用于筛选和鉴定的农药个人化合物数据库

Technical Note

Introduction

农药个人化合物数据库开发用于实现大量化合物的快速筛选。本文介绍了此数据库的内容及其使用方法。该数据库除包含农药筛选和鉴定必需的飞行时间质谱（TOF）信息外，还可通过更新保留时间对“目标”化合物信息进行快速的半自动定制。除筛选外，还可向农药分析员提供化合物的其他重要信息，包括结构式、指向 NIH PUBCHEM 数据库的链接以及大量有用的网络链接。

在过去一个世纪中，1000 多种农药被广泛用于农作物保护。一旦超出允许和建议的用量，其中的任何化合物就有可能残留在环境中，并因此而进入食品供应。在调查型监控中，目标化合物的检测和识别对于环境保护和人类健康而言非常重要。液相色谱/飞行时间质谱（LC/TOFMS）凭借其全谱精确质量数测量能力，能够提供灵敏且高度特异的检测。此外，安捷伦还开发了涵盖 1600 多种农药及相关化合物信息的精确质量数保留时间（AMRT）数据库，对这一功能进行补充。该数据库包括通用名称、分子式、结构式和 CAS 登记号。还可轻松使用用户条件下获得的色谱保留时间对库中的保留时间进行半自动更新。本技术指南将介绍如何使用安捷伦的个人化合物数据库（PCD）软件从该数据库中搜索经处理的 LC/TOFMS 数据。还介绍了如何通过安捷伦 MassHunter 定性数据处理软件手动和自动搜索该数据库。

Agilent Technologies

Description

Personal Compound Database

The Personal Compound Database software user interface is shown in Figure 1. Four tabs are available to the user: Single Search, Batch Search, Batch Summary, and Edit Compounds. The first tab is displayed in Figure 1 and demonstrates the functions and data available in the pesticide database. Functions on this tab include searching by mass (protonated, neutral, and proton abstraction), retention time (optional or required), formula, and CAS registry number. Any or all of these parameters can be included in a single search while allowing cations and/or anions. The data includes the common name of the pesticide or chemical, its molecular formula, exact mass, structure, and CAS registry number. The registry number is linked to the on-line National Institutes of Health database PUBCHEM and if clicked will launch the users' internet browser and search PUBCHEM for that CAS number. Please note that not all compounds are listed in PUBCHEM, or if there, may not contain the CAS registry number. In addition, not all compounds in the Pesticide database have been registered in Chemical Abstracts. For example, Figure 1 shows Atrazine D-5 with the CAS number missing. Two other important points need to be made about the Pesticide database. One is that it includes non-pesticides that may be commonly found in samples screened for pesticides such as bisphenol A. The other is that it also includes pesticides, such as hexachlorobenzene, that will not respond to an LC/MS analysis. This makes it a very inclusive database of information. The retention time column is not populated in the database as this would be highly dependent on the user's LC conditions and configuration. However, this provides a very powerful feature that will be described in detail.

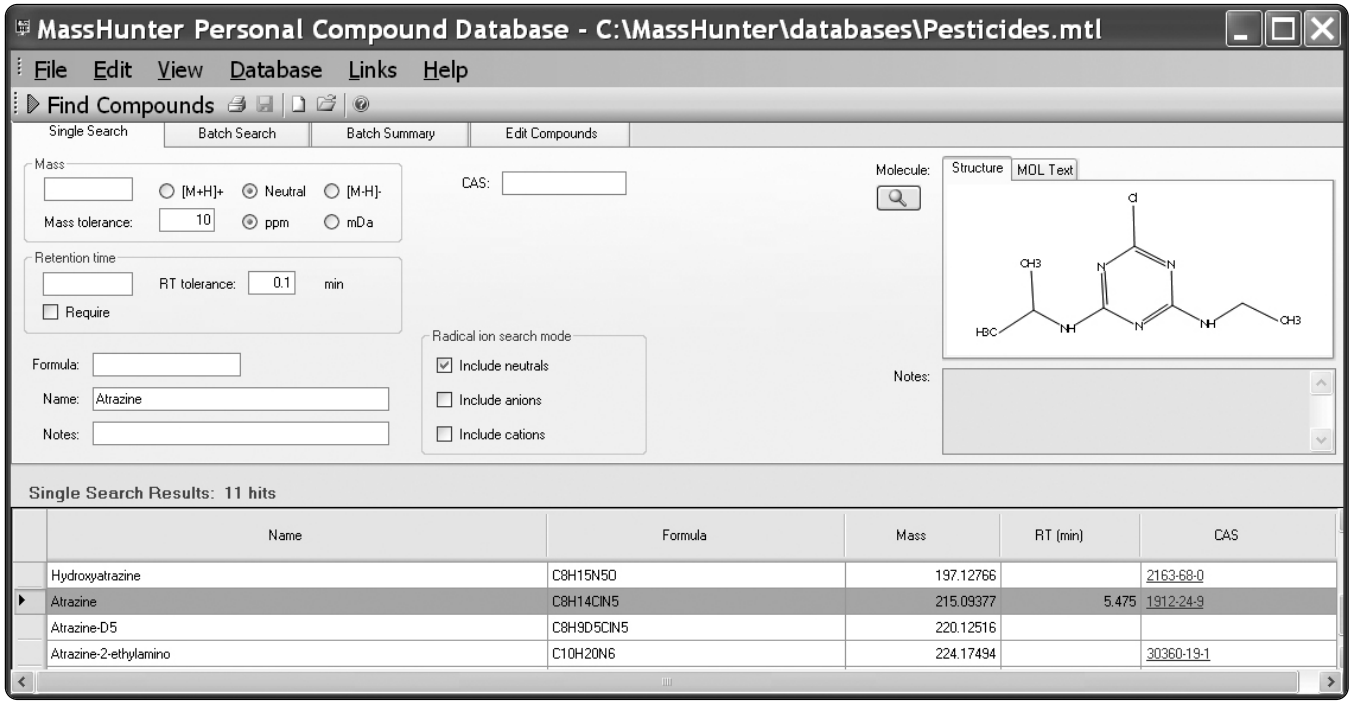

Figure 1. Single Search tab of the Personal Compound Database for Pesticides showing the results for the "Atrazine" name search.

Batch Search

The second tab, Batch Search, is shown in Figure 2. This tab allows the user to import a mass list with retention times from data collected on the user's instrumentation. Generating mass lists and searching the database was created for use with the Agilent LC/MS TOF or LC/MS QTOF systems collecting accurate mass data. Nominal mass lists collected with other instruments will provide too many hits as there are many nominal mass isobaric compounds in the database. In the example shown, isomers are listed. A mass list is typically generated from standards and samples that have been processed in the "Find Compounds - Molecular Feature Extractor" function of the Agilent MassHunter Qualitative Analysis software. The mass list can either be imported as a .csv or .txt file. The mass list can also be pasted directly from a copied list in the MassHunter Qualitative Analysis software as shown in Figure 2. Once a mass list, such as one from pesticide standards, is imported compounds found with similar masses will appear in red. This is indicated by arrows in Figure 2, a black-and-white screen shot of the Personal Compound Database software. These conflicting hits must be resolved manually by the user. In this example, three masses within the 10 ppm mass tolerance selected (mass 151.0631, 151.0641, and 151.0634) are shown having different retention times. It should be noted that the masses imported from MassHunter Molecular Feature Extractor are the calculated masses of the molecule, not the ion so all adduct possibilities are taken into account. These could be isomers or different compounds. The Agilent TOF and QTOF instruments typically provide mass accuracy better than 3 ppm, so a lower tolerance such as 3 ppm would avoid some conflicts. Since these are standards, the analyst should know the compounds injected and be able to select the correct compound and retention time.

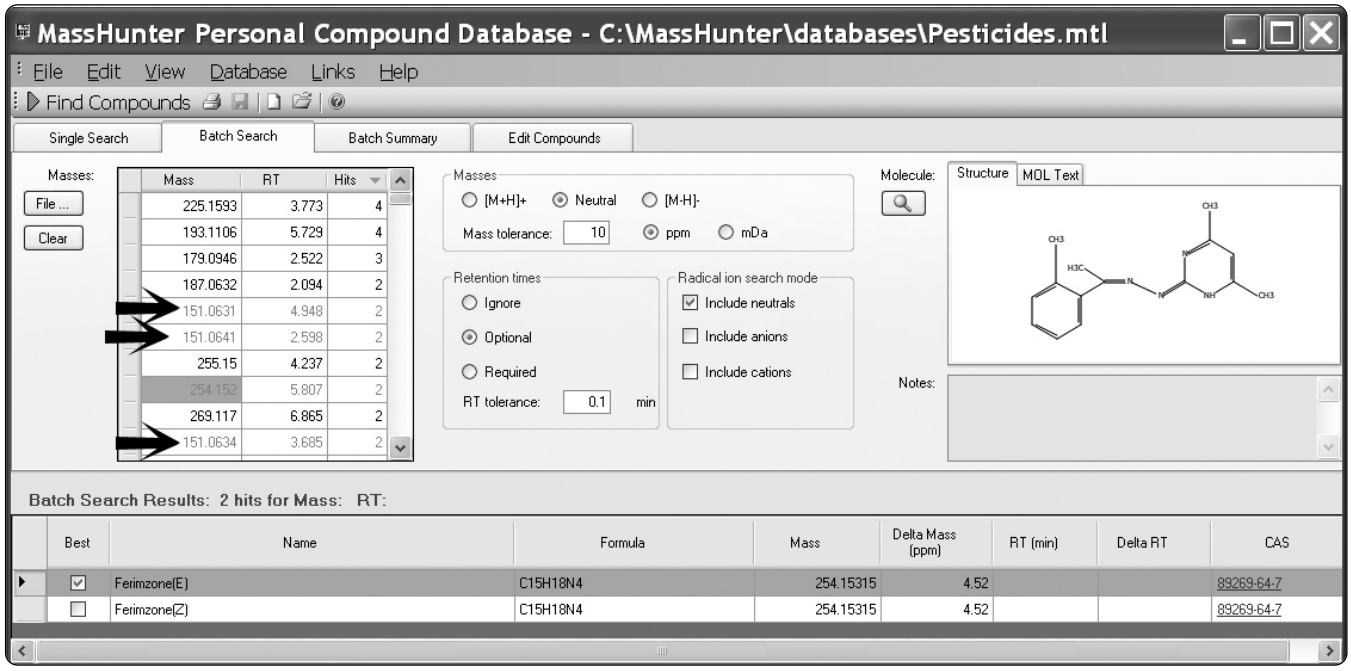

Figure 2. Batch Search tab of the Personal Compound Database for Pesticides showing the results for a mass list generated from a mixture of pesticide standards. Hits in red (indicated by the arrows) show multi-results of mass and retention times and represent conflicts.

Batch Summary

The third tab, Batch Summary, is shown in Figure 3. The capabilities provided in this tab are very powerful. Once all conflicts are resolved with the Batch Search, the user can update retention times of important targeted compounds under the chromatographic conditions selected by the user. This information is then added to the user's custom database. The original Agilent Pesticide Database is "Read Only" and cannot be edited. However, if the user creates a new database based on the Agilent Pesticide database, then this custom file can be edited, retention times updated and compounds added (or deleted). The process can be repeated creating a custom database for each chromatographic set of conditions that the user requires for each different analyses of matrix compound combinations. This provides the analyst "targeted" analyses tailored to their specific needs.

Edit Compounds

The last tab, Edit Compounds, allows the user to change the information in their custom database, individually edit names, structures, retention times, and other parameters. This tab is shown in Figure 4. Compounds can be added or deleted and the database must be updated from this screen. Once a custom database is created, it can be searched by the methods described above or searched directly in the MassHunter Qualitative Analysis software. This will be described in more detail. In summary, the Personal Compound Database for Pesticides provides an informative, user friendly interface that allows customization to meet the needs of the user. Links directly to the internet include direct PUBCHEM searching of each compound in the database with CAS numbers. This gives direct links to TOXNET and the literature enabling the user to efficiently access information about pesticides of interest or concern. The Links pull-down menu of the Personal Compound Database software provides access to useful websites, PUBCHEM, Chem Industry, Compendium of Pesticides, EPA - Pesticides, NIPC, NIH NLM, TOXNET, PAN Database, and WHO - Pesticides.

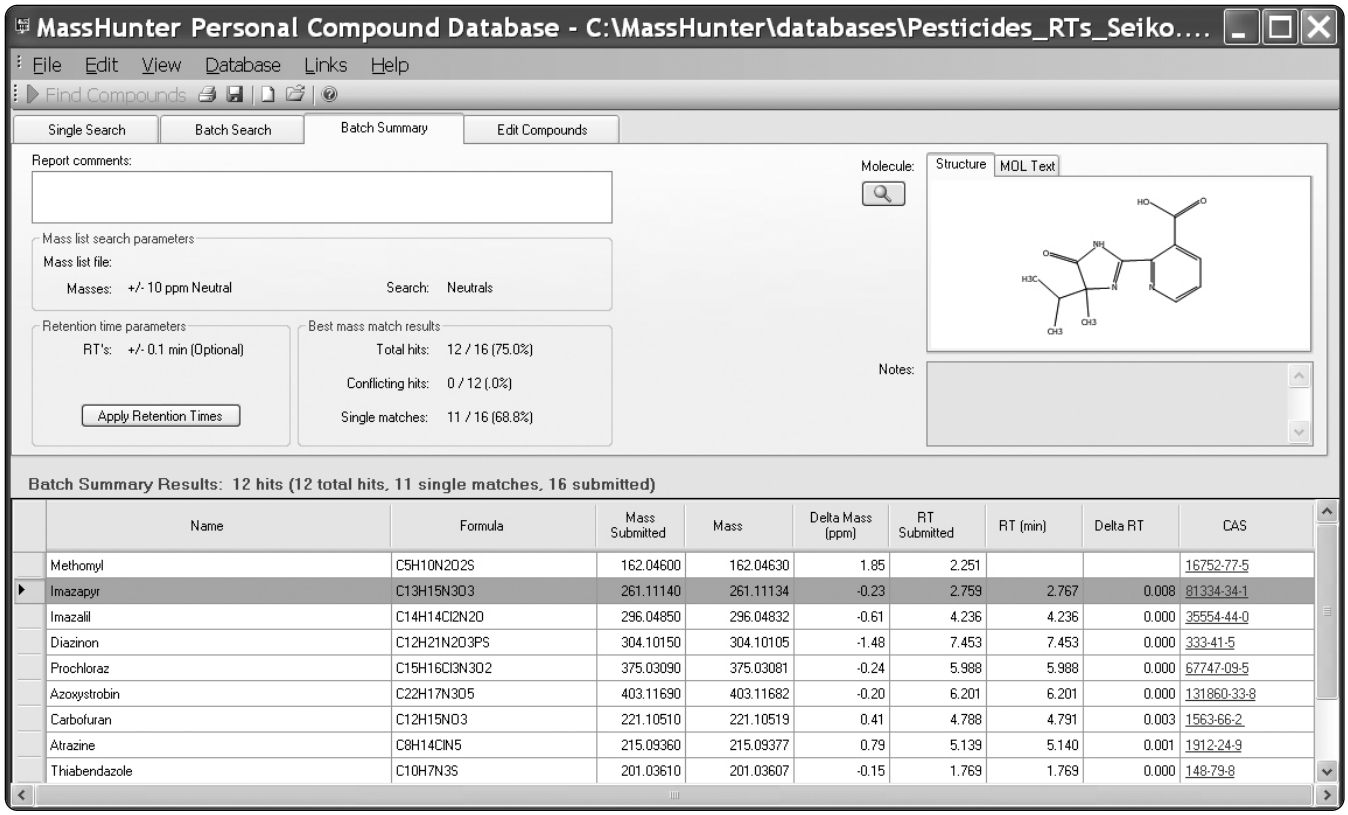

Figure 3. Batch Summary tab of the Personal Compound Database for Pesticides showing the update of retention times from the results obtained in the Batch Search tab.

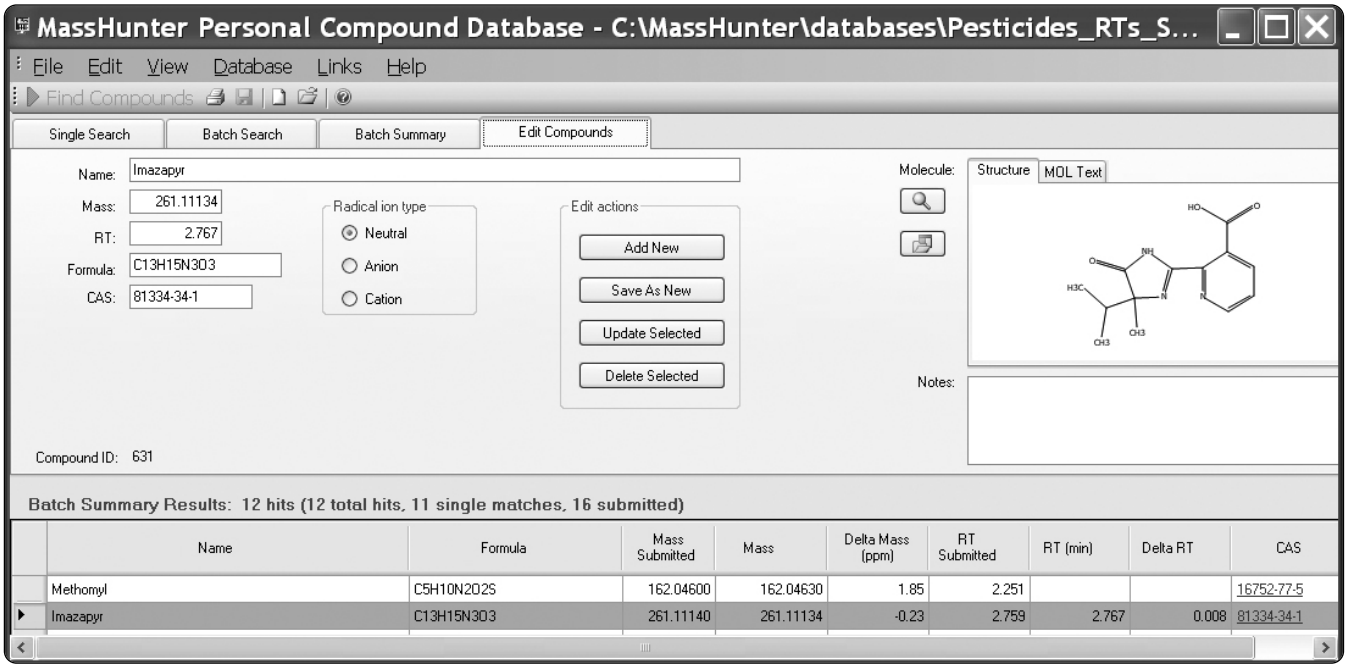

Figure 4. Edit Compounds tab of the Personal Compound Database for Pesticides that allows for editing entries, adding entries, deleting entries and updating a customized database.

Searching the PCD Pesticides

The power of the information rich database lies in the ability to manually, semi-automatically or automatically search LC/Single MS TOF or QTOF accurate mass data for the compounds contained therein. Having made this statement, the user is reminded that of the 1600 compounds provided in the database, there are pesticides that do not ionize by the ionization methods commonly employed by LC/MS. For example, compounds such as hexachlorobenzene will not be detected in a datafile collected by electrospray ionization LC/MS TOF. However, there are still more than a thousand compounds that would be detected at different detection limits in different matrices. Although obvious, it must also be stated that compounds that ionize well only in positive ionization mode will be detected in that mode and those ionized well only in negative ion mode will only be detected in that mode. Certainly, pesticides that have been analyzed under specific conditions where retention times and detection limits have been recorded, under good laboratory practices and quality control procedures will most likely be detected in samples that contain those compounds. Even with these caveats, there is still great analytical power in the ability to screen samples for such a large list of pesticides to determine if a compound not being sought (non-targeted) is there. The ability to collect data with mass accuracy routinely better than 3 ppm and search an exact mass database makes this possible. The technology to detect and identify compounds that may be in real samples at significant levels but are not being sought, is a needed and worthwhile endeavor.

Searching the Personal Compound Pesticide Database can be done with data from real samples in the following ways. Single *m/z* values from LC/MS TOF or QTOF data can be input directly into the Single Search screen of the Personal Compound Database software. Retention time can be included in the search. It should be made optional so that all "hits" with and without retention times that match in *m/z* value will be returned. Using the Personal Compound Database software with the provided or customized pesticide database, a mass list created in MassHunter Qualitative software from sample data can be searched using the Batch Search tab by either importing or pasting the mass list. This is the same procedure used for standards to update the retention times. However, the most efficient search of the database is from MassHunter Qualitative software. Once a search method has been developed, this method can be incorporated into the MassHunter Acquisition worklist so that the data analysis (DA) will be done automatically, and the search done after each data acquisition run. The ability to create customized databases with the chromatographic conditions needed for specific matrices allows the user to conduct targeted screening. Target screening is a specific search for pesticides where standards have been analyzed under defined conditions and

retention times entered into the custom database. In addition, searching the entire database with optional retention times provides the ability to detect non-targeted compounds, or those where no standards have been run but are in the database and have been ionized and detected.

Example of MassHunter Qualitive Analysis Search Methods

As described above, the pesticide analyst may be concerned with three types of compound identification: targeted, non-targeted, and unknowns. For targeted compound determination a custom database may be created starting with the supplied pesticide database of 1600 compounds. A standard or series of standards would then be run on the LC/MS TOF or QTOF. An example chromatogram of pesticide standards is shown in Figure 5. Using the "Find Compounds" and the "Molecular Feature Extractor" of MassHunter a masslist of compounds found in the standard analysis is created and copied to the "Batch Search" tab of the Personal Compound Database. If there are any conflicts as shown in Figure 2, they must be resolved by checking the correct isomer. In this example a conflict of two isomers, acetachlor and arachlor is shown in Figure 6 and are resolved. After resolving conflicts the retention times are updated on the Batch Summary tab as demonstrated in Figure 7 thus saving the updates to the custom database.

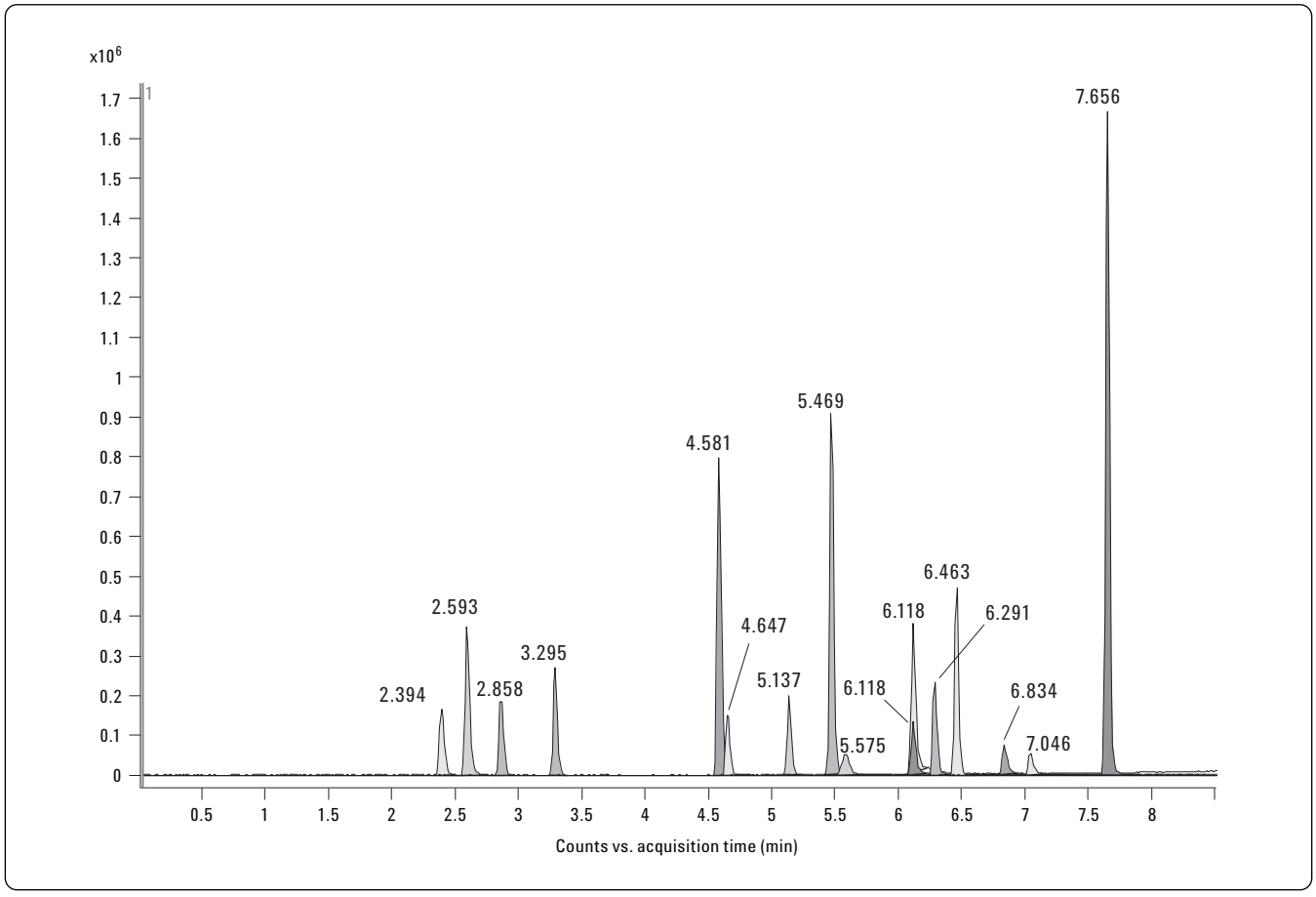

Figure 5. Total ion chromatogram of a pesticide standard mixture run on an Agilent LC/MS TOF system and processed with MassHunter Qualitative Analysis software.

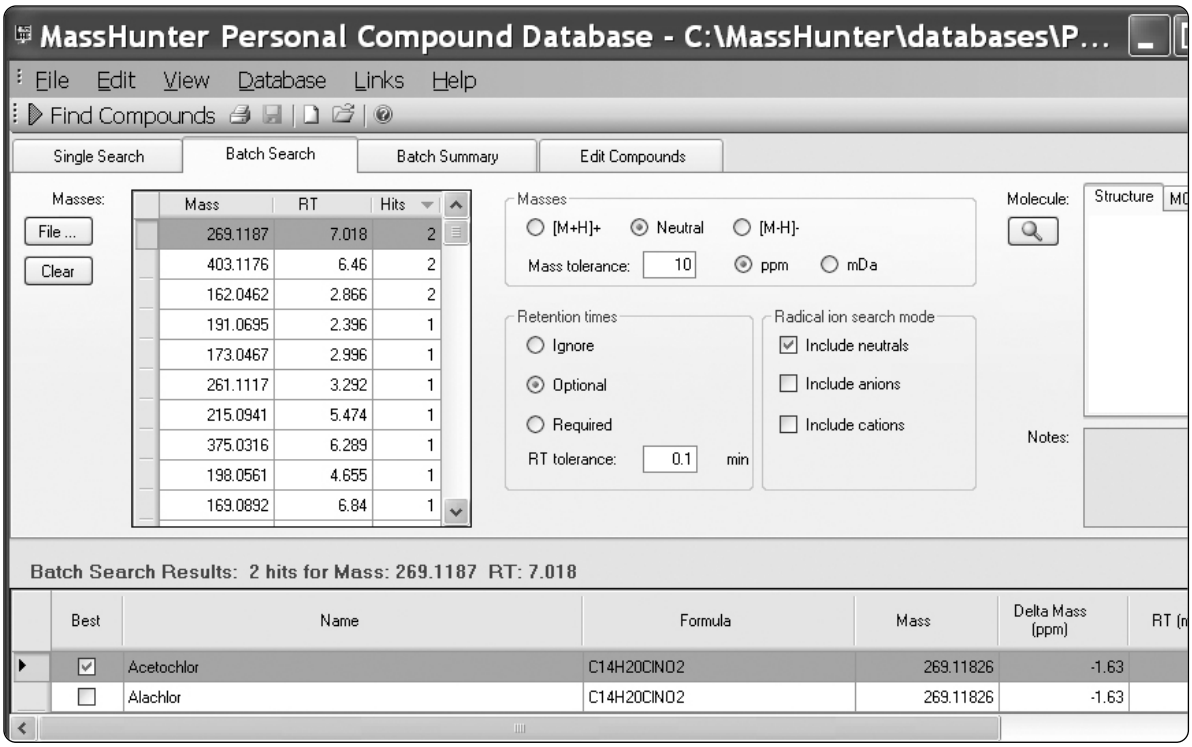

Figure 6. Batch search results of the data file represented by the chromatogram in Figure 5.

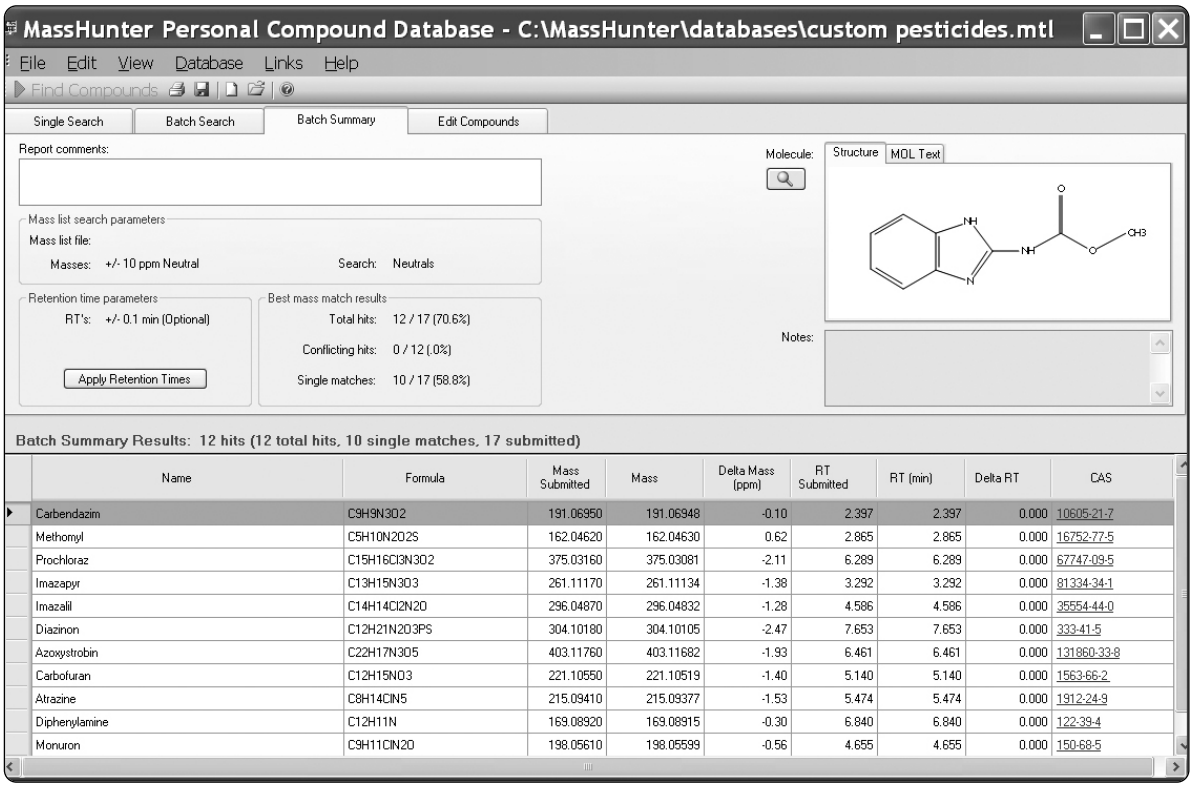

Figure 7. Batch summary results with retention times updated.

Once the analyst has customized his or her database with specific retention times for the chromatographic conditions used for targeted pesticides or has decided to simply search the database in a non-targeted analysis for all possible compounds that may be ionized, that search can be done automatically in MassHunter Qualitative Analysis. The search can be done automatically from a worklist or directly from MassHunter Qualitative Analysis software. In either case the first step is to create a DataAnalysis method (DA). Figure 8 shows the total ion chromatogram of a bell pepper extract run under the same analytical conditions as the standard. The chromatogram shows a very complex matrix. Using the "Molecular Feature Extractor" in MassHunter over 4000 compounds were found in this data file. A search of the customized pesticide database using mass and optional retention times (Figure 9) quickly provides a determination of all "targeted" compounds in the sample or those being sought with retention times. It also provides a determination of "non-targeted" compounds detected that are in the database. The results for this example are shown in Figure 10. Note that the compounds with retention times are scored > 90. Compounds with no retention times are scored 50 or less. Scores are based on mass accuracy of all isotopes, abundance of the isotopes as compared to theoretical values, and the spacing of the isotopes as compared to theoretical spacing. The scoring can be used by the analyst to access whether the hit is a real positive.

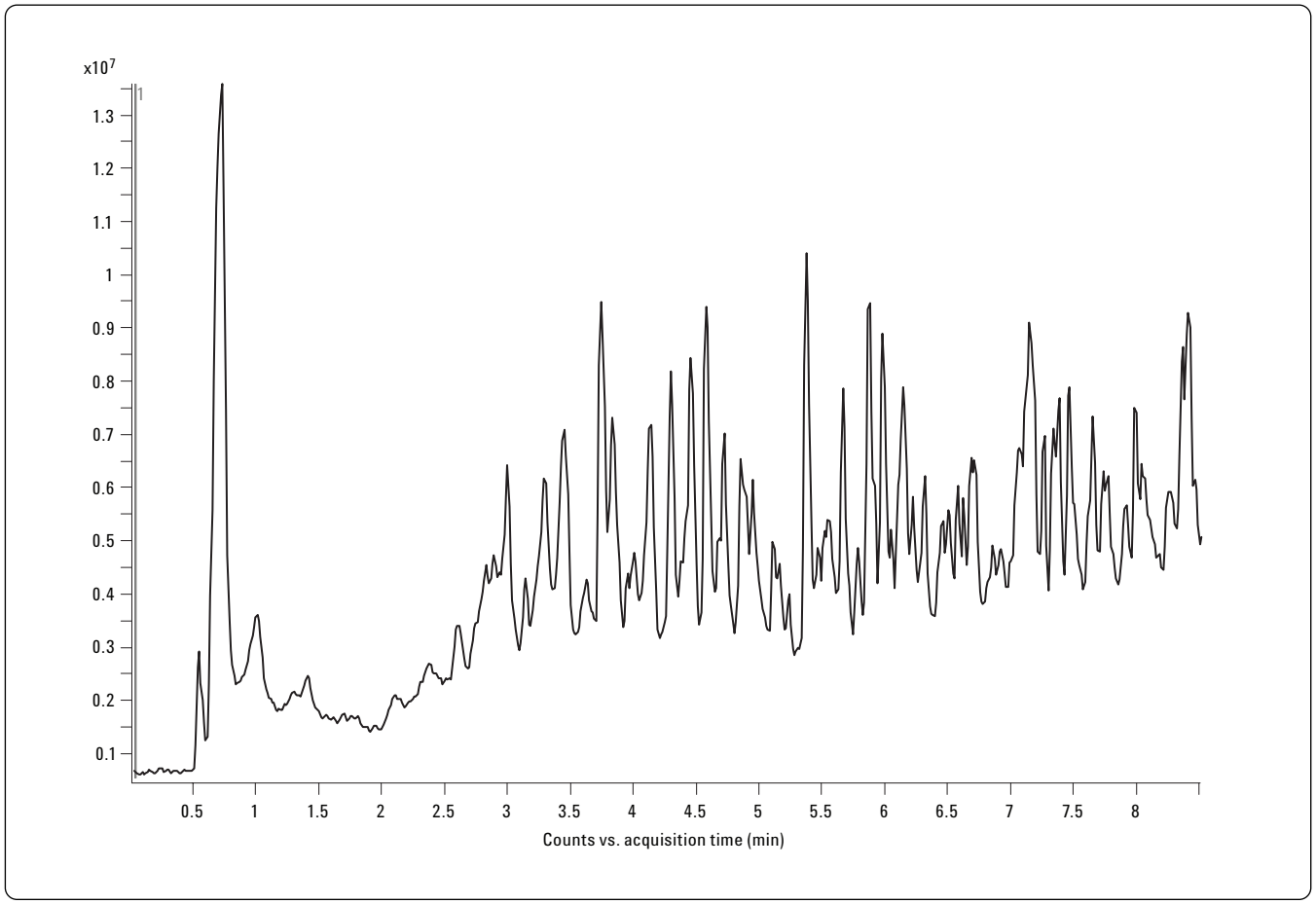

Figure 8. Total ion chromatogram of a bell pepper extract run on the same Agilent LC/MS TOF system.

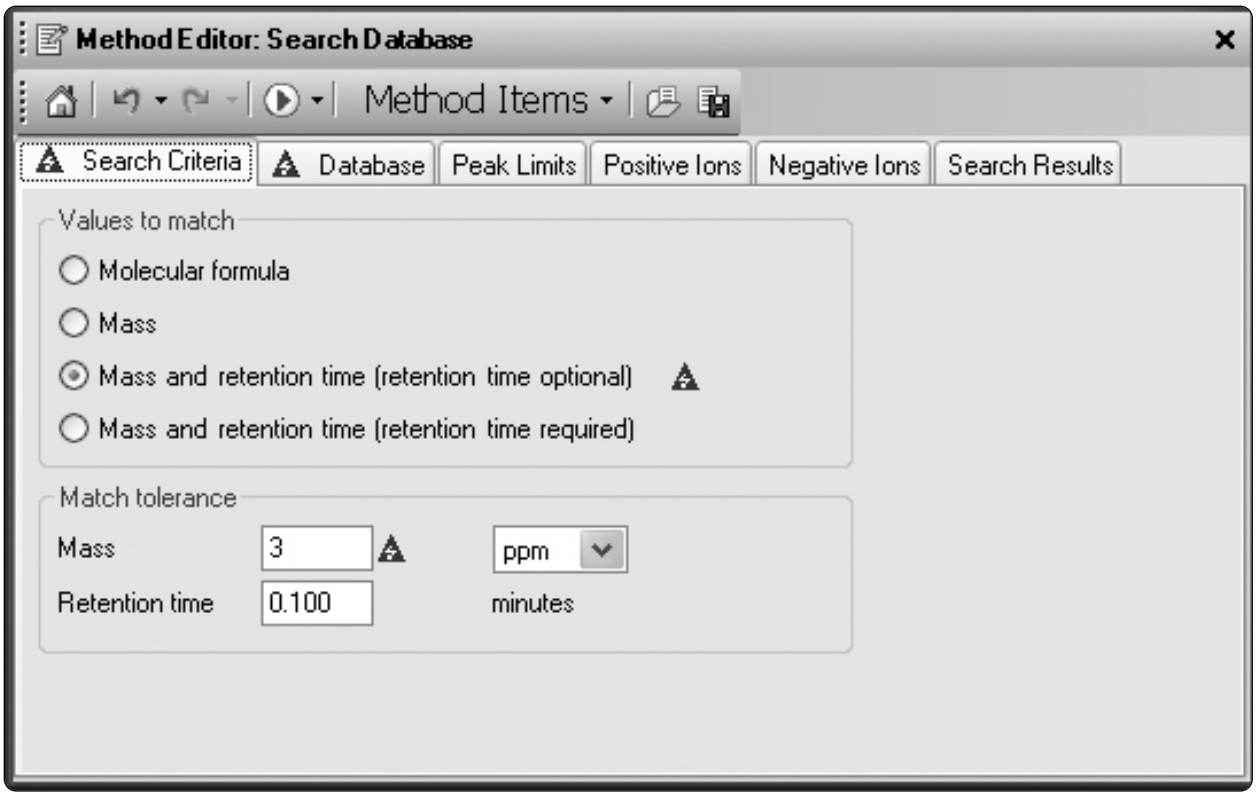

Figure 9. The database search criteria in MassHunter Qualitative Analysis using the customized pesticide database with > 1600 compounds and retention times added from standards analyses. Note these settings will provide for "targeted" (compounds sought with retention times) and "non-targeted" (compounds in database for which no standards have been run) determination.

Compound List

Name	RT	RT (DB)	RT Diff (DB)	Mass	Mass (DB)	Diff (DB, ppm)	Score (D	Hits (DB)	Ions	Height
Diphenylamine	6.843	6.84	-0.003	169.0893	169.0892	-0.62	99.8	1	2	44736
Atrazine	5.476	5.474	-0.002	215.094	215.0938	-1.09	99.68	1	4	814783
Thiabendazole	2.594	2.598	0.004	201.0362	201.0361	-0.61	99.64	1	3	230596
Carbofuran	5.142	5.14	-0.002	221.1055	221.1052	-1.22	99.56	1	5	114690
Monuron	4.65	4.655	0.005	198.0562	198.056	-1.25	99.09	1	3	84376
Azoxystrobin	6.463	6.461	-0.002	403.1175	403.1168	-1.64	98.76	1	11	595918
Carbendazim	2.386	2.397	0.011	191.0696	191.0695	-0.43	97.79	4	2	103038
Prochloraz	6.295	6.289	-0.006	375.0319	375.0308	-2.97	95.82	1	7	193455
Imazapyr	3.279	3.292	0.013	261.1119	261.1113	-1.95	95.63	1	2	206899
Methomyl	2.848	2.865	0.017	162.0465	162.0463	-1.15	94.09	2	18	460124
Imazalil	4.603	4.586	-0.017	296.0489	296.0483	-1.86	93.53	1	6	490117
Propamocarb	0.567			188.1525	188.1525	0.05	50	1	2	63608
Dimetan	1.238			211.1208	211.1208	0	50	1	2	8908
Aldicarb-oxime	2.128			133.0561	133.0561	0.09	50	1	3	331757
3,5-Xylyl methylcarb...	2.522			179.0946	179.0946	0.14	50	3	2	37673
ICIA0858	3.685			192.0899	192.0899	-0.07	50	2	2	92685
Sulfentrazone	5.345			385.9819	385.9819	-0.03	50	1	5	48976
Arecoline	0.739			155.0947	155.0946	-0.23	49.99	1	3	72940

Figure 10. Search results from MassHunter Qualitatve Analysis of bell pepper extract showing positive targeted analysis hits (those with retention times) and non-targeted analysis hits.

Summary

The Agilent Personal Compound Database for Pesticides has been created for analysts to screen for a large list of targeted pesticides and non-targeted compounds easily and accurately. The database provides:

- An information rich resource
- Easy and powerful customization
- Seamless integration with Agilent's MassHunter Qualitative analysis for Automated determinations

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
May 14, 2009
5990-3976EN

Agilent Technologies