An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using TOF or Q-TOF LC/MS with a Personal Forensic Toxicology Database

Application Note

Forensic Toxicology

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Abstract

A Forensic and Toxicological screening application kit has been developed for use with the Agilent TOF and Q-TOF Mass Spectrometers which contains an accurate mass database with a content of around 6700 analytes. The aim of the MassHunter Personal Forensic Toxicology Database Kit is to provide a user with a sufficient starting point for the analysis of samples for which the ability to detect and identify from a large array of forensic and toxicological analytes is necessary. The combined system allows the user to create custom databases containing retention times of compounds of interest for smaller and more specific suites of analytes according to specific requirements. A test mix containing analytes of forensic interest, to demonstrate the functionality of the MassHunter Personal Forensic Toxicology Database Kit, together with an example of a general screening method for common drugs of abuse is provided.

Agilent Technologies

Introduction

The application of high definition accurate mass spectrometers, such as time-of-flight (TOF) and quadrupole time-of-flight (Q-TOF), to screening, discovery and confirmation in the areas of forensics and toxicology has become more desirable given the indiscriminant and non-targeted nature of their full spectral data capture. Indeed, given the highly accurate and sensitive mass measurement of modern TOF and Q-TOF instruments (sub 2-ppm mass accuracy, pg on-column sensitivity and high resolution) in combination with powerful software data mining tools, post acquisition screening techniques are easier to perform reliably with a higher number of analytes in one analytical method. The lists of potential toxins are large and typically depend on the area of analytical focus such as work-place drug testing, doping control, post-mortem toxicology, or explosives.

Accurate single-stage mass spectrometry (MS) mass measurements identify monoisotopic adducts to a high confirmatory degree, and databases can be built to accommodate various suites of forensic and toxicological analytes of interest. They are obtained from both TOF and Q-TOF LC/MS instruments. In contrast LC/MS/MS with a triple quadrupole MS in its most sensitive mode, multi-reaction monitoring (MRM), provides targeted screening and confirmation only [1].

This application note describes the Agilent MassHunter Personal Forensic Toxicology Database Kit for Forensic and Toxicological Screening and Identification which contains the accurate mass (AM) details for around 6700 analytes of forensic and toxicological interest. The content was gathered upon advice from many leading institutions and knowledge bases world-wide and contains information such as common names, monoisotopic mass, compound formulas, CAS & Chemspider IDs, chemical structure and in most cases the IUPAC nomenclature. In addition to accurate mass, the ability to add retention time for a chromatographic method to every analyte for extra search confirmation is a built-in functionality of the MassHunter Personal Compound and Library (PCDL) program interfaces. This allows accurate mass retention time (AMRT) data mining routines. Furthermore, an analyst can use the database content 'as is' for non-targeted screening or create smaller custom and more targeted databases from the read-only supplied database. Custom databases can be edited by changing entries, adding, and deleting entries and semi-automatically updating retention times for particular analytes and methods. [2] The analyst can create as many custom databases with LC-dependent retention times as needed.

This application note describes the typical use of the MassHunter Personal Forensic Toxicology Database Kit through a few analytical screening work flow examples.

Experimental

The analysis results outlined in this application note were obtained using an Agilent 6230 Time-of-Flight LC/MS coupled to an Agilent 1200 SL Series LC system. The LC system consisted of a binary pump (G1312B), vacuum degasser (G1379B), automatic liquid sampler (G1367D), thermostatted column compartment (G1316B) and MassHunter Workstation equipped with the (G6855AA) MassHunter Personal Forensic Toxicology Database Kit.

Sample preparation

An ampoule from the LC/MS Toxicology Test Mix (p/n 5190-0470) which is included in the MassHunter Personal Forensic Toxicology Database Kit [G6855AA] was opened and 10 µL of the 1 µg/mL (1 ppm) solution was diluted to a concentration of 100 ng/mL (100 ppb) using 990 µL of pure LC/MS grade methanol to create a clean solvent standard for method checkout purposes.

Table 1 outlines the composition of the LC/MS Toxicology Test Mix (p/n 5190-0470) which is intended to cover a wide and representative range of forensic analyte classes.

Table 1. LC/MS Toxicology Test Mix Components (1 µg/mL)

Compound name	Formula	Mass
3,4-Methylendioxyamphetamine (MDA)	$C_{10}H_{13}NO_2$	179.09463
3,4-Methylenedioxyethamphetamine (MDEA)	$C_{12}H_{17}NO_2$	207.12593
Alprazolam	$C_{17}H_{13}ClN_4$	308.08287
Clonazepam	$C_{15}H_{10}ClN_3O_3$	315.04107
Cocaine	$C_{17}H_{21}NO_4$	303.14706
Codeine	$C_{18}H_{21}NO_3$	299.15214
delta9-Tetrahydrocannabinol (THC)	$C_{21}H_{30}O_2$	314.22458
Diazepam	$C_{16}H_{13}ClN_2O$	284.07164
Heroin	$C_{21}H_{23}NO_5$	369.15762
Hydrocodone	$C_{18}H_{21}NO_3$	299.15214
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	320.01193
Meperidine (Pethidine)	$C_{15}H_{21}NO_2$	247.15723
Methadone	$C_{21}H_{27}NO$	309.20926
Methamphetamine	$C_{10}H_{15}N$	149.12045
Methylendioxymethamphetamine (MDMA)	$C_{11}H_{15}NO_2$	193.11028
Nitrazepam	$C_{15}H_{11}N_3O_3$	281.08004
Oxazepam	$C_{15}H_{11}ClN_2O_2$	286.05091
Oxycodone	$C_{18}H_{21}NO_4$	315.14706
Phencyclidine (PCP)	$C_{17}H_{25}N$	243.1987
Phentermine	$C_{10}H_{15}N$	149.12045
Proadifen	$C_{23}H_{31}NO_2$	353.23548
Strychnine	$C_{21}H_{22}N_2O_2$	334.16813
Temazepam	$C_{16}H_{13}ClN_2O_2$	300.06656
Trazodone	$C_{19}H_{22}ClN_5O$	371.15129
Verapamil	$C_{27}H_{38}N_2O_4$	454.28316

Reagents and chemicals

Burdick & Jackson LC/MS grade acetonitrile together with de-ionized water (locally produced 18.1 MΩ) were used for mobile phases. Buffers were freshly prepared using a high purity source of formic acid and ammonium formate.

Instrument settings and MS acquisition method parameters

LC conditions

Column	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959764-902)			
Column temperature	60 °C			
Mobile phase	A) 5 mM NH_4 formate/0.01% formic acid in water B) 0.01% formic acid in acetonitrile			
Flow rate	0.5 mL/min			
Gradient program	Time (min)	%A	%B	Flow rate (mL/min)
	0	90	10	0.5
	0.5	85	15	0.5
	3.0	50	50	0.5
	4.0	5	95	0.5
	6.0	5	95	0.5
Injection volume	1 µL (with 5 second needle wash in flushport)			
Analysis time	6.0 minutes			
Post Time	2.0 minutes			
Overall cycle time	8.0 minutes			

Agilent 6230 TOF MS conditions

Source conditions

Electrospray AP-ESI (using Agilent Jet Stream Technology)	
Positive ionization polarity	
Sheath gas temperature and flow	380 °C, 12 L/min
Nozzle voltage	500 V
Drying gas temperature and flow	320 °C, 8 L/min
Nebulizer gas pressure	27 psi
Capillary voltage	3,750 V
Fragmentor voltage	150 V

Electrospray AP-ESI	
Positive ionization polarity	
Drying gas temperature and flow	350 °C, 12 L/min
Nebulizer gas pressure	30 psi
Capillary voltage	2,000 V
Fragmentor voltage	150 V

MS acquisition method parameters

Reference ion mass enabled	121.050873, 922.009798
Acquisition mode	MS1
Minimum mass value	50 *m/z*
Maximum mass value	1,050 *m/z*
Scan rate	3 Hz

All other instrument operating parameters were taken care of by Agilent's autotune functionality and subsequent mass calibration using standard settings.

Results and Discussion

Fast and easy start up with Agilent LC/MS Toxicology Test Mix

The LC/MS Toxicology Test Mix (p/n 5190-0470) is included in the MassHunter Personal Forensic Toxicology Database Kit (G6855AA) to rapidly implement the method and verify that acquisition and data analysis methodology is correctly set up. The LC/MS Toxicology Test Mix contains a representative range of components from 25 forensic analyte classes. (see Table 1). MS screening depends on accurate mass results from the TOF or Q-TOF. Therefore, the use of appropriate reference ions as outlined in the Experimental conditions section obtains the most accurate results.

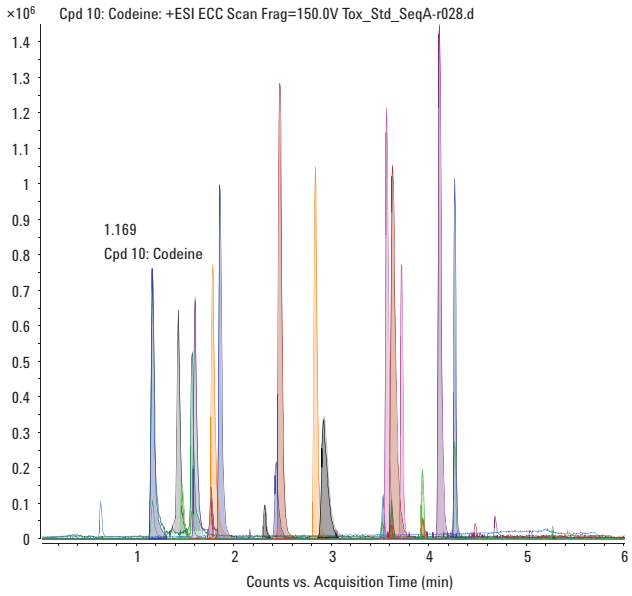


Figure 1. Extracted compound chromatogram of LC/MS Toxicology Test Mix.

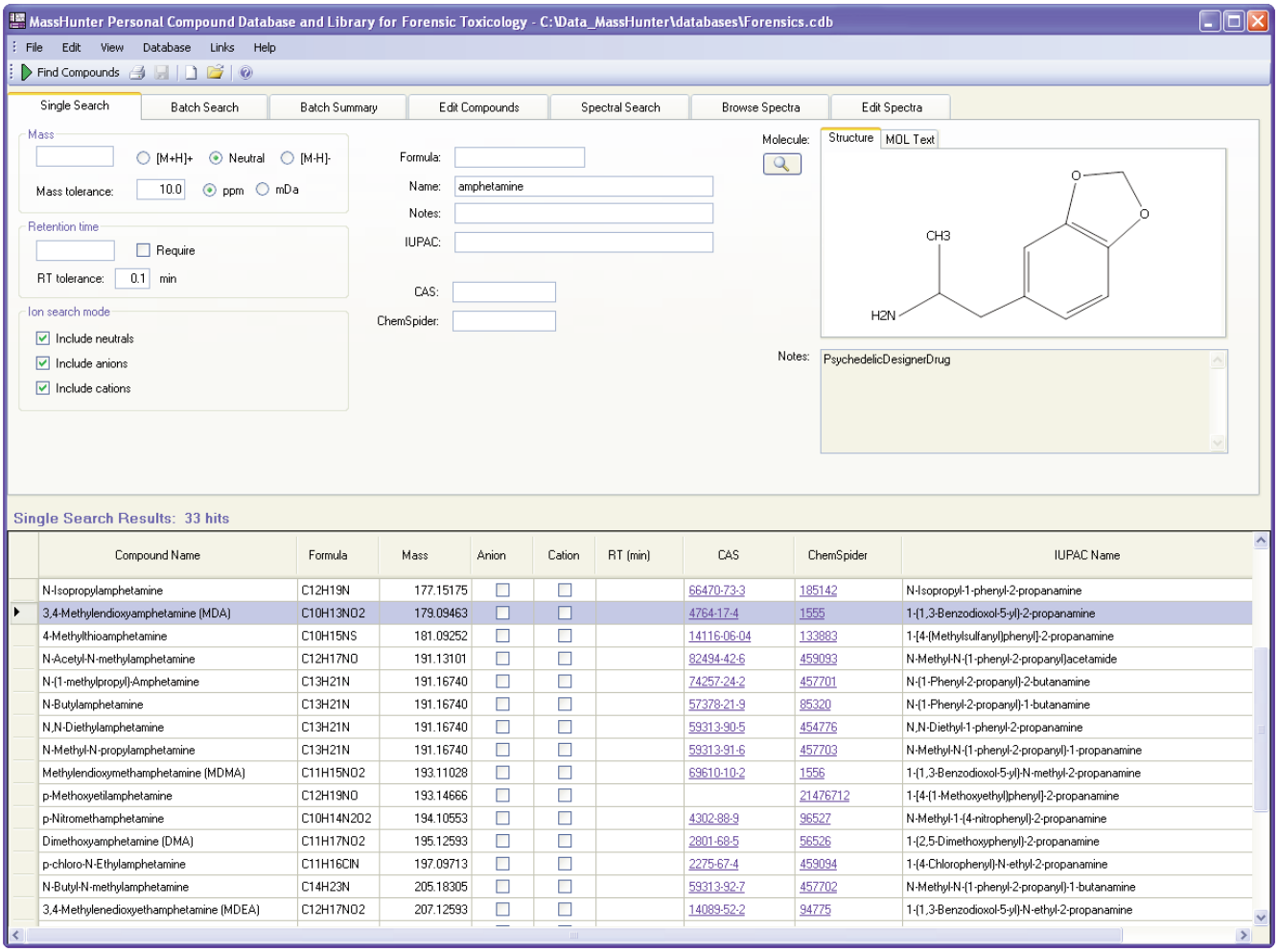
In compliance with the methodology outlined in the experimental section, a 1-µL injection of the 100 ng/mL LC/MS Toxicology Test Mix equates to a 100 pg on-column injection amount. Figure 1 shows an overlay of the expected extracted compound chromatograms for the LC/MS Toxicology Test Mix. A standard method is included for TOF and Q-TOF as part of the MassHunter Personal Forensic Toxicology Database Kit. These can be loaded so that all conditions are correct and the user can reproduce the analysis.

These methods are acquisition only methods and correspond to the instrument configuration as outlined in the experimental section of this application note. Appropriate settings must be manually input if a different instrument configuration is used. Similar results will demonstrate that the system is working properly.

Personal Compound Database and Library (PCDL) Software interface

Outline

An *open database* dialog box appears after invoking the PCDL interface from the desktop icon. It is best to choose the pre-installed Forensic.cdb from the MassHunter\database directory. Figure 2 illustrates the single search view of the software interface. The screen shows a list of search results for amphetamine. There are seven views available to the user, however, for the scope of this application note, only the first four (tabs to the left) that are directly applicable to AMRT functionality will be described. These views are switched on this flat user interface by clicking the appropriate tab: *Single Search*, *Batch Search*, *Batch Summary*, or *Edit Compounds*.



Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name
N-Isopropylamphetamine	C12H19N	177.15175	☐	☐		66470-73-3	185142	N-Isopropyl-1-phenyl-2-propanamine
3,4-Methylendioxyamphetamine (MDA)	C10H13NO2	179.09463	☐	☐		4764-17-4	1555	1-(1,3-Benzodioxol-5-yl)-2-propanamine
4-Methylthioamphetamine	C10H15NS	181.09252	☐	☐		14116-06-04	133883	1-[4-(Methylsulfanyl)phenyl]-2-propanamine
N-Acetyl-N-methylamphetamine	C12H17NO	191.13101	☐	☐		82494-42-6	459093	N-Methyl-N-(1-phenyl-2-propanyl)acetamide
N-(1-methylpropyl)-Amphetamine	C13H21N	191.16740	☐	☐		74257-24-2	457701	N-(1-Phenyl-2-propanyl)-2-butanamine
N-Butylamphetamine	C13H21N	191.16740	☐	☐		57378-21-9	85320	N-(1-Phenyl-2-propanyl)-1-butanamine
N,N-Diethylamphetamine	C13H21N	191.16740	☐	☐		59313-90-5	454776	N,N-Diethyl-1-phenyl-2-propanamine
N-Methyl-N-propylamphetamine	C13H21N	191.16740	☐	☐		59313-91-6	457703	N-Methyl-N-(1-phenyl-2-propanyl)-1-propanamine
Methylendioxymethamphetamine (MDMA)	C11H15NO2	193.11028	☐	☐		69610-10-2	1556	1-(1,3-Benzodioxol-5-yl)-N-methyl-2-propanamine
p-Methoxyetilamphetamine	C12H19NO	193.14666	☐	☐			21476712	1-[4-(1-Methoxyethyl)phenyl]-2-propanamine
p-Nitromethamphetamine	C10H14N2O2	194.10553	☐	☐		4302-88-9	96527	N-Methyl-1-(4-nitrophenyl)-2-propanamine
Dimethoxyamphetamine (DMA)	C11H17NO2	195.12593	☐	☐		2801-68-5	56526	1-(2,5-Dimethoxyphenyl)-2-propanamine
p-chloro-N-Ethylamphetamine	C11H16ClN	197.09713	☐	☐		2275-67-4	459094	1-(4-Chlorophenyl)-N-ethyl-2-propanamine
N-Butyl-N-methylamphetamine	C14H23N	205.18305	☐	☐		59313-92-7	457702	N-Methyl-N-(1-phenyl-2-propanyl)-1-butanamine
3,4-Methylenedioxyethamphetamine (MDEA)	C12H17NO2	207.12593	☐	☐		14089-52-2	94775	1-(1,3-Benzodioxol-5-yl)-N-ethyl-2-propanamine

Figure 2 Single Manual Search view of the PCDL software interface.

Any field or combination of fields in the upper portion of the Single Search tab (Figure 2.) can be used to manually search the loaded database. Table 2 lists all available search fields from the PCDL single search view. The powerful search algorithm also handles partial names (for example, 'amph' will return all database entries containing this letter string.)

Note: To view the entire contents of the loaded database, a single search invoked with all empty search fields will allow the user to display the entire database content.

Table 2. All Available Search Fields for PCDL Single Search

Search fields available (Single Search view)	Value
Mass	Measured mass (*m/z*)
Retention time	(minutes)
Formula	Empirical Formula
Name	Common name of compound (or part thereof)
Notes	Compound class or description
IUPAC	IUPAC or commonly recognized compound name
CAS	Unique CAS number
ChemSpider	Unique ChemSpider ID

Workflow A. Manual (Single Mass Search)

Using PCDL Program

Single search would normally be used manually by obtaining a measured mass from a measured or observed spectrum in MassHunter Qualitative Analysis program and typing it in to the mass search field. Figure 3 illustrates this manual application of the MassHunter Qualitative Analysis program and PCDL single search capability for observed masses.

In this example, a compound peak was identified in MassHunter Qualitative Analysis program from positive polarity TOF data, the spectrum was extracted, and the observed mass of 244.205770 *m/z* was searched against the PCDL database (including cations) for $[M+H]^+$ adducts using a mass tolerance of 10 ppm.

The search returns an accurate mass match with phencyclidine (PCP) and with a mass deviation (or delta mass) of 0.85 ppm between the measured and theoretical database values.

More detailed information of single search capability can be found in Agilent G6855AA MassHunter Personal Forensic Toxicology Database and Kit Quick Start Guides [3,4] .

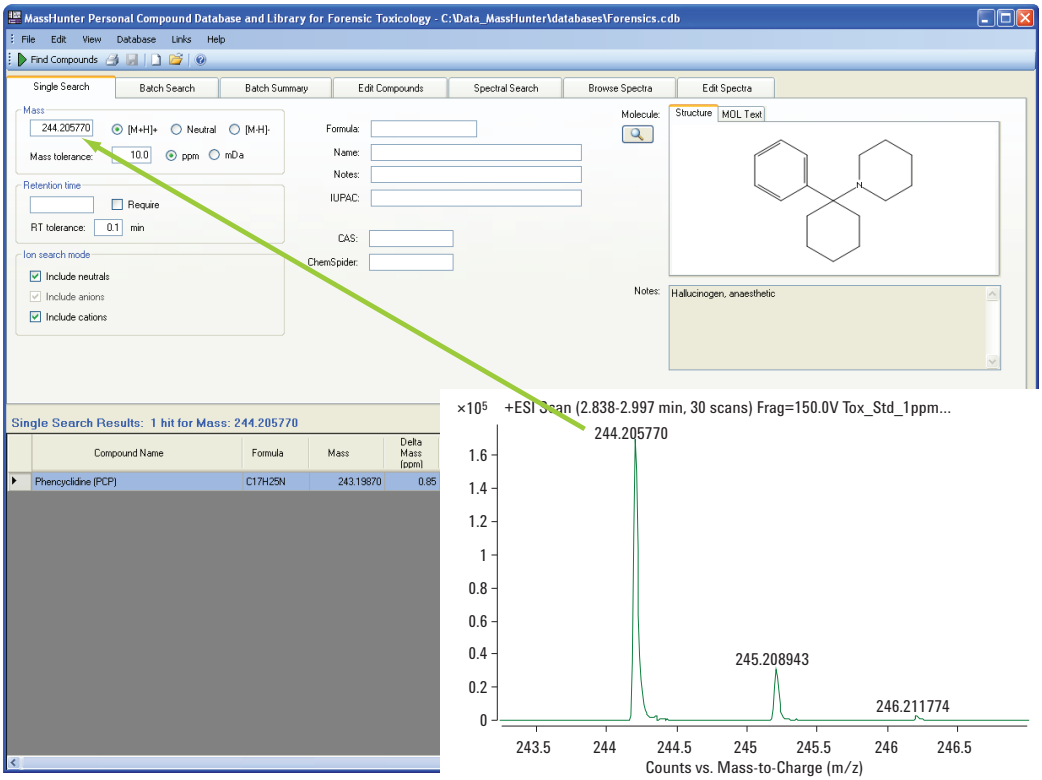


Figure 3. Manual search of observed mass.

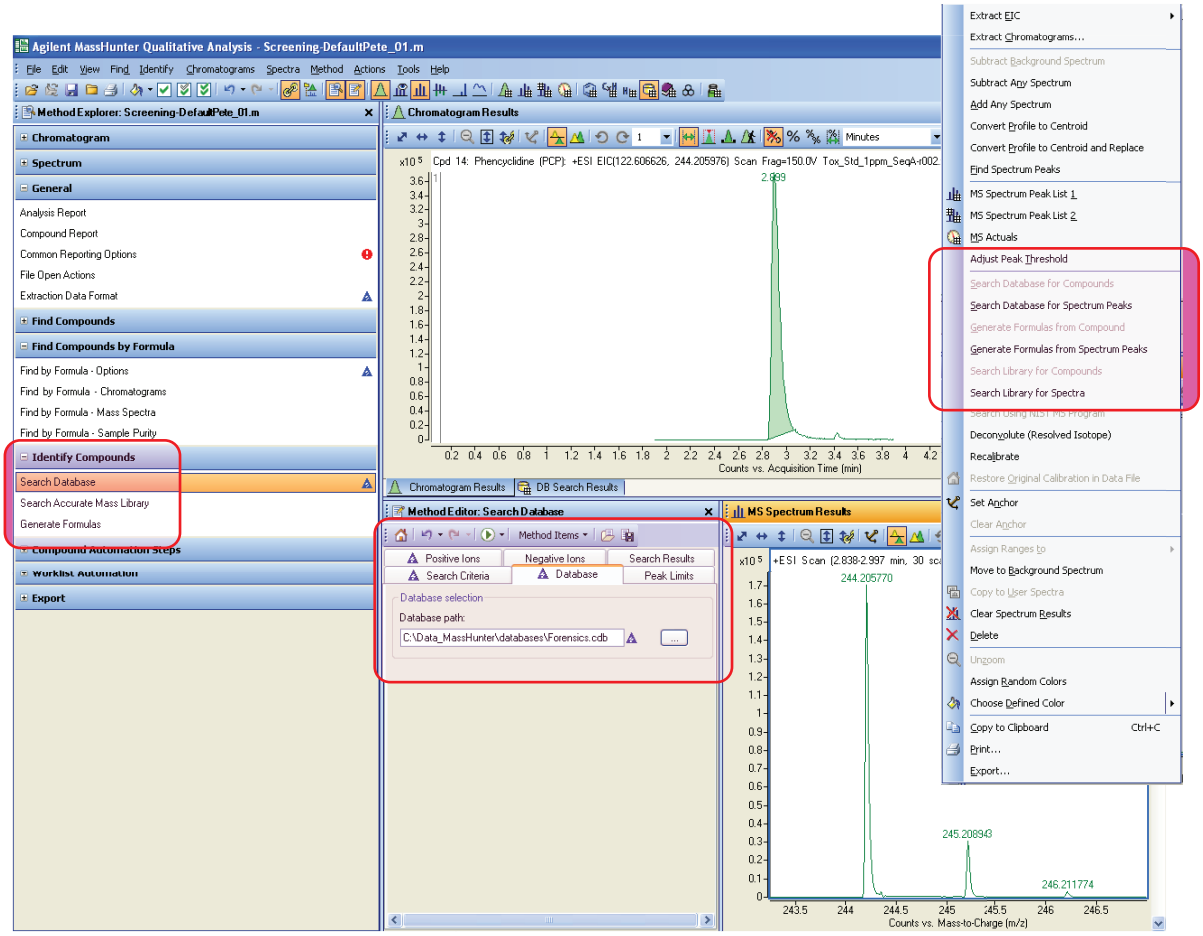


Figure 4A. Manual Search of observed mass using Agilent MassHunter Qualitative Analysis program.

Single manual search of database using Agilent MassHunter Qualitative Analysis program

To obtain a seamless single spectral peak database search via MassHunter Qualitative Analysis program, the database must be specified in the qualitative analysis method editor. Compatible software versions are B.03.01 or higher. Figures 4A through 4D illustrate the settings used for this example.

Figure 4a shows the typical MassHunter Qualitative Analysis program view containing the chromatographic peak in question together with its manually extracted spectrum. On the left side of the screenshot, the *Identify Compounds* method explorer options have been expanded and the *Search Database* method editor was selected. In the method editor, the required AMRT database was specified as forensic.cdb.

Figure 4B shows the mass tolerance window and the search criteria that can be selected, such as mass only or mass with retention time.

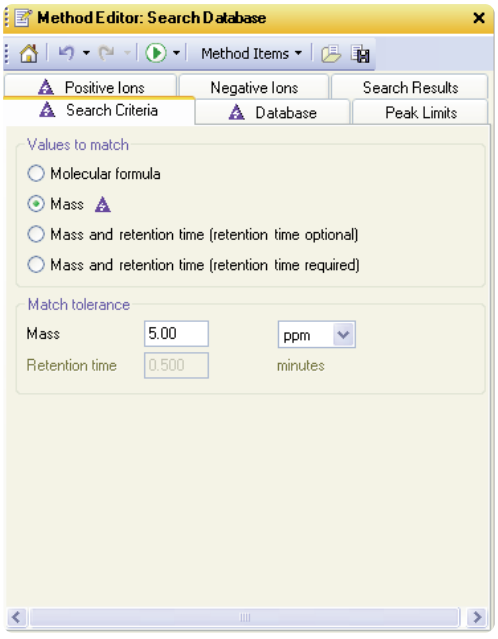


Figure 4B. Manual search criteria settings.

Figure 4C illustrates more adduct and charge state options required for the database search.

Right-click in the spectrum window and a shortcut menu appears against the specified AMRT database (Figure 4A.) This menu has various options including *Search database for spectrum peaks*. Selection of this option automatically invokes the database search. In Figure 4D the spectrum peak has been identified as PCP, with 0.87 ppm mass deviation and a spectral combined score of 99.36 out of 100, indicating extra confirmation of identity.

To calculate this score, three distinct score components were considered: Mass Match, Abundance Match, and Spacing Match with values of 99.61, 98.61, and 99.79, respectively. These are individually displayed in Figure 4D.

For trustworthy results, the software scores the database matches based on the similarity of each of the isotopic masses (Mass Match), isotope ratios (Abund Match), isotope spacing (Spacing Match), and optionally the retention time (RT Match).

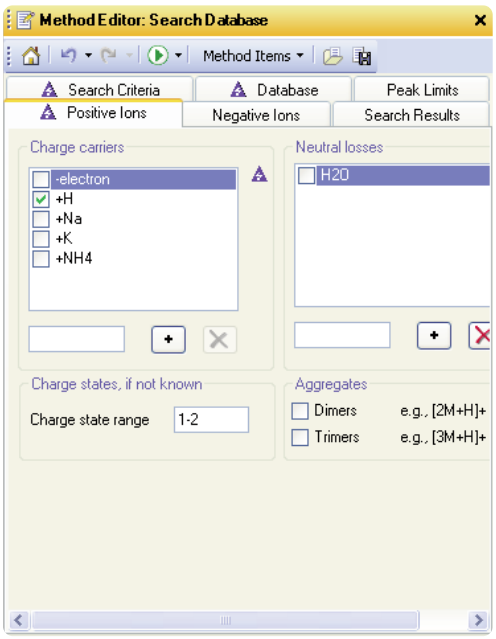


Figure 4C. Manual Search Adduct selection.

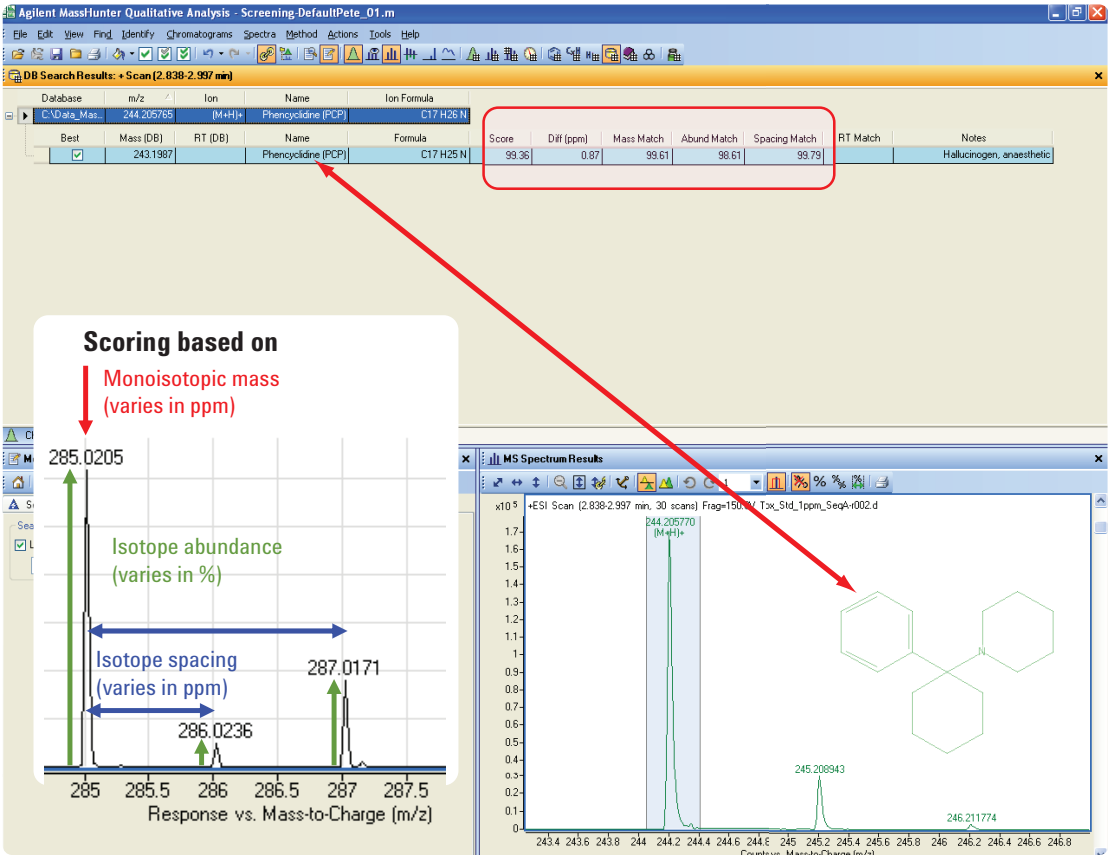


Figure 4D. Manual database search results using Agilent MassHunter Qualitative Analysis program.

Isotope spacing is another important component of the scoring algorithm. The mass spacing from the M to the M+1 and M+2 isotopes can be measured with low-ppm accuracy. Any small mass shifts affect all isotopes equally, so this measurement is independent of overall mass axis shifts. This is outlined graphically in Figure 4D.

In this example, a single AMRT database result of phencyclidine (PCP) was returned, together with its structure which is optionally overlaid on the peak spectrum as shown in Figure 4D and can be displayed if selected in the reporting options.

More detailed information about MassHunter Qualitative analysis program database searching can be found in the MassHunter Qualitative Analysis Program Help Files or user guides [5].

Workflow B. Data mining using Molecular Feature Extractor (MFE)

Batch PCDL searches (tabs 2 and 3) are designed for database searching and identification using an accurate mass list created from an automated data mining algorithm such as the Agilent Molecular feature extractor (MFE.) Such algorithms are extremely powerful, especially with complex data derived from difficult sample matrices, such as blood extracts. For the remainder of this application note, only batch searches invoked from inside the MassHunter Qualitative Analysis program interface will be outlined and described. For information on how to perform batch searches within the PCDL interface, please refer to the PCD application note [2].

Data mining algorithms such as MFE automatically search and mine complex sets of single-stage MS data to determine and distinguish most likely and real compound peaks from continuous background interferences. Combinations of adducts can be selected as part of the compound identification protocol to provide added assurance of compound validity.

Other data mining algorithms such as *find by MS/MS* and *find by Targeted MS/MS* are integral options included as part of the MassHunter Qualitative Analysis program software. The algorithms are dependent on the mode of operation and nature of the instrument being used. *Find by Formula* compound search routines are described in the Workflow C section of this application note.

For illustrative purposes, the LC/MS Toxicology Test Mix was analyzed under the conditions outlined in the experimental section. The data file was loaded into MassHunter Qualitative Analysis program. The *Find by Molecular Feature* method

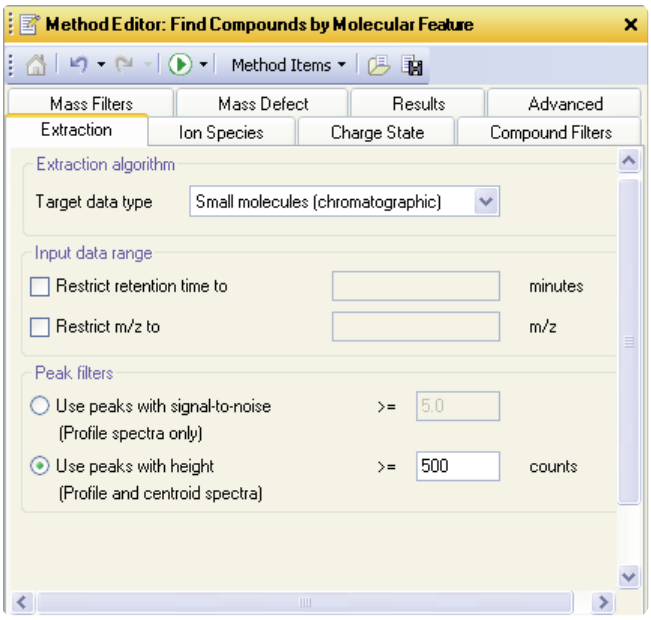


Figure 5A. MFE extraction parameters.

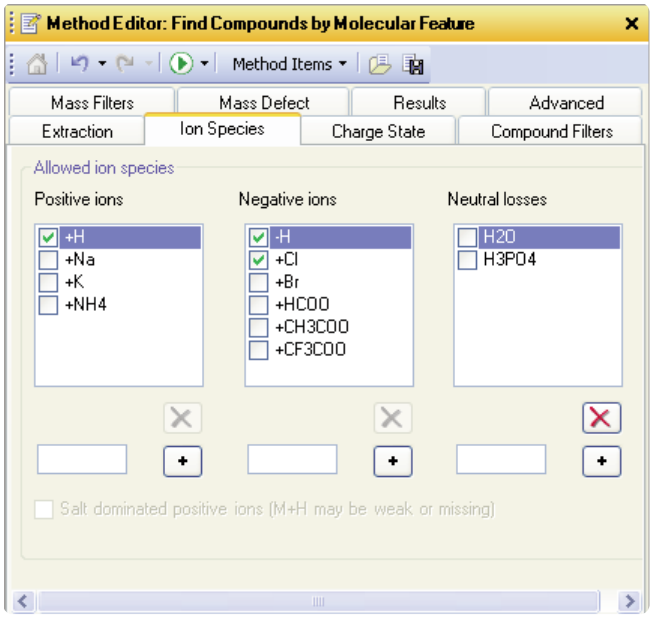


Figure 5B. MFE ion species setup.

editor was opened under the method explorer in the *Find Compounds* section (Figures 5A and B).

A very aggressive setting of absolute peak height threshold (>500 counts) was used in this example (Figure 5A), together with the small molecules algorithm (chromatographic) which yielded over 3,000 possible compound hits. By raising this threshold amount, less abundant analytes may remain undetected. Conversely with a higher threshold the number of potential false positives are greatly reduced. Only $[M+H]^+$

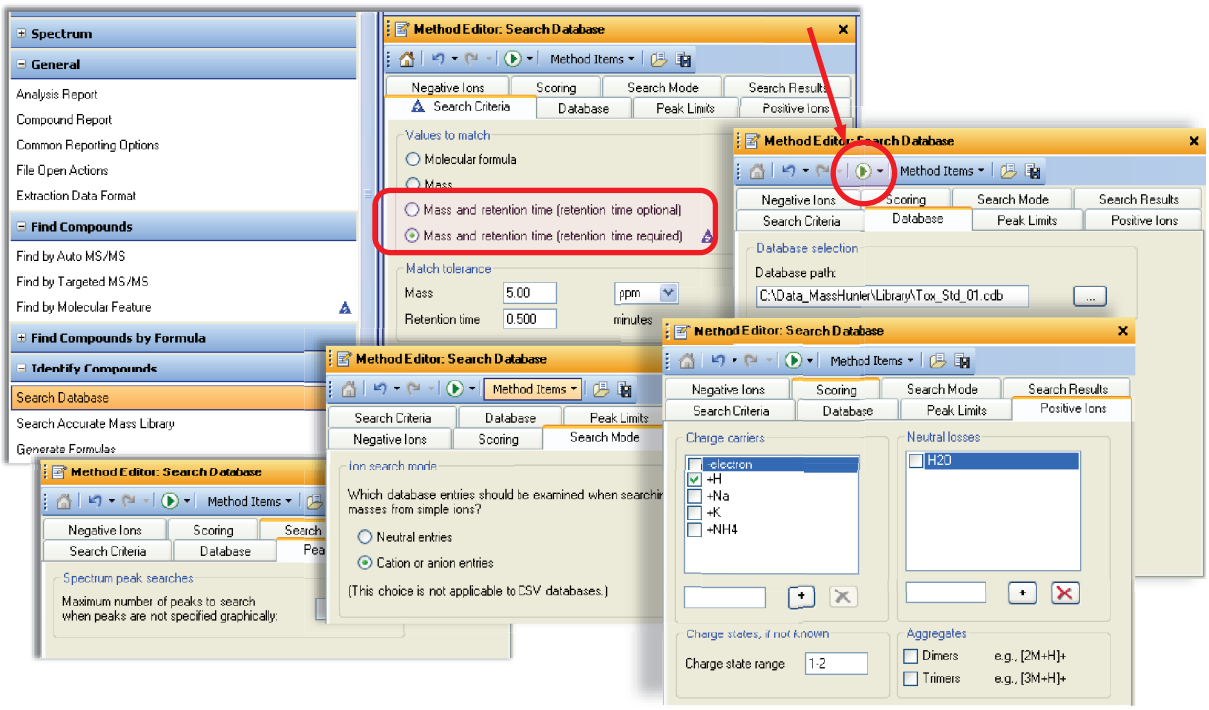


Figure 6. MFE compound database search settings.

adducts were searched in this instance, however, further confidence could have been sought (Figure 5B) by choosing additional adducts such as Na^+ and NH_4^+.

No compound, mass filters or mass defect filters were specified for this search and a maximum charge state of 1 was specified in the MFE method setup. The next step after MFE search was to specify the forensic AMRT database (Figure 6) in the identify compound/search database method editor, highlight all of the MFE-found compounds and search each compound against its content. A mass and retention time (RT) match was specified, since RT database values had already been pre-determined by analyzing individual standards and inserted into a customized compound database.

Figure 7 illustrates the results obtained from the MFE operation invoked by pressing the green *process* button highlighted in the title bar of the MFE method editor (Figure 6).

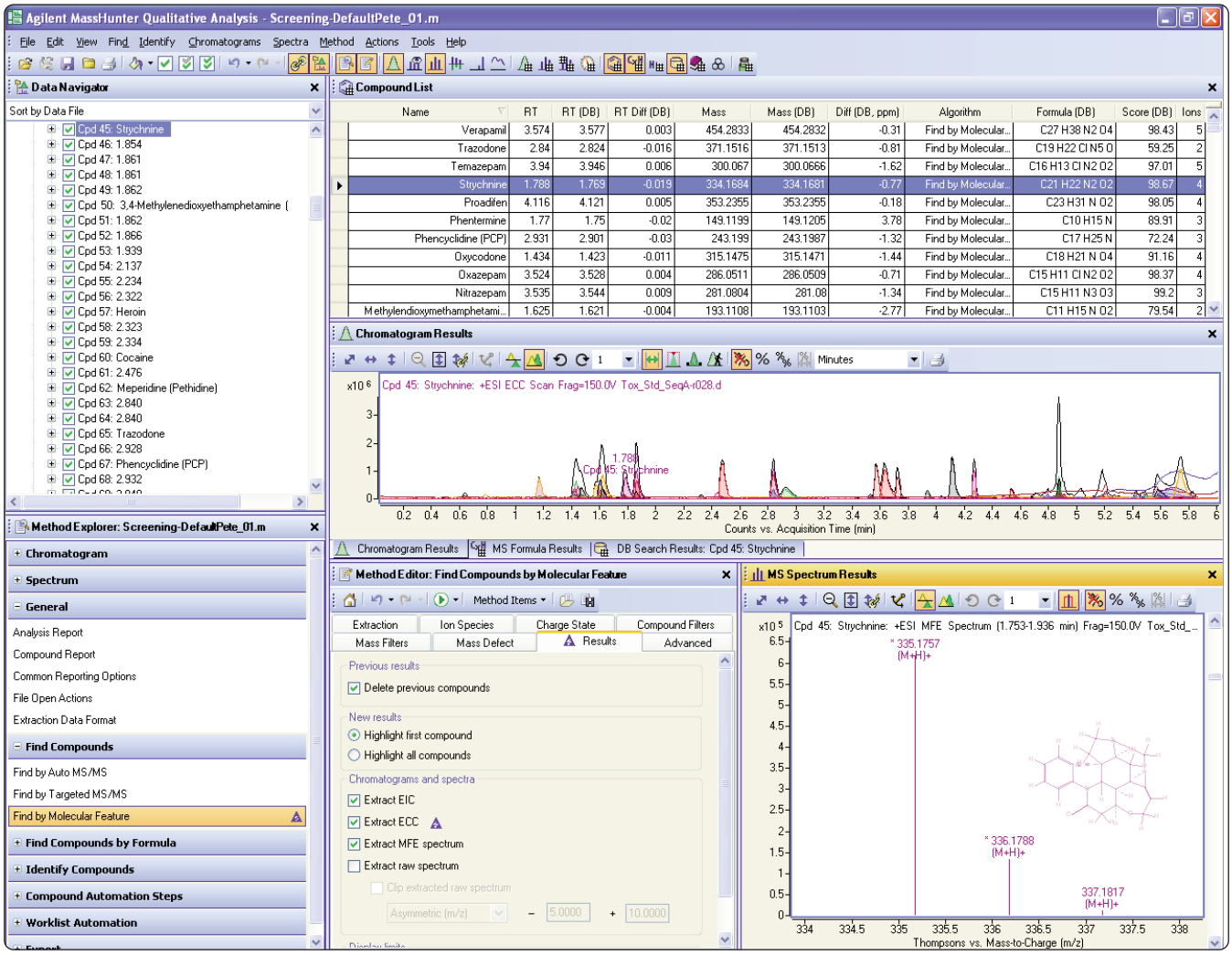


Figure 7. MFE compound database search results using Agilent MassHunter Qualitative Analysis program.

These results are detailed in Table 3 and show that all 25 compounds of the LC/MS Toxicology Test Mix were identified for this sample injection. This confirms that the data analysis settings for the find and identify steps are appropriate for the identification process. Many of the 3,000+ compounds identified by MFE did not find any PCDL matches as expected and the data analysis option of excluding non-positives was used to report only the database hits.

Isobaric compounds such as codeine/hydrocodone and methamphetamine/phentermine were also correctly identified and distinguished automatically, by using the retention capability of the PCDL database and by inputting the predetermined retention time of each analyte for this chromatographic methodology as outlined in the Agilent G6855AA MassHunter Personal Forensic Toxicology Database Quick Start Guide [3].

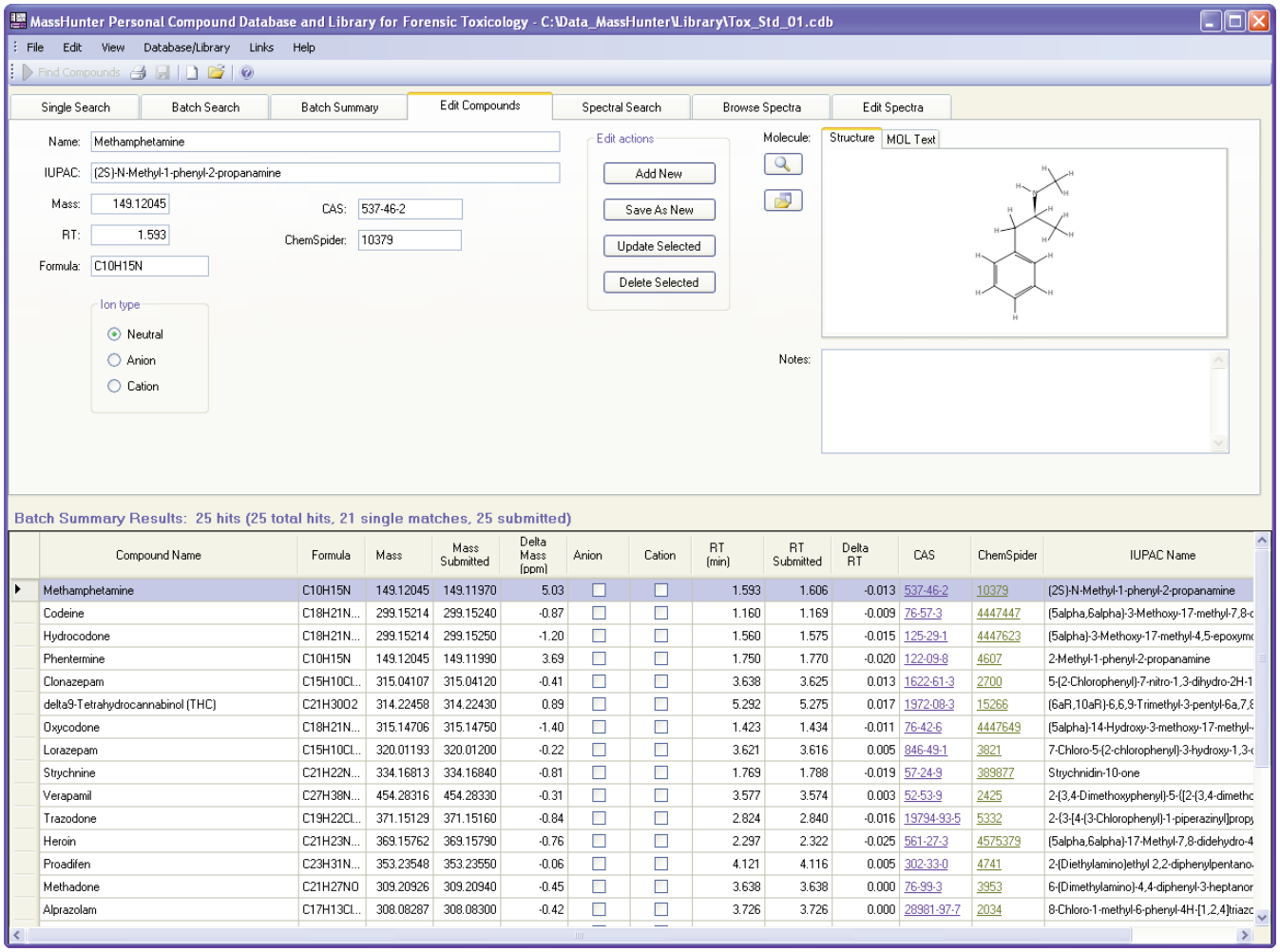
Table 3. MFE Compound and Database Search Results

Name	RT	RT (DB)	RT Diff (DB)	Mass	Mass (DB)	Diff (DB, ppm)	Formula (DB)	Score (DB)
Verapamil	3.574	3.577	0.003	454.2833	454.2832	-0.31	$C_{27}H_{38}N_2O_4$	98.43
Trazodone	2.84	2.824	-0.016	371.1516	371.1513	-0.81	$C_{19}H_{22}ClN_5O$	59.25
Temazepam	3.94	3.946	0.006	300.067	300.0666	-1.62	$C_{16}H_{13}ClN_2O_2$	97.01
Strychnine	1.788	1.769	-0.019	334.1684	334.1681	-0.77	$C_{21}H_{22}N_2O_2$	98.67
Proadifen	4.116	4.121	0.005	353.2355	353.2355	-0.18	$C_{23}H_{31}NO_2$	98.05
Phentermine	1.77	1.75	-0.02	149.1199	149.1205	3.78	$C_{10}H_{15}N$	89.91
Phencyclidine (PCP)	2.931	2.901	-0.03	243.199	243.1987	-1.32	$C_{17}H_{25}N$	72.24
Oxycodone	1.434	1.423	-0.011	315.1475	315.1471	-1.44	$C_{18}H_{21}NO_4$	91.16
Oxazepam	3.524	3.528	0.004	286.0511	286.0509	-0.71	$C_{15}H_{11}ClN_2O_2$	98.37
Nitrazepam	3.535	3.544	0.009	281.0804	281.08	-1.34	$C_{15}H_{11}N_3O_3$	99.2
Methylendioxymethamphetamine (MDMA)	1.625	1.621	-0.004	193.1108	193.1103	-2.77	$C_{11}H_{15}NO_2$	79.54
Methamphetamine	1.606	1.593	-0.013	149.1197	149.1205	4.82	$C_{10}H_{15}N$	81.88
Methadone	3.638	3.638	0	309.2094	309.2093	-0.61	$C_{21}H_{27}NO$	99.67
Meperidine (Pethidine)	2.477	2.456	-0.021	247.1577	247.1572	-1.7	$C_{15}H_{21}NO_2$	97.91
Lorazepam	3.616	3.621	0.005	320.012	320.0119	-0.19	$C_{15}H_{10}Cl_2N_2O_2$	98.27
Hydrocodone	1.575	1.56	-0.015	299.1525	299.1521	-1.2	$C_{18}H_{21}NO_3$	85.2
Heroin	2.322	2.297	-0.025	369.1579	369.1576	-0.63	$C_{21}H_{23}NO_5$	98.97
Diazepam	4.272	4.275	0.003	284.072	284.0716	-1.36	$C_{16}H_{13}ClN_2O$	58.97
delta9-Tetrahydrocannabinol (THC)	5.275	5.292	0.017	314.2243	314.2246	0.94	$C_{21}H_30O_2$	94.83
Codeine	1.169	1.16	-0.009	299.1524	299.1521	-0.72	$C_{18}H_{21}NO_3$	72.49
Cocaine	2.44	2.418	-0.022	303.1475	303.1471	-1.29	$C_{17}H_{21}NO_4$	98.03
Clonazepam	3.625	3.638	0.013	315.0412	315.0411	-0.42	$C_{15}H_{10}ClN_3O_3$	98.72
Alprazolam	3.726	3.726	0	308.083	308.0829	-0.33	$C_{17}H_{13}ClN_4$	96.77
3,4-Methylenedioxyethamphetamine (MDEA)	1.862	1.846	-0.016	207.1263	207.1259	-1.8	$C_{12}H_{17}NO_2$	97.4
3,4-Methylendioxyamphetamine (MDA)	1.474	1.473	-0.001	179.095	179.0946	-2.23	$C_{10}H_{13}NO_2$	86.15

Customized databases with user-added retention times

One of the benefits of the Agilent Personal Forensic Toxicology Database is that it can be saved to a user customized form. To create a read-write customizable database the user selects New Database from the PCDL File menu. The PCDL program then allows selection of an existing database and the naming of a new database. A description can also be given. When *Create* is selected, the database with the new name contains all the entries of the selected database. In this way multiple custom or smaller, more targeted databases can be created depending on the analytes of interest. A technical note on the Pesticide PCD [2] shows how users can run standards with unique chromatographic conditions and easily update or insert retention times in their custom database.

Customizing and updating PCDL AMRT compound data is accomplished by using tab 4 (from left) of the PCDL program interface. This is shown in Figure 8, where the options of *Add New*, *Save as New*, *Update Selected*, and *Delete Selected* are clearly present. When *Allow Editing* is activated from the *Database/Library* pull-down menu, any of the displayed information fields in the users' custom database can be changed, added to, or deleted. Furthermore, the ability to insert *.mol molecular diagrams to any new database entry is possible from the *Edit Compounds* tab.



Compound Name	Formula	Mass	Mass Submitted	Delta Mass (ppm)	Anion	Cation	RT (min)	RT Submitted	Delta RT	CAS	ChemSpider	IUPAC Name
Methamphetamine	C10H15N	149.12045	149.11970	5.03	☐	☐	1.593	1.606	-0.013	537-46-2	10379	(2S)-N-Methyl-1-phenyl-2-propanamine
Codeine	C18H21N...	299.15214	299.15240	-0.87	☐	☐	1.160	1.169	-0.009	76-57-3	4447447	(5alpha,6alpha)-3-Methoxy-17-methyl-7,8-d
Hydrocodone	C18H21N...	299.15214	299.15250	-1.20	☐	☐	1.560	1.575	-0.015	125-29-1	4447623	(5alpha)-3-Methoxy-17-methyl-4,5-epoxym
Phentermine	C10H15N	149.12045	149.11990	3.69	☐	☐	1.750	1.770	-0.020	122-09-8	4607	2-Methyl-1-phenyl-2-propanamine
Clonazepam	C15H10Cl...	315.04107	315.04120	-0.41	☐	☐	3.638	3.625	0.013	1622-61-3	2700	5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2H-1
delta9-Tetrahydrocannabinol (THC)	C21H30O2	314.22458	314.22430	0.89	☐	☐	5.292	5.275	0.017	1972-08-3	15266	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8
Oxycodone	C18H21N...	315.14706	315.14750	-1.40	☐	☐	1.423	1.434	-0.011	76-42-6	4447649	(5alpha)-14-Hydroxy-3-methoxy-17-methyl-
Lorazepam	C15H10Cl...	320.01193	320.01200	-0.22	☐	☐	3.621	3.616	0.005	846-49-1	3821	7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-d
Strychnine	C21H22N...	334.16813	334.16840	-0.81	☐	☐	1.769	1.788	-0.019	57-24-9	389877	Strychnidin-10-one
Verapamil	C27H38N...	454.28316	454.28330	-0.31	☐	☐	3.577	3.574	0.003	52-53-9	2425	2-(3,4-Dimethoxyphenyl)-5-{[2-(3,4-dimetho
Trazodone	C19H22Cl...	371.15129	371.15160	-0.84	☐	☐	2.824	2.840	-0.016	19794-93-5	5332	2-(3-[4-(3-Chlorophenyl)-1-piperazinyl]propy
Heroin	C21H23N...	369.15762	369.15790	-0.76	☐	☐	2.297	2.322	-0.025	561-27-3	4575379	(5alpha,6alpha)-17-Methyl-7,8-didehydro-4
Proadifen	C23H31N...	353.23548	353.23550	-0.06	☐	☐	4.121	4.116	0.005	302-33-0	4741	2-(Diethylamino)ethyl 2,2-diphenylpentano
Methadone	C21H27NO	309.20926	309.20940	-0.45	☐	☐	3.638	3.638	0.000	76-99-3	3953	6-(Dimethylamino)-4,4-diphenyl-3-heptanor
Alprazolam	C17H13Cl...	308.08287	308.08300	-0.42	☐	☐	3.726	3.726	0.000	28981-97-7	2034	8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazo

Figure 8. Edit Compounds PCDL interface tab.

Workflow C. Data mining using 'Find by Formula' (FBF)

The Find by Formula data-mining algorithm of the MassHunter Qualitative Analysis program uses a pre-defined empirical formula (or list of formulae) to search TOF and Q-TOF (MS) data files for evidence that peaks may be present. The PCDL-format databases can also be specified as the list of empirical formulae. Depending on the size and content of the database, FBF can take slightly longer than the MFE approach. However, FBF is highly accurate and sensitive especially at very low analyte concentration levels.

Figure 9 illustrates the results screen displayed after a Find by Formula search has been undertaken using the LC/MS Toxicology Test Mix data file. All 25 compounds were matched with accurate mass, abundance and isotopic spacing in a combined score (shown) together with retention time. The DA method editor settings used for this FBF analysis are shown in Figure 10, where Tox_std_01.cdb was a custom PCDL-format database.

When reporting the results, FBF assesses the chromatographic peak shape and isotopic match scores and returns the best match, even if there are several peaks displayed in the extracted compound chromatogram of similar mass.

Additional adducts $[M+Na]^+$, $[M+NH_4]^+$ and $[2M+H]^+$ were used during this FBF data screen. The extra information is displayed in the spectrum view and results table to provide added confirmatory evidence. Figure 9 shows the Temazepam spectrum which displays both $[M+H]^+$ and $[M+Na]^+$ adducts.

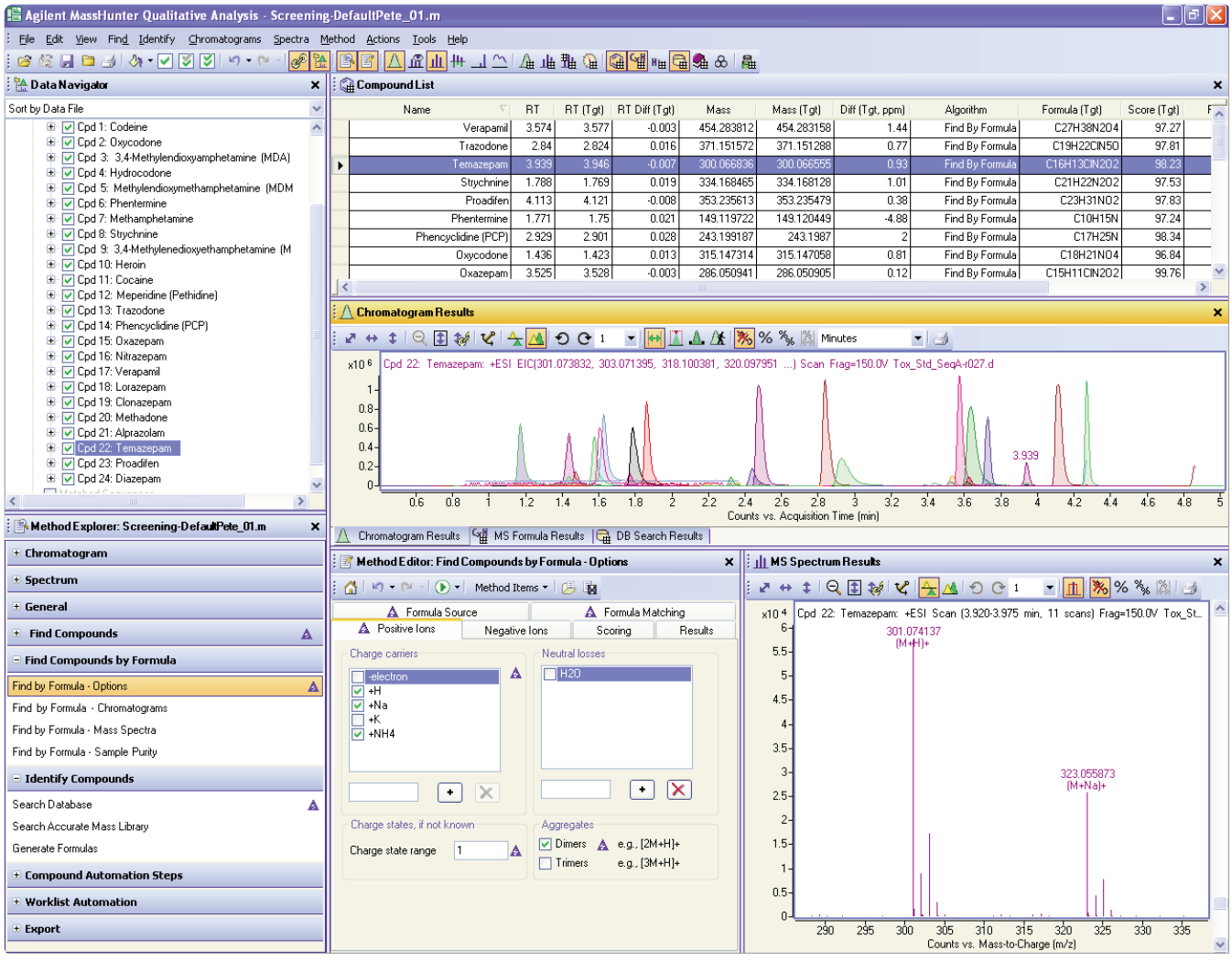


Figure 9. Find By Formula Database search results, Agilent MassHunter Qualitative Analysis program.

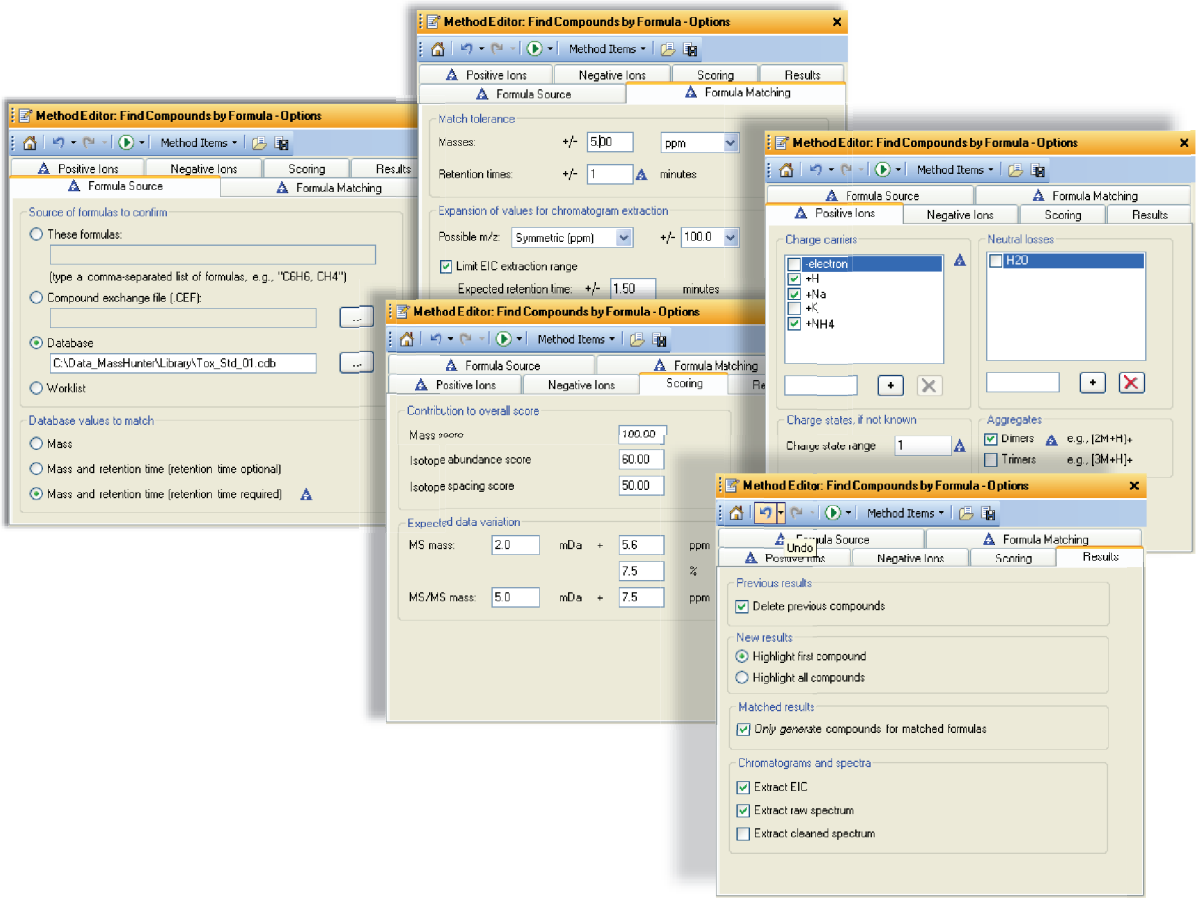


Figure 10. Find By Formula Database search - Method editor settings.

More in-depth information can be obtained from MassHunter Qualitative Analysis program Help files or Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide [5].

Reporting

Manual, MFE and FBF database searching all use the identical method of compound reporting options in the MassHunter Qualitative Analysis program software interface. Figure 11 details the reporting options which are based upon the standard compound report template CompoundReportWithIdentificationHits.xlsx. Under the General section of the method explorer, the 'Common reporting options' link opens the corresponding method editor pane, shown on the left side of Figure 11. MassHunter Qualitative Analysis program treats search algorithm data and database searches as compound-centric data. Therefore, to report the results the appropriate compound report template must be chosen. In this example, the correct report template is displayed.

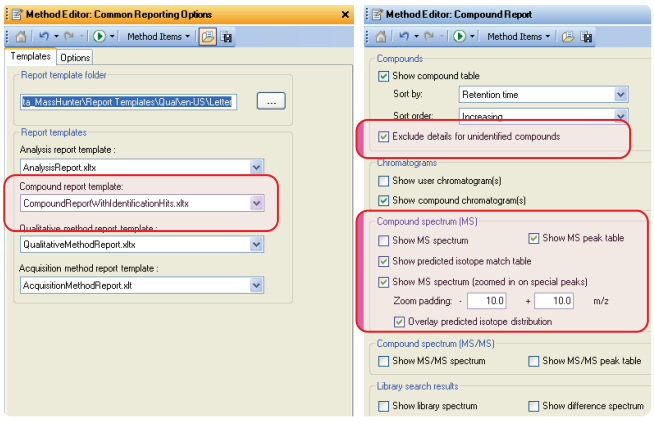


Figure 11. Common compound reporting options for Manual/MFE/FBF PCDL Searches.

More specific content can then be specified by choosing the information required for the Toxicology screen report using the Compound Report options of the method editor (shown on the right in Figure 11).

Decisions about the report content are decided here. For example, if the check box for *Exclude Details for Unidentified Compounds* is activated, then only positive PCDL identifications will be reported. The option to report compound extracted chromatograms, individual MS spectra, or summary results and individual compound tables is also determined from the compound report method editor.

Once all the correct settings have been achieved for the reporting of results, the green button (circled in Figure 12) activates the *printing dialogue* window which gives various options for directing the output of the data file results. The user can choose to send results directly to a specified printer or save the results in excel format or public distribution format (pdf). Alternatively, the results report can be processed by choosing the *Print Compound Report* option from the drop-down *File* menu.

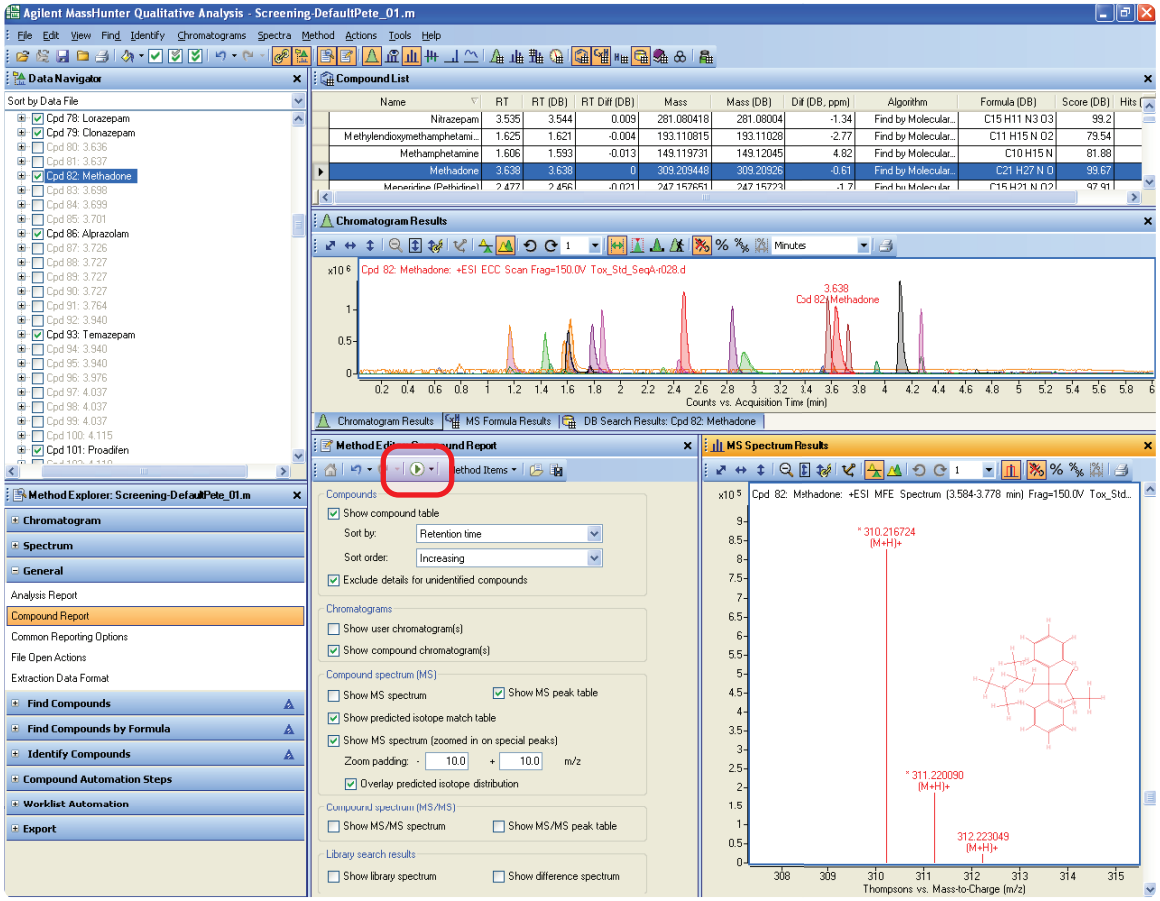


Figure 12. Compound Reporting for Manual/MFE/FBF PCDL Searches.

Figure 13 illustrates a typical report summary front page for the LC/MS Toxicology Test Mix.

Qualitative Compound Report

Data File	Tox_Std_SeqA-r028.d	**Sample Name**	Tox_Std_100pg
Sample Type	Sample	**Position**	Vial 2
Instrument Name	SEIKO-90500107	**User Name**	Pete Stone
Acq Method	PStone_Tox_Std_01.m	**Acquired Time**	7/17/2009 9:41:03 PM
IRM Calibration Status	Success	**DA Method**	Screening-DefaultPete_01.m
Comment			

Compound Table

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 10: Codeine	1.169	299.1524	Codeine	C18 H21 N O3	-0.72	2
Cpd 17: Oxycodone	1.434	315.1475	Oxycodone	C18 H21 N O4	-1.44	1
Cpd 23: 3,4-Methylendioxyamphetamine (MDA)	1.474	179.095	3,4-Methylendioxyamphetamine (MDA)	C10 H13 N O2	-2.23	1
Cpd 29: Hydrocodone	1.575	299.1525	Hydrocodone	C18 H21 N O3	-1.2	2
Cpd 34: Methamphetamine	1.606	149.1197	Methamphetamine	C10 H15 N	4.82	2
Cpd 39: Methylendioxymethampheta mine (MDMA)	1.625	193.1108	Methylendioxymethampheta mine (MDMA)	C11 H15 N O2	-2.77	1
Cpd 41: Phentermine	1.77	149.1199	Phentermine	C10 H15 N	3.78	2
Cpd 45: Strychnine	1.788	334.1684	Strychnine	C21 H22 N2 O2	-0.77	1
Cpd 50: 3,4-Methylenedioxyethamphetami ne (MDEA)	1.862	207.1263	3,4-Methylenedioxyethamphetami ne (MDEA)	C12 H17 N O2	-1.8	1
Cpd 57: Heroin	2.322	369.1579	Heroin	C21 H23 N O5	-0.63	1
Cpd 60: Cocaine	2.44	303.1475	Cocaine	C17 H21 N O4	-1.29	1
Cpd 62: Meperidine (Pethidine)	2.477	247.1577	Meperidine (Pethidine)	C15 H21 N O2	-1.7	1
Cpd 65: Trazodone	2.84	371.1516	Trazodone	C19 H22 Cl N5 O	-0.81	1
Cpd 67: Phencyclidine (PCP)	2.931	243.199	Phencyclidine (PCP)	C17 H25 N	-1.32	1
Cpd 73: Oxazepam	3.524	286.0511	Oxazepam	C15 H11 Cl N2 O2	-0.71	1
Cpd 74: Nitrazepam	3.535	281.0804	Nitrazepam	C15 H11 N3 O3	-1.34	1
Cpd 76: Verapamil	3.574	454.2833	Verapamil	C27 H38 N2 O4	-0.31	1
Cpd 78: Lorazepam	3.616	320.012	Lorazepam	C15 H10 Cl2 N2 O2	-0.19	1
Cpd 79: Clonazepam	3.625	315.0412	Clonazepam	C15 H10 Cl N3 O3	-0.42	1
Cpd 82: Methadone	3.638	309.2094	Methadone	C21 H27 N O	-0.61	1
Cpd 86: Alprazolam	3.726	308.083	Alprazolam	C17 H13 Cl N4	-0.33	1
Cpd 93: Temazepam	3.94	300.067	Temazepam	C16 H13 Cl N2 O2	-1.62	1
Cpd 101: Proadifen	4.116	353.2355	Proadifen	C23 H31 N O2	-0.18	1
Cpd 110: Diazepam	4.272	284.072	Diazepam	C16 H13 Cl N2 O	-1.36	1
Cpd 243: delta9-Tetrahydrocannabinol (THC)	5.275	314.2243	delta9-Tetrahydrocannabinol (THC)	C21 H30 O2	0.94	1

Compound Label	Name	RT	Algorithm	Mass
Cpd 10: Codeine	Codeine	1.169	Find by Molecular Feature	299.1524

Figure 13. Output Report from MFE/Database search.

Worklist Automation

Once the analyst or operator has decided on the correct settings for all aspects of the data mining routines, the PCDL search options and reporting options (outlined in this application note) can be saved to one convenient data analysis method. This method can be used for repetitive and consistent data manipulation from week to week. This is achieved by choosing the *Save As* option from the drop-down *Method* menu in the MassHunter Qualitative Analysis program interface. This method will then open as the default DA method when the MassHunter Qualitative Analysis program is started until another DA method is saved or loaded.

An added advantage to saving reprocessing options is the *Worklist Automation* functionality built into the MassHunter Qualitative Analysis program. Figure 14 outlines the setup of Worklist automation and specifically addresses a routine that would automatically interrogate a data file using MFE and PCDL database search followed by reporting of results to the specified printer or data file location.

In this example, a list of automatic data analysis steps are defined in order of operation, as they would be undertaken manually.

First, the sample data file is loaded, and all previous results (if any) are cleared. Next, the *Find by MFE* routine according to the saved DA method setup is performed with the compound results searched against the PCDL database specified in the DA method. Finally, any results are automatically sent to a final report, the format of which has been determined and also saved to the DA method.

Two further steps must be performed to run such a worklist automation routine automatically during sample data acquisition.

First, the DA analysis method and the Worklist Automation routine must be saved into the acquisition method by using the *Save As* option from the *Method* menu and selecting the MassHunter acquisition method name. Once *OK* is selected, the data analysis method becomes an integral part of the Acquisition method.

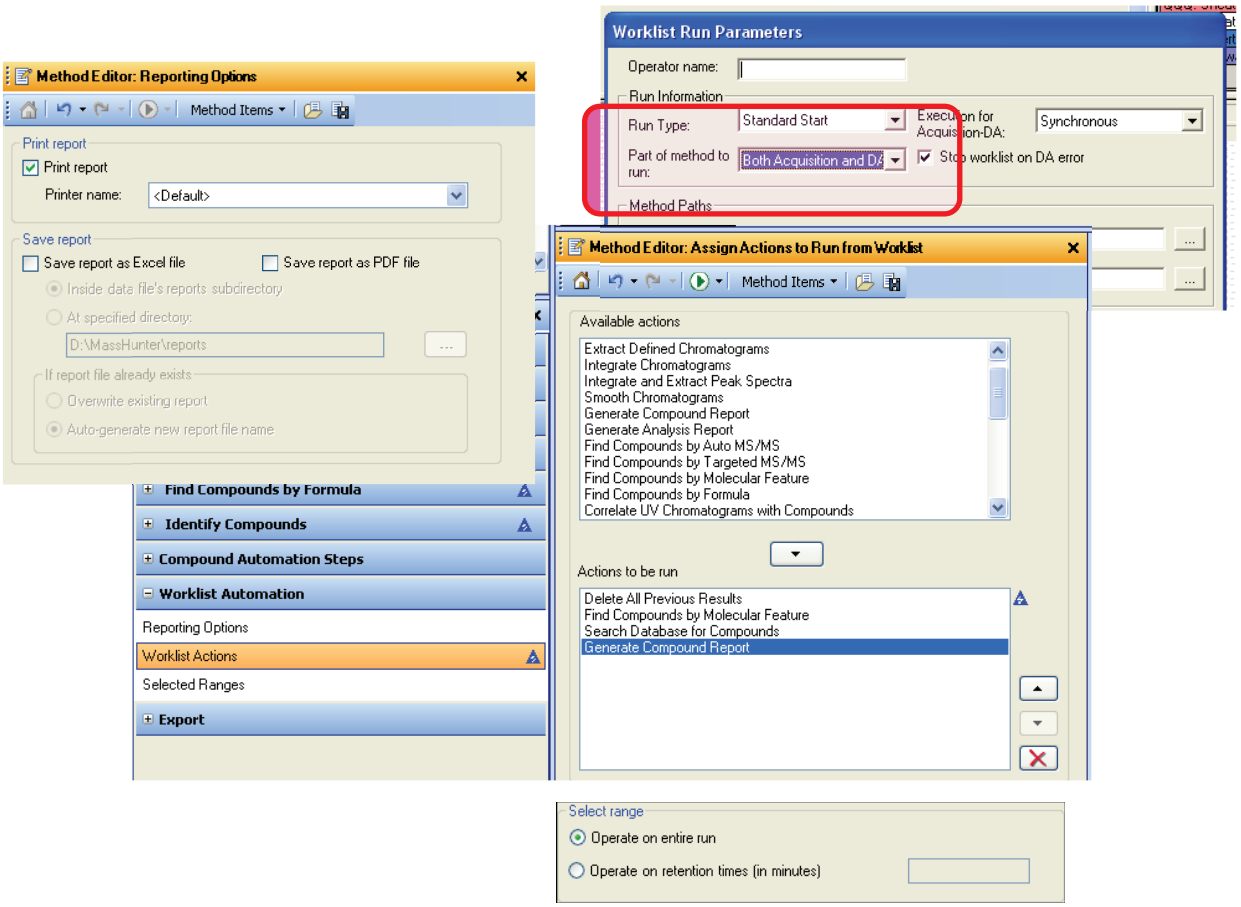


Figure 14. Worklist automation method setup.

Finally, to automatically perform Worklist Data Analysis during data acquisition, the *Worklist Run Parameters* window must be opened from the *Worklist* menu of MassHunter Acquisition software. Figure 14 shows a screen capture of this window with the settings highlighted so that the DA routine will operate *Parts of method to Run - Both Acquisition and DA*. The data analysis has the option to be run *Synchronously* or *Asyncronously*.

Conclusions

The Agilent MassHunter Personal Forensic Toxicology Database Kit has been developed to provide comprehensive screening of samples for both targeted and non-targeted approaches. The database includes accurate mass data for around 6,700 compounds of potential interest and gives the user flexibility in its use.

The MassHunter Personal Forensic Toxicology Database Kit offers:

- Fast and easy startup of complex analyses
- A comprehensive database of around 6,700 compounds including
    - Chemical structures, formulas and exact masses
    - Direct Chemical Internet links to PUBCHEM and ChemSpider
    - IUPAC names
    - The ability to create MS/MS spectral libraries
    - Complete customization with additions/deletions of retention time for chromatographic conditions developed by the user
- Results can be searched from within the PCDL software interface or directly from the MassHunter Qualitative Analysis program.
- Results can be data-mined with powerful searching tools, such as the Molecular Feature Extractor and Find by Formula
- Searches of the database can be partially or completely automated using MassHunter Qualitative Analysis program and the MassHunter Acquisition Worklist

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4. Agilent G6855AA MassHunter Personal Forensic Toxicology Database Kit Quick Start Guide, *Agilent Technologies*, publication number 5990-4264EN
5. Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide, *Agilent Technologies*, publication number G3335-90060.

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