



MassHunter METLIN Metabolite PCD/PCDL

Quick Start Guide

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What is the MassHunter METLIN Metabolite PCD/PCDL?

Where to find more information

What is the MassHunter METLIN Metabolite PCD/PCDL?

The MassHunter METLIN Metabolite PCD (Personal Compound Database) and PCDL (Personal Compound Database and Library) are amongst the best-known and most-comprehensive metabolite databases in the world today. They currently include annotated lists of over 64,000 endogenous and exogenous metabolites, as well lipids, drugs, drug metabolites, and di- and tripeptides repeats. Each entry may include mass, chemical formula, and structure information, as well as ID numbers that link to more information about the compound, such as the CAS and ChemSpider IDs.

The MassHunter METLIN Metabolite PCD/PCDL can be used with preloaded METLIN content that is periodically updated. It can also be used as the basis for your own customized PCD or PCDL.

You can use the Agilent Personal Compound Database and Library Manager to add, remove and edit the compounds in your PCD/PCDL to meet the specific needs of your laboratory and your analyses. You can add retention times to your customized PCD/PCDL based on standards and/or retention times for compounds you analyze. You can also add your own spectra to your customized PCDL, in addition to those provided in the master PCDL.

The high mass accuracy of the Agilent tandem quadrupole time-of-flight (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.

Terminology Note

A **PCD** is an accurate mass compound database, which may or may not contain retention times. A **PCDL** contains both an accurate mass compound database and an MS/MS accurate mass spectral database, which is often referred to as a spectral library or library.

Where to find more information

Go to <http://www.chem.agilent.com/> for the most current information on Agilent products.

Kit Content

You will receive the following manuals and media when you purchase the MassHunter METLIN Metabolite PCD/PCDL.

- *MassHunter METLIN Metabolite PCD/PCDL Quick Start Guide*, which tells you how to set up methods to acquire MS data that can be searched in the MassHunter METLIN Metabolite PCD/PCDL.
- *MassHunter Personal Compound Database and Library Manager Quick Start Guide*, which tells you how to install and use the MassHunter Personal Compound Database and Library Manager.

MassHunter Personal Compound Database and Library Manager disc This disc contains:

- MassHunter Personal Compound Database and Library Manager
- *MassHunter Personal Compound Database and Library Manager Quick Start Guide* (PDF)
- Software license agreements
- Example data

PCD version **MassHunter METLIN Metabolite PCD disc** This disc contains:

- MassHunter METLIN Metabolite PCD files:
 - **Metlin_AM_PCD.cdb** (accurate mass compound database)
 - **Metlin_AMRT_PCD.cdb** (accurate mass compound database with retention times)
 - **Metlin_Metabolites_AM_PCD.cdb** (subset of **Metlin_AM_PCD.cdb** that includes only the metabolites that have a KEGG or HMP ID)
 - **Metlin_Lipids_AM_PCD.cdb** (subset of **Metlin_AM_PCD.cdb** that includes only lipids)
- *MassHunter METLIN Metabolite PCD/PCDL Quick Start Guide* (PDF)
- MassHunter METLIN Metabolite PCD compound listing (PDF)
- Example metabolite data files

What is the MassHunter METLIN Metabolite PCD/PCDL?

Kit Content

- PCDL version** **MassHunter METLIN Metabolite PCDL disc** This disc contains:
- MassHunter METLIN Metabolite PCDL files:
 - **Metlin_AM_PCDL.cdb** (accurate mass compound database and accurate mass MS/MS spectral library)
 - **Metlin_AMRT_PCDL.cdb** (accurate mass compound database with retention times and accurate mass MS/MS spectral library)
 - **Metlin_Metabolites_AM_PCDL.cdb** (subset of **Metlin_AM_PCDL.cdb** that includes only the metabolites that have a KEGG or HMP ID)
 - **Metlin_Lipids_AM_PCDL.cdb** (subset of **Metlin_AM_PCDL.cdb** that includes only lipids)
 - *MassHunter METLIN Metabolite PCD/PCDL Quick Start Guide* (PDF)
 - MassHunter METLIN Metabolite PCDL compound listing (PDF)
 - Example metabolite data files

Installation

Follow these instructions to install MassHunter PCDL Manager and the MassHunter METLIN Metabolite PCD or PCDL on your computer.

Before you begin

To acquire data

- 1 Check that the Agilent 1200 Series Infinity LC is properly installed and verified.
- 2 Check that *one* of the following instruments is properly installed and verified:
 - Agilent 6200 Series Time-of-Flight LC/MS (TOF), *or*
 - Agilent 6500 Series Quadrupole Time-of-Flight (Q-TOF)

NOTE

A Q-TOF LC/MS instrument is needed to use the MS/MS features in a PCDL. A TOF LC/MS instrument does not produce MS/MS spectra.

To analyze data

- 1 Check that the following programs are properly installed:
 - MassHunter Data Acquisition B.04.00 or higher
 - MassHunter Qualitative Analysis B.05.00 or higher
- 2 Install MassHunter PCDL Manager (B.04.00 SP1 or higher), if it is not currently installed on your computer. Refer to the *MassHunter Personal Compound Database and Library Manager Quick Start Guide* for installation instructions.

If PCDL Manager B.04.00 or lower is installed, remove the program before you install PCDL Manager B.04.01 SP1 or higher.
- 3 Proceed to install *either* the MassHunter METLIN Metabolite PCD or PCDL as described on the next page.

Install MassHunter METLIN Metabolite PCD or PCDL

- 1 Place the *MassHunter METLIN Metabolite PCD* (or *PCDL*) disc in your computer drive.

The Contents and Information screen appears. If it does not, double-click **Start.htm** on the installation disc.

- 2 In the Contents and Information screen:
 - a Click **METLIN Metabolite PCD** (or **PCDL**) **Installation**.
 - b Follow the on-screen instructions to install all four PCD or PCDLs, two files at a time (Step 1 and Step 2).

For PCD, these files are installed on your computer:

- **MassHunter\PCDL\Metlin_AM_PCD.cdb**
- **MassHunter\PCDL\Metlin_AMRT_PCD.cdb**
- **MassHunter\PCDL\Metlin_Metabolites_AM_PCD.cdb**
- **MassHunter\PCDL\Metlin_Lipids_AM_PCD.cdb**

For PCDL, these files are installed on your computer:

- **MassHunter\PCDL\Metlin_AM_PCDL.cdb**
- **MassHunter\PCDL\Metlin_AMRT_PCDL.cdb**
- **MassHunter\PCDL\Metlin_Metabolites_AM_PCDL.cdb**
- **MassHunter\PCDL\Metlin_Lipids_AM_PCDL.cdb**

- 3 When the installation is complete, remove the disc from the drive.

Searching and managing the METLIN Metabolite PCD/PCDL

Identifying compounds and spectrum peaks using Qualitative Analysis

The following capabilities are available for a *MassHunter METLIN Metabolite PCD/PCDL*:

If you want to...	See this online Help topic in Qualitative Analysis:	Click this menu item:
Search an accurate mass database (PCD) to identify compounds (with or without retention times)	<i>Identifying Compounds > Search database for a compound</i>	Identify > Search Database for Compounds
Search an accurate mass database (PCD) to identify compounds from spectrum peaks	<i>Spectrum Tasks > Search database from a spectrum</i>	Identify > Search Database for Spectrum Peaks

The following capabilities are available for a *MassHunter METLIN Metabolite PCDL-only*:

If you want to...	See this online Help topic in Qualitative Analysis:	Click this menu item:
Search an accurate mass spectral library (PCDL) to identify compounds	<i>Identifying Compounds > Search accurate mass library for compounds</i>	Identify > Search Library for Compounds
Search an accurate mass spectral library (PCDL) for spectra	<i>Spectrum Tasks > Search accurate mass library for spectra</i>	Identify > Search Library for Spectra

Managing METLIN Metabolite PCD/PCDL content with PCDL Manager

Manage the content of your MassHunter METLIN Metabolite PCD/PCDL using the Agilent Personal Compound Database and Library (PCDL) Manager:

- Create and edit custom PCDs and PCDLs, including adding proprietary compounds, retention times, and MS/MS spectra.
- Search and store MS/MS centroid spectra acquired on a Q-TOF instrument.
- Search or browse MS/MS spectra.
- Search for compounds in PCDs and PCDLs, using text, formula, accurate mass, and retention time (optional or required).
- Import mass lists from MassHunter Mass Profiler, Mass Profiler Professional, and MassHunter Qualitative Analysis software (for accurate mass and optional retention time searching only).
- Load spectra from either a **.CEF** file or by copy-and-pasting mass spectra from MassHunter Qualitative Analysis software and search for those spectra in the current PCDL.
- Do private, on-site searches, which keep intellectual property safe.
- Link to web sites for more information on many compounds.

For more information, see the *MassHunter Personal Compound Database and Library Manager Quick Start Guide* and PCDL Manager online Help.

Chromatography Conditions

This section provides the LC/MS operating conditions that will let you successfully search and identify compounds and spectra in your data files using the MassHunter METLIN Metabolite PCD/PCDL with both accurate mass and retention time matching. The Agilent HPLC method is based on reverse phase separation. Note that this separation does not work well for the very hydrophilic compounds such as sugars, amino acids and organic acids, which are best analyzed by a HILIC type of analysis.

- 1** To track retention time stability and mass spectrometer abundance drift during analysis, add an internal standard (9-Anthracene carboxylic acid at 1 ng/ μ L) to the dissolution solvent.

9-Anthracene carboxylic acid is detectable in ESI and APCI in both positive and negative ion modes.

- 2** To resuspend dried samples:

- a** First add 50 μ L of a solution of 9-Anthracene carboxylic acid at 2 ng/ μ L in methanol.
- b** Then add 50 μ L of a solution of 0.4% (v/v) acetic acid in water.
- c** Vortex the sample after each addition of solvent to facilitate good recovery of hydrophobic metabolites such as the free fatty acids.

- 3** Use the following instrumentation to analyze the samples:

- Agilent 1260 SL System with Binary Pump (1312B), plumbed for minimum delay volume as described in the pump manual. For more information, see “[Capillary tubing ordering information](#)” on page 11.
- Agilent Solvent Degasser (G1379B)
- Agilent Autosampler (G1367C) and Thermostat (G1330B)
- Agilent Column Compartment (G1316C)
- Agilent 6200 Series TOF LC/MS or 6500 Series Q-TOF LC/MS instrument with Dual ESI Source (G3251B)

- 4** Set up an acquisition method in the MassHunter Data Acquisition program for your sample analyses, using the parameters given in the following sections:

- “[Positive Ion Polarity Analysis](#)” on page 12
- “[Negative Ion Polarity Analysis](#)” on page 15

Chromatography Conditions

Managing METLIN Metabolite PCD/PCDL content with PCDL Manager

- 5 Identify compounds using the MassHunter METLIN Metabolite PCD by accurate mass and retention time matching (AMRT).

See *MassHunter Qualitative Analysis online Help* for information on searching accurate mass databases.

- 6 (*PCDL-only*) Identify compounds using the MassHunter METLIN Metabolite PCDL by accurate mass library spectral matching.

See *MassHunter Qualitative Analysis online Help* for information on searching accurate mass MS/MS libraries.

Capillary tubing ordering information

This section provides the information to order the capillary tubing that is used to plumb the system for minimum delay volume, which is required for the Universal RP-AMRT method.

Description	Part Number
SS connecting capillary, 700 mm, 0.17mm (green) as outlet capillary from pump to injector	G1312-87304
Capillary connect from autosampler to column compartment: flexible capillary tubing (<i>one</i> of the following)	
<ul style="list-style-type: none"> • 340 mm, 0.12 mm id (red), connect directly to the heater block in the TCC • 300 mm, 0.12 mm id (red), connect directly to the switching valve in the TCC 	<ul style="list-style-type: none"> • G1316-87319 <i>or</i> • G1316-87318
Stainless steel guard column: Zorbax-SB-C8 Rapid resolution cartridge (2.1×30mm 3.5 μm)	873700-936
Rapid resolution cartridge holder - hardware kit	820555-901
Separation column: Zorbax SB-Aq 1.8 μm 2.1×50mm	827700-914
Attached to the rapid resolution system using (2) capillary connects: stainless steel tubing: 70mm, 0.12 mm ID (red label)	G1316-87303
<ul style="list-style-type: none"> • Heater to guard column • Guard column to analytical column 	
PEEK tubing (red), 0.005"/0.13mm, 1/16 OD, 5.0M; cut to 650 mm in length to connect the analytical column to MS equipped with dual ESI source	5042-6461

Positive Ion Polarity Analysis

The following conditions were used to determine the retention time data for many metabolite standards, which are contained in the MassHunter METLIN Metabolite PCD.

Table 1 Autosampler and Column

Parameter or Component	Value or Description
Injection volume	2 μ L
Autosampler temperature	4°C
Column temperature	60°C
Guard column	Zorbax-SB-C8 (p/n 873700-936) Rapid Resolution Cartridge (2.1 \times 30mm 3.5 μ m) using hardware kit (p/n 820555-901)
Analytical column	Zorbax SB-Aq 1.8 μ m 2.1 \times 50mm (p/n 827700-914)

Table 2 Pump

Parameter	Value								
Flow rate	0.6 mL/minute								
Mobile phase	A = 0.2% (v/v) acetic acid in water B = 0.2% (v/v) acetic acid in methanol 2% A / 98% B								
Gradient	<table border="1"> <thead> <tr> <th>Time</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>2</td> </tr> <tr> <td>13</td> <td>98</td> </tr> <tr> <td>19</td> <td>98</td> </tr> </tbody> </table>	Time	%B	0	2	13	98	19	98
Time	%B								
0	2								
13	98								
19	98								
Run time	19 minutes								
Post time	5 minutes								

Table 3 Dual ESI Source Settings

Parameter	Value
Gas Temp	325°C
Drying Gas	10 L/minute
Nebulizer pressure	45 psig
VCap	4000V
Fragmentor	140V
Skimmer	65V
Oct 1 RF Vpp	750 V

Table 4 TOF / Q-TOF Acquisition Settings

Parameter	Value
Mode	Positive Ion Mode
Mass Range	1700 <i>m/z</i>
Instrument Mode	Extended Dynamic Range
Data Storage	Centroid
Abs. threshold	100
Rel. threshold(%)	0.001

Table 5 Ion Funnel Settings

Parameter	Value
Funnel DC	30V
Funnel Voltage Drop	High Pressure Funnel = 100V Low Pressure Funnel = 50V
RF Voltage	High Pressure Funnel = 90V Low Pressure Funnel = 40V

Chromatography Conditions

Positive Ion Polarity Analysis

Table 6 Dual ESI Source Settings

Parameter	Value
Gas Temp	325°C
Drying Gas	10 L/minute
Nebulizer pressure	45 psig
VCap	4000V
Fragmentor	140V
Skimmer	65V
Oct 1 RF Vpp	750 V

Table 7 Spectral Parameters

Parameter	Value
Stored Mass Range	50 - 1600 <i>m/z</i>
Acquisition Rate	1.5 spectra /sec
Reference Mass Correction	Enabled
Reference Masses	121.050873, 922.009798 To achieve optimal results, introduce the reference masses via a separate isocratic pump that uses a CE splitter at 1:100.

Negative Ion Polarity Analysis

The following conditions were used to determine the retention time data for many metabolite standards, which are contained in the MassHunter METLIN Metabolite PCD.

Table 8 Autosampler and Column

Parameter or Component	Value or Description
Injection volume	2 μ L
Autosampler temperature	4°C
Column temperature	60°C
Guard column	Zorbax-SB-C8 (p/n 873700-936) Rapid Resolution Cartridge (2.1×30mm 3.5 μ m) using hardware kit (p/n 820555-901)
Analytical column	Zorbax SB-Aq 1.8 μ m 2.1×50mm (p/n 827700-914)

Table 9 Pump

Parameter	Value								
Flow rate	0.6 mL/minute								
Mobile phase	A = 0.2% (v/v) acetic acid in water B = 0.2% (v/v) acetic acid in methanol 2% A / 98% B								
Gradient	<table border="1"> <thead> <tr> <th>Time</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>2</td> </tr> <tr> <td>13</td> <td>98</td> </tr> <tr> <td>19</td> <td>98</td> </tr> </tbody> </table>	Time	%B	0	2	13	98	19	98
Time	%B								
0	2								
13	98								
19	98								
Run time	19 minutes								
Post time	5 minutes								

Chromatography Conditions

Negative Ion Polarity Analysis

Table 10 TOF / Q-TOF Acquisition Settings

Parameter	Value
Mode	Negative Ion Mode
Mass Range	1700 <i>m/z</i>
Instrument Mode	Extended Dynamic Range
Data Storage	Centroid
Abs. threshold	100
Rel. threshold(%)	0.001

Table 11 Ion Funnel Settings

Parameter	Value
Funnel DC	30V
Funnel Voltage Drop	High Pressure Funnel = 100V Low Pressure Funnel = 50V
RF Voltage	High Pressure Funnel = 90V Low Pressure Funnel = 40V

Table 12 Dual ESI Source Settings

Parameter	Value
Gas Temp	325°C
Drying Gas	10 L/minute
Nebulizer pressure	45 psig
VCap	3500V
Fragmentor	140V
Skimmer	65V
Oct 1 RF Vpp	750 V

Table 13 Spectral Parameters

Parameter	Value
Stored Mass Range	50 - 1600 <i>m/z</i>
Acquisition Rate	1.5 spectra /sec
Reference Mass Correction	Enabled
Reference Masses	119.03632, 980.016375 To achieve optimal results, introduce the reference masses via a separate isocratic pump that uses a CE splitter at 1:100.

www.agilent.com

In This Guide

This Quick Start Guide describes how to install and use the Agilent MassHunter METLIN Metabolite PCD/PCDL.

This information is subject to change without notice. Agilent Technologies shall not be liable for errors contained herein or for incidental or consequential damages with the furnishing, performance or use of this material. Agilent specifically disclaims any warranties for any implied warranties of merchantability or fitness for a particular purpose.

This guide is valid for the B.05.00 revision or higher of the MassHunter METLIN Metabolite PCD/PCDL, until superseded.

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