

Using Your QTRAP[®] LC/MS/MS System at Full Potential

A Quick-Start Guide to Activate and Perform MS/MS Library Searching for Identification and Confirmation MasterView™ and MultiQuant™ Software

Overview

This document outlines the 6 easy steps you can follow to analyze full scan MS/MS spectra (MRM-triggered MS/MS scans collected on a QTRAP[®] LC/MS/MS system) and compare those results to MS/MS compound libraries to identify and confirm positive peaks in unknown samples.

The benefits of this workflow include:

- Improved selectivity multiple fragment ions are detected (beyond just 2 MRM transitions) meaning additional confidence in identification of positive findings
- Improved sensitivity Enhanced MS/MS scans are called 'enhanced' because fragment ions are accumulated in Q3 of your QTRAP[®], giving you better signal-to-noise for the detected MS/MS spectra
- Improved data processing dual injection approach with automatic quantitation, identification and confirmation using MasterView[™] software and MultiQuant[™] software
- Improved confidence ability to automatically calculate ion ratios and compare results to MS/MS mass spectral libraries





The QTRAP[®] Software Workflow in MasterView[™] Software



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Create a new or open an existing XIC list

- Create a new XIC list by simply copying Name, Mass, Fragment Mass and Retention Time (RT) from your Scheduled MRM[™] acquisition method into the MasterView[™] software.
- The XIC list can be saved and opened for future processing.

Edit settings for data processing and library searching 0.8

- Data processing settings can be edited by clicking the 'Settings...' button at the top of the XIC list
- Define thresholds for intensity and S/N in the calculations tab.
- In the 'Library Searching' tab select the libraries to search, the search algorithm, and specify other criteria such as mass tolerance, Collision Energy tolerance, and the use of polarity and Collision Energy Spread when searching

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C T R	Wiff file Name	Sample Name	Number of positive		V HARD R	water large formula	Name	Mass (Da)	Fragment Mass (Da)	Width (Da)	Expected BT (min)	RT Width	Calculated	Library Hit	Library	Known
L			results	4		/ > > >	Acetaminrid	222	126	0	66	0.5			0	
	Data Scheduled MRM-E	Sample 1	0	2			Acetamipric	223	120	0	9.5	0.5			0	
0	Data Scheduled MRM-E	Sample 2	0				Bromuconazole A	378	159	0	9.8	0.5			0	
0	Data Scheduled MRM-E	Sample 3	0	4			Bromuconazole B	378.1	159	0	10.2	0.5			0	
0	Data Scheduled MRM-E S	Sample 4	0	5			Clothianidin	250	132	0	4.5	0.5			0	
C	Data Scheduled MRM-E	Sample 5	0	6			Cyproconazole	292.12	70.04	0	8.8	0.5			0	
0	Data Scheduled MRM-E S	Sample 6	0	7	V		Epoxiconazole	330.08	121	0	9.6	0.5			0	
0	ata Scheduled MRM-E	Sample 7	0	• 8	V 00		Etaconazole	328.1	159	0	9.7	0.5			0	
-	ata Scheduled MRM-E	Sample 8	-	9	V 00		Fenarimol	331.04	268	0	9.2	0.5			0	
	Data Cahadulad MDM E	Cample 0	0	10	V 00		Flutriafol	302.11	70.04	0	8	0.5			0	
	All Scheduled MRM-E 3	sample 3	0	11	V •		Imazalil	297	158.9	0	9.9	0.5			0	
	Jata Scheduled MRM-E S	sample 10	0	12	V •		Imidacloprid	256	209	0	6	0.5			0	
				13	V 00		Metribuzin	215.1	187.1	0	6.9	0.5			0	
				14	 ✓ ● 		Myclobutanil	289	70	0	9	0.5			0	
				15	 ✓ ● 		Nitenpyram	271.2	225.1	0	4.4	0.5			0	
citina racu	ulti aqual as battas		50 -	16	 ✓ ● 		Nuarimol	315	252.1	0	8.8	0.5			0	
silive resu	nic equal of Detter 🤍 🦲		, 30	17			Paclobutrazol	294.14	70.04	0	8.4	0.5			0	►
			-//2	Control	Nene					74				_	_	

0.6 0.4 0.2 0.0 0.60 0.85 0.45 0.55 0.65 0.75 0.80 0.90 0.40 0.50 0.95 MasterView P MQ MV New Session RT Width (min) Mass (Da) Fragment Mass (Da) Width (Da) Expected RT (min) Calculated Concentr. Library Hit Library Score Known Concentr 223 126 6.6 0.5 0

ulations Ubrary Searching Columns	Confidence Settings	User Settings		
Perform Library Search				
Algorithm To Use During Library Search	Confirmation Searc	h		•
Results Sorted By	Fit			•
Libraries To Search	Search All Libra	ries		^
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Algorithm Parameters				
Constraint			Tolerance	
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Retention Time		+/-	0.5	min
Mass Tolerance		+/-	0.4	min
Use Polarity		Intensity Threshold	0.05	
Use Collision Energy Spr	ead	Minimal Purity	10 %	
I Use Compared Specific	Purity Threshold	Interaction Fronte	5	





- Define criteria for compound identification for RT and library searching in the 'Confidence Settings' tab.
- Note: All settings will be saved with the XIC list.



- Confidence in identification is visualized using the RT and Library 'traffic light'
- Numeric values can be found in the RT % Error and Library Score column. Any of these columns can be used to sort results.
- The MS/MS spectrum can be visually compared to the library spectrum by clicking the 'Show MS/MS' button.

	Mass Error Mass Error (ppm)	Retention Time % Error	Isotope Isotope Ratio % Difference	Library Hit Library Score	Formula Finder Formula Finder Score
\checkmark	< 5.0	< 2.5	< 20.0	> 70.0	> 50.0
A	< 10.0	< 5.0	< 40.0	> 50.0	> 20.0
•	>= 10.0	>= 5.0	>= 40.0	<= 50.0	<= 20.0
ined Score lual scores mbined into a	0 %	50 %	0 %	50 %	0 %



Data example: Identification of Azoxystrobin, Carbendazim, Imidacloprid, and Thiabendazole in an avocado sample (QuEChERS extract 10x diluted)

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- Findings in unknown samples are automatically compared against a standard injection of known concentration.
- The MRM transitions can be normalized for easy comparison of peak intensity.
- Results with a concentration higher than the standard are highlighted in green. Results can be filtered using the 'Display highlighted XIC only'.
- Results can be reported using customizable report templates or can be exported in MultiQuant[™] for further processing including the automatic calculation of ion ratios.

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		Data Sched	uled MRM	Sample 1		0		43	\checkmark		•	•	Fenh	examid	302.1	9	7.2	0.1	8.9		2	258.4118	Fenhexamid	0.49	10	0
~		Data Sched	uled MRN	Sample 2		4		71	\checkmark				Triflox	ystrobin	409	1	86	0.1	10.4		2	4.3781	Trifloxystrobin	0.29	99	.5
\checkmark		Data Sched	uled MRN	Sample 3		2		18	$\mathbf{\mathbf{v}}$				Pyrim	ethanil	200.1	1	00	0	8.5		0.5	145.0609	Pyrimethanil	0.76	99	4
~		Data Sched	uled MRN	Sample 4		1		20	\mathbf{x}				Carte	oxytéň	308	19	0.30	0.1	10.8		2	3.6899	Carbondaria	0.14	99	4
5		Data Sched	aled MRM	Sample 5	-	1		30	\mathbf{x}				Carbe	nuazim	220.1		00	0.1	5.3		2	1.5914	Currodial	0.07	99	2
*		Data Sched	and MDI	Sample 6	-			35	×.				Tehuo		226.1		70	0.1	3.6		2	1.000	Tehuconazole	0.59	56	9
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<u>،</u>		Data Sched	aed MRM	Sample /	-	3		49	ž				Methan	nidophos	142		34	0.1	11		2	0.2068	Methamidonho	0.09	23	7
<u>_</u>		Uata Sched	ned MRM	Sample 8	-	1		10	ž	٠.			Flut	riafol	302.11	70	.04	0	8		0.5	0.1847	Flutriafol	1.17	16	.5
/		Data Sched	uled MRM	Sample 9		3		3	Ĵ,				Bromuco	onazole A	378	1	59	0	9.8		0.5				C	1
/		Data Sched	led MRM	Sample 10)	2		5	v			•	Cloth	ianidin	250	1	32	0	4.5		0.5	0.019	No Acquired M	2.09	C	
1		Data Sched	led MRM	Sample 11	1	3		7	V			•	Epoxic	onazole	330.08	1	21	0	9.6		0.5	0.3101	No Acquired M	0.71	C	
1		Data Sched	led MRM	Sample 12	2	2		11	~	~~			Ima	iliszalil	297	15	8.9	0	9.9		0.5	0.344	No Acquired M	0.97	C	
		Data Sched	uled MRM	Sample 13	3	0	-	13	 Image: A second s				Metr	ibuzin	215.1	18	7.1	0	6.9		0.5	0.692	No Acquired MS	2.93	C	
	va re	rult agust a	r hatter			50 -		15	~		••		Niten	ipyram	271.2	22	5.1	0	4.4		0.5	0.2426	No Acquired M	3.39	C	
siu	ive re	suic equal o	Detter			JU •	-1	19		~~			Thiad	cloprid	253.2	1	26	0	7.4		0.5	0.0075	No Acquired M	6 0.47	C	×
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Data example: Identification of Carbendazim, Cyprodinil, Fenhexamid, Pyrimethanil, Quinoxyfen, and Trifloxystrobin in a grapes sample (QuEChERS extract 10x diluted), however, only Fenhexamid and Pyrimethanil were present at a concentration higher than 10 μg/kg)

Confirmation of Fenhexamid using a second analysis and automatic ion ratio calculation in MultiQuant[™] software

Additional library searching and reporting functionality is available in the LibraryView[™] and Cliquid[®] software.

For additional support on implementing this workflow in your own lab, or for support on other AB SCIEX products, visit our website or email us at support@absciex.com.

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