MassHunter Forensics and Toxicology PCD Kit

Quick Start Guide

What is the MassHunter Forensics and Toxicology PCD Kit?

The MassHunter Forensics and Toxicology PCD Kit lets you screen over 7300 analytes of forensic and toxicological interest with accurate mass measurement, all in a single LC/MS analysis.

The MassHunter Forensics and Toxicology PCD Kit helps minimize method development time for your Forensics and Toxicology analysis. The database can store your retention times for compounds you target along with all entries in new

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databases that you name. You can add, remove and change the compounds in the database to meet the specific needs of your laboratory and your analyses.

The high mass accuracy of the Agilent time-of-flight (TOF) and tandem quadrupole time-of-flight (Q-TOF) LC/MS instrument provides the capability to screen and identify all compounds in the database that are detected by their exact mass and retention time (if known). Retention times can be a search criterion specified as not required (non-targeted screen), as optional providing a targeted and non-targeted forensic and toxicological screen, or required (targeted screen only).

Kit Content

MassHunter Forensics and Toxicology PCD Kit Quick Start Guide The Quick Start Guide gives an overview of the MassHunter Forensics and Toxicology PCD Kit and tells you how to use it.

MassHunter Personal Compound Database and Library Manager Quick Start Guide

The Quick Start Guide gives you an overview of the MassHunter Personal Compound Database and Library Manager and tells you how to use it with the MassHunter Forensics and Toxicology PCD.

MassHunter Forensics and Toxicology PCD Kit Disk This disk contains:

- MassHunter Forensics and Toxicology PCD
- *MassHunter Forensics and Toxicology PCD Kit Quick Start Guide* (PDF)
- Technical note on accurate mass database
- The TOF/Q-TOF LC/MS method **Forensic_Tox_Test_Mix_TOF.m** for running the test mix (positive ion mode)
- Example methods for acquisition and data analysis
- A sample chromatogram and database screening report obtained with the test mix
- A file that contains a list of the Forensics and Toxicology PCD compounds

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

ZORBAX LC Column (p/n 959764-902) Eclipse Plus C18, 2.1mm x 100, 1.8 µm.

LC/MS Toxicology Test Mix (p/n 5190-0470) Test Mix containing 25 analytes of forensic and toxicological interest (4 ampoules) for your test runs. The contents of this are listed in Table 1.

Table 1 LC/MS Forensics and Toxicology Test Mix Content

Compound Name	Formula	Mass
3,4-Methylendioxyamphetamine (MDA)	C10H13NO2	179.09463
3,4-Methylenedioxyethamphetamine (MDEA)	C12H17NO2	207.12593
Alprazolam	C17H13ClN4	308.08287
Clonazepam	C15H10ClN3O3	315.04107
Cocaine	C17H21NO4	303.14706
Codeine	C18H21NO3	299.15214
delta9-Tetrahydrocannabinol (THC)	C21H30O2	314.22458
Diazepam	C16H13ClN2O	284.07164
Heroin	C21H23NO5	369.15762
Hydrocodone	C18H21NO3	299.15214
Lorazepam	C15H10Cl2N2O2	320.01193
Meperidine (Pethidine)	C15H21NO2	247.15723
Methadone	C21H27NO	309.20926
Methamphetamine	C10H15N	149.12045
Methylendioxymethamphetamine (MDMA)	C11H15NO2	193.11028

Table 1 LC/MS Forensics and Toxicology Test Mix Content (continued)

Compound Name	Formula	Mass
Nitrazepam	C15H11N3O3	281.08004
Oxazepam	C15H11ClN2O2	286.05091
Oxycodone	C18H21NO4	315.14706
Phencyclidine (PCP)	C17H25N	243.1987
Phentermine	C10H15N	149.12045
Proadifen	C23H31NO2	353.23548
Strychnine	C21H22N2O2	334.16813
Temazepam	C16H13ClN2O2	300.06656
Trazodone	C19H22ClN5O	371.15129
Verapamil	C27H38N2O4	454.28316

Where to find more information

Application Notes and Publications You can find information about the MassHunter Forensics and Toxicology PCD in the application notes and publications included on the MassHunter Forensics and Toxicology PCD disk.

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

Before You Begin

Installation

1. Check that the Agilent 1200 Infinity Series LC is properly installed and verified.
2. On the Agilent 1200 Series Binary Pump SL, check that the mixer and damper are bypassed. See "To bypass mixer and damper" on page 22 for details.
3. Check that the Agilent 6200 Series Time-of-Flight LC/MS or Agilent 6500 Series Quadrupole Time-of-Flight instrument is properly installed and verified.
4. Check that the following programs are properly installed:
   - MassHunter Data Acquisition B.04.00 or higher
   - MassHunter Qualitative Analysis B.04.00 or higher
   - MassHunter Quantitative Analysis B.04.00 SP3 or higher
5. Install the MassHunter Personal Compound Database and Library Manager. Refer to the *MassHunter Personal Compound Database and Library Manager Quick Start Guide*.
6. Install the MassHunter Forensics and Toxicology PCD.
   a. Insert the MassHunter Forensics and Toxicology PCD disk into the disk drive.
   b. In the MassHunter Forensics and Toxicology PCD welcome screen, click **Forensics and Toxicology PCD Installation**.
   c. Read the instructions to install the database, then click **Install the Forensics and Toxicology *Broecker, Herre & Pragst* PCD and Test Mix PCD**.

Required Reagents and Parts

- LC/MS grade acetonitrile, methanol and water
- ZORBAX LC Column, p/n 959764-902
- Formic Acid (highest purity)
- Ammonium Formate (highest purity)

Getting Started

The sample data files provided in the MassHunter Forensics and Toxicology PCD disk were acquired with the test mix on a system with the LC/MS system configured as described in "Installation" on page 5. Along with the sample data files are the methods with which these data files were acquired. If you review the acquisition method and sample data, you will get an idea of the data acquisition, data processing, and result interpretation from using the MassHunter Forensics and Toxicology PCD Kit.

To review the Data Acquisition method, use the MassHunter Data Acquisition program to open the method file **Forensic_Tox_Test_Mix_TOF.m** or **Forensic_Tox_Test_Mix_QTOF.m**, depending on your instrument. The following Data Acquisition settings for the positive ion compounds are listed:

- Data Acquisition method information
- TOF/Q-TOF LC/MS settings
- Wellplate sampler settings
- Binary pump settings
- Thermostatted column compartment settings

Note that the method uses two reference ions, which are dispensed from reference bottle A of the calibration delivery system. The two compounds used are from the API-TOF Reference Mass Solution (p/n G1969-85001) and are purine and HP-0921. Prepare the reference ion solution as recommended in the installation guide for your instrument. Do not use the trifluoracetic acid (TFA) found in the reference kit.

Make sure little residual or no signal from the TFA in the calibration mix comes from tuning or calibrating.

To run the test mix

Run the LC/MS Forensics and Toxicology Test Mix to get a better idea of how the MassHunter Forensics and Toxicology PCD Kit will work for you.

1 Do a check tune to verify that the instrument operates properly.

Refer to the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide* for instructions to tune the instrument.

2 Prepare the LC/MS Forensics and Toxicology Test Mix.

The concentration of the test mix stock solution is 1 µg/mL (1 ppm).

a Dilute 1 mL of the stock solution to 10.0 mL with methanol to create the final solution concentration.

b Transfer 1 mL of the final sample solution to a standard 2 mL sample vial for analysis.

The final solution is a 100 ng/mL (100 ppb) working solution.

3 Prepare mobile phases A and B.

- A= 5 mM ammonium formate/0.01% formic acid in water
- B= 0.01% formic acid in acetonitrile

4 Verify the system configuration.

For the analysis of the LC/MS Forensics and Toxicology Test Mix, load the method **Forensic_Tox_Test_Mix_TOF.m** or **Forensic_Tox_Test_Mix_QTOF.m**, depending on instrument that you use. This method uses the HPLC system configuration as listed below. Systems that deviate from this configuration may not work with this method.

Column	2.1 x 100 ZORBAX Eclipse Plus C18 1.8 µm, p/n 959764-902
Wellplate Sampler	h-ALS-SL+, model# G1367D
Pump	Binary Pump – SL, Model 1312B configured with damper and mixer bypassed.
Column Compartment	Column – SL, Model G1316B

5 Check that your method is set up to make a 1 µL injection.

6 Click **Run > Interactive Sample** to do a single sample run, or create a worklist to make multiple injections.

7 If you do not see all the peaks after you process your data:

a Extend your Stop time in the method to 10 minutes.

b Check that you detect both reference ions between 10,000 and 100,000 counts, and that their *m/z* values are within a few mDa of the expected *m/z* values.

c Make sure your system is tuned and calibrated correctly.

d Run the test mix again.

This will not affect your results but will show if retention times are different on your system. There are a number of reasons your retention times can change from those determined by Agilent, such as different instrument delay volume, dead volumes or configuration.

To process and interpret test mix data (Defined Extracted Ion Chromatograms)

In this topic, you process the data file that you created when you ran the test mix. The figures in this task are based on the example data file **Forensic_Tox_Test_Mix_100pg.d** found in the **Example Data** folder on the MassHunter Forensics and Toxicology PCD disk. Your results may differ slightly.

1 Open the Agilent MassHunter Qualitative Analysis program.

Click **Cancel** if you are asked to open a data file.

2 Process the data file for the positive ion test mix:

a Load the method **Forensic_Tox_Test_Mix_DA_Method.m**.

b Open the data file that you created when you ran the test mix.

You can also use example data file **Forensic_Tox_Test_Mix_100pg.d** in the **Example Data** folder on the MassHunter Forensics and Toxicology PCD disk. See Figure 1.

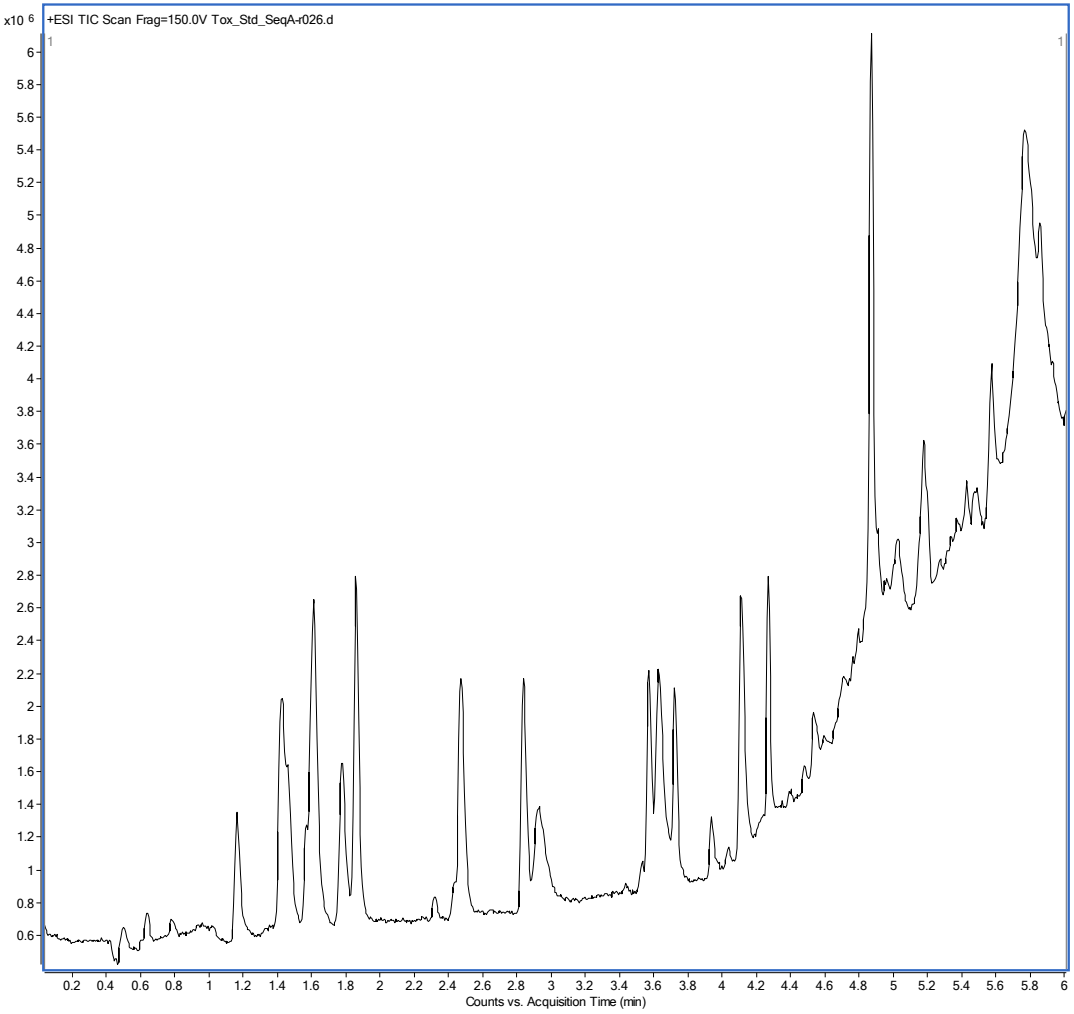


Figure 1 Example Test Mix Total Ion Chromatogram

c In Method Explorer, click **Chromatogram > Define Chromatograms**. See Figure 2.

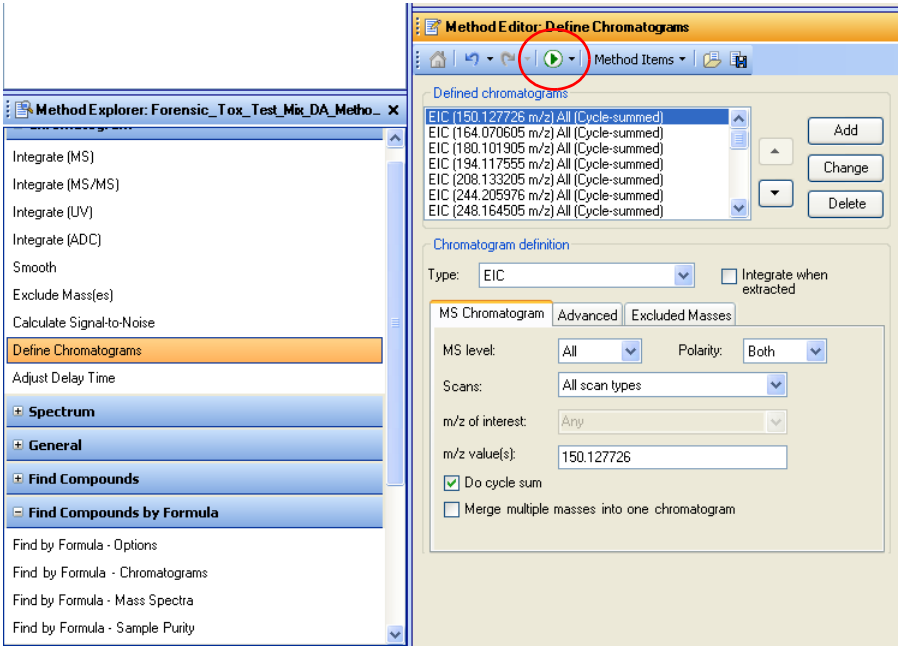


Figure 2 Define Chromatograms section selected. Click the green arrow (circled) to extract the ions.

A list of the exact *m/z* values of the compounds in the mixture is displayed in the Chromatograms > Define Chromatograms section.

3 Click the green arrow in the Method Editor toolbar to extract the ions.

After the chromatograms are extracted, they are displayed in the Chromatogram Results window, as seen in Figure 3, if the view is in List Mode. In Figure 3, you can see the major peak in each EIC. The text mix data collected on your system will show a similar result.

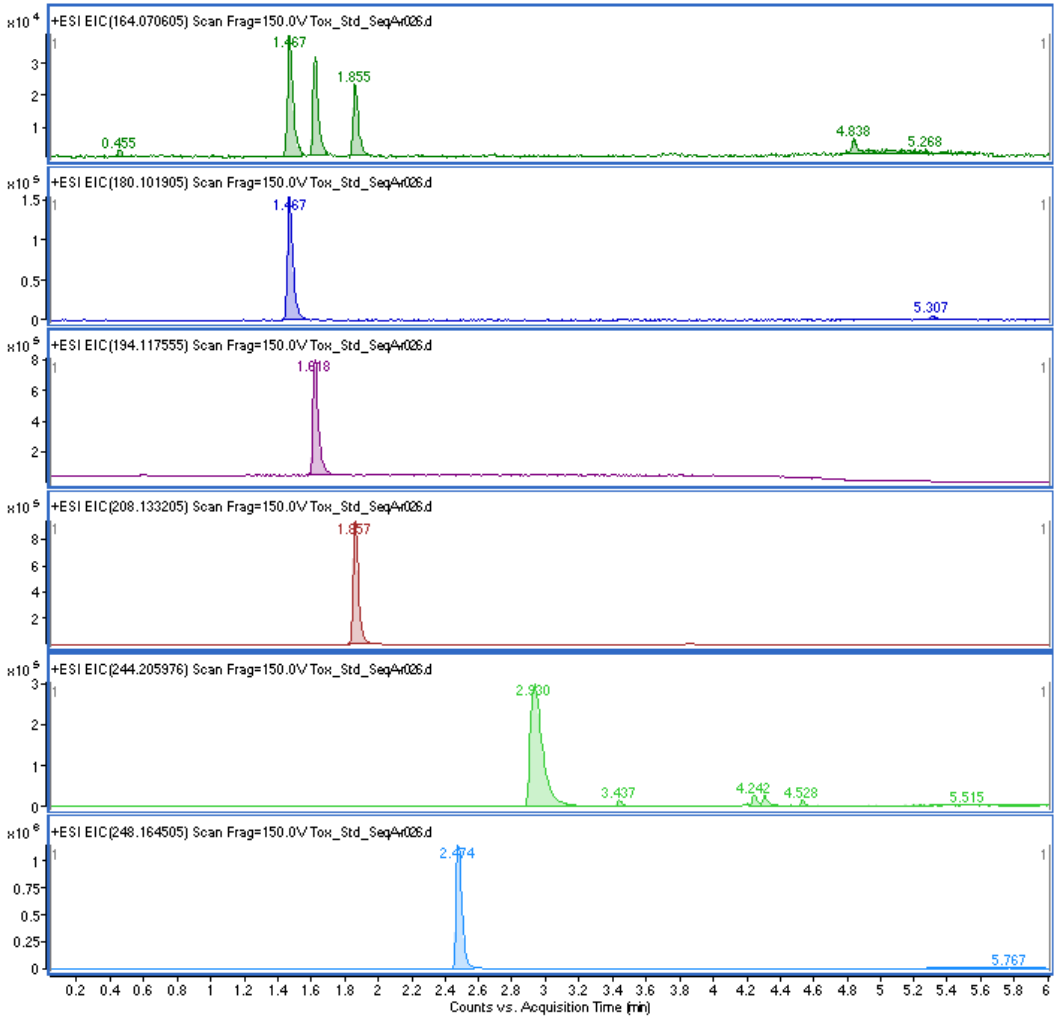


Figure 3 Extracted Ion Chromatograms

To process and interpret test mix data (Find by Formula)

1 Locate the **Find Compounds by Formula** section in the Method Explorer.

2 From the MassHunter Forensics and Toxicology PCD disk, copy the custom database **Tox_Std_01.cdb** to **D:\MassHunter\PCDL**, or wherever MassHunter databases are stored. See Figure 4.

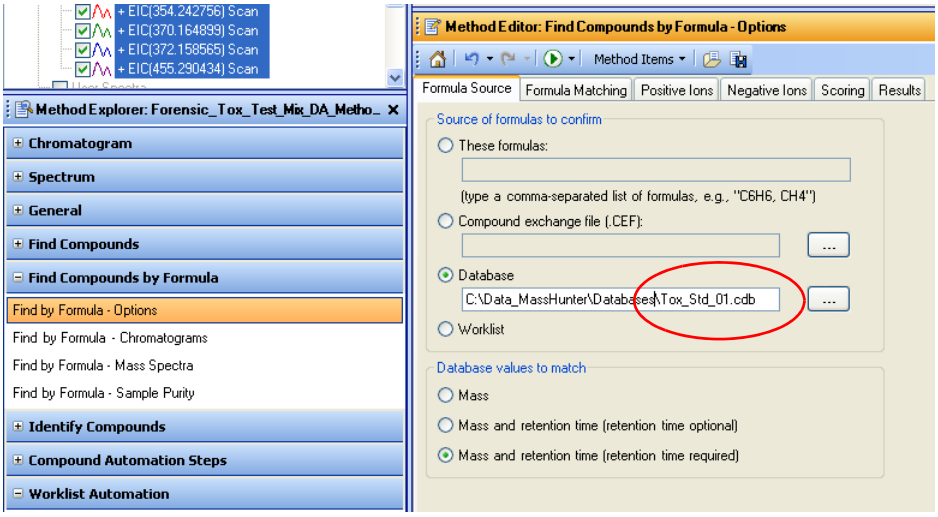


Figure 4 Find by Formula Method Editor Options (Custom Database)

3 Review the settings in this method to become familiar with peak detection, mass tolerances and other settings. These settings may need to be adjusted for specific matrices.

If the retention times are not the same in your sample, click the **Mass** option instead.

4 Find compounds in the data file **Forensic_Tox_Test_Mix_100pg.d** by using the Forensics and Toxicology database. Use **Find by formula** and click the green arrow ( ) in the Method Editor toolbar.

The Qualitative Analysis program searches each entry in the custom Forensics and Toxicology database to find compounds in the data file.

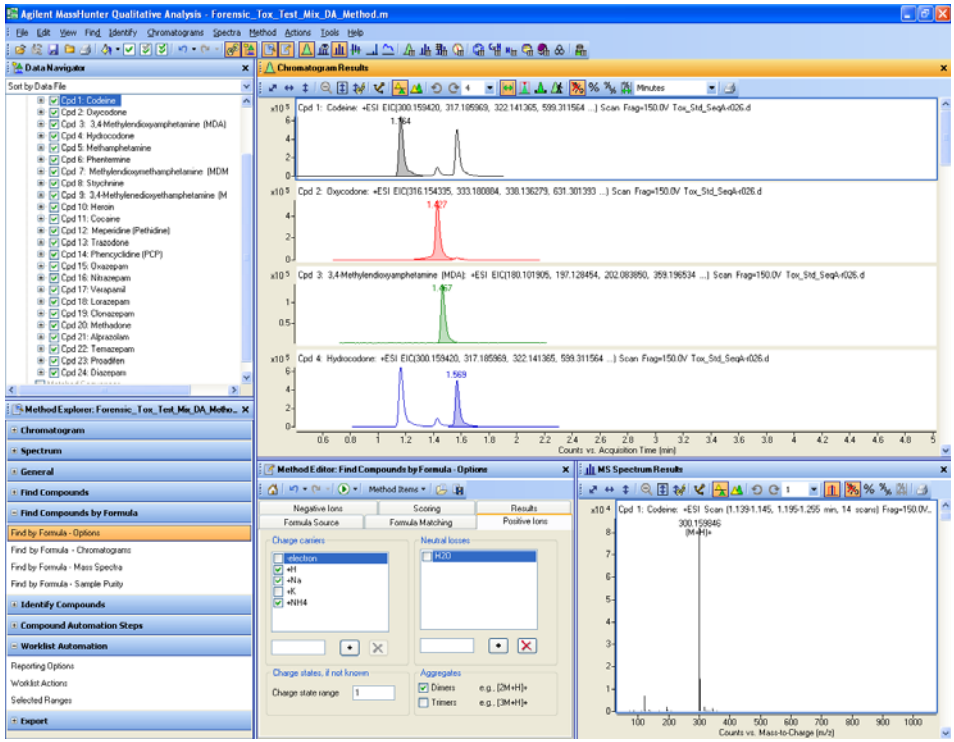


Figure 5 Find By Formula Results using an example Custom Forensics and Toxicology Database with added Retention Times.

Note that codeine and hydrocodone have been correctly identified using the retention time information. These analytes are isobaric and accurate mass alone could not distinguish between each.

To process and interpret test mix data (Find by Molecular Feature Extractor)

1. In the Method Explorer, locate the Find Compounds > Find by Molecular Feature section and review the settings in all tabs.
2. In the Method Editor, review all settings in the Find Compounds by Molecular Feature tabs. These will have to be adjusted per sample type and according to sample matrices.
3. Click the green arrow () in the Method Editor toolbar to search the data file, using the model settings.

   The Molecular Feature Extractor (MFE) “mines” the data file for all possible compounds and uses a “first principle” approach. Once the possible compounds have been separated and identified from probable background interferences, a compound list is generated.

   All possible analytes according to the method settings will be extracted.

   Figure 6 illustrates the results for Find by Molecular Feature.
4. In the Data Navigator, click the **Compounds** line to select all compounds that were generated by MFE and which are shown. When all the compounds are selected, right-click the selected compounds and click **Search Database for Compounds** from the shortcut menu (Figure 6).

   If the Advanced tab is not visible, click **Tools > User Interface Configuration** and then mark the **Accurate mass (TOF, Q-TOF)** and **Show advanced parameters** check boxes.

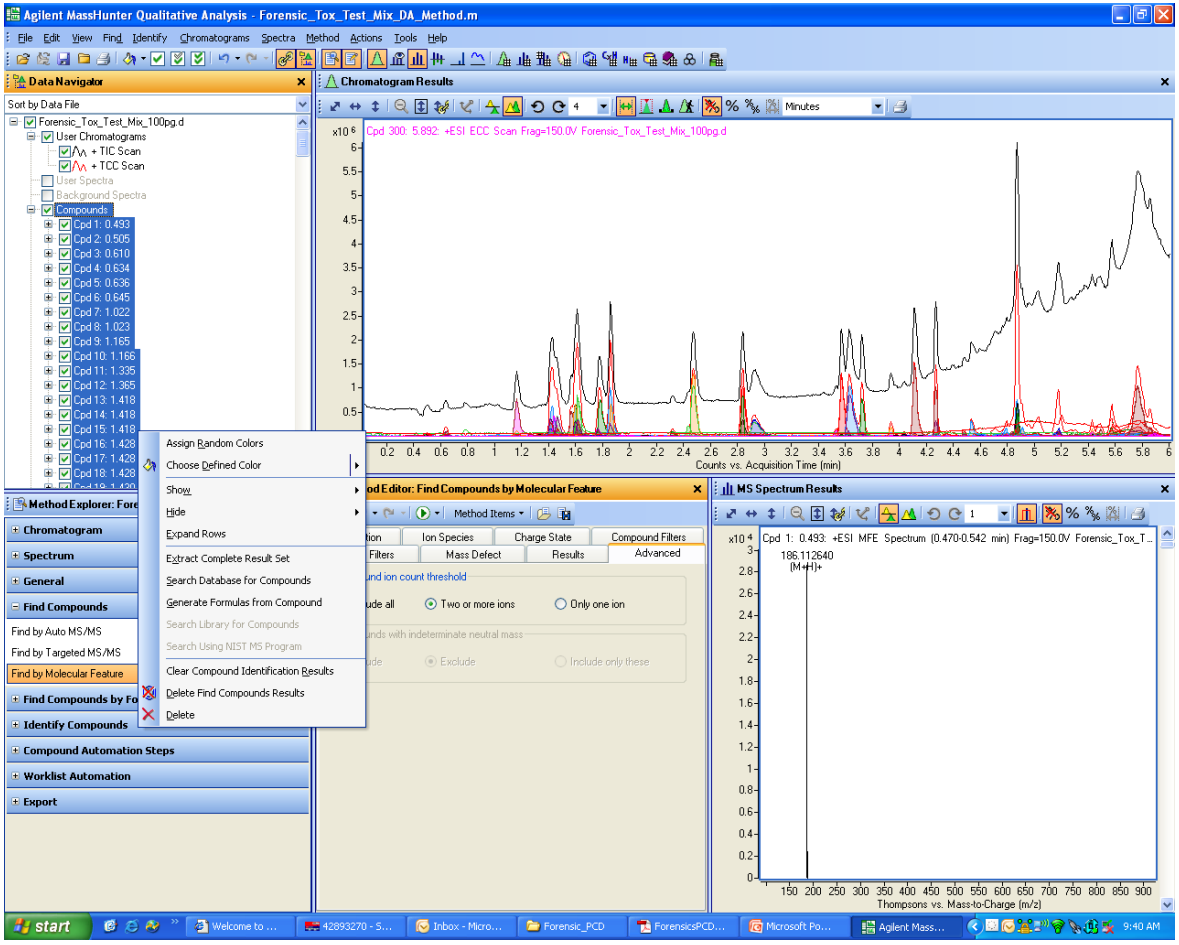


Figure 6 Database Search Results on Find by Molecular Feature compounds

The custom database is searched against each MFE result. Figure 7 shows the compound identification results obtained from a search on the MassHunter Forensics and Toxicology PCD.

An example of the Molecular Feature Extractor Report for the LC/MS Forensics and Toxicology Test Mix is included on the MassHunter Forensics and Toxicology PCD disk in this kit.

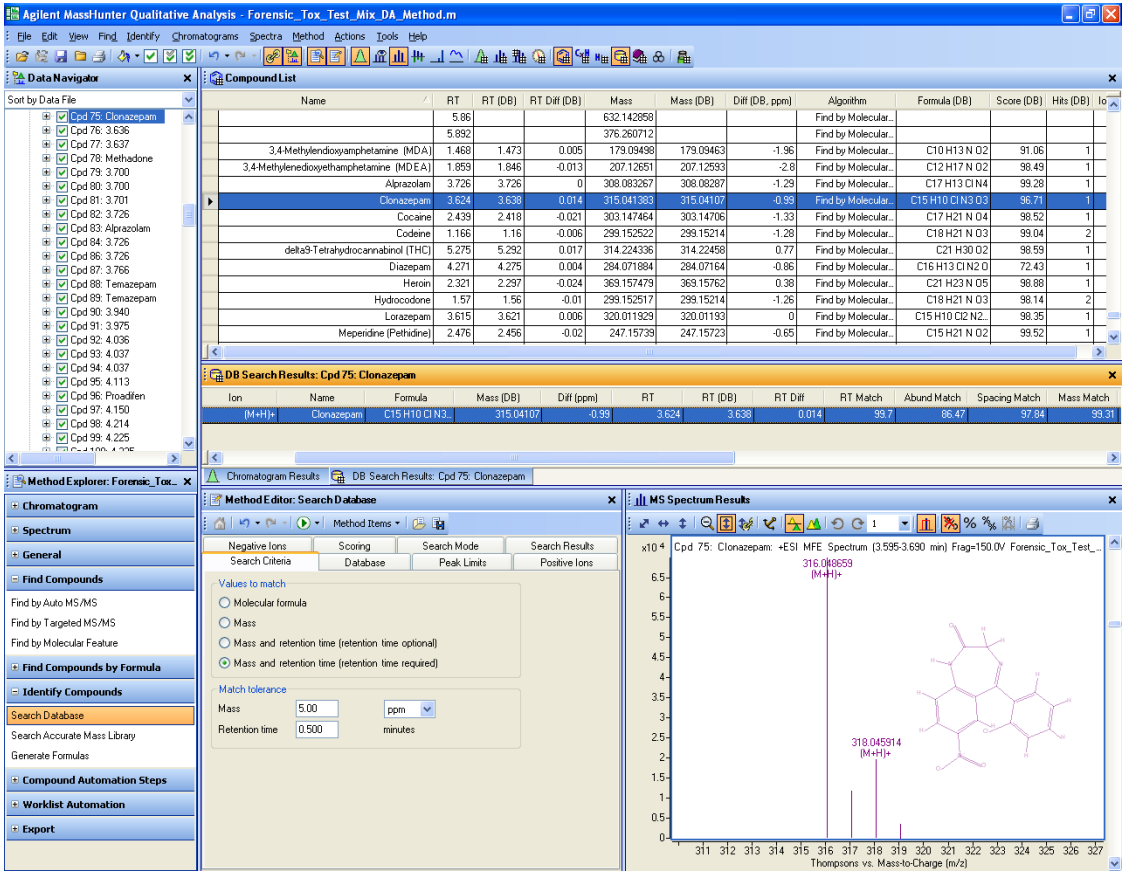


Figure 7 Find By Molecular Feature Database Search Results

To process data automatically using Worklist Automation

After you decide the correct settings for all aspects of the Find Compounds algorithms and Search Database algorithms (such as those described in the application note 5990-4252EN), you can save these settings to one convenient Qualitative Analysis method for repetitive and consistent data manipulation from week to week.

The Worklist Automation feature of the MassHunter Qualitative Analysis program lets you take advantage of the ability to save reprocessing options. This topic describes how you can set up Worklist Automation to automatically process a data file with the Find by Molecular Feature algorithm, search the MassHunter Forensics and Toxicology PCD, and send the report of results to a specific printer or data file location.

1 In the Method Explorer, click **Worklist Automation > Worklist Actions**.

The Method Editor shows a list of automatic Qualitative Analysis actions that will be executed in the order shown.

2 Copy the actions that you want the method to do from the **Available actions** list to the **Actions to be run** list. See Figure 8.

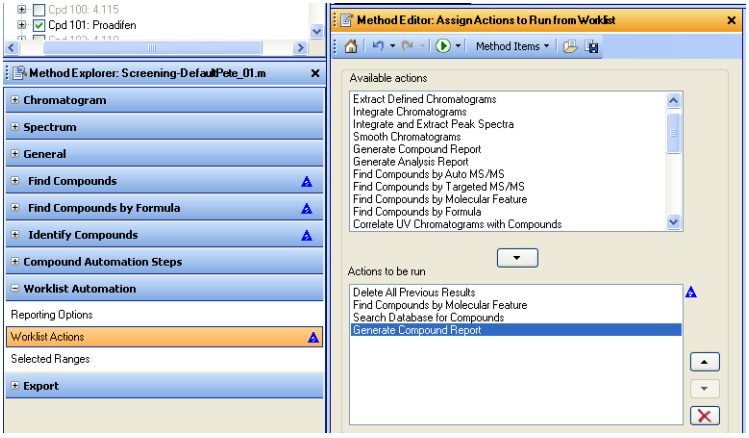


Figure 8 Method Editor with list of selected actions

Note that if Search Database for Compounds is selected as an action to be run, then make sure that in the **Find Compounds by Molecular Feature > Results** tab, the **Highlight All Compounds** option is selected.

3 If you chose **Generate Compound Report**, then modify the reporting options:

   a From the **Worklist Automation** list, click **Reporting Options**.

   b In the Method Editor, in the Reporting Options section, set your reporting options. See Figure 9.

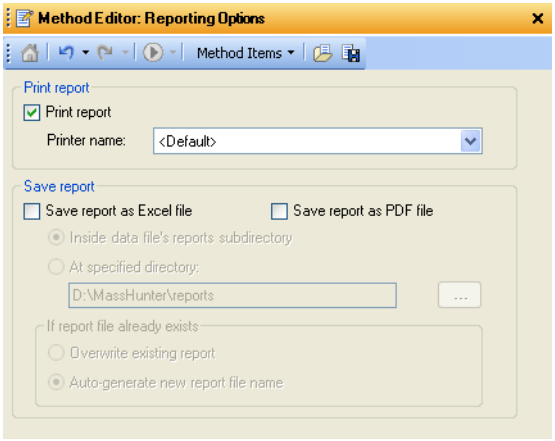


Figure 9 Reporting Options

4 In the MassHunter Qualitative Analysis program, click **Method > Save As**.

5 Browse to the folder on your system that contains the Data Acquisition method that you want to automate.

6 Click the name of the Data Acquisition method that you want to automate and click **Save**.

   The Qualitative Analysis method is now attached and is an integral part of the Data Acquisition method.

7 Create a Data Acquisition worklist:

   a In the MassHunter Data Acquisition program, click **Worklist > Worklist Run Parameters**.

   b For **Part of method to run**, select **Both Acquisition and DA**.

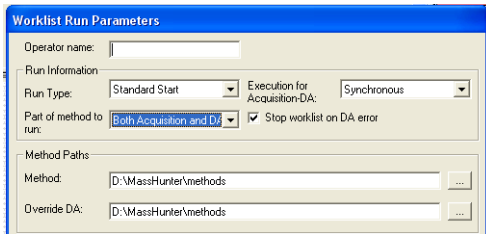


Figure 10 Worklist Run Parameters window

c Select whether **Execution for Acquisition-DA** is to be **Synchronous** or **Asynchronous**.

8 Save the worklist.

9 Run the worklist.

The Qualitative Analysis steps defined and set up under **Actions to be Run** in the Method Editor will run automatically during the sample acquisition without any user intervention.

Using worklist automation, features of the MassHunter Data Acquisition program for TOF and Q-TOF with the MassHunter Qualitative Analysis program and in combination with the MassHunter Forensics and Toxicology PCD, samples can be screened for and reported automatically.

You can create smaller and more focussed custom databases from the larger MassHunter Forensics and Toxicology PCD for a specific industry needs such as work-place drug testing.

NOTE Some compounds in the database will only ionize using specific LC/MS sources, such as electrospray or APCI.

To develop a custom database

The use of a smaller and more focussed database to screen samples can be a powerful tool to detect and identify specific analytes that are required by various regulatory organizations, such as governmental work-place drug testing. Once a custom database of targeted compounds is created, then single standards of those compounds must be analyzed using a standard chromatography method, retention times recorded, and detection limits determined.

- Run standards of targeted compounds and create custom databases from the MassHunter Forensics and Toxicology PCD.

  The Technical Note (p/n 5990-3976EN) included on the MassHunter Forensics and Toxicology PCD disk describes how to create a custom database, and to add retention times for your compounds and chromatographic conditions to the database.

  An example of the addition of retention times to a custom database for a negative ion test mix is given in the application note p/n 5990-4251EN.

  The MassHunter Personal Compound Database and Library (PCDL) Manager, supplied with the MassHunter Forensics and Toxicology PCD Kit (G6855AA), is an updated version of the MassHunter Personal Compound Database program described in the technical note. The PCDL Manager contains additional fields such as IUPAC names and ChemSpider links (and the ability to add spectra to a spectral library), but the functionality is identical to that described in the technical note on the MassHunter Forensics and Toxicology PCD disk (p/n 5990-4252EN).

To bypass mixer and damper

The Binary Pump SL is delivered in standard configuration (damper and mixer connected). This step shows how to bypass the damper and mixer and convert the pump to low delay volume mode.

Configurations where only the damper or the mixer is disconnected while the other part is still in line are not supported by Agilent Technologies.

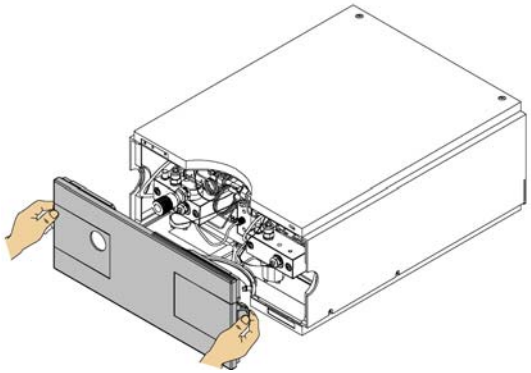
Tools required

- Wrench, 1/4-inch x 5/16-inch (p/n 8710-0510)
- Wrench, open end, 14-mm (p/n 8710-1924)
- Hex Driver, 1/4-inch, slitted (p/n 5023-0240)

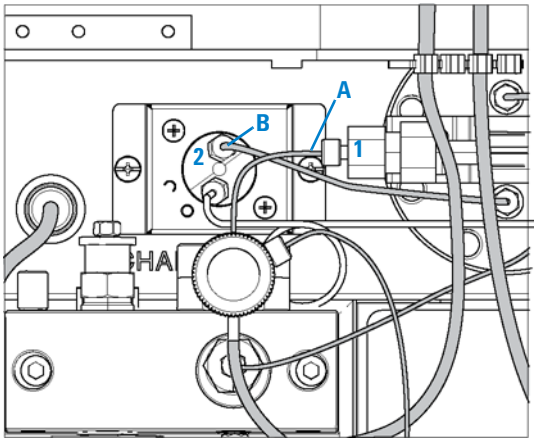
Preparations for this procedure

- Flush the system (water if buffers were used, otherwise IPA).
- Turn the flow off.

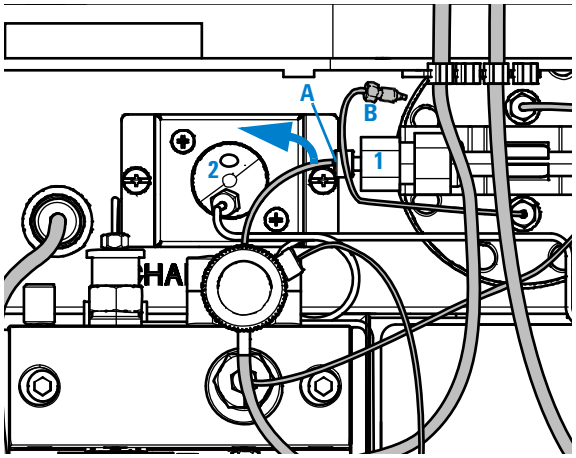
1 Remove the front cover by pressing the clip fastener on both sides of the cover.



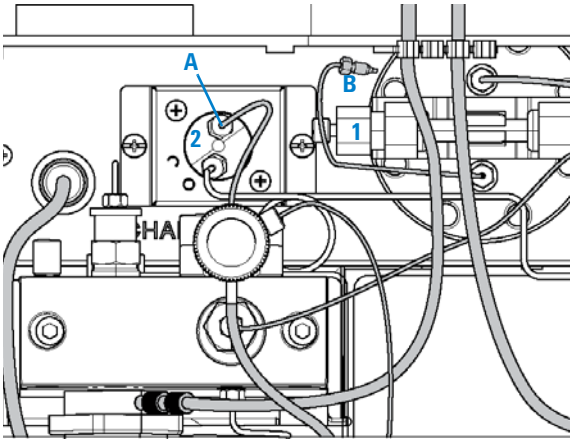
2 Use the 1/4 inch hex driver to remove fitting **B** from port *2* of the pressure sensor.



3 Fold capillary end **B** away. It remains unconnected. Disconnect fitting **A** from outlet **1** of the mixer.



4 Connect fitting **A** to port **2** of the pressure sensor. Seal port **1** of the mixer with a plastic blank nut.



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In This Guide

This Quick Start Guide describes how to use the MassHunter Forensics and Toxicology PCD Kit.

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This guide is valid for the B.04.00 revision or higher of the MassHunter Forensics and Toxicology PCD, until superseded.

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