Agilent 6400 Series
Triple Quadrupole
LC/MS/MS
Users Session

QQQ Method Development and Optimization

MassHunter Quant:
Method setup
Peak detection optimization
Quant troubleshooting

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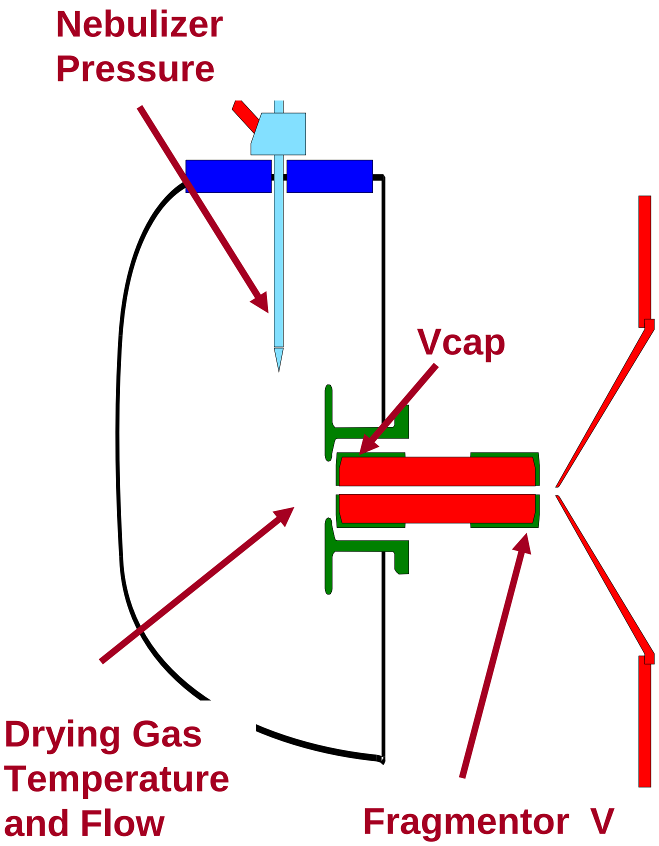
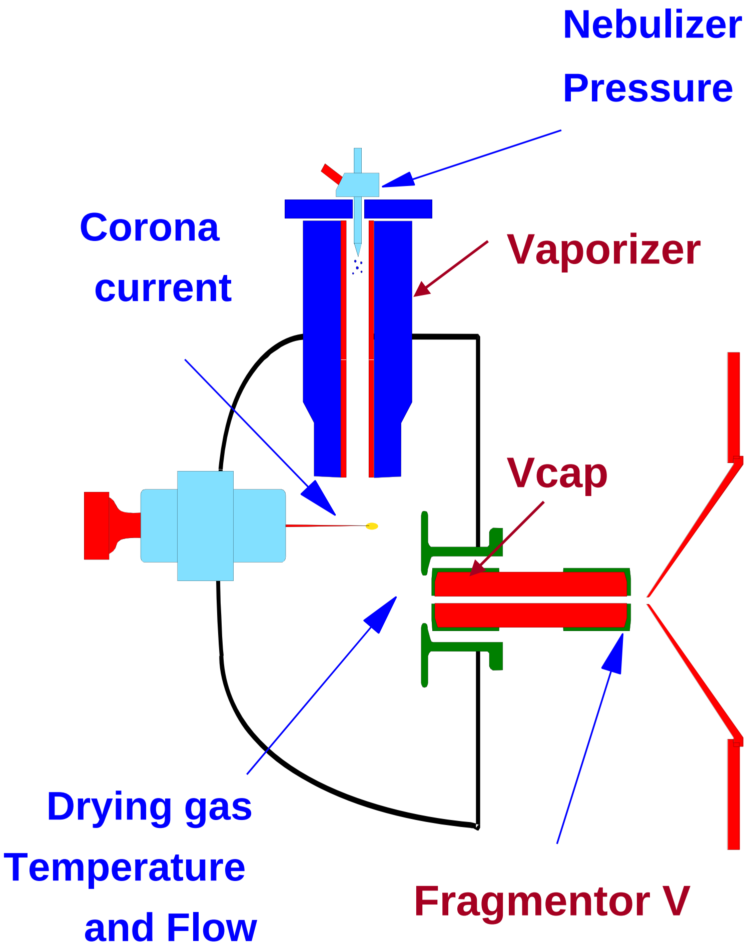
Application Scientist

Agenda

- QQQ method development and optimization
  - Source optimization for standard and Agilent Jetstream sources
  - Compound optimization using flow injection or Optimizer software
- MassHunter Quant software
  - Current revision B.03.02: What's New
  - Review of Quant methods: creation from acquired data, existing batch
  - Global and Advanced parameters
  - Optimizing peak identification and rejection
  - Quant method troubleshooting: identification and integration

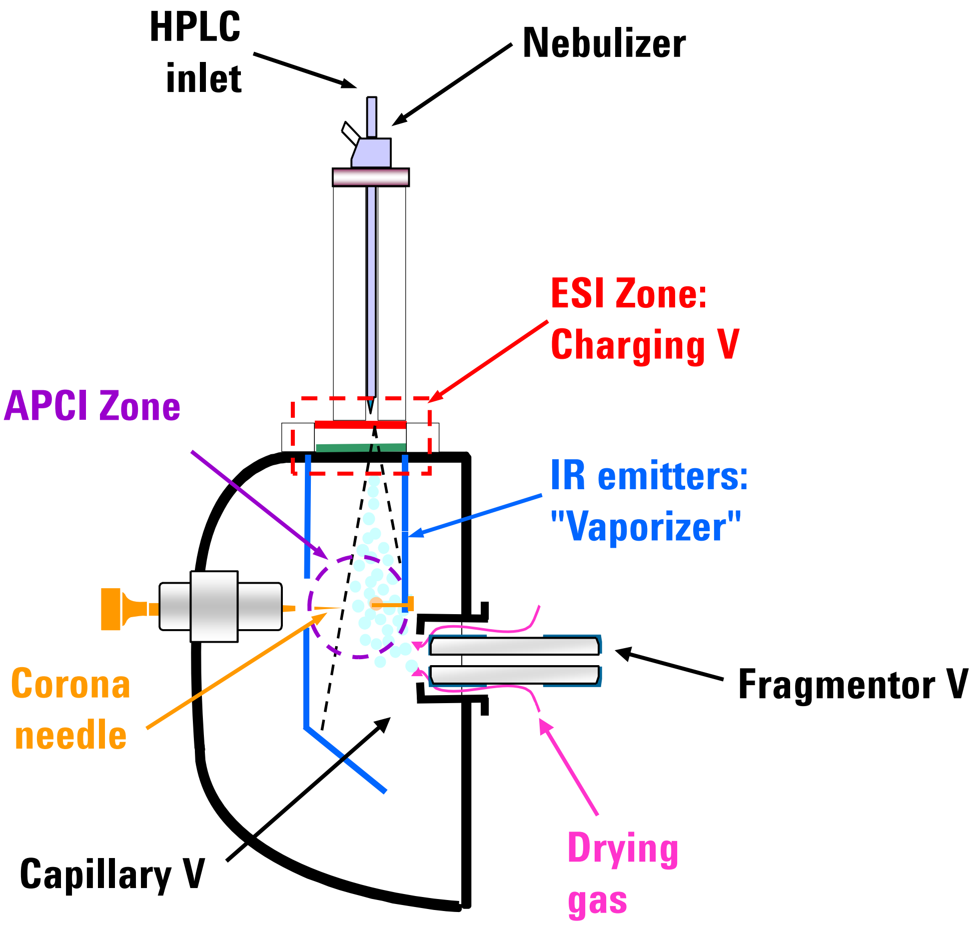
Agilent ESI and APCI sources:
for polar to non-polar compounds





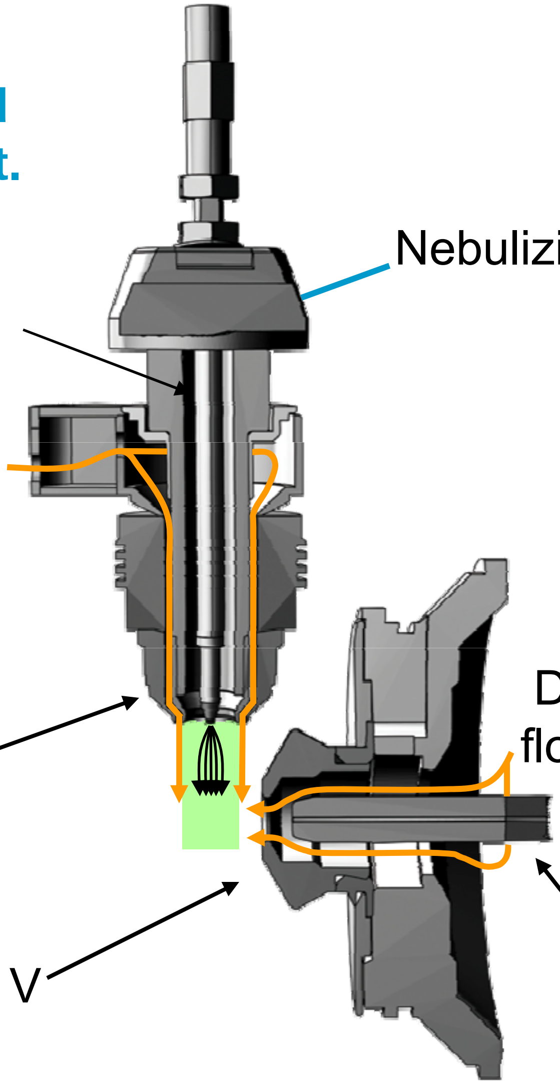
Agilent Multimode source for flexibility

ESI or APCI or Mixed mode (simultaneous ESI/APCI)



Agilent Jetstream Technology (AJT)

The super-heated sheath gas collimates the nebulizer spray and presents more ions to the MS inlet.



Nebulizing gas: pressure

Enhanced efficiency nebulizer

*Sheath gas: flow and temperature

Drying gas: flow and temperature

*Nozzle voltage

Resistive sampling capillary entrance: Capillary V

Resistive sampling capillary exit: FragV

*New parameters unique to AJT source

Source optimization for Agilent LC/MS systems

ESI, APCI, MM and Agilent Jetstream Technology (AJT)

Flow dependent parameters:

- Nebulizer pressure
- Drying gas temperature and flow
- Vaporizer temperature (MM)
- Sheath gas flow (AJT)

Compound dependent parameters:

- Capillary voltage
- Fragmentor voltage
- Collision Energy (QQQ, QTOF)
- Vaporizer temp (APCI, MM, AJT)
- Sheath gas temperature (AJT)
- Nozzle voltage (AJT)

Starting parameters for Agilent sources (small molecules)

Parameter	ESI source	APCI source
Nebulizer pressure	25 psi 0.2 mL/min 50 psi 1 mL/min	60 psi
Drying gas flow	10 Lpm ≤ 0.2 mL/min 12 Lpm > 0.4 mL/min	5 Lpm
Drying gas temp	325-350°C	350°C
Capillary voltage	3500V	3500V
Fragmentor voltage compound dependent	70-100 V Single quads 120-140V QQQ, TOF 140-170V 6230, 6530	70-100 V Single quads 120-140V QQQ, TOF 140-170V 6230, 6530

Starting parameters for Agilent sources (small molecules)

Parameter	Multimode source	Agilent Jetstream
Nebulizer pressure	60 psi ESI 20 psi APCI 40 psi Mixed mode	45 psi
Drying gas flow	5 Lpm	7 Lpm
Drying gas temp	250°C ESI, Mixed 300°C APCI	300°C
Capillary voltage	2000V	3500V
Charging voltage (MM)	2000V	
Nozzle voltage (AJT)		500V
Fragmentor voltage Compound dependent	70-100 V Single quads 120-140V QQQ, TOF 140-170V 6230, 6530	70-100 V Single quads 120-140V QQQ, TOF 140-170V 6230, 6530

Tuning and Calibration for Agilent QQQ systems

•Agilent QQQ systems are very stable and require infrequent Autotuning (if not, place a service call!).

•Current software uses "ESI-Low" tune mix for all QQQs.

•Routine use only requires resolution and mass axis verification for polarity and resolution modes to be used.

  - Run performance check sample (lab SOP)
  - Do Checktune (checks all three resolution modes)
  - In Manual Tune, turn on calibrant and observe MS1 and MS2 profiles. Use *Adjust Gain and Offset* button if width or mass axis requires adjustment.

•If using negative ion, Autotuning should be done monthly or less to minimize source exposure to TFA.

  - Flush source extensively with LC flow after 30-minute Autotune before running samples in negative ion.

- Autotune at least quarterly to optimize ion transmission and update EM voltage.

Optimizing the AJT source beginning with recommended starting values ( )

Order of effect on sensitivity	Things to note
Sheath gas temperature (250°C)	Requires time to stabilize
Sheath gas flow (8 Lpm)	Generally 10-12 Lpm for 0.2-0.7mL
Nebulizer pressure (45 psi)	LC flow- and mobile phase- dependent
Capillary voltage (3500V)	Somewhat MW dependent
Nozzle voltage (0V)	Very compound- and polarity-dependent
Drying gas temperature (250°C)	Interaction with flow and sheath parms
Drying gas flow (5 Lpm)	Generally 5-7 Lpm, higher when in doubt

Optimizing the AJT source
Typical values ( ) and Increments

Order of effect on sensitivity	Increments and ranges to test
Sheath gas temperature (325°C)	50°C, 250-400°C
Sheath gas flow (10 Lpm)	2 Lpm, 8 -12 Lpm
Nebulizer pressure (40 psi)	5 psi, 25 – 50 psi
Capillary voltage (3500V)	500V, 2500-4500V
Nozzle voltage (0V)	500V, 0 - 2000V
Drying gas temperature (300°C)	50°C, 250-350°C
Drying gas flow (7 Lpm)	2 Lpm, 5-11 Lpm

Optimization techniques

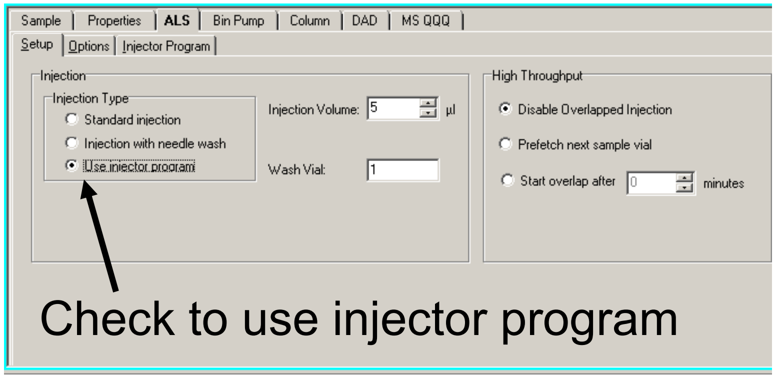
•Fragmentor V and Collision Energy (for MS/MS systems) should be optimized first using recommended starting parameters for source.

•Voltages, gas flows change quickly and can be optimized with series of flow injections using method with injector program and time segments.

•Temperatures (sheath gas, drying gas, vaporizers) require equilibration time between injections; perhaps best done with final chromatography conditions.

• Template flow injection methods also come with Optimizer software

Injector program for series of flow injections



Injection Type
○ Standard injection
○ Injection with needle wash
● Use injector program

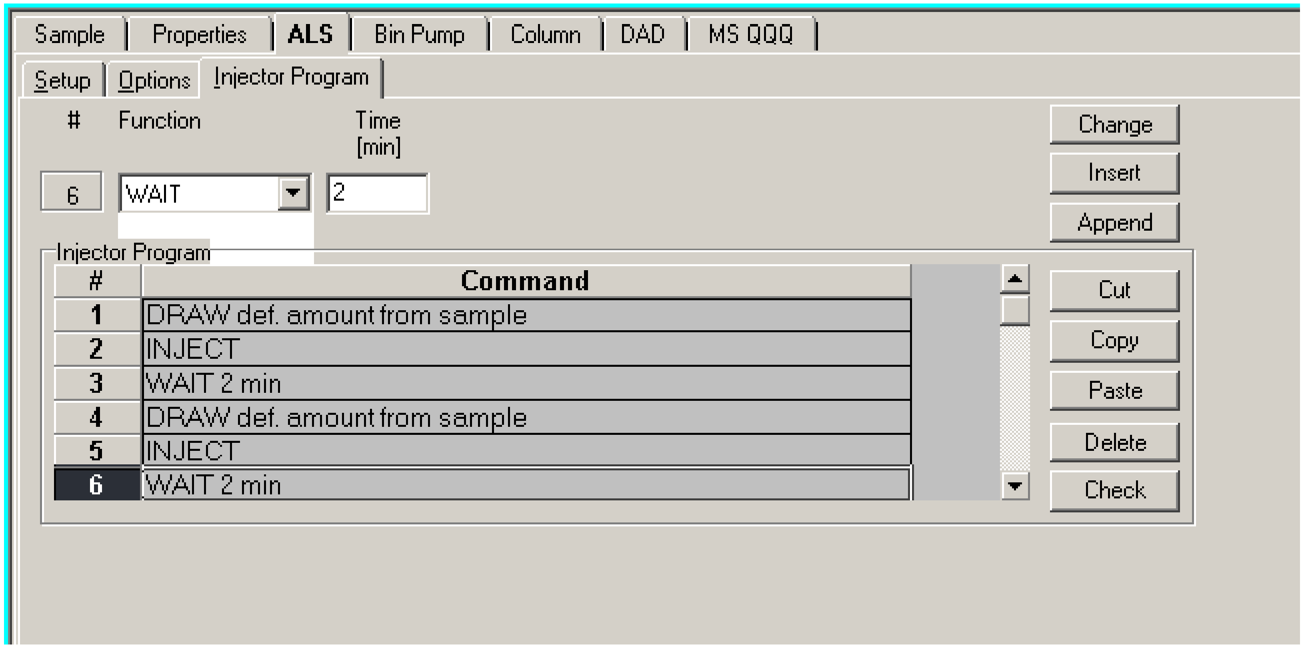
Injection Volume: 5 µl
Wash Vial: 1

● Disable Overlapped Injection
○ Prefetch next sample vial
○ Start overlap after 0 minutes

Check to use injector program

Injector program steps:
Valve to Bypass
Remote Startpulse
Repeat n times [n = number of time segments]
Draw default amount from sample
Valve to Mainpass
Wait 0.25 min [adjust to sync with acq time segments]
End repeat

Example Injector Program with 2-min spacing and explicit repeats



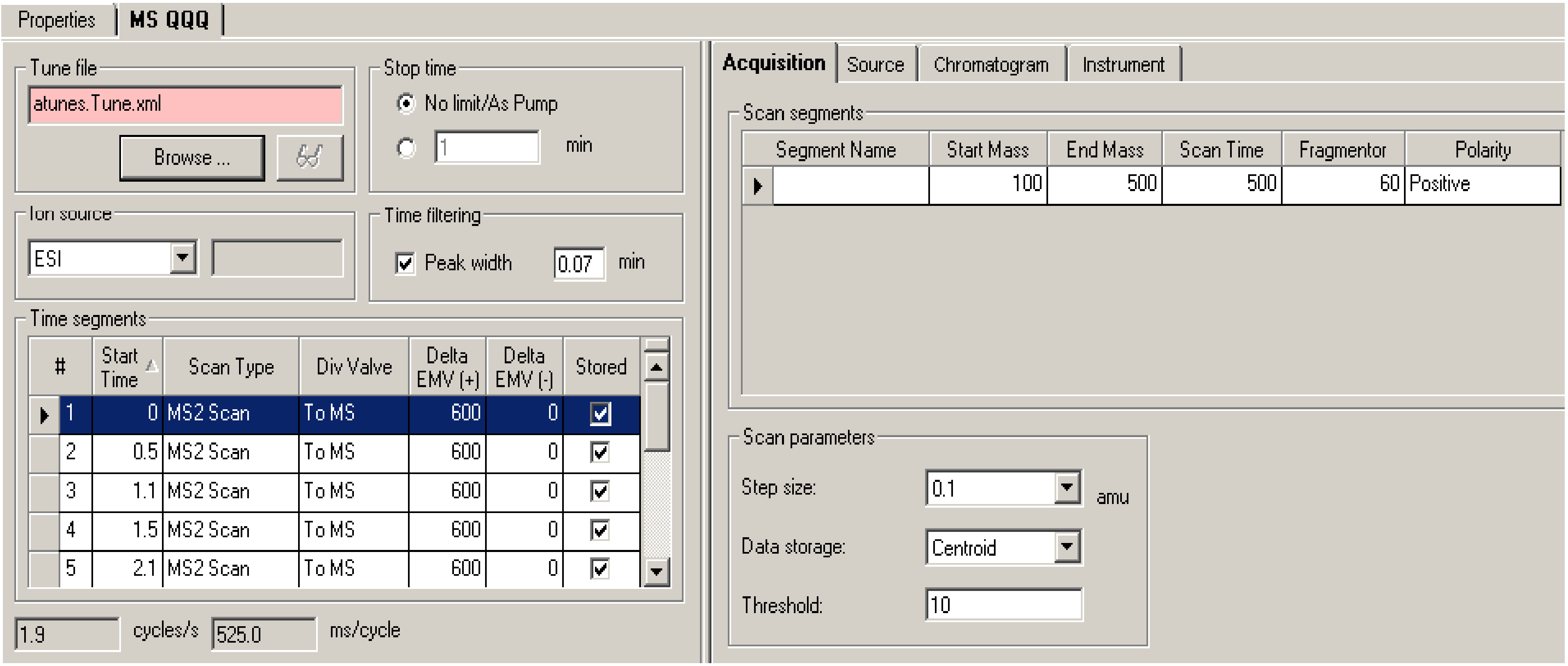
#	Function	Time [min]
6	WAIT	2

Injector Program

#	Command
1	DRAW def. amount from sample
2	INJECT
3	WAIT 2 min
4	DRAW def. amount from sample
5	INJECT
6	WAIT 2 min

Note: *Inject* and *Remote Startpulse* commands work differently on QQQ and TOF/QTOF.

Injector program for series of flow injections
Synchronize with MS time segments



Tune file: atunes.Tune.xml

Stop time: ● No limit/As Pump ○ 1 min

Ion source: ESI

Time filtering: ☑ Peak width 0.07 min

Time segments

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	MS2 Scan	To MS	600	0	☑
2	0.5	MS2 Scan	To MS	600	0	☑
3	1.1	MS2 Scan	To MS	600	0	☑
4	1.5	MS2 Scan	To MS	600	0	☑
5	2.1	MS2 Scan	To MS	600	0	☑

1.9 cycles/s 525.0 ms/cycle

Scan segments

Segment Name	Start Mass	End Mass	Scan Time	Fragmentor	Polarity
	100	500	500	60	Positive

Scan parameters

Step size: 0.1 amu

Data storage: Centroid

Threshold: 10

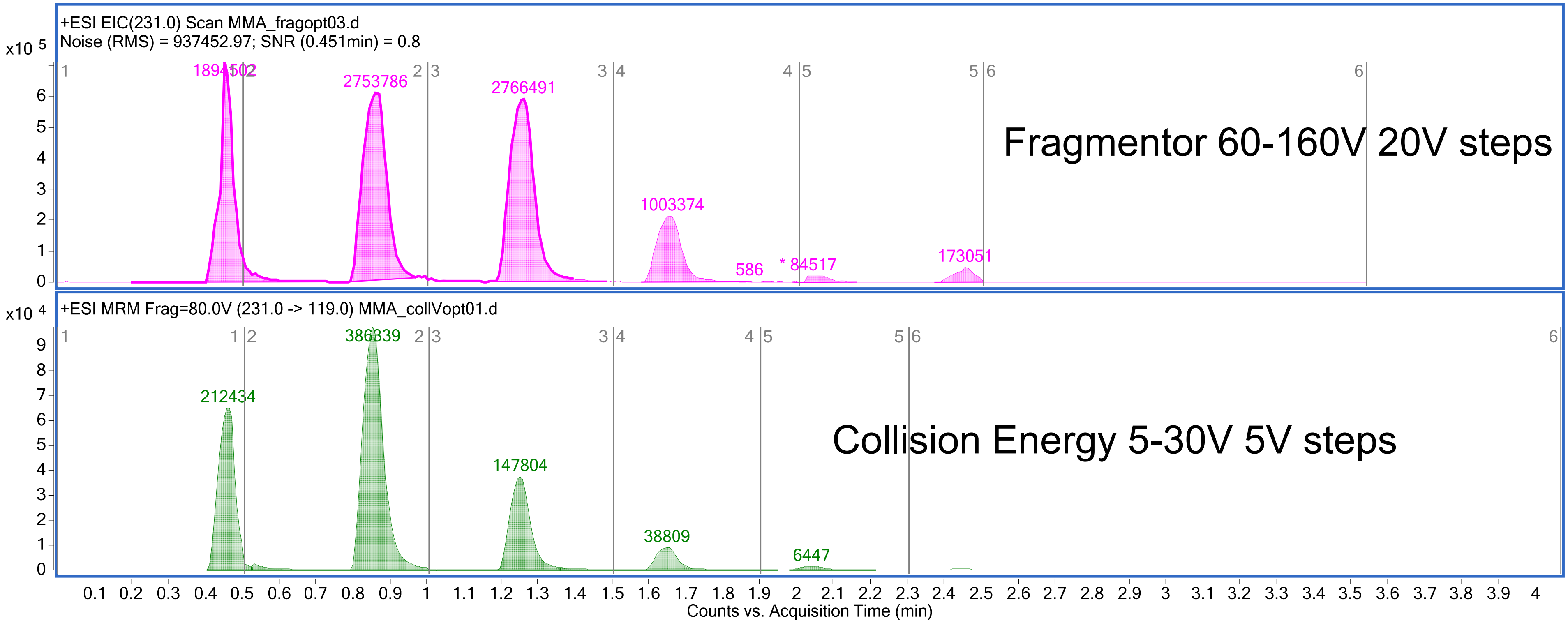
Fragmentor and Collision Energy optimization using flow injection with injector program

Methylmalonic acid, dibutyl ester

Maximize MH+ ion transmission, minimize CID with Fragmentor voltage

Maximize product ion signal(s) with Collision Energy

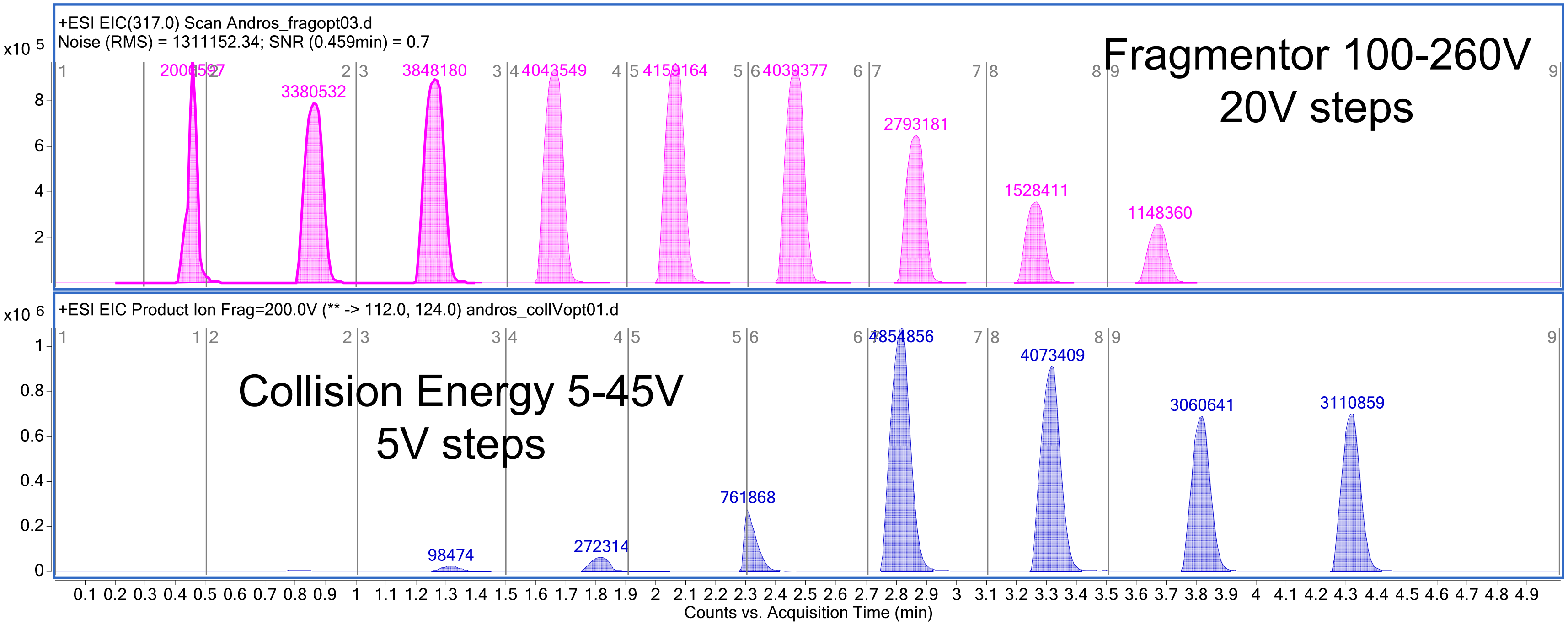
Can fine tune each with smaller steps after initial experiments



Fragmentor and Collision Energy optimization using flow injection with injector program

Androstenedione

Maximize MH+ ion transmission, minimize CID with Fragmentor voltage
Maximize product ion signal(s) with Collision Energy
Can fine tune each with smaller steps after initial experiments



Fragmentor and Collision Energy Determination with Optimizer software

•Optional add-on to MassHunter QQQ acquisition software

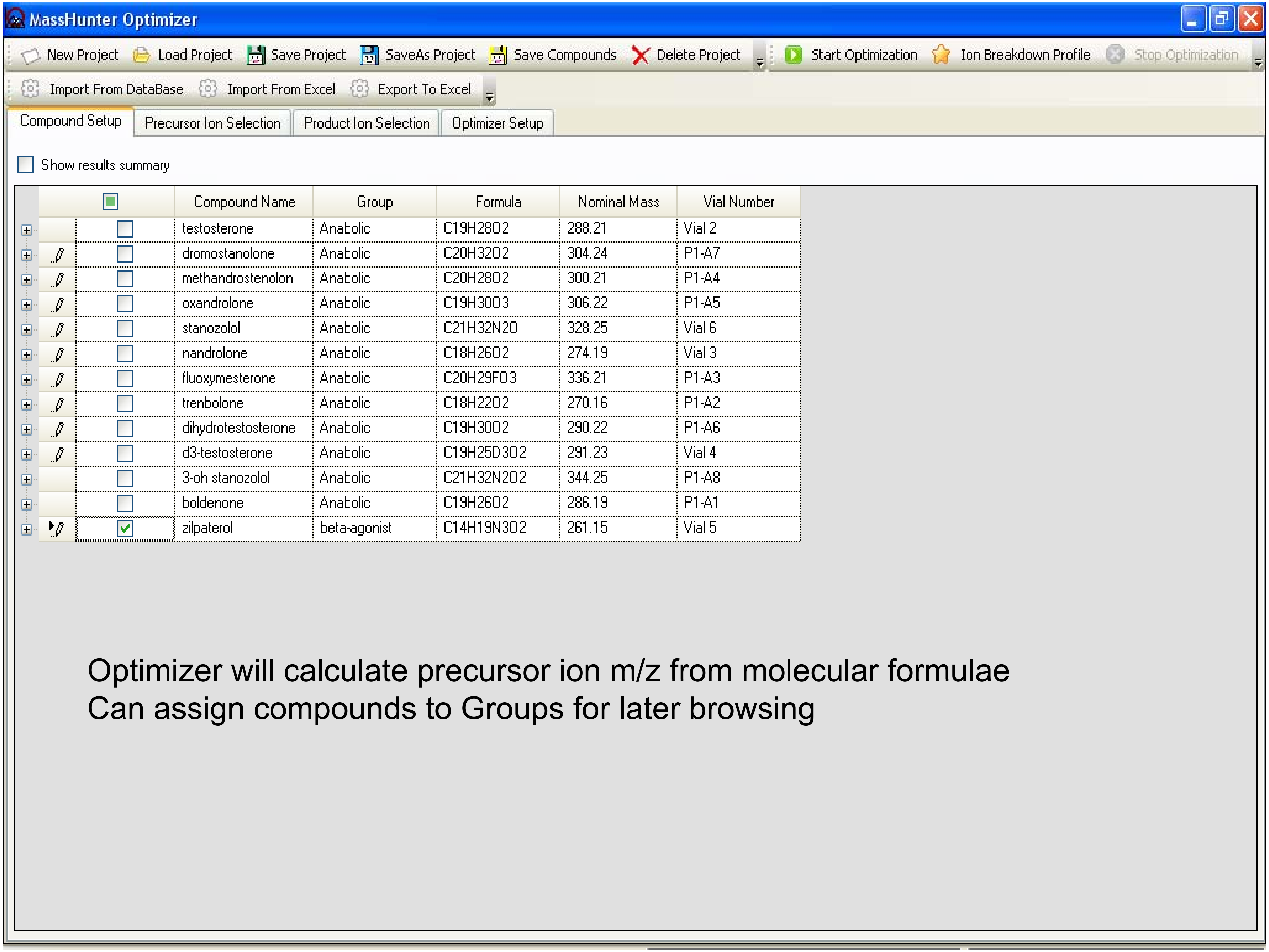
•Current version: B.02.01 (requires B.02.01 Acquisition)

•Useful for frequent method development [new compound each week] or for multi-compound methods [e.g. toxicology, environmental applications]

•Many toxicology and pesticide transitions are available in new Agilent application kits

•Can use Flow Injection before LC separation is developed

•Can use LC method to optimize multiple compounds in a few injections



☐ Show results summary

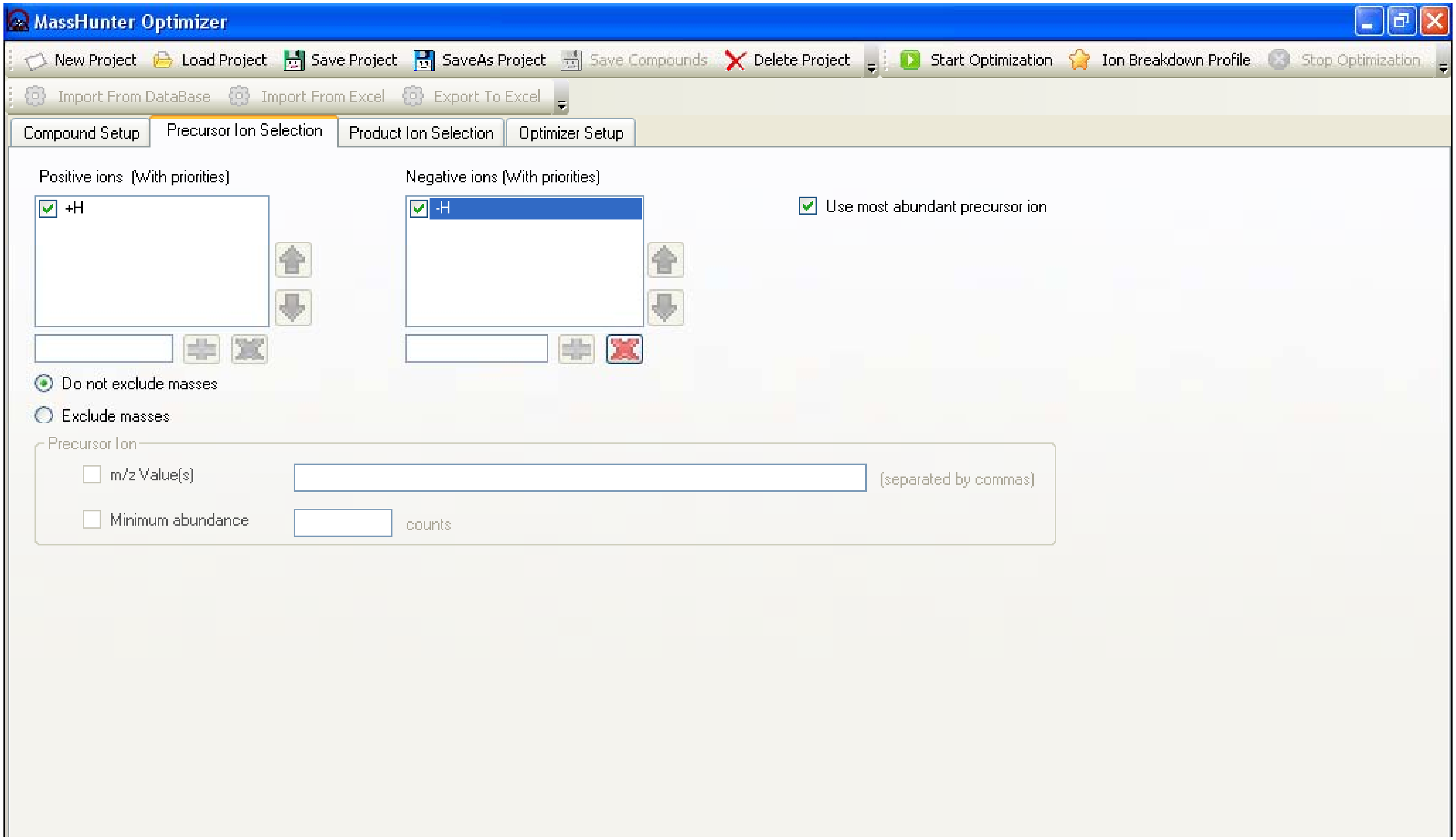
■	Compound Name	Group	Formula	Nominal Mass	Vial Number
☐	testosterone	Anabolic	C19H28O2	288.21	Vial 2
☐	dromostanolone	Anabolic	C20H32O2	304.24	P1-A7
☐	methandrostenolon	Anabolic	C20H28O2	300.21	P1-A4
☐	oxandrolone	Anabolic	C19H30O3	306.22	P1-A5
☐	stanozolol	Anabolic	C21H32N2O	328.25	Vial 6
☐	nandrolone	Anabolic	C18H26O2	274.19	Vial 3
☐	fluoxymesterone	Anabolic	C20H29FO3	336.21	P1-A3
☐	trenbolone	Anabolic	C18H22O2	270.16	P1-A2
☐	dihydrotestosterone	Anabolic	C19H30O2	290.22	P1-A6
☐	d3-testosterone	Anabolic	C19H25D3O2	291.23	Vial 4
☐	3-oh stanozolol	Anabolic	C21H32N2O2	344.25	P1-A8
☐	boldenone	Anabolic	C19H26O2	286.19	P1-A1
☑	zilpaterol	beta-agonist	C14H19N3O2	261.15	Vial 5

Optimizer will calculate precursor ion m/z from molecular formulae
Can assign compounds to Groups for later browsing

MassHunter Optimizer – precursor ion selection

User-selectable adducts (H, Na, K, OAc$^-$, HCOO$^-$, etc.)

Can run both positive and negative ion methods in same Project



Positive ions (With priorities)

☑ +H

Negative ions (With priorities)

☑ -H

☑ Use most abundant precursor ion

● Do not exclude masses

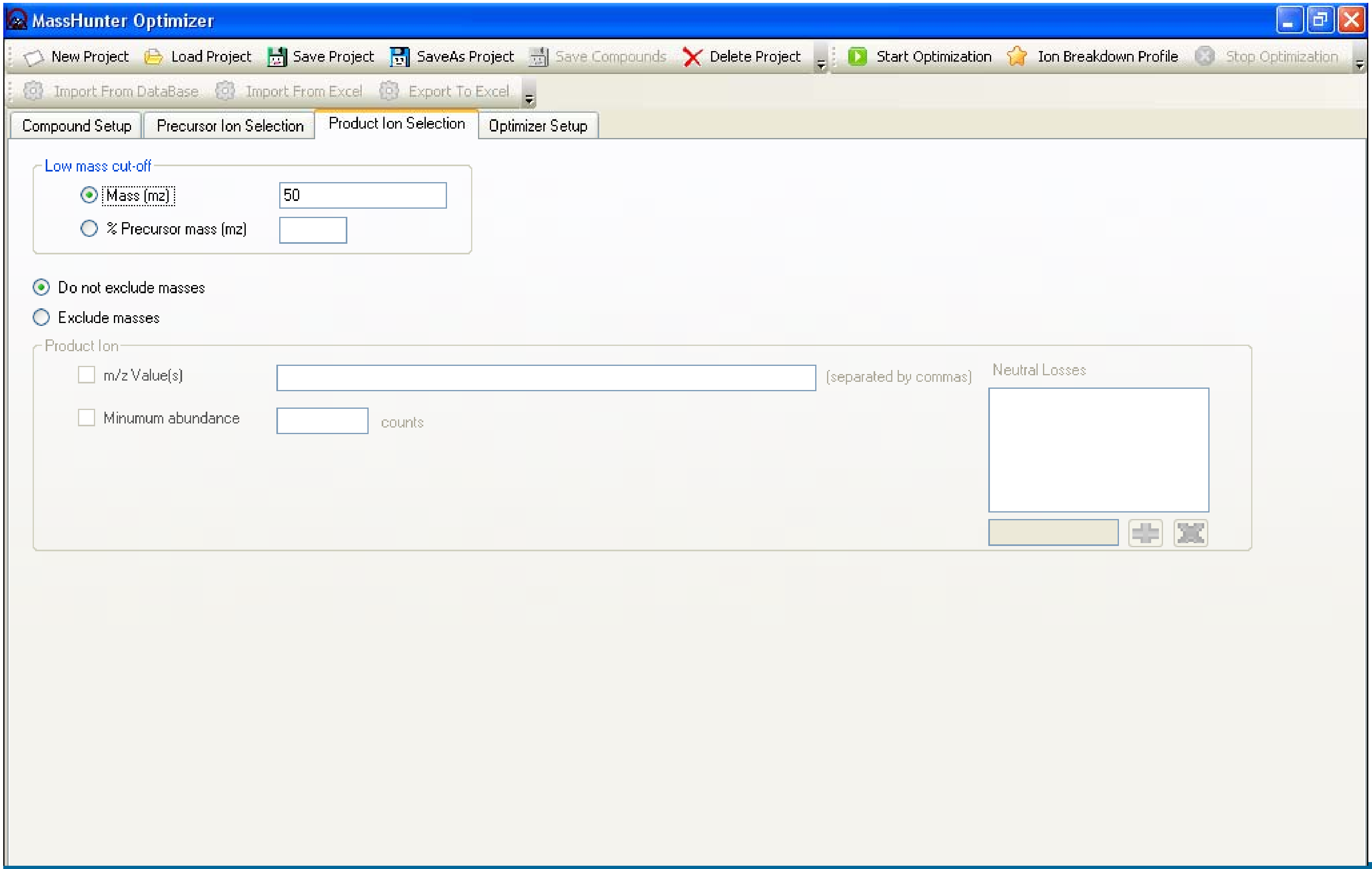
○ Exclude masses

Precursor Ion

☐ m/z Value(s) (separated by commas)

☐ Minimum abundance counts

Optimizer – product ion selection and rejection (set mass range, minimum abundance)



Low mass cut-off

● Mass (mz) 50

○ % Precursor mass (mz)

● Do not exclude masses

○ Exclude masses

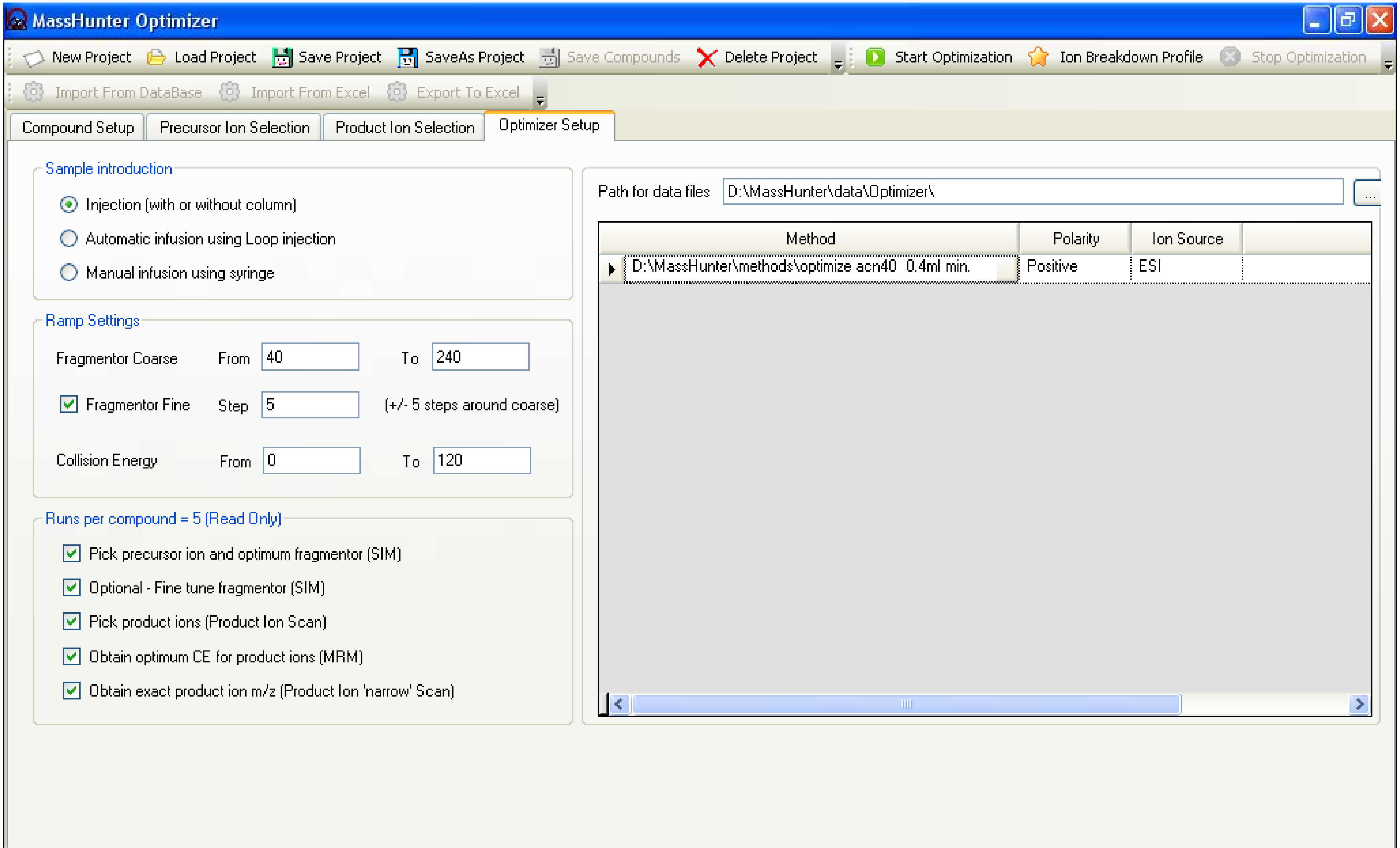
Product Ion

☐ m/z Value(s) (separated by commas)

☐ Minumum abundance counts

Neutral Losses

Optimizer – method type, parameter step selection



Sample introduction

- ● Injection (with or without column)
- ○ Automatic infusion using Loop injection
- ○ Manual infusion using syringe

Ramp Settings

Fragmentor Coarse From 40 To 240

☑ Fragmentor Fine Step 5 (+/- 5 steps around coarse)

Collision Energy From 0 To 120

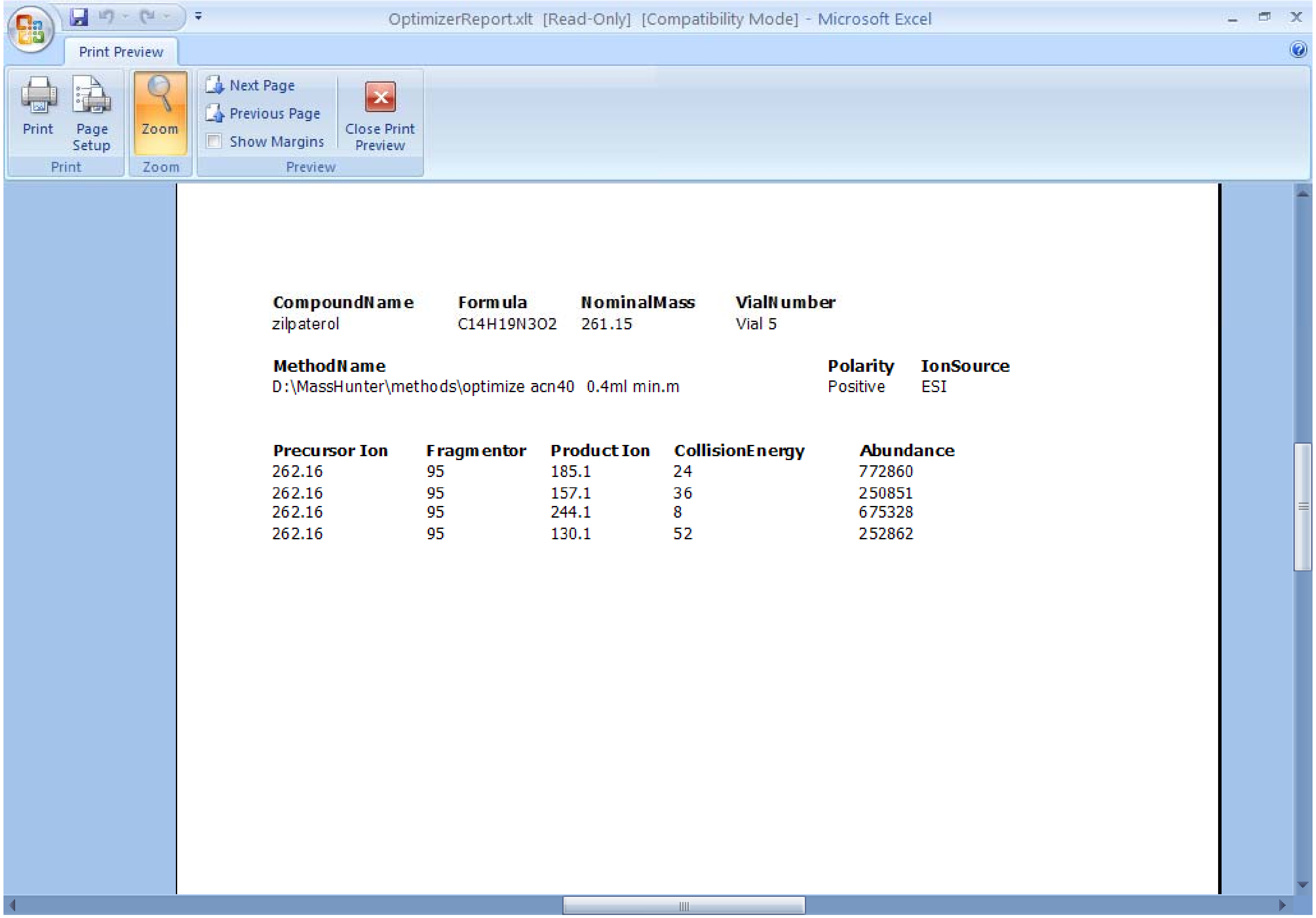
Runs per compound = 5 (Read Only)

- ☑ Pick precursor ion and optimum fragmentor (SIM)
- ☑ Optional - Fine tune fragmentor (SIM)
- ☑ Pick product ions (Product Ion Scan)
- ☑ Obtain optimum CE for product ions (MRM)
- ☑ Obtain exact product ion m/z (Product Ion 'narrow' Scan)

Path for data files D:\MassHunter\data\Optimizer\

Method	Polarity	Ion Source
D:\MassHunter\methods\optimize acn40 0.4ml min.	Positive	ESI

Optimizer results
Automatically added to MRM database

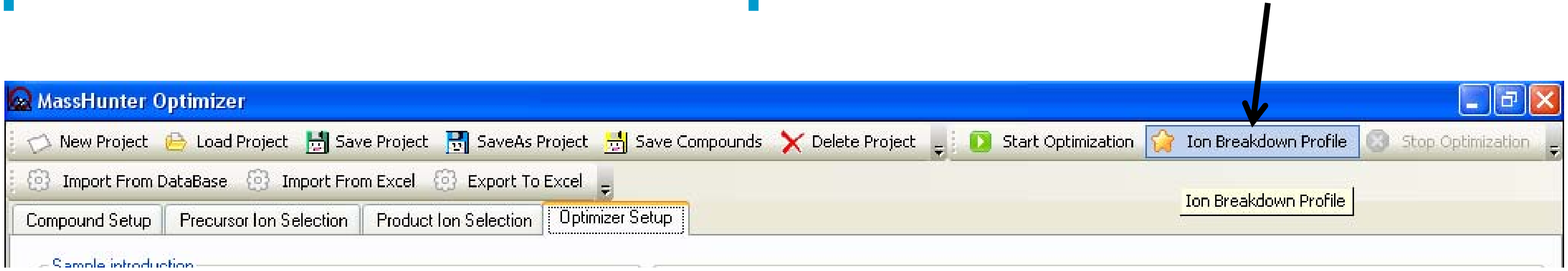


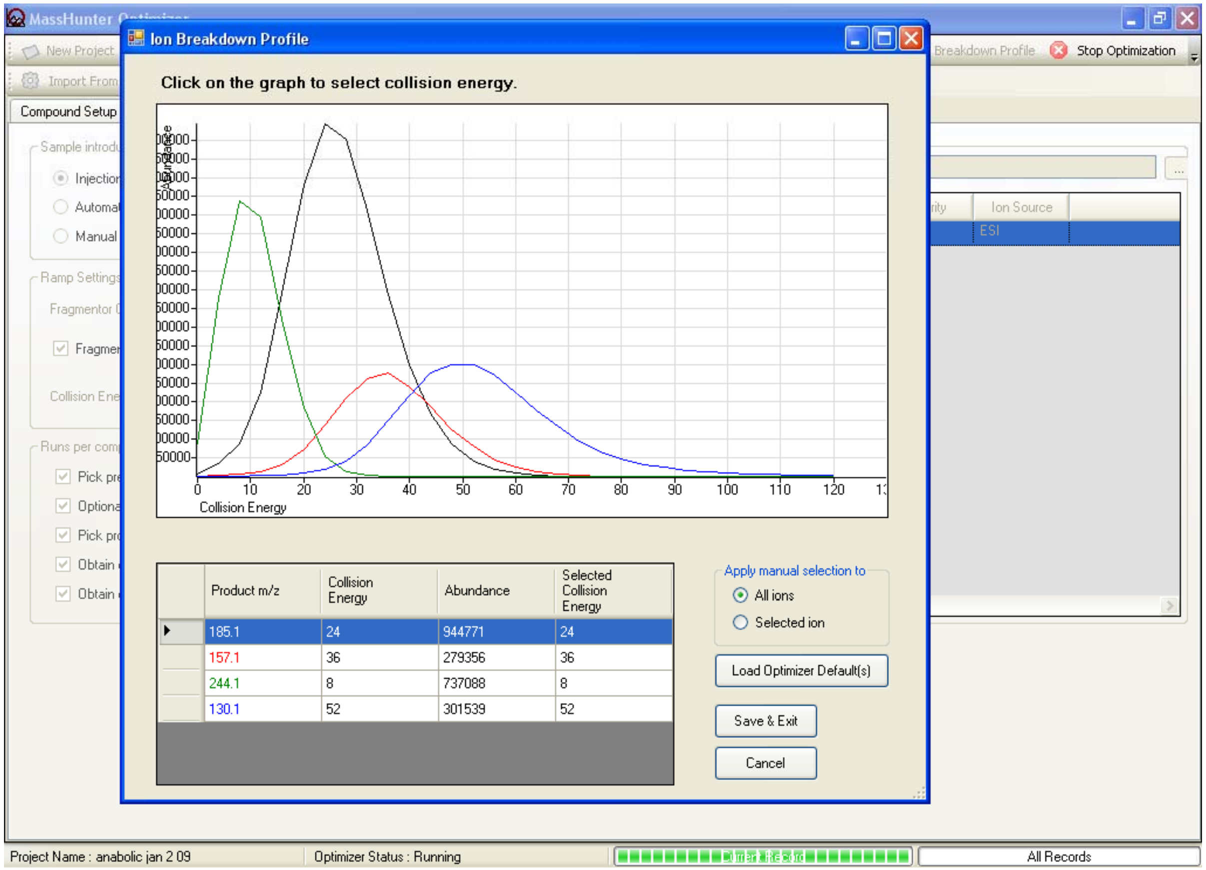
CompoundName	**Formula**	**NominalMass**	**VialNumber**
zilpaterol	C14H19N3O2	261.15	Vial 5

MethodName	**Polarity**	**IonSource**
D:\MassHunter\methods\optimize acn40 0.4ml min.m	Positive	ESI

Precursor Ion	**Fragmentor**	**Product Ion**	**CollisionEnergy**	**Abundance**
262.16	95	185.1	24	772860
262.16	95	157.1	36	250851
262.16	95	244.1	8	675328
262.16	95	130.1	52	252862

Optimizer – interactive parameter selection





Click on the graph to select collision energy.

	Product m/z	Collision Energy	Abundance	Selected Collision Energy
▶	185.1	24	944771	24
	157.1	36	279356	36
	244.1	8	737088	8
	130.1	52	301539	52

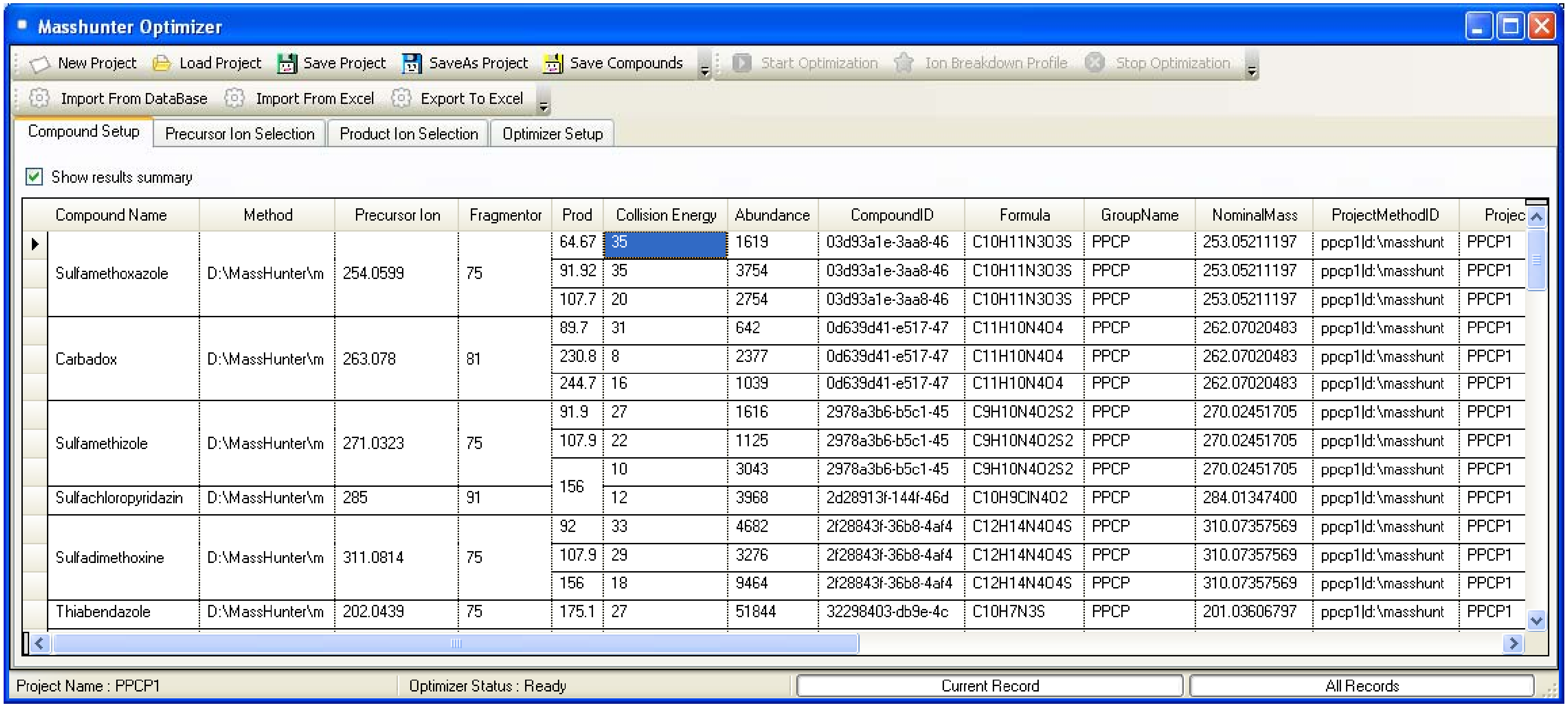
Apply manual selection to
● All ions
○ Selected ion

Project Name : anabolic jan 2 09

Optimizer Status : Running

MassHunter Optimizer

Results for the project can be viewed immediately after run: precursor and product ions, Fragmentor and Collision Energies



☑ Show results summary

Compound Name	Method	Precursor Ion	Fragmentor	Prod	Collision Energy	Abundance	CompoundID	Formula	GroupName	NominalMass	ProjectMethodID	Projec
Sulfamethoxazole	D:\MassHunter\m	254.0599	75	64.67	35	1619	03d93a1e-3aa8-46	C10H11N3O3S	PPCP	253.05211197	ppcp1|d:\masshunt	PPCP1
				91.92	35	3754	03d93a1e-3aa8-46	C10H11N3O3S	PPCP	253.05211197	ppcp1|d:\masshunt	PPCP1
				107.7	20	2754	03d93a1e-3aa8-46	C10H11N3O3S	PPCP	253.05211197	ppcp1|d:\masshunt	PPCP1
Carbadox	D:\MassHunter\m	263.078	81	89.7	31	642	0d639d41-e517-47	C11H10N4O4	PPCP	262.07020483	ppcp1|d:\masshunt	PPCP1
				230.8	8	2377	0d639d41-e517-47	C11H10N4O4	PPCP	262.07020483	ppcp1|d:\masshunt	PPCP1
				244.7	16	1039	0d639d41-e517-47	C11H10N4O4	PPCP	262.07020483	ppcp1|d:\masshunt	PPCP1
Sulfamethizole	D:\MassHunter\m	271.0323	75	91.9	27	1616	2978a3b6-b5c1-45	C9H10N4O2S2	PPCP	270.02451705	ppcp1|d:\masshunt	PPCP1
				107.9	22	1125	2978a3b6-b5c1-45	C9H10N4O2S2	PPCP	270.02451705	ppcp1|d:\masshunt	PPCP1
				156	10	3043	2978a3b6-b5c1-45	C9H10N4O2S2	PPCP	270.02451705	ppcp1|d:\masshunt	PPCP1
Sulfachloropyridazin	D:\MassHunter\m	285	91		12	3968	2d28913f-144f-46d	C10H9ClN4O2	PPCP	284.01347400	ppcp1|d:\masshunt	PPCP1
Sulfadimethoxine	D:\MassHunter\m	311.0814	75	92	33	4682	2f28843f-36b8-4af4	C12H14N4O4S	PPCP	310.07357569	ppcp1|d:\masshunt	PPCP1
				107.9	29	3276	2f28843f-36b8-4af4	C12H14N4O4S	PPCP	310.07357569	ppcp1|d:\masshunt	PPCP1
				156	18	9464	2f28843f-36b8-4af4	C12H14N4O4S	PPCP	310.07357569	ppcp1|d:\masshunt	PPCP1
Thiabendazole	D:\MassHunter\m	202.0439	75	175.1	27	51844	32298403-db9e-4c	C10H7N3S	PPCP	201.03606797	ppcp1|d:\masshunt	PPCP1

Project Name : PPCP1

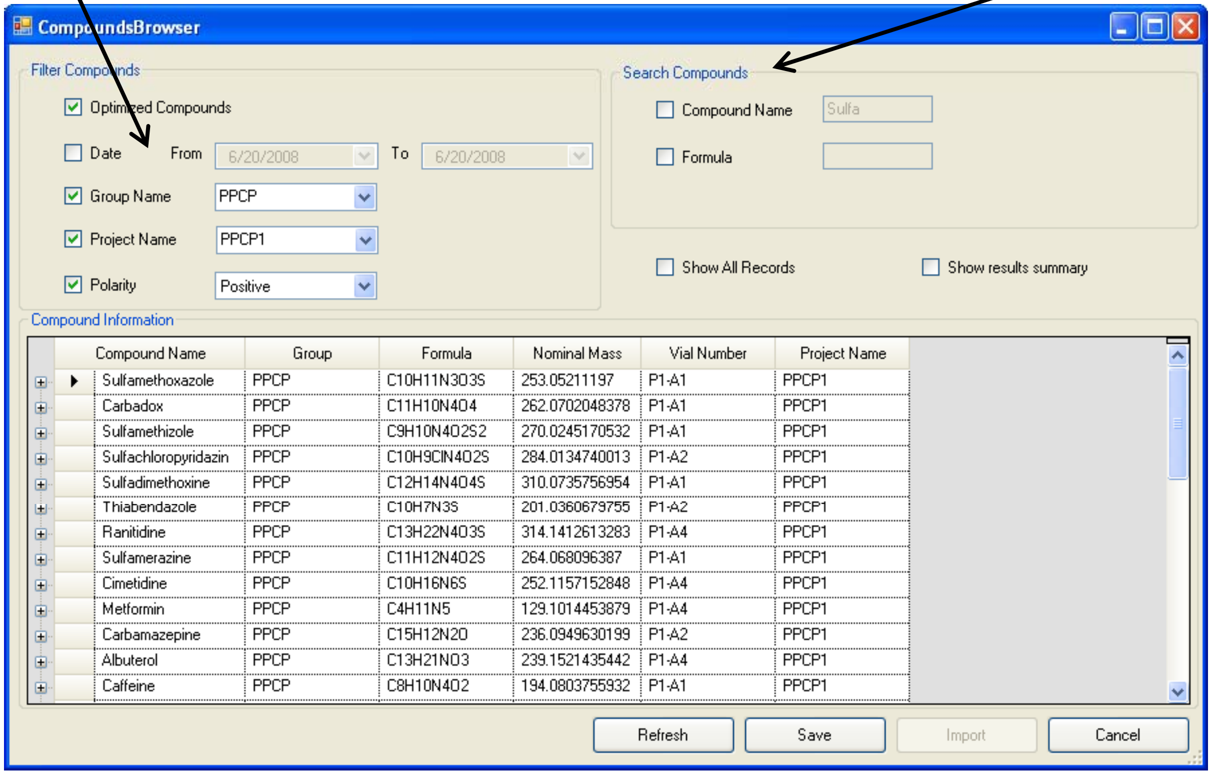
Optimizer Status : Ready

Optimizer

Browse compounds in Acquisition for import

Compounds can be filtered by project, group, polarity or date

Compounds can be searched for by name or formula



☑ Optimized Compounds

☐ Date From 6/20/2008 To 6/20/2008

☑ Group Name PPCP

☑ Project Name PPCP1

☑ Polarity Positive

☐ Compound Name Sulfa

☐ Formula

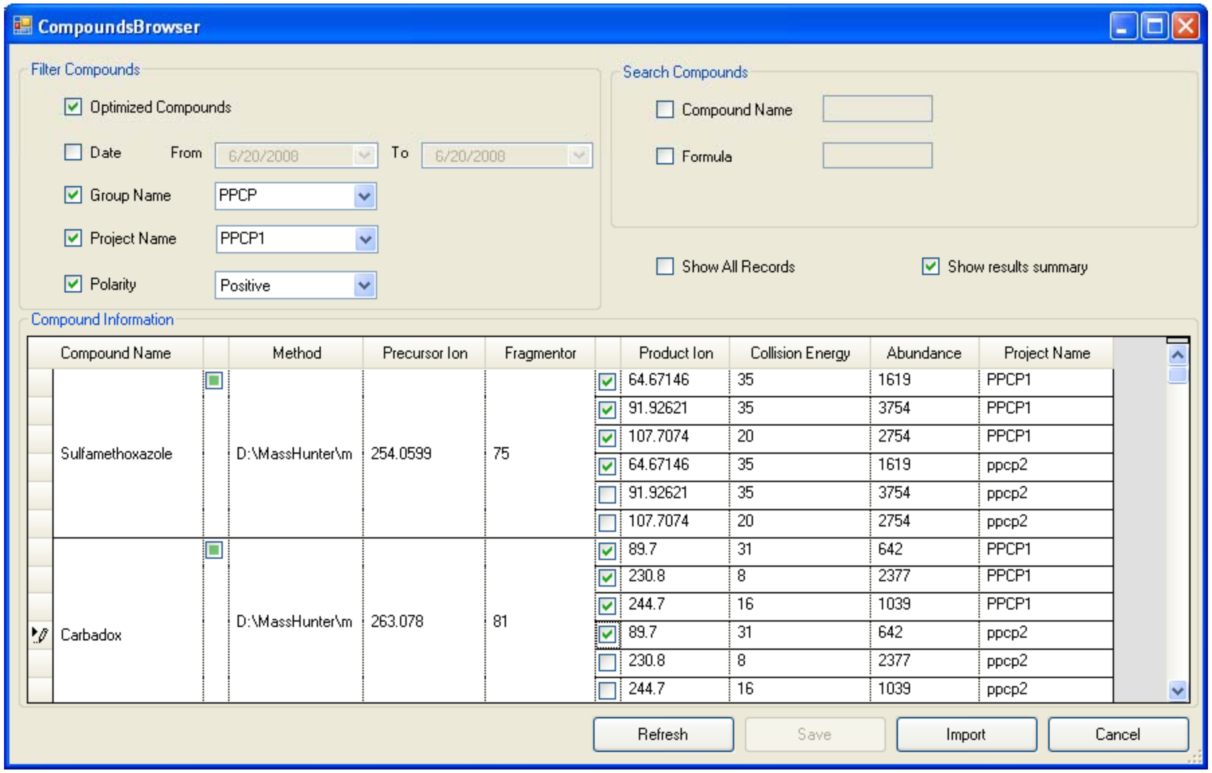
☐ Show All Records

☐ Show results summary

Compound Name	Group	Formula	Nominal Mass	Vial Number	Project Name
Sulfamethoxazole	PPCP	C10H11N3O3S	253.05211197	P1-A1	PPCP1
Carbadox	PPCP	C11H10N4O4	262.0702048378	P1-A1	PPCP1
Sulfamethizole	PPCP	C9H10N4O2S2	270.0245170532	P1-A1	PPCP1
Sulfachloropyridazin	PPCP	C10H9ClN4O2S	284.0134740013	P1-A2	PPCP1
Sulfadimethoxine	PPCP	C12H14N4O4S	310.0735756954	P1-A1	PPCP1
Thiabendazole	PPCP	C10H7N3S	201.0360679755	P1-A2	PPCP1
Ranitidine	PPCP	C13H22N4O3S	314.1412613283	P1-A4	PPCP1
Sulfamerazine	PPCP	C11H12N4O2S	264.068096387	P1-A1	PPCP1
Cimetidine	PPCP	C10H16N6S	252.1157152848	P1-A4	PPCP1
Metformin	PPCP	C4H11N5	129.1014453879	P1-A4	PPCP1
Carbamazepine	PPCP	C15H12N2O	236.0949630199	P1-A2	PPCP1
Albuterol	PPCP	C13H21NO3	239.1521435442	P1-A4	PPCP1
Caffeine	PPCP	C8H10N4O2	194.0803755932	P1-A1	PPCP1

MassHunter Optimizer

Build LC/MS/MS method from compound database



Filter Compounds

☑ Optimized Compounds

☐ Date From 6/20/2008 To 6/20/2008

☑ Group Name PPCP

☑ Project Name PPCP1

☑ Polarity Positive

Search Compounds

☐ Compound Name

☐ Formula

☐ Show All Records ☑ Show results summary

Compound Information

Compound Name		Method	Precursor Ion	Fragmentor		Product Ion	Collision Energy	Abundance	Project Name
Sulfamethoxazole	🟩	D:\MassHunter\m	254.0599	75	☑	64.67146	35	1619	PPCP1
					☑	91.92621	35	3754	PPCP1
					☑	107.7074	20	2754	PPCP1
					☑	64.67146	35	1619	ppcp2
					☐	91.92621	35	3754	ppcp2
					☐	107.7074	20	2754	ppcp2
Carbadox	🟩	D:\MassHunter\m	263.078	81	☑	89.7	31	642	PPCP1
					☑	230.8	8	2377	PPCP1
					☑	244.7	16	1039	PPCP1
					☑	89.7	31	642	ppcp2
					☐	230.8	8	2377	ppcp2
					☐	244.7	16	1039	ppcp2

- Select compounds in database after filtering
- Select transitions for each compound (all or individual)
- Easily import into template LC/MS method

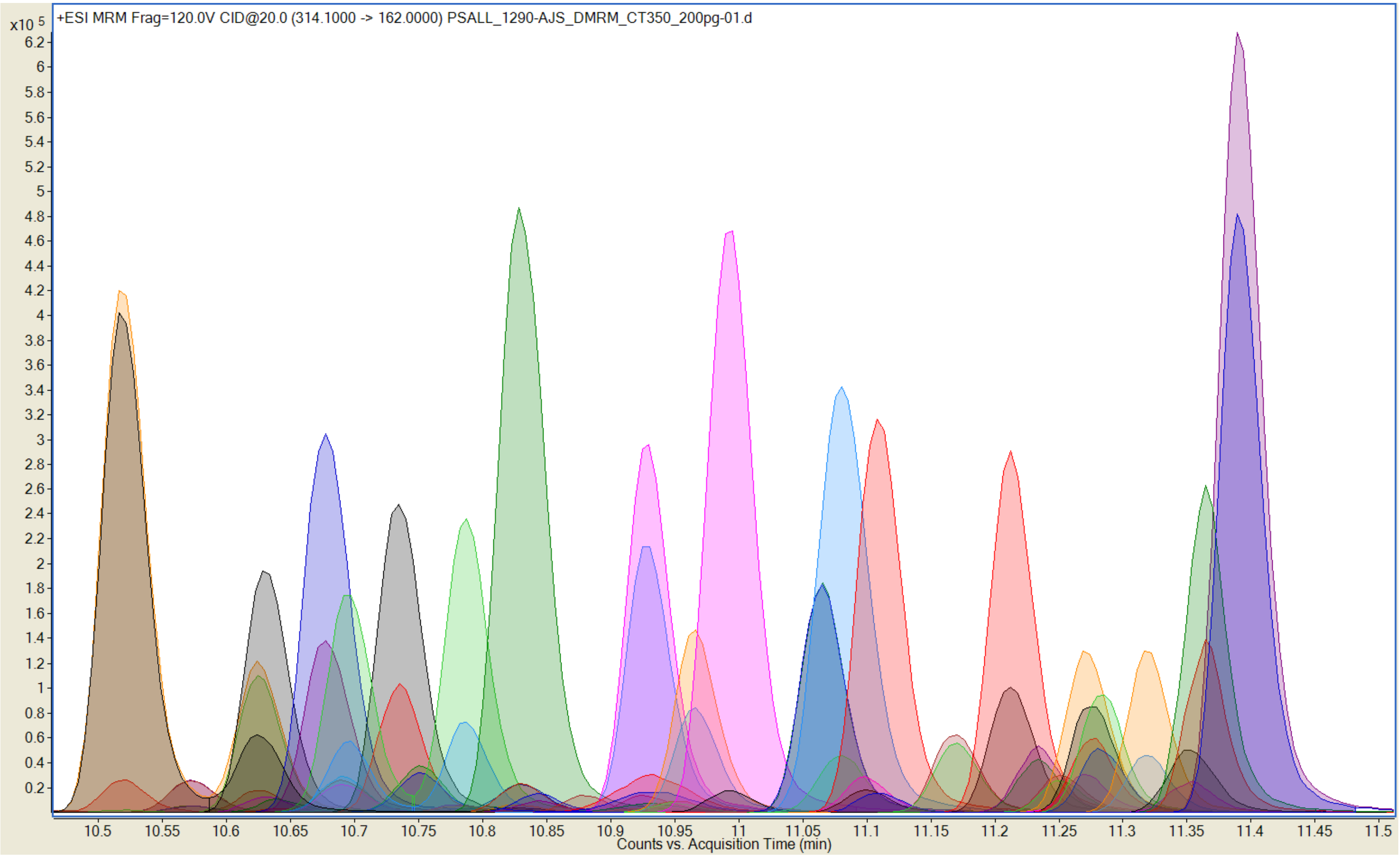
Dynamic MRM

Optimization of MRM Acquisition

- Good quantitation requires adequate number of data points across each peak (ideally 10-20)
- High confidence or regulated identification requires > 1 MRM per compound.
- Monitoring many compounds simultaneously lowers dwell time per MRM.
- Therefore for best sensitivity, only monitor compounds in retention time window where they elute.
- Traditional approach of time segments has limitations and is tedious to setup up and maintain.

Peak Capacities are very high with UHPLC

40 MRM Transitions in 1 minute time window



The solution: MassHunter Dynamic MRM

Included in QQQ Acquisition B.02.01

For applications requiring quantitation of 100 – 1000 compounds in one run; some examples:

- Food and environmental analysis (e.g. pesticides)
- Targeted quantitation of proteins via peptides (proteomics)

WITHOUT Dynamic MRM:

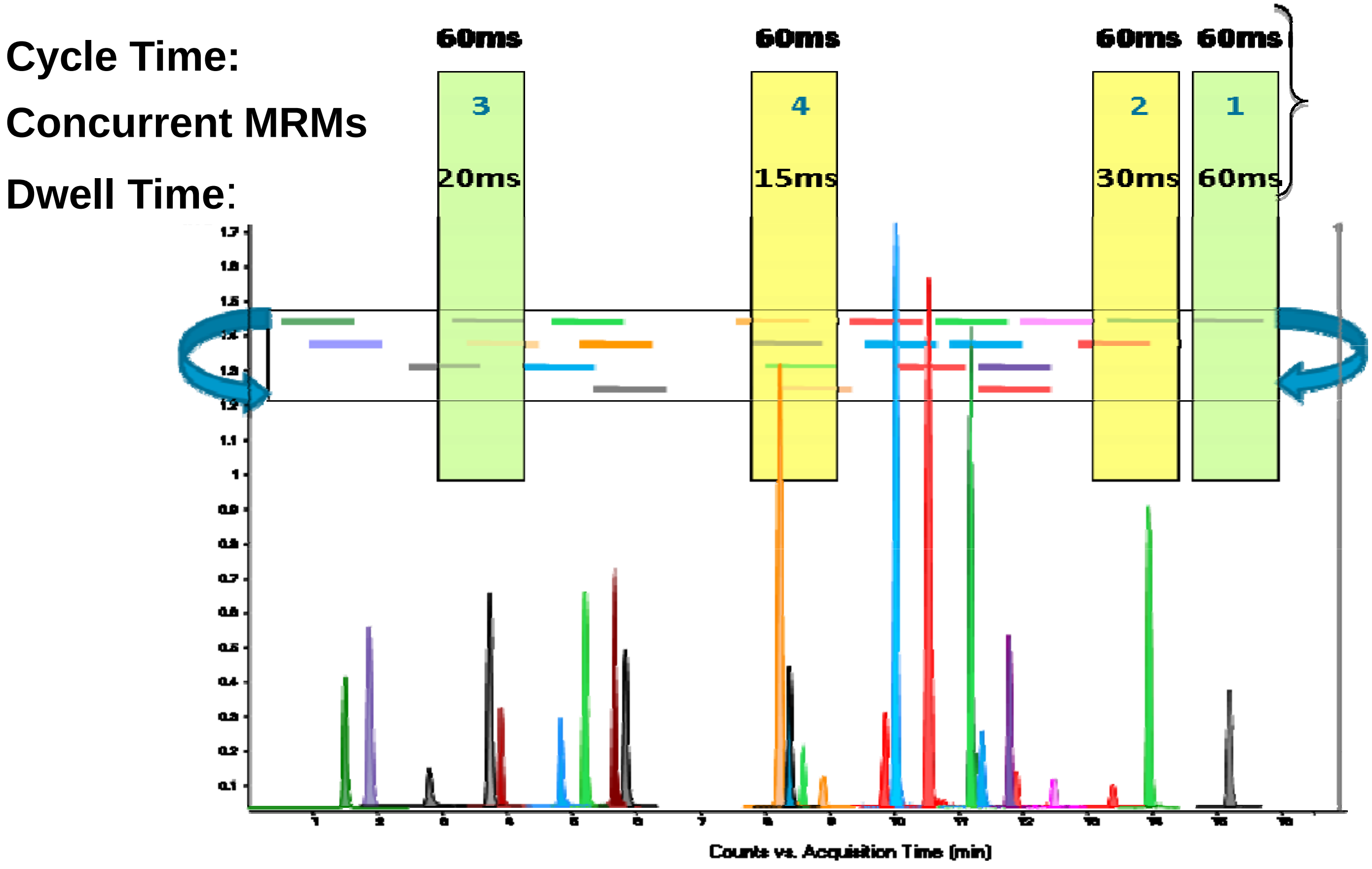
- Need to manually set up multiple time segments to maximize dwell times
- Tedious to set up; problematic if changes in retention times

WITH Dynamic MRM:

- Automatic setup of overlapping time segments without user intervention
- Fewer MRMs per unit time results in longer dwell time => incr sensitivity
- Unaffected by minor chromatographic time shifts

Dynamic MRM for 6400 Series Triple Quads

monitors transitions only when compounds elute

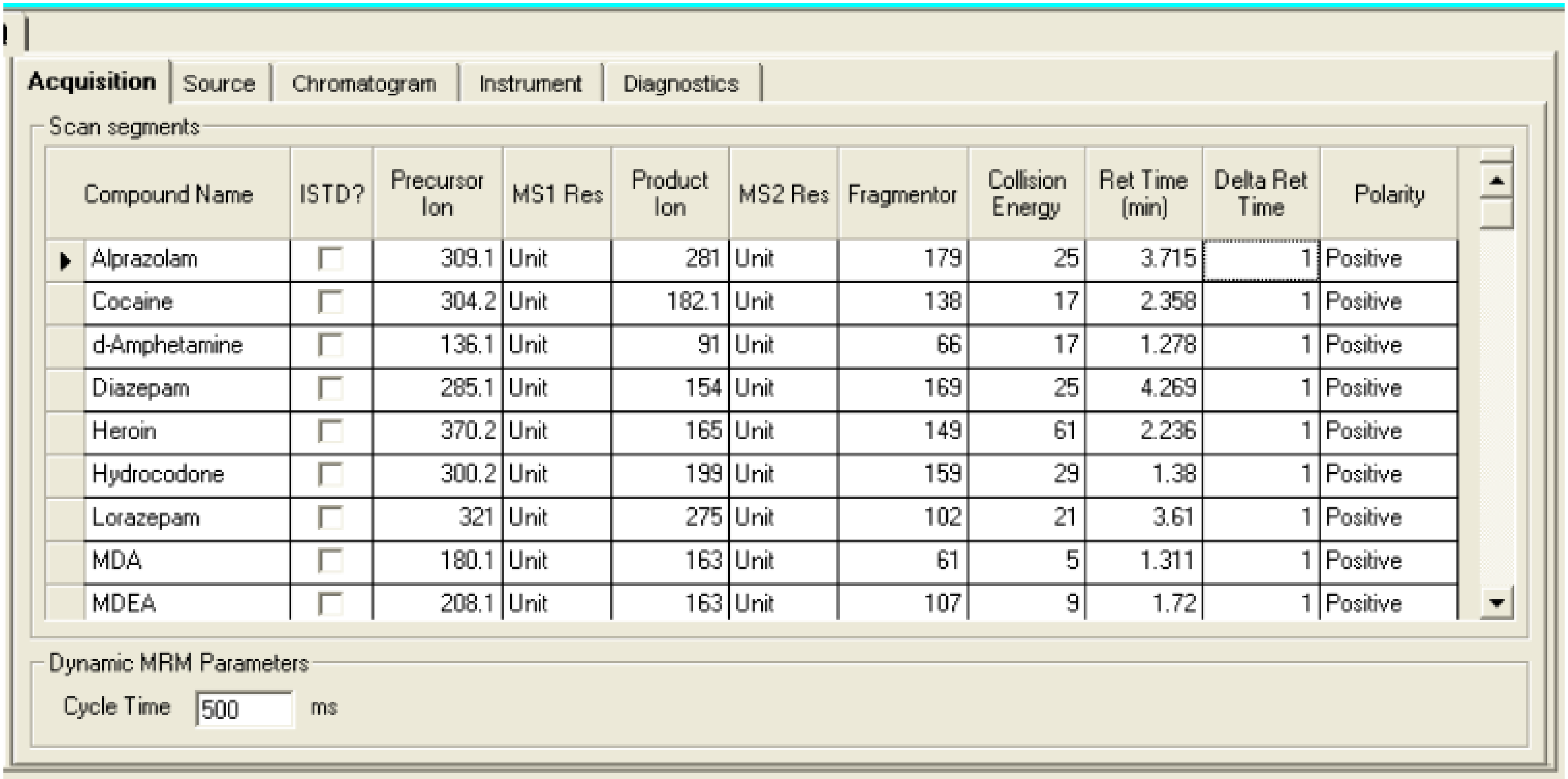


1. # Concurrent MRMs fewer than with time segments → more data points across each peak
2. Allows longer dwell times → better sensitivity, S/N

Acquisition setup with Dynamic MRM:

No time segments, instead

Retention Time and "Delta Ret Time" [MRM time range]



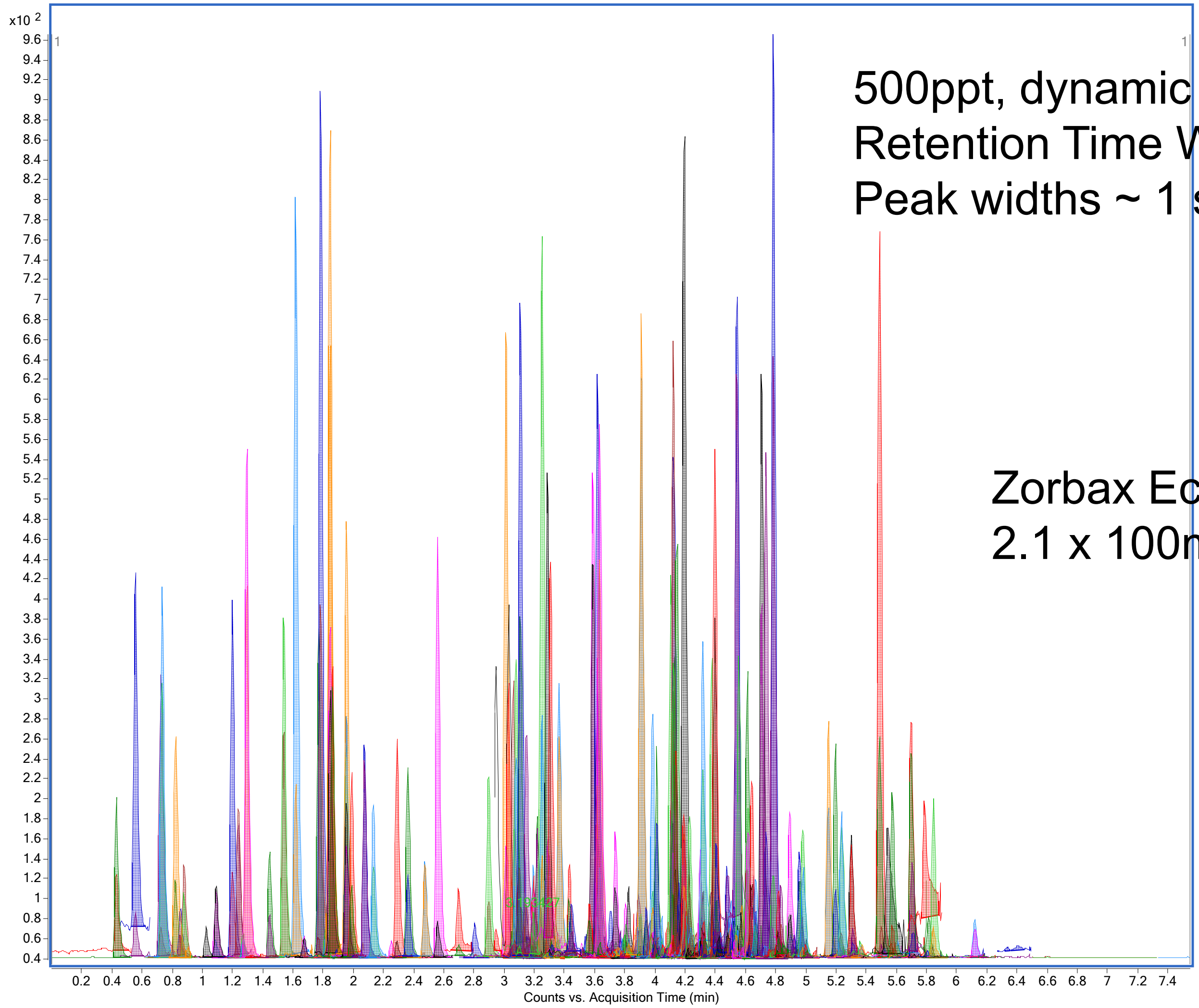
Scan segments

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time	Polarity
Alprazolam	☐	309.1	Unit	281	Unit	179	25	3.715	1	Positive
Cocaine	☐	304.2	Unit	182.1	Unit	138	17	2.358	1	Positive
d-Amphetamine	☐	136.1	Unit	91	Unit	66	17	1.278	1	Positive
Diazepam	☐	285.1	Unit	154	Unit	169	25	4.269	1	Positive
Heroin	☐	370.2	Unit	165	Unit	149	61	2.236	1	Positive
Hydrocodone	☐	300.2	Unit	199	Unit	159	29	1.38	1	Positive
Lorazepam	☐	321	Unit	275	Unit	102	21	3.61	1	Positive
MDA	☐	180.1	Unit	163	Unit	61	5	1.311	1	Positive
MDEA	☐	208.1	Unit	163	Unit	107	9	1.72	1	Positive

Dynamic MRM Parameters

Cycle Time 500 ms

8 Minute Dynamic MRM Analysis with 6460 QQQ - 250-compound Pesticide Screen.



500ppt, dynamic MRM
Retention Time Window = 12 sec
Peak widths ~ 1 second

Zorbax Eclipse Plus C18
2.1 x 100mm (1.8µm)

Summary – Optimization of QQQ Acquisition

- Source optimization: flow and compound dependent parameters
- Agilent Jetstream Technology source: increased sensitivity vs regular ESI source if parameters optimized correctly:
  - Sheath gas temperature and flow are most important
  - Other parameters have less effect on response
  - Need less drying gas flow and temperature, but keep capillary clean
- Dynamic MRM is available for all 6400 models
  - More data points across peak
  - Longer dwell times for better sensitivity, S/N
  - Easier to set up and maintain than time segment methods

MassHunter Quantitative Software
Review and Quant Method Optimization

Topics

- Method setup from acquired data
- Method re-use and updating
- Peak detection optimization
- Peak identification troubleshooting
- What's new in MH Quant B.03.02

Important MassHunter Quant concepts and rules: Batches

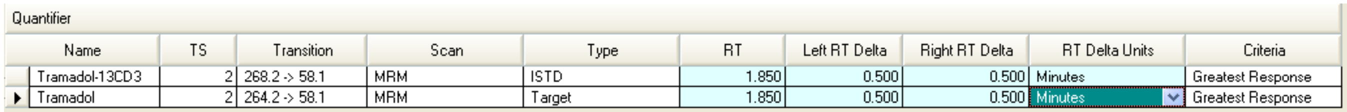
- A Batch is a file which contains all the Quant results from a set of data files AND the Quant method used. Very convenient for backup and moving data around.
- All the data files in a Batch must reside in a single directory, so put them all together <u>before</u> creating New Batch.
- Select the data directory BEFORE naming the new Batch
- Using the *Browse to Copy Samples* button when creating the Batch can be dangerous: you will have two copies of the same files (one with Quant results and one without)!

Important MassHunter Quant concepts and rules: Quant methods

- THE most common method problem: Calibration/QC level names in Batch and Quant method do not match, e.g.
  - Batch: levels are 1,2,…5, QC-Lo, QC-Hi
  - Method: levels are L1, L2…L5, QC-Low, QC-High
- 2nd most common problem: a hidden column in the Method with a key parameter, e.g. Criteria in MRM Compound Setup for peak selection, or Ion Polarity if method created manually.
- Many Quant parameters can be copied between compounds with *Apply to All* button.
- When in doubt, Right-Click to look for convenient features and shortcuts, like Fill Down or Fill Column.

MassHunter Quant – optimizing target compound identification

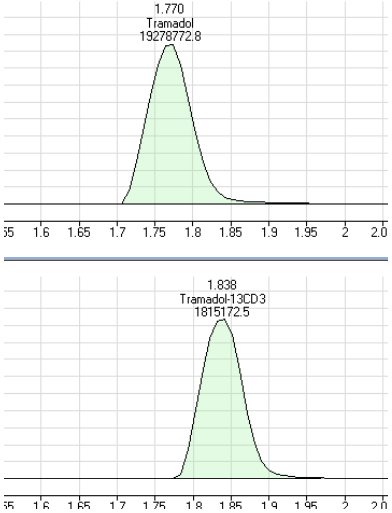
Make the Criteria column visible in MRM Compound Setup:



Quantifier

	Name	TS	Transition	Scan	Type	RT	Left RT Delta	Right RT Delta	RT Delta Units	Criteria
	Tramadol-13CD3	2	268.2 -> 58.1	MRM	ISTD	1.850	0.500	0.500	Minutes	Greatest Response
▶	Tramadol	2	264.2 -> 58.1	MRM	Target	1.850	0.500	0.500	Minutes	Greatest Response

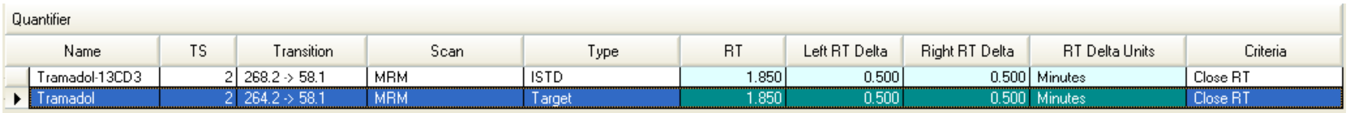
Using the default of Greatest Response may result in the wrong peak being chosen as the target compound:



Endogenous interference with wrong retention time incorrectly identified as tramadol (no qualifier)

MassHunter Quant – optimizing target compound identification

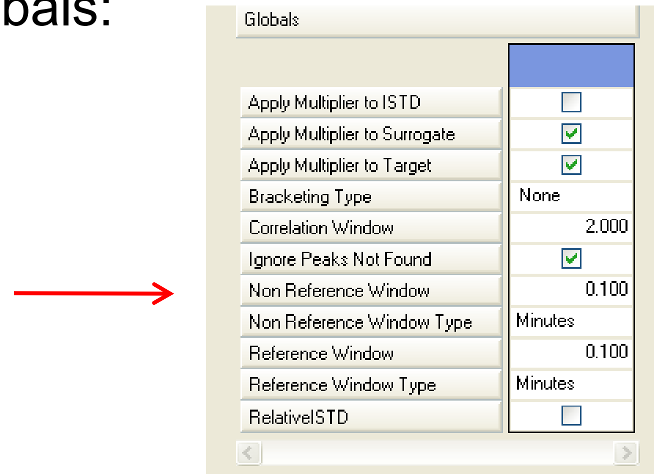
Change the Criteria to be more specific:



Quantifier

	Name	TS	Transition	Scan	Type	RT	Left RT Delta	Right RT Delta	RT Delta Units	Criteria
	Tramadol-13CD3	2	268.2 -> 58.1	MRM	ISTD	1.850	0.500	0.500	Minutes	Close RT
▸	Tramadol	2	264.2 -> 58.1	MRM	Target	1.850	0.500	0.500	Minutes	Close RT

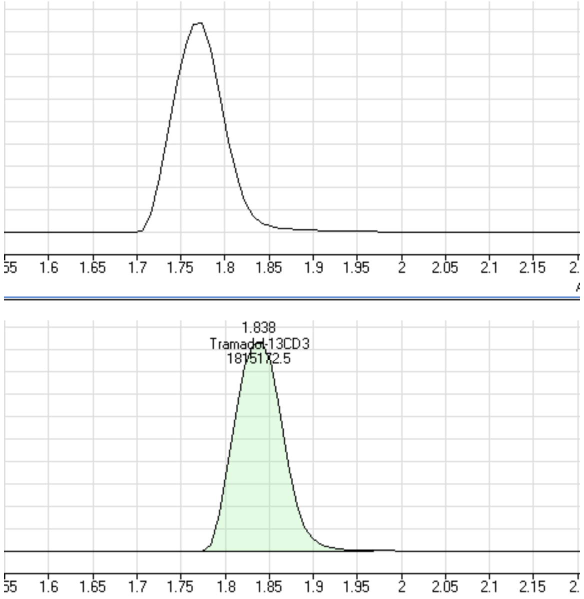
This might still not be selective enough, so specify how close the retention time must match the calibrated RT with Non-reference Window parameter in Globals:



Globals

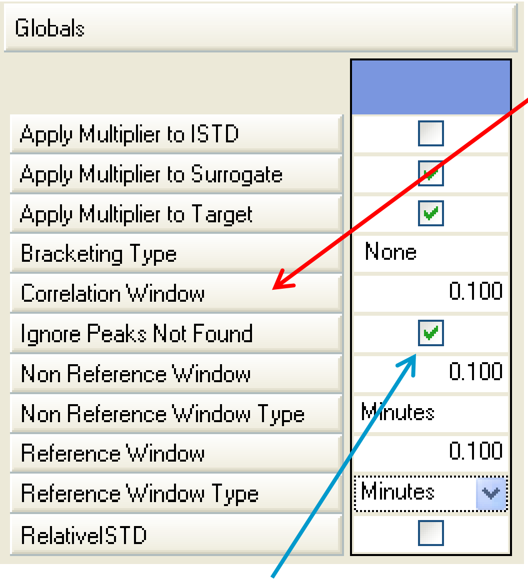
Apply Multiplier to ISTD	☐
Apply Multiplier to Surrogate	☑
Apply Multiplier to Target	☑
Bracketing Type	None
Correlation Window	2.000
Ignore Peaks Not Found	☑
Non Reference Window	0.100
Non Reference Window Type	Minutes
Reference Window	0.100
Reference Window Type	Minutes
RelativeISTD	☐

Now the offending peak with the wrong RT is ignored:



MassHunter Quant – optimizing target compound identification

Qualifying the Qualifier:
For qualifiers, you can ensure that the qualifier and the quant ion have the same retention time (i.e. line up properly as they should if coming from the same chromatographic peak), by setting the **Correlation Window** in Globals correctly. The default of 2 minutes is WAY too large.



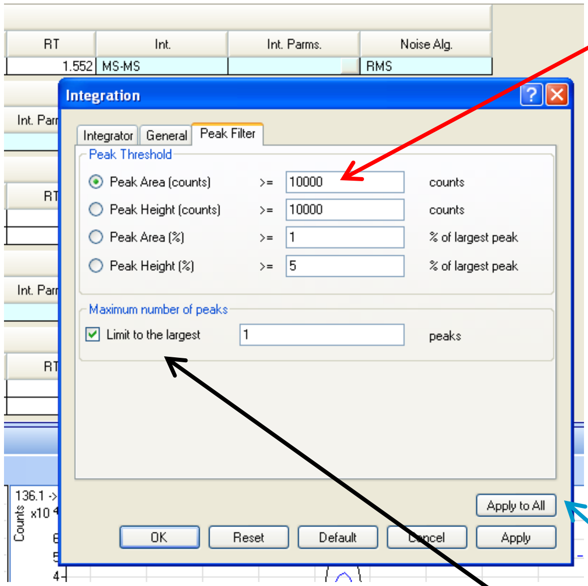
Globals

Apply Multiplier to ISTD	☐
Apply Multiplier to Surrogate	☑
Apply Multiplier to Target	☑
Bracketing Type	None
Correlation Window	0.100
Ignore Peaks Not Found	☑
Non Reference Window	0.100
Non Reference Window Type	Minutes
Reference Window	0.100
Reference Window Type	Minutes
RelativeISTD	☐

The Ignore Peaks Not Found checkbox will prevent reporting "Amount=0.00" for target compounds not present with your criteria.

MassHunter Quant –
Setting a reporting threshold:

You can also make Quant ignore peaks that meet these criteria but are below your desired detection or reporting threshold, or that really are background (if you can't use Qualifiers). Set a Peak Filter (area reject) value in *Advanced Tasks …Integration Parameters*.



RT	Int.	Int. Parms.	Noise Alg.
1.552	MS-MS		RMS

Peak Threshold

● Peak Area (counts) >= 10000 counts

○ Peak Height (counts) >= 10000 counts

○ Peak Area (%) >= 1 % of largest peak

○ Peak Height (%) >= 5 % of largest peak

Maximum number of peaks

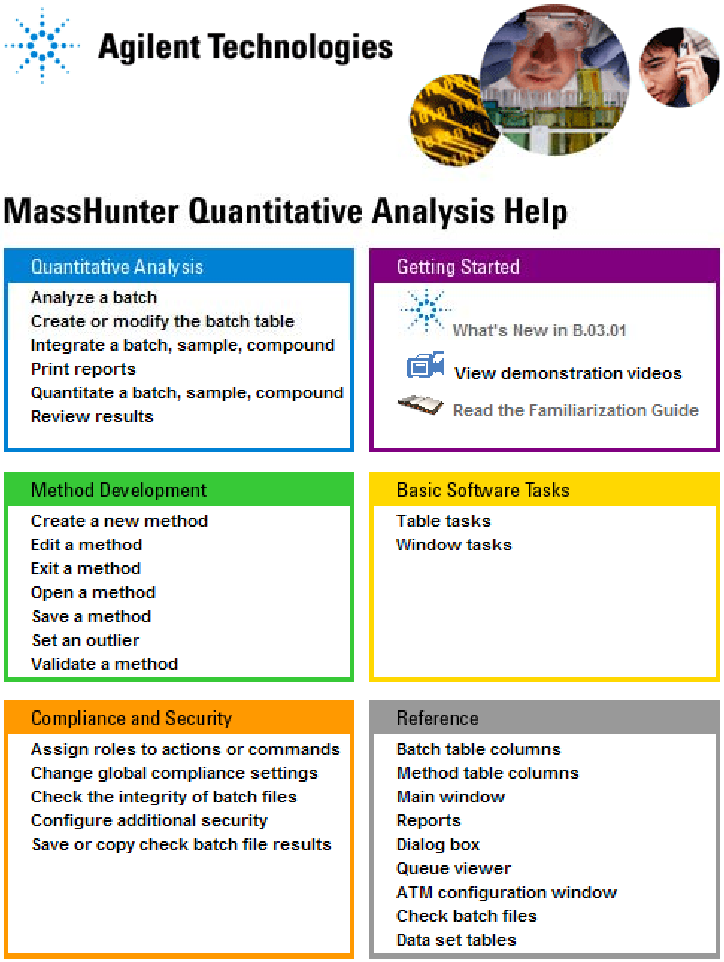
☑ Limit to the largest 1 peaks

You can have the same Peak Filter for all compounds with Apply to All, or specific values for each compound. You can also limit integration to the N largest peak(s), like only one in this example.

MassHunter Quantitation Integrators:

General
Universal
MS/MS – requires 64 data points within window

What's new in MassHunter Quant B.03.02 (just type 'What's new' in the Online Help!)



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MassHunter Quantitative Analysis Help

Quantitative Analysis
- Analyze a batch
- Create or modify the batch table
- Integrate a batch, sample, compound
- Print reports
- Quantitate a batch, sample, compound
- Review results

Getting Started
- What's New in B.03.01
- View demonstration videos
- Read the Familiarization Guide

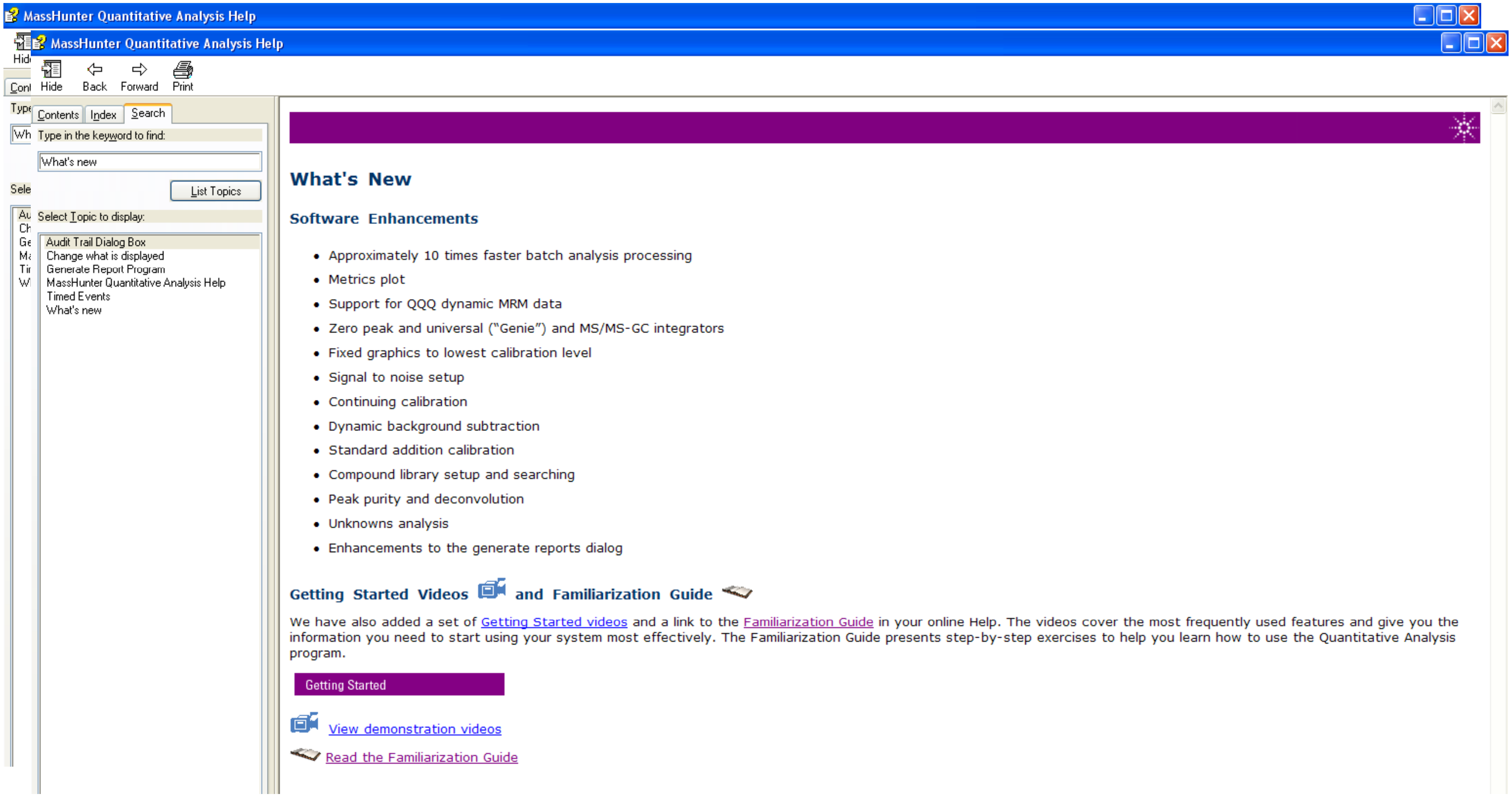
Method Development
- Create a new method
- Edit a method
- Exit a method
- Open a method
- Save a method
- Set an outlier
- Validate a method

Basic Software Tasks
- Table tasks
- Window tasks

Compliance and Security
- Assign roles to actions or commands
- Change global compliance settings
- Check the integrity of batch files
- Configure additional security
- Save or copy check batch file results

Reference
- Batch table columns
- Method table columns
- Main window
- Reports
- Dialog box
- Queue viewer
- ATM configuration window
- Check batch files
- Data set tables

What's new in MassHunter Quant B.03.02
Something for everybody



Type in the keyword to find:

What's new

Select Topic to display:

- Audit Trail Dialog Box
- Change what is displayed
- Generate Report Program
- MassHunter Quantitative Analysis Help
- Timed Events
- What's new

What's New

Software Enhancements

- Approximately 10 times faster batch analysis processing
- Metrics plot
- Support for QQQ dynamic MRM data
- Zero peak and universal ("Genie") and MS/MS-GC integrators
- Fixed graphics to lowest calibration level
- Signal to noise setup
- Continuing calibration
- Dynamic background subtraction
- Standard addition calibration
- Compound library setup and searching
- Peak purity and deconvolution
- Unknowns analysis
- Enhancements to the generate reports dialog

Getting Started Videos and Familiarization Guide

We have also added a set of Getting Started videos and a link to the Familiarization Guide in your online Help. The videos cover the most frequently used features and give you the information you need to start using your system most effectively. The Familiarization Guide presents step-by-step exercises to help you learn how to use the Quantitative Analysis program.

Getting Started

View demonstration videos

Read the Familiarization Guide

What's new in MassHunter Quant B.03.02
More legibly:

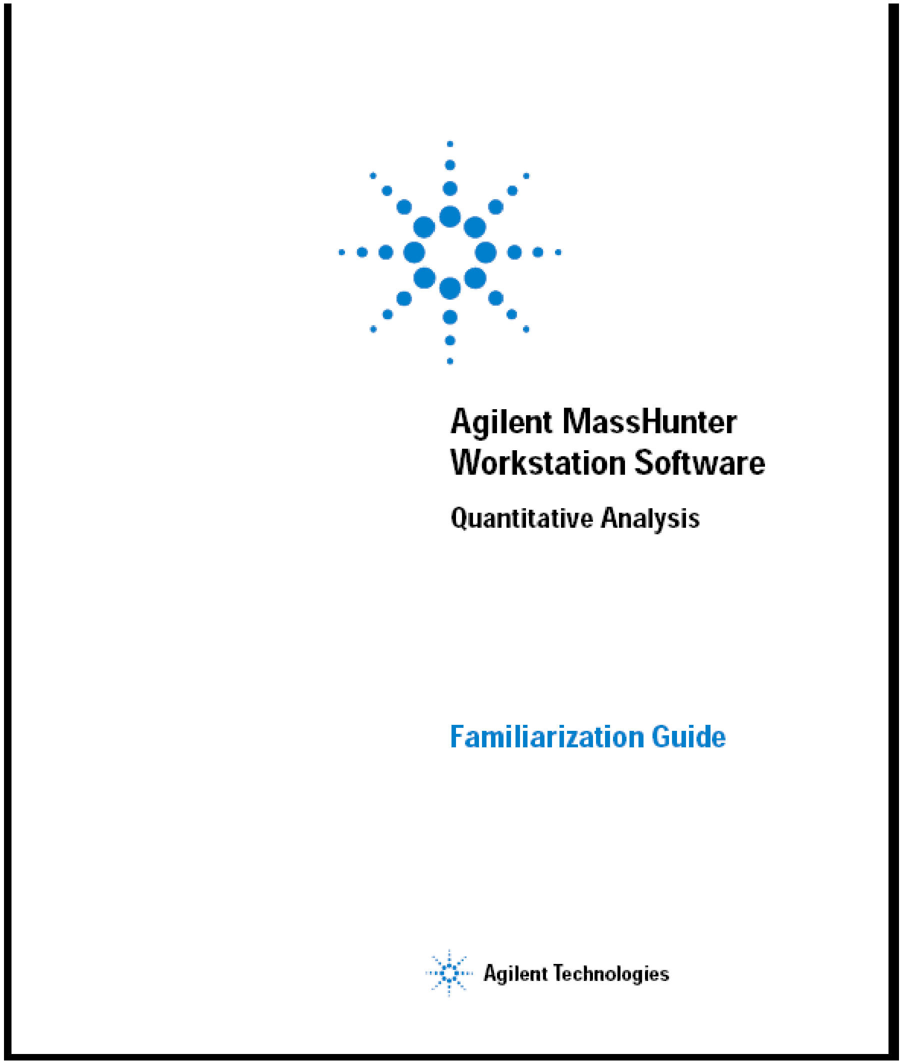
Software Enhancements (some are instrument-specific)

- Approximately 10 times faster batch analysis processing
- Compound Math !
- Metrics plot (e.g. for IS areas)
- Support for QQQ dynamic MRM data
- More integrator choices: “Universal" (ChemStation) and GC/MS/MS integrators
- Fixed graphics to lowest calibration level
- Signal to noise setup
- Continuing calibration
- Dynamic background subtraction
- Standard addition calibration
- Compound library setup and searching
- Peak purity and deconvolution
- More choices in the Generate Reports dialog (e.g. no graphics)

- **Direct Link to *Getting Started Videos* and *Familiarization Guide***

Final recommendation for learning MassHunter Quant:

Uses data files included with the software



Agilent MassHunter Workstation Software

Quantitative Analysis

Familiarization Guide

Agilent Technologies