 5067-5961)
- Column to Q-TOF Inlet Filter: Tubing PEEK, 1.6 mm od, 0.12 mm id, about 700 mm long

Column and mobile phases

- Agilent ZORBAX RRHD SB-Aq column, 2.1 × 150 mm, 1.8 µm (p/n 859700-914) thermostatted to 40°C
- 1 mM ammonium acetate and 0.1% acetic acid (v/v) in water as mobile phase (A)
- 0.1% acetic acid (v/v) in acetonitrile as mobile phase (B)

Environmental contaminant standards

Retention times were estimated based on mixed standard solutions of pesticides, pharmaceuticals, and drugs of abuse provided by Agilent collaborative research groups or included in:

- LC/MS Pesticide Comprehensive Test Mix (p/n 5190-0551)
- LC/MS Forensic Toxicology Comprehensive Test Mix (p/n 5190-0555)

A number of factors can cause your retention times to differ from those determined by Agilent. These factors include different instrument delay volumes, dead volumes or configuration changes. Any deviation from the configuration described in this document or the method described in application note 5991-6627EN can change the retention times significantly. With the configuration described here, retention times are expected to be stable within a window of less than ± 0.5 minutes for the majority of compounds. To account for possible RT drifts during compound identification, adjust these parameters in LC/Q-TOF Qualitative Data Analysis.

- For Database Search or Find by Formula, in the Scoring tab set the Retention time expected data variation to ±1.
- For Find by Formula, in the Formula Matching tab set the Retention times match tolerance to ±1.25.
- For Find by Formula, in the Formula Matching tab set the Expected retention time to ±1.25.

If expected compounds are not included with retention time information in the database, set the Values to Match to Mass and retention time (retention time optional) to see all possible mass matches. If retention times are expected to

vary significantly compared with the retention time information in the database, set the Values to Match to Mass to see all possible mass matches over the entire chromatogram.

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