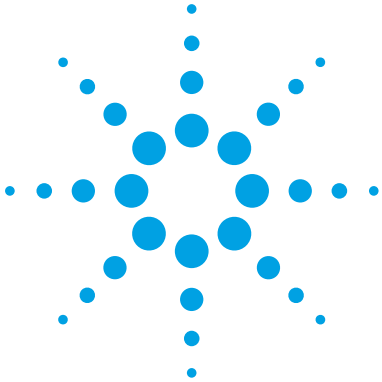

MassHunter METLIN Metabolite PCD/PCDL

Quick Start Guide

Agilent Technologies

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. The databases currently include annotated lists of:

- over 30,000 metabolites and metabolite-related compounds with KEGG, HMDB, ChEBI, or BioCyc IDs
- Approximately 39,000 lipids and lipid-related compounds
- 169,400 theoretical di-, tri- and tetrapeptides

MassHunter METLIN Metabolite PCD and PCDL are also provided in three convenient subsets; Metabolites, Lipids and Theoretical Peptides.

Each entry can include mass, chemical formula, and structure information, as well as ID numbers that link to more information about the compound, such as the KEGG, HMDB, Lipid MAPS, ChEBI, BioCyc, PubChem, 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 add your own spectra to your customized PCDL, in addition to those provided in the master PCDL. In addition, you can import, edit and view ion mobility information, such as collision cross section values for use with the Agilent IM-QTOF mass spectrometer.

With MassHunter Qualitative Analysis B.07.00 and higher, you can send GC/MS and MS/MS spectra to PCDL Manager. After you do a Find by Formula search to identify compounds in your sample data, you can then send the GC/MS or MS/MS spectra to your custom PCDL. However, when you send spectra, make sure to use compatible software versions, such as version B.08 for both Qualitative Analysis and PCDL Manager. You can also filter spectral noise and correct the fragment ions to their theoretical accurate mass.

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 fragment 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; contains the complete METLIN content except for theoretical tetrapeptides)
  - **Metlin_AMRT_PCD.cdb** (accurate mass compound database with retention times; contains the complete METLIN content except for theoretical tetrapeptides)
  - **Metlin_Metabolites_AM_PCD.cdb** (subset of **Metlin_AM_PCD.cdb** that includes only the metabolites that have a KEGG, HMDB, ChEBI, or BioCyc ID)
  - **Metlin_Lipids_AM_PCD.cdb** (subset of **Metlin_AM_PCD.cdb** that includes only lipids)
  - **Metlin_Peptides_AM_PCD.cdb** (accurate mass compound database; contains only theoretical di-, tri- and tetrapeptides)
- *MassHunter METLIN Metabolite PCD/PCDL Quick Start Guide* (PDF)
- MassHunter METLIN Metabolite PCD compound listing (PDF)
- Example metabolite data files

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; contains the complete METLIN content except for theoretical tetrapeptides)
  - **Metlin_AMRT_PCDL.cdb** (accurate mass compound database with retention times and accurate mass MS/MS spectral library; contains the complete METLIN content except for theoretical tetrapeptides)
  - **Metlin_Metabolites_AM_PCDL.cdb** (subset of **Metlin_AM_PCDL.cdb** that includes only the metabolites that have a KEGG, HMDB, ChEBI, or BioCyc ID)
  - **Metlin_Lipids_AM_PCDL.cdb** (subset of **Metlin_AM_PCDL.cdb** that includes only lipids)
  - **Metlin_Peptides_AM_PCD.cdb** (accurate mass compound database containing only theoretical di-, tri- and tetrapeptides)
- *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)

3 Check that the MassHunter Data Acquisition software that is appropriate for your instrument is installed.

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

To analyze data

1 Check that MassHunter Qualitative Analysis B.07.00 or higher is properly installed.

2 Check that MassHunter PCDL Manager (B.08.00 SP1 or higher) is properly installed. Refer to the *MassHunter Personal Compound Database and Library Manager Quick Start Guide* for installation instructions. (If SP1 is not found on the PCDL Manager installation media, look for it on the METLIN Metabolite PCD or PCDL installation media.)

If PCDL Manager B.04.01 SP1 or lower is installed, remove the program before you install PCDL Manager B.08.00 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. Insert the installation media into the installation drive.

   If the installation screen does not open, double-click **Start.bat** on the installation media.

2. On the **Installation** page, click **Install**.

3. Click **Complete** to install all PCDs or PCDLs, and supplemental files.

   The complete installation can take several minutes to complete.

   For PCD, these files are installed on your computer in the indicated pairs:

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

   For PCDL, these files are installed on your computer in the indicated pairs where applicable:

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

4. 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 MassHunter Qualitative Analysis

Table 1 lists ways to use the MassHunter Qualitative Analysis B.08.00 programs to search the PCDL to identify compounds and spectrum peaks.

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)	In the MassHunter Qualitative Analysis **Workflows** program: **Target/Suspect Screening > Find by Formula**	Find compounds by formula
Search the library and database based on MS and MS/MS spectral information from compound features (with or without retention times)	In the MassHunter Qualitative Analysis **Workflows** program: **Compound Identification > Search Library/DB for All or Selected Compounds**	Search library/database for a compound
Identify compounds from spectrum peaks (with or without retention times)	In the MassHunter Qualitative Analysis **Navigator** program: **Identify Spectra > Search Library/DB for Selected Spectra**	Search library/database for a spectrum

Managing METLIN Metabolite PCD/PCDL content with PCDL Manager

Use the MassHunter Personal Compound Database and Library (PCDL) Manager to manage the content of your PCDL:

- Create custom PCDLs, specific to your analysis by searching using compound name, formula, and mass, for IDs such as KEGG, HMDB, Lipid MAPS, ChEBI, BioCyc, PubChem or ChemSpider, CAS registry number or IUPAC name.
- Edit custom PCDLs, including adding proprietary compounds, retention times, collision cross sections, and raw experimental or theoretically corrected MS/MS spectra.
- Search and view MS/MS centroid spectra acquired on a Q-TOF instrument.
- Search for compounds in PCDLs, using text, formula, accurate mass, and retention time (optional or required).
- Import retention times and collision cross sections from CSV files.
- Send spectra to your customized PCDL directly from the Qualitative Analysis programs to create your own custom library. Choose from options to filter spectral noise and/or to correct the fragment ions to their theoretical accurate mass.
- 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.
- Create new spectra from published work by adding m/z versus relative abundance values.
- 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 retention times in the METLIN_AMRT_PCD/PCDL were determined based on the chromatographic conditions described in this section. 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. To account for possible retention time drifts during compound identification, adjust the retention related parameters in the MassHunter Qualitative Data Analysis Workflows program.

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/µL) 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 µL of a solution of 9-Anthracene carboxylic acid at 2 ng/µL in methanol.
   b. Then add 50 µL 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) or newer, plumbed for minimum delay volume as described in the pump manual. For more information, see “Capillary tubing ordering information” on page 12.
   - Agilent Solvent Degasser (G1379B or newer)

- Agilent Autosampler (G1367C or newer) and Thermostat (G1330B or newer)
- Agilent Column Compartment (G1316C or newer)
- Agilent 6200 Series TOF LC/MS or 6500 Series Q-TOF LC/MS instrument with Dual ESI Source (G3251B or newer)

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 13
- "Negative Ion Polarity Analysis" on page 16

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.

Table 2 Capillary tubing parts

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 (*one* of the following) • 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	 • G1316-87319 *or* • 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) • Heater to guard column • Guard column to analytical column	G1316-87303
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 3 Autosampler and Column

Parameter or Component	Value or Description
Injection volume	2 µL
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 µm) using hardware kit (p/n 820555-901)
Analytical column	Zorbax SB-Aq 1.8 µm 2.1×50mm (p/n 827700-914)

Table 4 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	**Time** **%B** 0 2 13 98 19 98
Run time	19 minutes
Post time	5 minutes

Table 5 TOF / Q-TOF Acquisition Settings

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

Table 6 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 7 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 8 Spectral Parameters

Parameter	Value
Stored Mass Range	50 - 1600 *m/z*
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 9 Autosampler and Column

Parameter or Component	Value or Description
Injection volume	2 µL
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 µm) using hardware kit (p/n 820555-901)
Analytical column	Zorbax SB-Aq 1.8 µm 2.1×50mm (p/n 827700-914)

Table 10 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	**Time** **%B** 0 2 13 98 19 98
Run time	19 minutes
Post time	5 minutes

Table 11 TOF / Q-TOF Acquisition Settings

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

Table 12 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 13 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 14 Spectral Parameters

Parameter	Value
Stored Mass Range	50 - 1600 *m/z*
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.

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In This Guide

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

This guide is valid for the B.08.00 revision or higher of the MassHunter METLIN Metabolite PCD/PCDL, until superseded.

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