Increased Productivity for Target Compound Screening Using TOF-MS and Accurate-Mass Databases with Optional Retention Time

Part 1: Unique Capabilities of Agilent Software

Technical Overview

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Abstract

Accurate-mass databases can be used with time-of-flight (TOF) mass spectrometry (MS) for rapid, cost-effective screening of samples for target compounds. Agilent provides the most extensive, locally-installable, accurate-mass (AM) databases for endogenous metabolites, pesticides, and forensics/toxicology compounds on the market. These Agilent Personal Compound Databases enable customizable searches and addition of proprietary compounds. The unique capability to add HPLC retention times (RTs) enables use of both accurate mass and greatly increased specificity. Unique scoring algorithms further increase confidence in results. The seamless integration of these databases with multiple Agilent software packages enables new levels of automation and productivity.

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Overview

With increased sample loads, many laboratories are searching for ways to improve productivity for target compound analysis. TOF instruments are ideal for screening applications because accurate-mass spectra provide high specificity. Agilent TOF and quadrupole-TOF (Q-TOF) LC/MS systems deliver an industry-leading combination of mass accuracy, resolution, sensitivity, dynamic range, acquisition speed, and ease of use. To complement these systems, Agilent offers accurate-mass databases, called Personal Compound Databases (PCDs), which can be searched from within the Agilent Personal Compound Database and Library (PCDL) software, as well as from Agilent MassHunter Qualitative Analysis, Mass Profiler, and Mass Profiler Professional. The integration of these powerful software products with the Personal Compound Databases tremendously increases the speed and specificity of screening analyses. This technical overview describes the advantages of the Agilent software solutions for target compound screening using accurate-mass databases. The specificity can be further increased by using accurate mass with optional retention time (AMRT) databases.

Unique advantages of the Agilent solution for searching of accurate-mass databases

More content

Agilent provides accurate mass (AM) databases that have the greatest amount of application specific content in the industry. The following databases are available:

- **Agilent METLIN Personal Metabolite Database,** which contains more than 23,000 compounds related to metabolomics, including 8000 lipids for lipidomics studies
- **MassHunter Personal Pesticide Database** with over 1600 pesticides gathered from five regional regulatory laboratories from Europe and Asia
- **MassHunter Personal Forensic and Toxicology Database** with about 6700 compounds, including controlled substances (for example, drugs of abuse), explosives, compounds from the World Anti-Doping Agency (WADA) Exclusion List, toxins, and mycotoxins

Agilent allows confidential searches

Personal Compound Databases (PCDs) reside and can be searched on a local computer, keeping all searches completely confidential. This eliminates privacy concerns associated with Web-based searches. Because the databases are *personal*, users can add proprietary compounds to customize them. For example, they can add newly identified endogenous metabolites (potential biomarkers) to a custom METLIN database.

Definitions

- Accurate-mass database – **AM database** – contains the calculated exact masses, molecular formulas, and names of compounds, but no spectra
- Accurate-mass/retention time database – **AMRT database** – same as AM database, plus, optionally, contains retention times from a defined set of chromatographic conditions
- **Library** – same as AM database, but contains MS/MS spectra and optional RT
- **Personal Compound Database (PCD)** – AM or AMRT database in MassHunter format, located on the local PC or intranet, provided by Agilent or created by a user
- **Personal Compound Database and Library (PCDL)** – same as PCD, but in addition contains MS/MS spectra for some or all compounds
- **Personal Compound Database and Library (PCDL) software** – MassHunter software module that allows conducting manual searches, adding MS/MS spectra to a library, and editing of AMRT information and MS/MS spectra
- **Target compounds** – compounds being sought
- **Non-target compounds** – compounds not being sought but in database
- **Unknown compounds** – compounds not being sought and not in database

Optional retention time adds specificity to searches

Agilent AM databases are unique because they can include retention times for use in the search. Retention time provides significantly improved specificity and can allow the clear distinction between isomers, most of which can be separated chromatographically. Users can analyze standards under a defined set of chromatographic conditions and then add retention times to the database, converting an AM database to an AMRT database. They can then easily update retention times if chromatographic conditions change. The combination of accurate mass and retention time provides selectivity that is intermediate between an AM-only database search and MS/MS library searching on a Q-TOF instrument. Use of retention time enhances confidence in results.

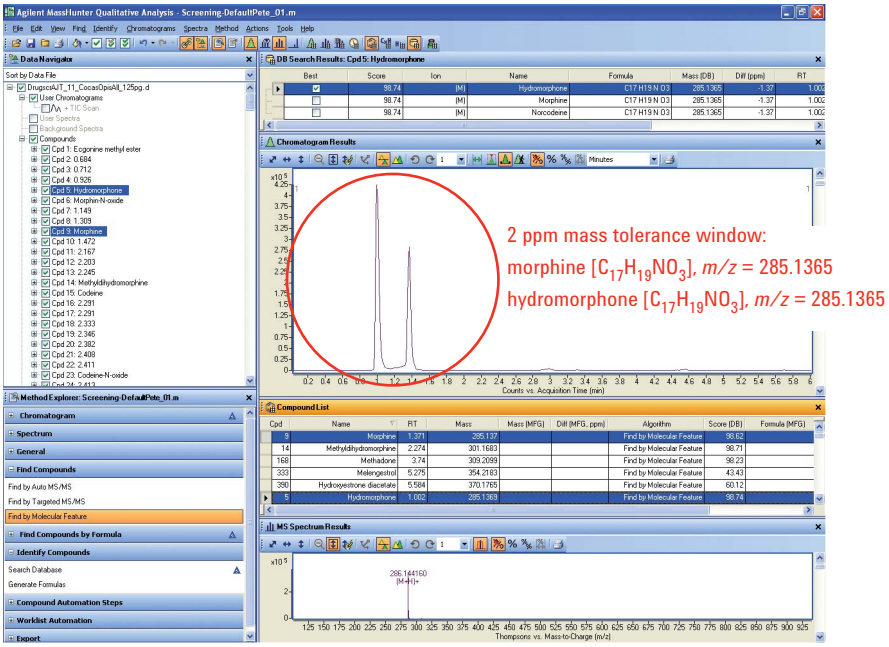


Figure 1. Addition of retention time to the MassHunter Forensic and Toxicology Database enables definitive AMRT screening for these two isomeric target compounds.

With an AMRT database, the analyst has the flexibility to search by:

- Mass
- Mass with LC retention time (useful when only a subset of the compounds in the database have an associated retention time)
- Mass plus LC retention time

New, powerful PCDL software

The user interface for the new Agilent Personal Compound Database and Library (PCDL) software is unique and provides more capabilities than competitive solutions. Users can perform manual searches of a single accurate mass, and searches from mass lists of all compounds detected in a data file. They can view results of manual searches and resolve conflicts (for example, two compound hits for the same mass). When the chromatography changes, the PCDL software allows easy update of all retention times. In addition, users can browse databases, view and add structures, and click links to PUBCHEM and other useful websites. An Agilent technical note describes these capabilities in greater detail.[1]

The PCDL software not only provides the ability to edit and search AMRT databases, but also to add MS/MS spectra to the compound entries so investigators can conduct MS/MS library searches (not covered in this technical overview).

Scoring that better discriminates between good and bad hits

Agilent's scoring algorithm for database searches provides excellent differentiation between good and poor compound hits, because it takes into account not only accurate mass, but also isotope abundance and spacing. (See **Figure 2**.) The use of isotope abundance pattern is very powerful for compounds with elements such as halogens, which have multiple naturally occurring isotopes with significant intensities. Scoring by isotope abundance works well only because detection in the Agilent TOF and Q-TOF systems uses an analog-to-digital converter (ADC), which provides accurate isotope ratios. Some other TOF systems and orbital trapping instruments do not provide the accurate isotope ratios that are needed for the algorithm to work correctly[2].

Isotope spacing is another important component of the scoring algorithm, enhancing confidence even further. The mass position of the M+1 and M+2 isotopes is calculated based on the number and type of elements contributing to them, and the mass spacing from the M to the M+1 and M+2 isotopes can be measured with low- to sub-ppm accuracy. Any small mass shifts affect all isotopes equally, so this measurement is independent of overall mass axis shifts.

This sophisticated scoring algorithm is also used by the MassHunter Molecular Formula Generation (MFG) and Find by Formula (FBF) algorithms.

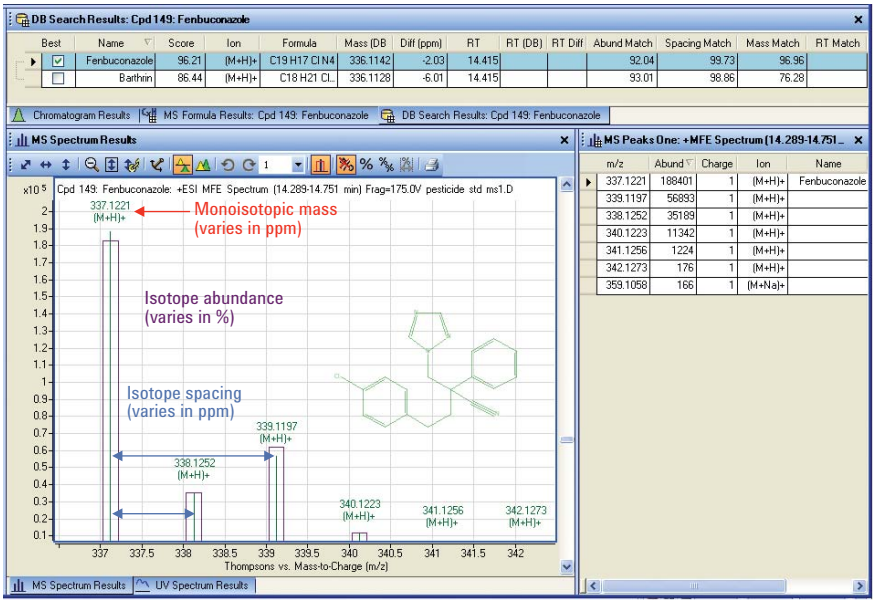


Figure 2. For confident results, PCDL software scores the database matches based on the similarity of the monoisotopic masses (Mass Match), isotope ratios (Abundance Match), isotope spacing (Spacing Match), and optionally, the retention time (RT Match). The above example shows two hits when searching the compounds found in a water sample against the Pesticides AM database. While the Abundance Match and Spacing Match are comparable, Fenbuconazole has a significantly better Mass Match.

Full automation

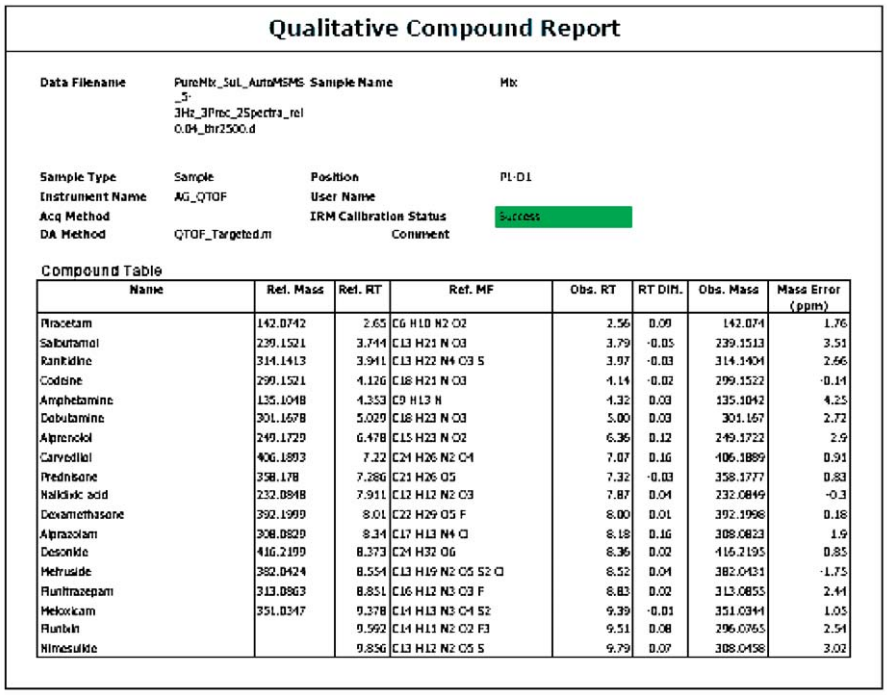
For ultimate productivity, the entire analysis process for target compound screening can be completely automated. Automation can include everything from sample injection and data acquisition to compound searching and reporting. Automated batch analysis is set up via a worklist – a list of all samples to be run with an LC/MS method. The worklist incorporates a data acquisition method, as well as a data processing method. All data processing parameters for the various steps are stored with the data processing method that runs automatically after data acquisition. Agilent has unique compound finding algorithms that can precede searches of the Personal Compound Databases. For example, the Molecular Feature Extraction (MFE) algorithm finds all detectable compounds in the sample, even if they coelute. As described in more detail in Part 2 of this technical overview,[3] multiple compound-finding algorithms allow users to choose the best approach for a given application.

Compound identification via PCDs in differential analysis workflows

The identification of compounds via Agilent PCDs provides unique and seamless integration between search of an accurate-mass database and differential analysis with Agilent Mass Profiler and Mass Profiler Professional. Investigators use the Agilent MassHunter Qualitative Analysis software to find all detectable compounds in each sample. Mass Profiler and Mass Profiler Professional then perform differential analysis on the resulting feature lists, after alignment via RT and accurate mass and then compare feature sets between study sample groups. Without leaving these software applications, users can search an AMRT database to identify target compounds (for example, endogenous metabolites) in the list of differentially expressed compounds, and can annotate their results.

Custom reporting

For maximum flexibility, reports can include all results from accurate-mass database searches with customizable Excel reporting. Preconfigured report templates that meet the needs of many users are part of the product. Analysts can customize the reports to include the desired level of detail and exactly the results they want.



Qualitative Compound Report

Data Filename	PureMix_5uL_AutoMSMS_5-3Hz_3Proc_2Spectra_rel0.04_thr2500.d	**Sample Name**	Mix
Sample Type	Sample	**Position**	P1-D1
Instrument Name	AG_QTOF	**User Name**	
Acq Method		**IRM Calibration Status**	Success
DA Method	QTOF_Targeted.m	**Comment**	

Compound Table

Name	Ref. Mass	Ref. RT	Ref. MF	Obs. RT	RT Dlff.	Obs. Mass	Mass Error (ppm)
Piracetam	142.0742	2.65	C6 H10 N2 O2	2.56	0.09	142.074	1.76
Salbutamol	239.1521	3.744	C13 H21 N O3	3.79	-0.05	239.1513	3.51
Ranitidine	314.1413	3.941	C13 H22 N4 O3 S	3.97	-0.03	314.1404	2.66
Codeine	299.1521	4.126	C18 H21 N O3	4.14	-0.02	299.1522	-0.14
Amphetamine	135.1048	4.353	C9 H13 N	4.32	0.03	135.1042	4.25
Dobutamine	301.1678	5.029	C18 H23 N O3	5.00	0.03	301.167	2.72
Alprenolol	249.1729	6.478	C15 H23 N O2	6.36	0.12	249.1722	2.9
Carvedilol	406.1893	7.22	C24 H26 N2 O4	7.07	0.16	406.1889	0.91
Prednisone	358.178	7.286	C21 H26 O5	7.32	-0.03	358.1777	0.83
Nalidixic acid	232.0848	7.911	C12 H12 N2 O3	7.87	0.04	232.0849	-0.3
Dexamethasone	392.1999	8.01	C22 H29 O5 F	8.00	0.01	392.1998	0.18
Alprazolam	308.0829	8.34	C17 H13 N4 Cl	8.18	0.16	308.0823	1.9
Desonide	416.2199	8.373	C24 H32 O6	8.36	0.02	416.2195	0.85
Mefruside	382.0424	8.554	C13 H19 N2 O5 S2 Cl	8.52	0.04	382.0431	-1.75
Flunitrazepam	313.0863	8.851	C16 H12 N3 O3 F	8.83	0.02	313.0855	2.44
Meloxicam	351.0347	9.378	C14 H13 N3 O4 S2	9.39	-0.01	351.0344	1.05
Flunbin		9.592	C14 H11 N2 O2 F3	9.51	0.08	296.0765	2.54
Nimesulide		9.856	C13 H12 N2 O5 S	9.79	0.07	308.0458	3.02

Figure 3. This custom report shows all of the drugs found in a sample by search of the Agilent MassHunter Forensic and Toxicology Database.

Conclusion

Target compound screening can be accomplished very efficiently using Agilent TOF and Q-TOF MS results and databases with accurate mass and optional LC retention time. The combination of Agilent MassHunter Workstation software, Agilent Personal Compound Databases, and the PCDL software greatly increases the speed of screening by providing:

- The industry's most extensive accurate-mass databases for endogenous metabolites, pesticides, and forensics/toxicology compounds
- The ability to perform confidential searches and add proprietary compounds
- The unique capability to add retention times to databases and easily update them, for greatly increased specificity
- Unique scoring algorithms which use the mass accuracy of Agilent TOF and Q-TOF systems, plus isotope intensities and spacing to differentiate between good and poor hits for highly confident results
- Complete automation of the analysis, from sample injection to a final customizable report
- The ability to search databases and view results from Agilent's differential analysis software modules – the Mass Profiler and Mass Profiler Professional software applications
- A preconfigured, intuitive software layout for target compound screening

These features enable the rapid, low-cost, reliable screening that is required for many targeted analysis applications.

References

1. J. Zweigenbaum, "Pesticide Personal Compound Database for Screening and Identification," Agilent publication number 5990-3976EN, **2009**.

2. "Agilent 6540 UHD Q-TOF produces more accurate isotope ratios than Thermo LTQ Orbitrap XL," Agilent publication number 5990-4337EN, **2009**.

3. F. Kuhlmann, P. Stone, and K. Imatani, "Increased Productivity for Target Compound Screening Using TOF-MS and Accurate-Mass Databases with Optional Retention Time, Part 2: Integrated, Automated Workflows," Agilent publication number 5990-4829EN, **2009**.

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