

Pesticide Dynamic MRM Compound **Database for Screening and Identification Using the Agilent Triple Quadrupole LC/MS Systems**

Technical Note

Introduction

Over the last century more than 1000 pesticides have been in common use for crop protection. Beyond approved and recommended usage, there always exists the possibility that any of these chemicals can be found in the environment and make their way into the food supply. To protect both the environment and human health, detection and identification in survey type monitoring is very important. Liquid chromatography/tandem mass spectrometry with a triple quadrupole LC/MS meets this need by providing the most sensitive and highly selective detection in complex samples. The system must be run in multiple reaction monitoring (MRM) mode, in order to obtain the maximum sensitivity and selectivity from this technology.

Although the triple quadrupole LC/MS is the most sensitive method for multiresidue analysis, the technique can only detect the pesticides that have been included in the methodology. Each pesticide must contain its predetermined precursor ion and an indicative product ion. This single precursor/product ion pair or transition is required for screening in the MRM mode. For confirmation of a compound at least two transitions must be included in the method so that their presence and correct ratio in a sample can be determined along with the correct chromatographic retention time. Because every compound is different there are specific instrument conditions that will provide an appropriate response for each transition. On the Agilent systems this includes both fragmentor voltage, optimizing transmission of the precursor ion into the mass spectrometer, and the collision energy, optimizing the maximum intensity for a specific product ion. Excellent results are obtained using all other mass spectrometer settings provided by the system's Autotune program.

A powerful tool called MassHunter Optimizer has been added to the Agilent 6400 Series triple quadrupole LC/MS systems. This software allows automated optimization of compound specific parameters, and it is within this tool that the Pesticide Database operates. Any compound that the user optimizes can be saved to a Project or to a Database. Agilent has created a pesticide database containing the operating parameters for over 700 pesticides. The Pesticide Database is read-only and can be saved to any name for customization by re-optimization of compounds in



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the database or addition/deletion of those present. Presently, modifications can be done only in MassHunter Optimizer or by saving method conditions in MassHunter Acquisition, not in the database browser. The power of the database is due to the fact that the conditions included will provide good results without the user needing to optimize each and every compound. In addition, methods are included that now contain retention times for Dynamic MRM [1,2] acquisition. For analysts needing customized methods with hundreds of pesticides per method, the database will allow fast startup and provide good results without the need to optimize the compounds in the database. This does not negate the need to run standards and validate results with good $\Omega C/\Omega A$ procedures. Compounds that the user needs to analyze but are not in the database will need to be optimized. This is readily facilitated by MassHunter Optimizer, and the compounds can then be added to the user's customized database.

Description

The Pesticide Dynamic MRM Database requires MassHunter Acquisition and MassHunter Optimizer 3.01 or later. The link to the database is from the MassHunter Acquisition software or MassHunter Optimizer. It is here that the user can import selected compounds to rapidly develop a customized method, which meets the analytical needs of a specific analysis. This can include compounds in the supplied Pesticide MRM Database and those added by the user, importing to a custom designed chromatographic method for a specific matrix, or a host of other needs for customization. Figure 1 is a screen capture of the MS QQQ Acquisition setup tab for controlling any of the Agilent 6400 Series triple quadrupole LC/MS systems. Right-clicking on the white or grey area of the "Scan Segments" section of the screen displays the pull-down menu where "Import from optimizer" is accessed. When this is selected, the database browser is opened with the default database. The Agilent pesticide database or a customized version of it can be made the default (please note again that the supplied database is read-only). The user can then select the compounds and product ions to import into the acquisition method of his or her choice. The user can also save the compounds in the method to the database with the retention times used in Dynamic MRM.

Sample	Prope	rties h-ALS-SL	.+ BinPu	mp-SL2	Colum	n-SL M	IS QQ									
Tune file Stop time						Acq	uisition Source	Chroma	togram In	strument	Diagnostic	5				
Browse 65 C 1 min							Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy		
- Ion sou	Ion source							Bentazon		239.1	Unit	132	Unit	80	32	
ESI	ESI 🔽 Agilent Jet Stream 🔽 Peak width 0.04 min							2,4,5-T		252.9	Unit	194.8	Unit	76	S	
- Time or	The second							Silvex		266.9	Unit	194.9	Unit	90	5	
Time se	I me segments							Acifluorfen		360	Unit	315.9	Unit	78	5	
#	Time	Scan Type	Div Valve	EMV (+)	EMV (-)	Stored			Dinoseb		239.1	Unit	207	Unit	154	21
▶ 1	▶ 1 0 Dynamic MRM To MS 0 0 🔽							Hexaflumuron		459	Unit	438.9	Unit	102	5	
											Add Row Delete Row Sort					
								Dunamia MPM Parameters			1	Import from optimizer				
							Dynamic MRM Parameters				Update Method					
1	cycles/s ms/cycle							Cycle rime 300 ms				Cut				
												Сору				
												Paste Paste from Cl	ipboard			

Figure 1. MS QQQ Acquisition tab of MassHunter showing the "Import from optimizer" function.

Once in the Database Browser the Pesticide Database can be opened. Figure 2 shows the Database Browser with the Pesticide Database loaded. The pull-down menu at the top left of the screen in Figure 2 or the Database Save icon allows the user to save the database to a new name. This is a necessary step for changes to be made because the Pesticide Dynamic MRM Database is read-only. Compounds can be deleted from the database in the Database Browser, but they cannot be added. That must be done from MassHunter Optimizer (see below). The copied database can now be customized and set to the default. The database contains the compound name, its formula, the nominal monoisotopic mass (nominal mass plus one decimal place where the second is not significant) of the compound, and the method(s) that were used for analyses. The parameters for analysis include the precursor that gave the optimal signal and its associated fragmentor voltage, at least two product ions (if the compound did produce two significant product ions), and the optimized collision energy for each product ion. In addition, the abundance and response factor of each ion is shown so that the user can distinguish between the quantitation ion and the qualifier by their response. In addition, acquisition methods and retention times with retention time windows are given for Dynamic MRM. The user may select a method and import compounds and their associated retention times with that method. The LC portion of this method must be used or the retention times become invalid. If not all the compounds desired are found in a specific method, the user must find the retention times for those compounds using the desired method and then import the other compounds from the database with the same method. The method must also use the same LC configuration used in the database method. Therefore, if a user has a 1200 SL pump, a method using the Agilent Infinity 1290 LC should not be used without expecting to modify the retention times. If only some of the compounds in a method or compounds from various methods are desired, they can be added to the "Import List" as shown in Figure 3. This shopping cart of compounds allows mixing sources of the compounds found by the various search filters provided (Figure 2).

Database Browser								
File Edit View								
Filter Compounds			Search Compounds					
Enable Filters			Sear	ch Text		Colum	nns	
E a state a						Select (Columns	^
Uptimized Compounds					Sec.	Project Name	Loiumis	
Date From 3/17/2010	To 3/17/2010 💌				100	Compound Nam	e	
E Group Name	~				IN	Formula		
		AND						
Project Name	×					CAS		
Method Base-Neutral Pesticides	Test Mix DMRM.m 🔽					Chemical Classe	\$	
						Synonyms		~
Polarity Positive	*		Match entire	word for each stri	ng	- Cuci Liko		
Show All Records Select T	op transitions. Use 🕫	Abundance C	Response Factor			Add to Import List	Searc	h
Compound Name Formula	MW Polarity	Species	Precursor	Product	Frag	CE	BT	BT
Aminocarb C11H16N2O2	208.26 Positive	[M+H]+	209.1	137.1	120	20	3.409	
Aminocarb C11H16N2O2	208.26 Positive	[M+H]+	209.1	152.1	120	10	3.409	
Atrazine C8H14CIN5	215.68 Positive	[M+H]+	216.1	174.1	120	15	8.282	_
Atrazine C8H14CIN5	215.68 Positive	[M+H]+	216.1	132	120	20	8.282	
Carbofuran C12H15N03	221.25 Positive	[M+H]+	222.1	123	120	15	7.896	
Carbofuran C12H15N03	221.25 Positive	[M+H]+	222.1	165.1	120	10	7.896	
Diazinon C12H21N2O3PS	304.35 Positive	[M+H]+	305.1	169.1	160	20	11.845	_
		Ind.in.	205.1	160.1	100	20	11 046	>
Current Database : D:\MassHunter\databases\Pes	ticide_DynamicMRM_Database_Ver_	03.01 (ReadOnly	ı)			Import	Close	

Figure 2. Database Browser view of Pesticide Database.

A good way to search for compounds is to make a list in Excel and copy that list to the search text window (Figure 3). However, the search filter desired MUST be checked. For example, if the user has a list of compound names, then the compound name box must be checked. Likewise if the list is CAS numbers then the "CAS" box must be checked. Selected compounds can be added to the Import list and removed from that list if necessary. However the database itself cannot be edited from the database browser, even in a user-created database.

Edic View										
	6									
ch/Filter Import Li	st									
C		14.7	D.L.N.	Ci	D	Bulut		05	PT	
Compound Name	Formula	200.2C	Polarity	Species	Precursor	Product	Frag	120	2 409	1
Aminocarb	C11H16N202	208.26	Positive	[M+H]+	209.1	152.1	120	10	3.403	1
Atrazine	C8H14CIN5	215.68	Positive	[M+H]+	216.1	1741	120	15	8 282	1
Átrazine	C8H14CIN5	215.68	Positive	[M+H]+	216.1	132	120	20	8 282	1
Carbofuran	C12H15NO3	221.25	Positive	[M+H]+	222.1	123	120	15	7.896	1
Carbofuran	C12H15N03	221.25	Positive	[M+H]+	222.1	165.1	120	10	7.896	1
Diazinon	C12H21N2O3PS	304.35	Positive	[M+H]+	305.1	169.1	160	20	11.845	1
Diazinon	C12H21N2O3PS	304.35	Positive	[M+H]+	305.1	153.1	160	20	11.845	1
Dimethoate	C5H12N03PS2	229.26	Positive	[M+H]+	230	199	80	5	5.858	1
Dimethoate	C5H12NO3PS2	229.26	Positive	[M+H]+	230	171	80	10	5.858	1
mazalil	C14H14Cl2N2O	297.18	Positive	[M+H]+	297.1	159	110	20	7.344	1
mazalil	C14H14Cl2N2O	297.18	Positive	[M+H]+	297.1	255	110	15	7.344	1
Imazapyr	C13H15N3O3	261.28	Positive	[M+H]+	262.1	234.1	160	15	4.733	1
Malathion	C10H1906PS2	330.36	Positive	[M+H]+	331	99	80	10	10.646	1
Malathion	C10H1906PS2	330.36	Positive	[M+H]+	331	127	80	5	10.646	1
Metazachlor	C14H16CIN30	277.75	Positive	[M+H]+	278.1	134.1	100	15	8.96	1
Metazachlor	C14H16CIN30	277.75	Positive	[M+H]+	278.1	210.1	100	15	8.96	1
Metosulam	C14H13Cl2N5O4	418.26	Positive	[M+H]+	418	175	120	20	8.472	1
1	C14H13Cl2N5O4	418.26	Positive	[M+H]+	418	228	120	15	8.472	1

Figure 3. Import list where compounds selected from different search filters can be added to a Dynamic MRM method.

Finally, the other access point for the database is from MassHunter Optimizer program. It is from this access that the user can add compounds to their customized database. Figure 4 shows the initial screen for the MassHunter Optimizer. The icons across the top allow import and export to and from Excel, naming and saving projects and compounds, starting an optimization or breakdown profile, and access to the databases. The circled icon in Figure 4 allows the import from an acquisition method. Figure 5 with "show results" from an import demonstrates that not only the operating parameters, but the retention times and the retention time windows are imported from the method. By selecting the icon circled in Figure 5 the user can then export this to the default database (their custom database). Again, the Pesticide Dynamic MRM database is read-only but once accessed it can be saved as a customized database and then set as the default. As the default, compounds and projects can be saved from MassHunter Optimizer to the database. Compounds can be deleted directly from the Database Browser but can only be added or changed using MassHunter Optimizer. It will be useful for the user to save compounds not in the provided database and at times to re-optimize certain compounds for specific user conditions.

📓 MassHunter Optimizer (dehydroepi)										
File Edit View Import/Export Optimization Tools Help										
Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup										
C Sample introduction										
Injection (with or without column) Path for data files D:\MassHunter\data\Optimizer\										
	Acq Me	hod	Polarity Ion Source	Comments						
 Automatic infusion using Loop injection 	D:\MassHunter\Methods\0	ptimizer_pos1.m Po	isitive ESI+Agilent J							
Manual infusion using syringe										
Fragmentor										
Coarse range From 50 To 200										
Fine Step (+/- 5 steps around coarse)										
E Fact										
Collision Energy										
CE range From 5 To 40										
				2						
Start from predicted value Overwrite Existing										
100										
				✓						
Project Name : dehydroepi Optimizer Status : Read	dy	Current Re	ecord)	All Records						

Figure 4. First tab of MassHunter Optimizer where parameter range and method settings are made. Note icon "Import from Database" provides access to Database Browser.

🖬 MassHunter Optimizer (dehydroepi)										
File Edit View Import/Export Optimization Tools Help										
Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup										
Show results summary										
Compound Name	Formula	Mass	Precursor	Product	Frag	CE	RT	RT Window	^	
Dehydroepiandroste C19	9H28O2	288.21	271.2	253.1	140	5			Perform multi-compound run	
Dehydroepiandroste C19	9H28O2	288.21	271.2	105	140	40				
Dehydroepiandroste C19	3H28O2	288.21	271.2	81	140	25			Curle Time	
Dehydroepiandroste C19	9H28O2	288.21	271.2	142.7	140	29			Cycle Time	
Aminocarb			209.13	137.08	120	20	1.222	0.62	Expected peak width (base) 0 s	
Imazapyr			262.12	234.12	150	20	Ĭ.734	0.81		
Imazapyr			262	217	160	15	1.734	0.81	Frag conversionale time	
Thiabendazole			202.04	175.03	120	30	1.832	0.62	Prag coarse cycle une	
Dimethoate			230	171	80	10	2.482	0.44		
Metoxuron			229.1	72.1	93	14	3.189	0.50	Frag fine cycle time 0 ms	
Bentazone			239.05	132.05	100	25	3.398	0.49		
Bentazone			239.05	197	100	20	3.398	0.49	Product scan cycle time ms	
Imazalil			297	159	160	20	3.670	0.89		
Carboturan			222.11	123.04	120	15	3.931	0.50	Collision Energy cycle time 0 ms	
Larboruran			222.11	165.09	120	10	3.931	0.50		
Atrazine			216.1	132.03	120	20	4.195	0.62		
Atrazine			216.1	229.02	120	15	4.195	0.62		
Meteorulam			418.01	228.02	120	15	4.460	0.47		
metosulam			410.01	354	120	10	4.460	0.47	Fill Values	
	Ш							>		
Project Name : dehydroepi Optimizer Status : Ready Current Record All Records										

Figure 5. Import from an Acquisition method with "Show Results" displayed. The circled icon allows export of all compound information to the default database.

Summary

The Dynamic MRM Pesticide Database provides over 700 compounds with specific parameters for all Agilent 6400 Series triple quadrupole LC/MS systems. It is designed to meet the needs of laboratories analyzing hundreds of pesticides in one analysis. It allows re-optimization of compounds through the MassHunter Optimizer program and incorporation of the compounds into data acquisition methods for multi-residue analysis where Dynamic MRM is most useful. Its benefits to the analyst are:

- Fast method development with compound-specific parameters for hundreds of compounds
- Storage and retrieval of compounds added to the supplied database
- · Customization to meet specific needs of laboratories and their analyses

The database and its functionality will continue to evolve to provide greater search and retrieval capabilities and faster development for Dynamic MRM methods.

For More Information

The Dynamic MRM Pesticide Database is included in only the Pesticide Application kit (Agilent part number, G1733AA). Details of how to use the database are given in the Quick Start Guide that is included in the application kit.

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