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) is one of the best-known and most-comprehensive metabolite databases in the world today. It currently includes annotated lists of over 24,000 endogenous and exogenous metabolites, as well as di- and tripeptides. 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 as is, or as the basis of your own customized PCD or PCDL. You can add, remove and change the compounds in your PCD/PCDL to meet the specific needs of your laboratory and your analyses. Your customized PCD/PCDL can include the 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 disk This disk 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 disk This disk 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)
- *MassHunter METLIN Metabolite PCD/PCDL Quick Start Guide* (PDF)
- MassHunter METLIN Metabolite PCD compound listing (PDF)
- Example metabolite data files
- A file that contains a list of the METLIN Metabolite PCD compounds.

PCDL version

MassHunter METLIN Metabolite PCDL disk This disk 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)
- *MassHunter METLIN Metabolite PCD/PCDL Quick Start Guide* (PDF)
- MassHunter METLIN Metabolite PCDL compound listing (PDF)
- Example metabolite data files
- A file that contains a list of the METLIN Metabolite PCDL compounds

Installation

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

Before you start

1. Check that the Agilent 1200 Series 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 instrument is needed to use the MS/MS features in a PCDL. A TOF instrument does not produce MS/MS spectra.

3. Check that the following programs are properly installed:
   - MassHunter Data Acquisition B.04.00 or higher
   - MassHunter Qualitative Analysis B.04.00 or higher
4. Install MassHunter PCDL Manager, 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.
5. Proceed to install *either* the MassHunter METLIN Metabolite PCD or PCDL as described on the next page.

Install MassHunter METLIN Metabolite PCD

1. Place the *MassHunter METLIN Metabolite PCD* disk in your computer drive.
2. When the Contents and Information screen appears:
   a. Click **METLIN Metabolite PCD Installation**.
   b. Click **Install the METLIN Metabolite AM and AMRT PCD files**.

   The following files are installed on your computer:
   - **MassHunter\PCDL\Metlin_AM_PCD.cdb**
   - **MassHunter\PCDL\Metlin_AMRT_PCD.cdb**
3. When the installation is complete, remove the disk from the drive.

Install MassHunter METLIN Metabolite PCDL

1. Place the *MassHunter METLIN Metabolite PCDL* disk in your computer drive.
2. When the Contents and Information screen appears:
   a. Click **METLIN Metabolite PCDL Installation**.
   b. Click **Install the METLIN Metabolite AM and AMRT PCDL files**.

   The following files are installed on your computer:
   - **MassHunter\PCDL\Metlin_AM_PCDL.cdb**
   - **MassHunter\PCDL\Metlin_AMRT_PCDL.cdb**
3. When the installation is complete, remove the disk 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)	*Identifying Compounds* > *Search database for a compound*	**Identify > Search Database for Compounds**
Search an accurate mass database (PCD) to identify compounds from spectrum peaks	*Spectrum Tasks* > *Search database from a spectrum*	**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	*Identifying Compounds* > *Search accurate mass library for compounds*	**Identify > Search Library for Compounds**
Search an accurate mass spectral library (PCDL) for spectra	*Spectrum Tasks* > *Search accurate mass library for spectra*	**Identify > Search Library for Spectra**

Managing METLIN Metabolite PCD/PCDL content with PCDL Manager

PCDL Manager lets you do the following to manage the content of your MassHunter METLIN Metabolite PCD/PCDL:

- 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 Agilent MassHunter Mass Profiler, GeneSpring MS, and 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/ l) to the dissolution solvent.

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

2. To re-suspend 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), 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 TOF (62XX series) or Q/TOF (65XX series) 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 14

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.

Part Number	Description
G1312-87304	SS connecting capillary, 700 mm, 0.17mm (green) as outlet capillary from pump to injector
Capillary connect from autosampler to column compartment: flexible capillary tubing (*one* of the following)	
• G1316-87319	• 340 mm, 0.12 mm id (red), connect directly to the heater block in the TCC
• G1316-87318	• 300 mm, 0.12 mm id (red), connect directly to the switching valve in the TCC
873700-936	Stainless steel guard column: Zorbax-SB-C8 Rapid resolution cartridge (2.1X 30mm 3.5 µm)
820555-901	Rapid resolution cartridge holder - hardware kit
827700-914	Separation column: Zorbax SB-Aq 1.8 µm 2.1 X 50mm
G1316-87303	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
5042-6461	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

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.

Autosampler and Column

Thermostat temperature: 4°C

Injection volume: 2 µL

Column temperature: 60°C

Guard column: Zorbax-SB-C8 (P/N: 873700-936) Rapid Resolution Cartridge (2.1X 30mm 3.5 µm) using hardware kit (P/N: 820555-901).

Analytical column: Zorbax SB-Aq 1.8 µm 2.1 X 50mm (P/N: 827700-914)

Pump

Flow rate: 0.6 mL/minute

Solvent A: 0.2% (v/v) acetic acid in water

Solvent B: 0.2% (v/v) acetic acid in methanol

Initial solvent composition: 2% A / 98% B

Gradient

Time	%B
0	2
13	98
19	98

Stop Time: 19 minutes

Post Time: 5 minutes

TOF / QTOF Acquisition Settings

Positive Ion Mode

Mass Range: 1700 *m/z*

Instrument Mode: Extended Dynamic Range

Data Storage: Centroid

Abs. threshold: 100

Rel. threshold(%): 0.001

Dual ESI Source Settings

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

Spectral Parameters

Stored Mass Range: 50 - 1600 m/z

Acquisition Rate: 1.5 spectra /sec

Reference Mass Correction: Enabled

Reference Masses: 121.050873, 922.009798

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.

Autosampler and Column

Thermostat temperature: 4°C

Injection volume: 2 µL

Column temperature: 60°C

Guard column: Zorbax-SB-C8 (P/N: 873700-936) Rapid Resolution Cartridge (2.1X 30mm 3.5 µm) using hardware kit (P/N: 820555-901).

Analytical column: Zorbax SB-Aq 1.8 µm 2.1 X 50mm (P/N: 827700-914)

Pump

Flow rate: 0.6 mL/minute

Solvent A: 0.2% (v/v) acetic acid in water

Solvent B: 0.2% (v/v) acetic acid in methanol

Initial solvent composition: 2% A / 98% B

Gradient

Time	%B
0	2
13	98
19	98

Stop Time: 19 minutes

Post Time: 5 minutes

TOF / QTOF Acquisition Settings

Negative Ion Mode

Mass Range: 1700 *m/z*

Instrument Mode: Extended Dynamic Range

Data Storage: Centroid

Abs. threshold: 100

Rel. threshold(%): 0.001

Dual ESI Source Settings

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

Spectral Parameters

Stored Mass Range: 50 - 1600 m/z

Acquisition Rate: 1.5 spectra /sec

Reference Mass Correction: Enabled

Reference Masses: 119.03632, 980.016375

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In This Guide

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

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This guide is valid for the B.04.00 revision or higher of the MassHunter METLIN Metabolite PCD/PCDL, until superseded.

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