Agilent

METLIN PCDL

User Guide

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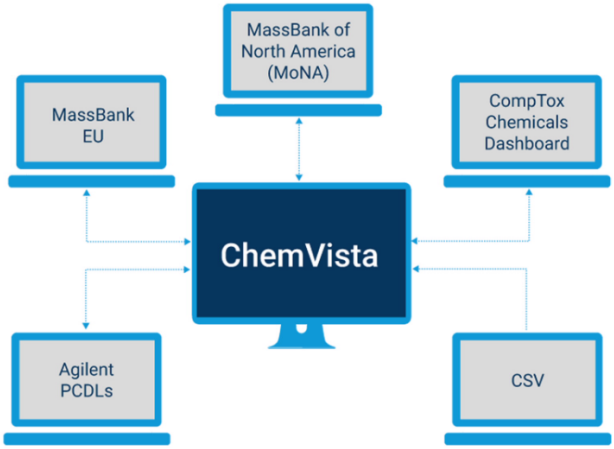

Notices

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Software Revision

This guide is valid for the C.01.00 revision or higher of the METLIN PCDL program and compatible METLIN PCDL programs, until superseded.

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In This Book

This book describes the METLIN Metabolite Personal Compound Database and Library (PCDL).

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1 Overview

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This chapter provides an overview of the MassHunter METLIN Metabolite Personal Compound Database and Library.

What is the METLIN PCDL?

The METLIN PCDL (Personal Compound Database and Library) is 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

METLIN 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. When used in tandem with Agilent ChemVista Library Manager, subsets are easily created using class tags (e.g. Estrogen, Designer Drug, Polyphenol), regulatory information, etc.

Working with your MassHunter PCDL

You can use the MassHunter METLIN Metabolite 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 refine 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 lists 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

Table 1. Identifying Features

If you want to edit the method to...	Select this Method Editor section	Refer to Online Help topic
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. It may be desirable in certain circumstances to edit or turn off the classification feature in ChemVista 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 individual compound 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. If desired, 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:

- METLIN 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)
- The METLIN PCDL compound listing (PDF)
- Example metabolite 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 Method Setup

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This chapter describes the METLIN PCDL configuration and setup.

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 METLIN PCDL with both accurate mass and retention time matching.

The retention times in the METLIN_AMRT_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 resuspension.
3. Use the following instrumentation to analyze the samples:
   - **a** 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 15.
   - **b** Agilent Solvent Degasser (G1379B or newer).
   - **c** Agilent Autosampler (G1367C or newer) and Thermostat (G1330B or newer).
   - **d** Agilent Column Compartment (G1316C or newer).
   - **e** 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:
   a **"Positive Ion Polarity Analysis"** on page 16
   b **"Negative Ion Polarity Analysis"** on page 18
5 Identify compounds using the MassHunter METLIN Metabolite PCDL 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 METLIN 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	61312-87304
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	 - 61316-87319 *or* - 61316-87318
Stainless steel guard column: Zorbax-SB-C8 Rapid resolution cartridge (2.1x30mm 3.5 μm)	873700-936
Rapid resolution cartridge holder - hardware kit	820555-901
Separation column: Zorbax SB-Ag 1.8 μm 2.1x50mm	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	61316-87303
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 ES! 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 PCDL.

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 (2.1x30mm 3.5 pm) using hardware kit (p/n 820555-901)
Analytical column	Zorbax SB-Aq 1.8 µm 2.1x50mm (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	750V

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 PCDL.

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 (2.1x30mm 3.5 pm) using hardware kit (p/n 820555-901)
Analytical column	Zorbax SB-Aq 1.8 µm 2.1x50mm (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

Table 10. Pump

Parameter	Value
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

Table 13. Dual ESI Source Settings

Parameter	Value
VCap	3500V
Fragmentor	140V
Skimmer	65V
Oct 1 RF Vpp	750V

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	119.053632, 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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