Agilent 6400 Series Triple Quadrupole Users Workshop

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QQQ Method Development and Optimization

MassHunter Quantitation: Batch and Method setup QC's, Outliers, Data Review

Agilent 6400 Series Triple Quadrupole Users Workshop

David Presser
John Hughes
Anabel Fandino
David Weil
Doug McIntyre
Shane Tichy
Peter Stone
Laura Phillips
André Szczesnieski

Agenda

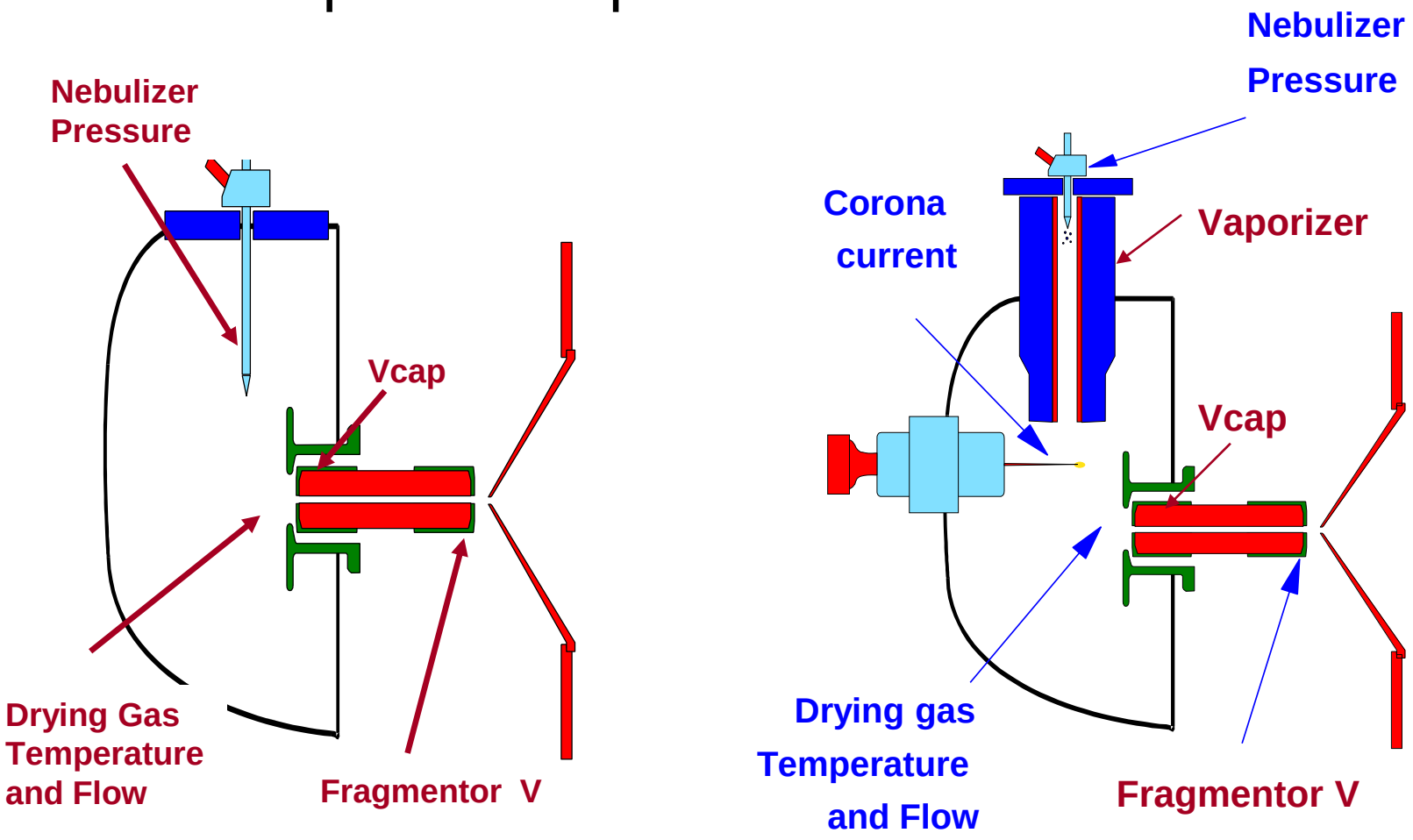
QQQ method development and optimization

- Acquisition overview
- Source optimization for standard and Agilent JetStream sources
- Compound optimization using flow injection or Optimizer software
- Dynamic MRM

MassHunter Quant software – current revision B.06.00 SP1

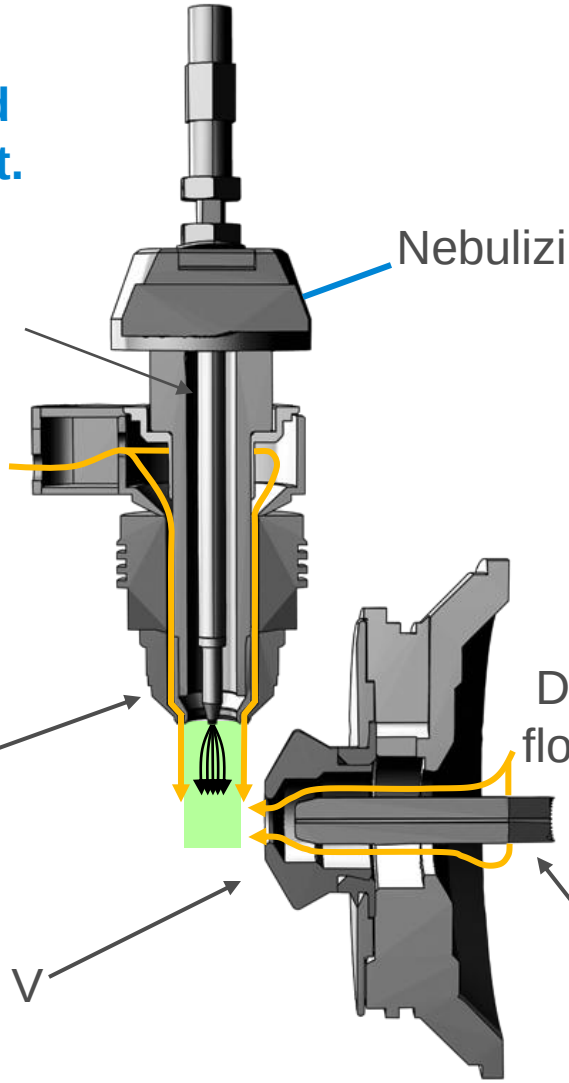
- Creating a Batch and Quantitation method
  - Setting up QC's
  - Outliers
  - Reviewing data
  - Reporting and Exporting data

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



Agilent Jetstream Technology (AJT)

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



*New parameters unique to AJT source

Tuning and Calibration for Agilent QQQ systems

Agilent QQQ systems are very stable and require infrequent Autotuning

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 Autotune before running samples in negative ion.

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

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)

6410 starting parameters for small molecules

6410 Parameter	Standard ESI source	APCI source
Capillary voltage	3500 V	3500 V
Drying gas flow	10 Lpm ≤ 0.2 mL/min 12 Lpm > 0.4 mL/min	5-7 Lpm
Drying gas temp	350°C	350°C
Fragmentor voltage	135 V	135 V
Nebulizer pressure	25 psi 0.2 mL/min 50 psi 1 mL/min	60 psi
Vaporizer temp		350°C

Multi-Mode Source starting parameters

Multimode	ESI	APCI	Mixed mode
Capillary voltage	2000 V	2000 V	1000 V
Charging voltage	2000 V	2000 V	2000 V
Corona current	0	5	1
Drying gas flow	5 Lpm	5 Lpm	5 Lpm
Drying gas temp	250°C	300°C	300°C
Fragmentor voltage	135 V	135 V	135 V
Nebulizer pressure	60 psi	20 psi	40 psi
Vaporizer temp	150-200°C	200-250°C	150-250°C

6460 starting parameters for small molecules

6460 Parameter	Starting value
Capillary voltage	3500 V
CAV – acceleration	7 volts
Drying gas flow	10 L/min
Drying gas temp	300°C
Fragmentor voltage	135 V
Nebulizer pressure	45 psi
Nozzle voltage	0 V pos, 2000 V neg
Sheath Gas flow	11 L/min
Sheath Gas temp	300°C

6490 starting parameters for small molecules

6490 Parameter	Starting value
Capillary voltage	3500 V
CAV – acceleration voltage	5 volts
Drying gas flow	15 L/min
Drying gas temp	150°C
Fragmentor voltage	380 V fixed
iFunnel high pressure RF	110 V pos, 90 V neg
iFunnel low pressure RF	60 V pos, 40 V neg
Nebulizer pressure	30 psi (flow dependent)
Nozzle voltage	0 V pos, 2000 V neg
Sheath gas temp	225°C
Sheath gas flow	11 L/min

Optimizing the Agilent JetStream Technology beginning with recommended starting values ( )

Order of effect on sensitivity

- Sheath gas temperature (225°C)
- Sheath gas flow (11 Lpm)
- Nebulizer pressure (30 psi)
- Capillary voltage (3500V)
- Nozzle voltage (0V)
- Drying gas temperature (150°C)
- Drying gas flow (15 Lpm)

Things to note

- Typically 50°C above DGT
- Generally 10-12 Lpm for 0.2-0.7mL
- LC flow and mobile phase dependent
- Somewhat MW dependent
- Very compound and polarity dependent
- Interaction with flow and sheath parms
- Higher when in doubt

Optimizing the Agilent JetStream Technology Typical values ( ) and Increments

Order of effect on sensitivity	Increments and ranges to test
Sheath gas temperature (225°C)	50°C, 225-400°C
Sheath gas flow (11 Lpm)	2 Lpm, 8 -12 Lpm
Nebulizer pressure (30 psi)	5 psi, 25 – 50 psi
Capillary voltage (3500V)	500V, 2500-4500V
Nozzle voltage (0V)	500V, 0 - 2000V
Drying gas temperature (150°C)	50°C, 200-350°C
Drying gas flow (15 Lpm)	2 Lpm, 5-20 Lpm

Source Optimization 6490

Types	PreWait(min)	Replicate	StepWait (min)	StartValue	EndValue	StepSize
High Pressure RF	0	1	0	70	210	10
Low Pressure RF	0	1	0	40	210	10
Sheath Gas Temp	20	1	20	200	400	50
Sheath Gas Flow	20	1	20	9	12	1
Gas Temp	20	1	20	120	250	30
Gas Flow	20	1	20	14	20	2
Nebulizer	0	1	0	15	50	5
Capillary	0	1	0	2000	4500	500
Nozzle Voltage	0	1	0	0	2000	500

Optimization techniques

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

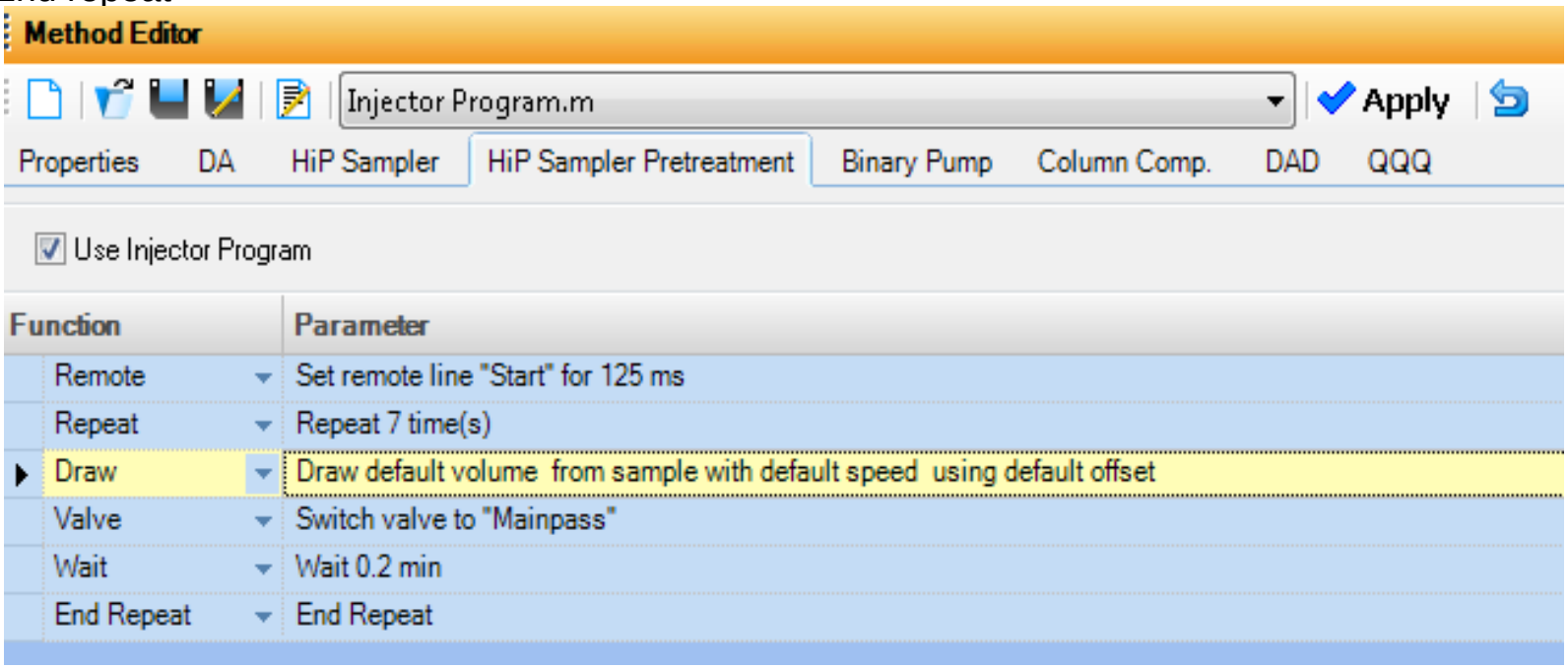
Voltages, gas flows change quickly and can be optimized with series of flow injections using method with injector program and time segments.

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

Injector program for series of flow injections

Injector program steps:

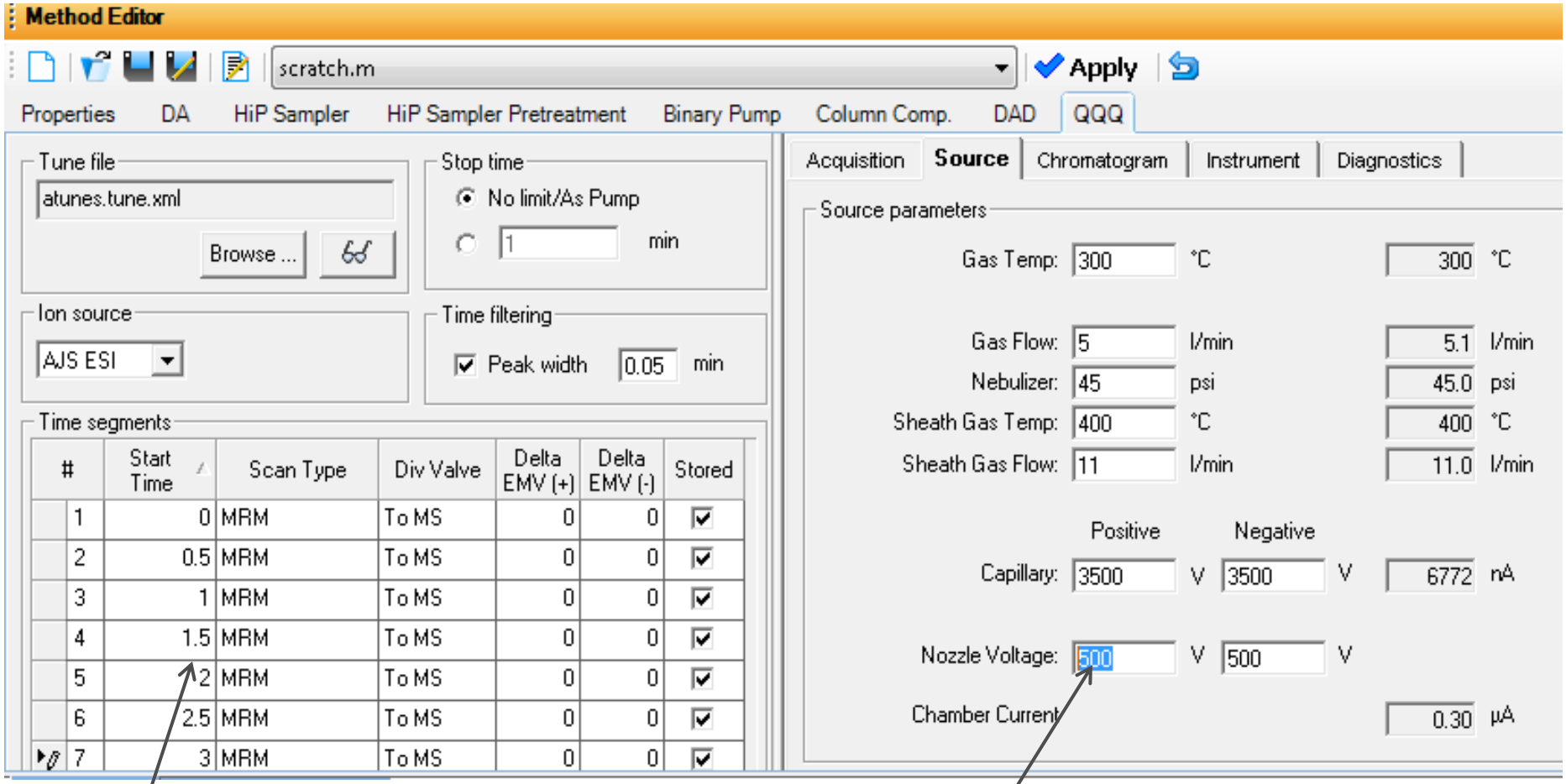
Remote Startpulse [Starts the instrument]
Repeat n times
Draw default amount from sample
Valve to Mainpass [injects sample without start signal]
Wait 0.20 min [allow sample to elute from needle before repeating]
End repeat



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

MS acquisition for series of flow injections:

Synchronize MS time segments with peak spacing

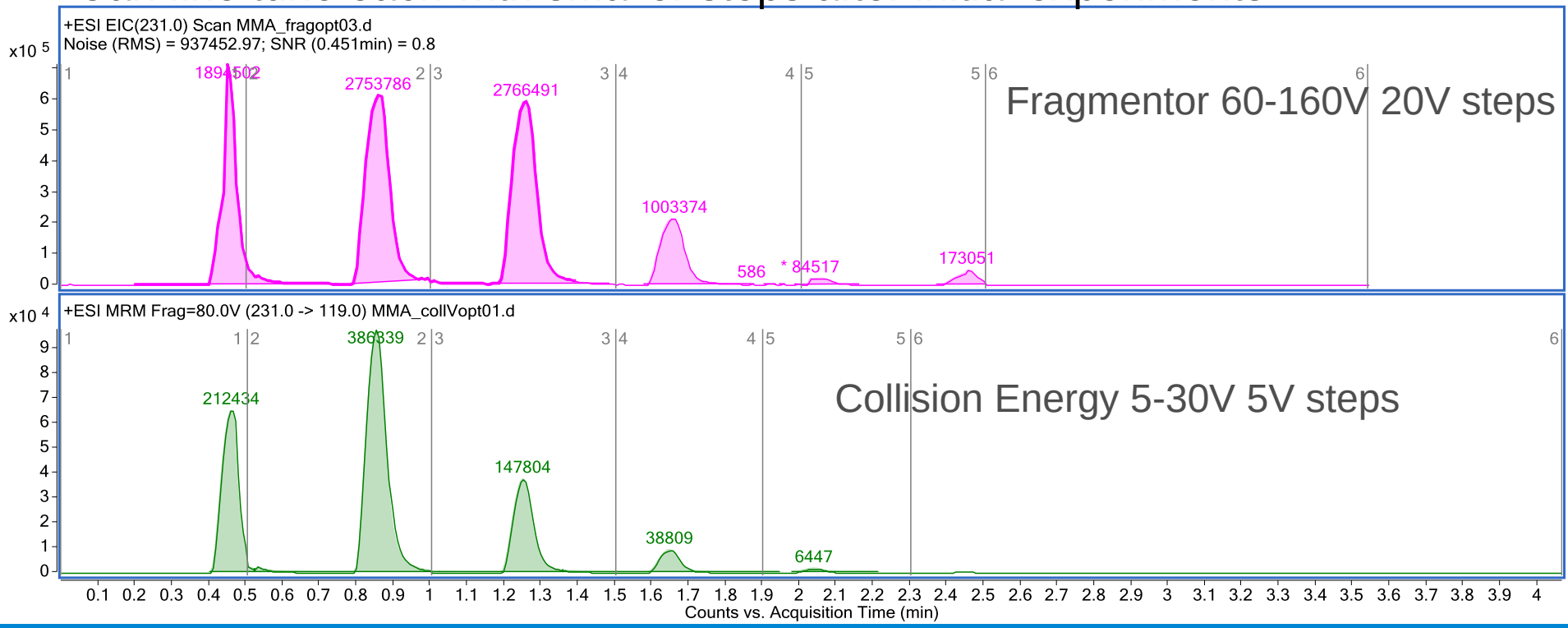


Example: MS time segments increment nozzle voltage

Fragmentor and Collision Energy optimization

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



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.

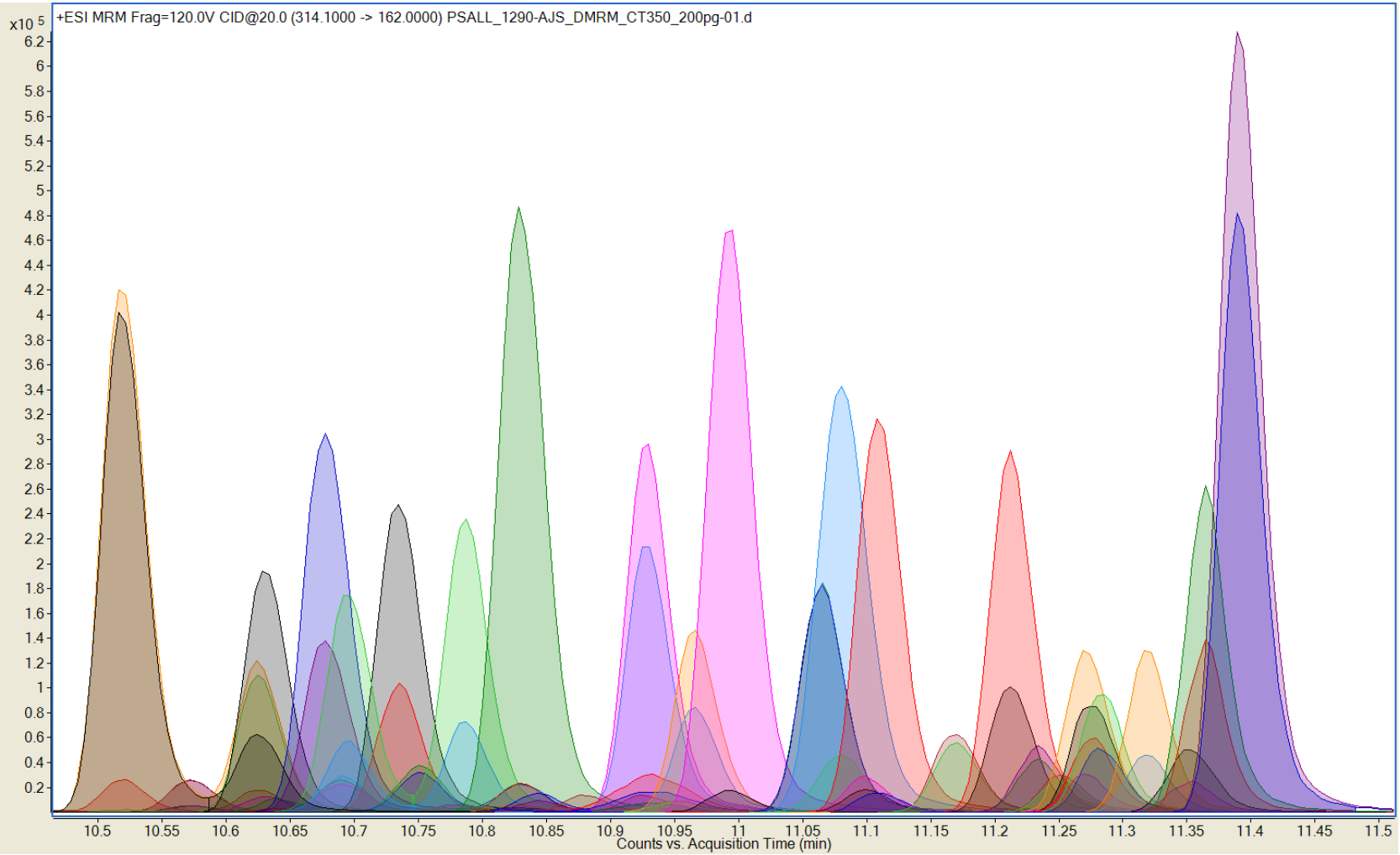
Monitoring many compounds simultaneously lowers dwell time per MRM.

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.

UHPLC peak capacities are very high

40 MRM transitions in a one minute window



The solution: MassHunter Dynamic MRM

Included in QQQ Acquisition B.02.01 and later

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)

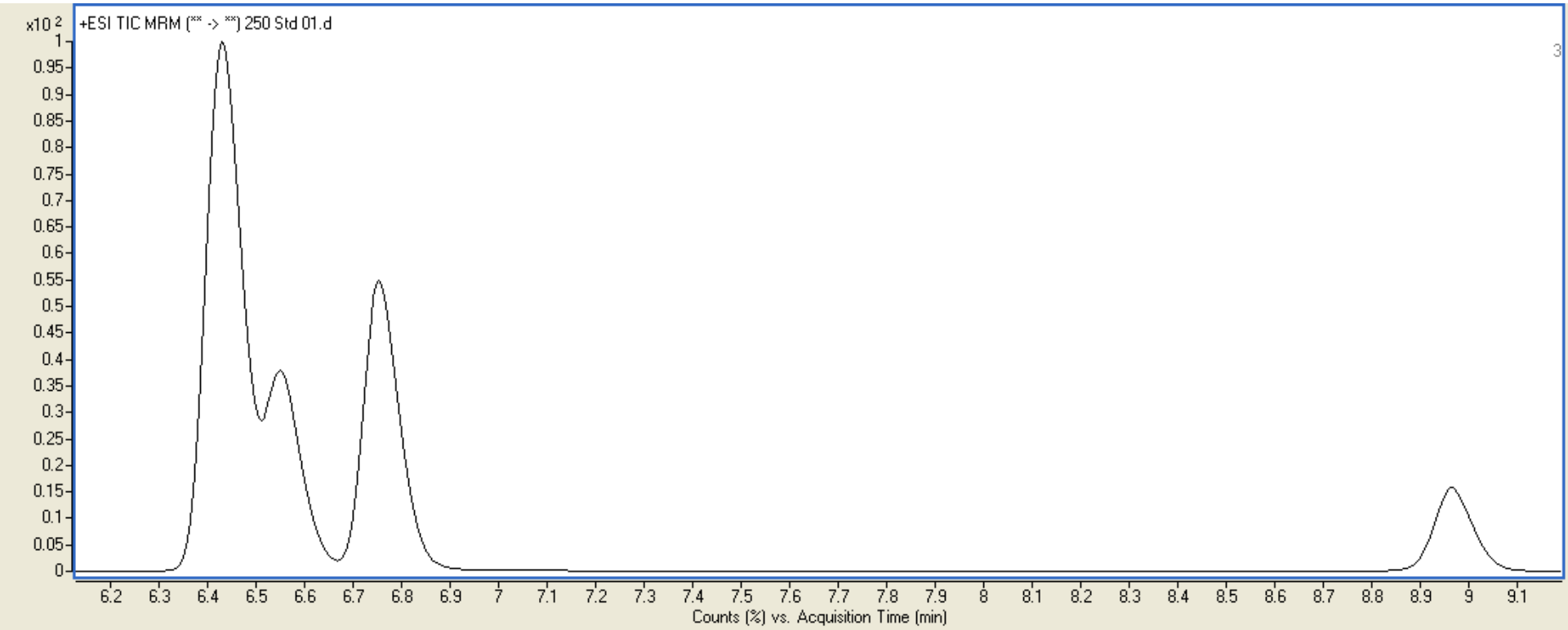
Without Dynamic MRM:

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

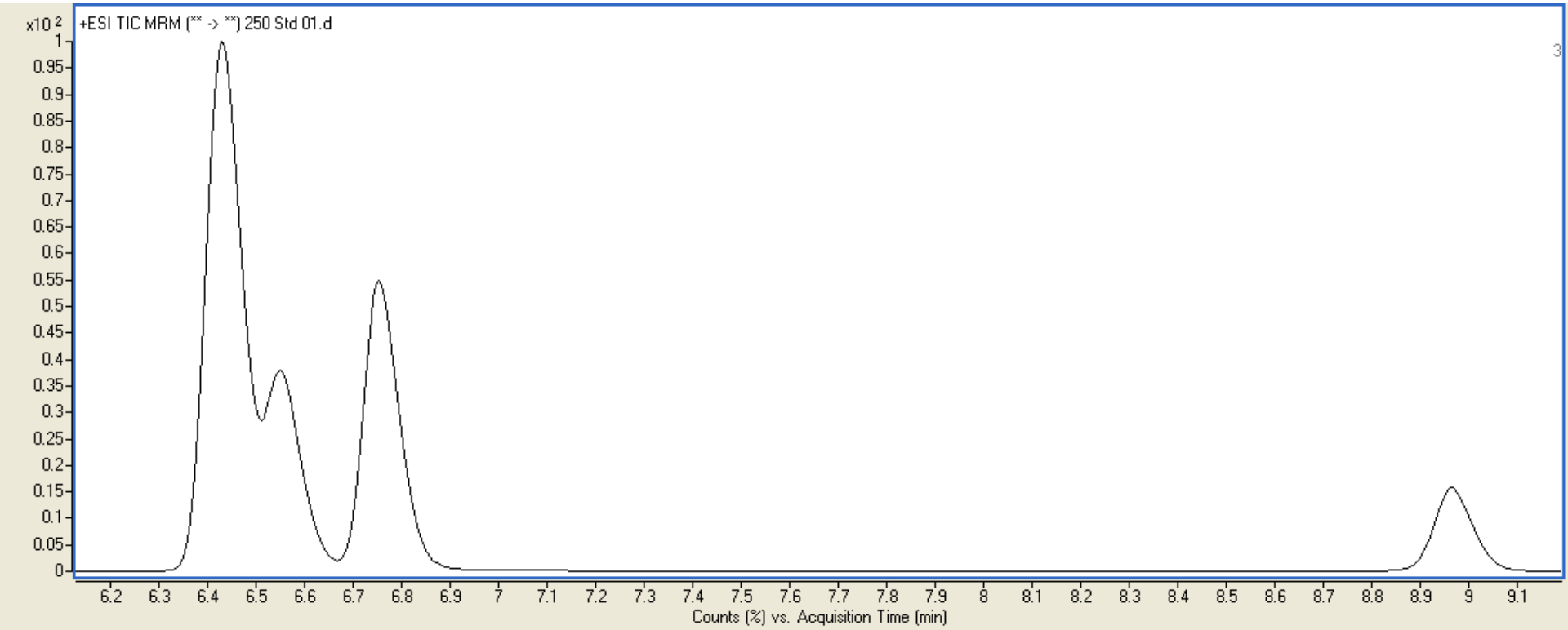
With Dynamic MRM:

- Automatic setup of overlapping time segments without user intervention
- May increase signal to noise
- Can update all retention times using latest run

MRM: width approximates dwell time

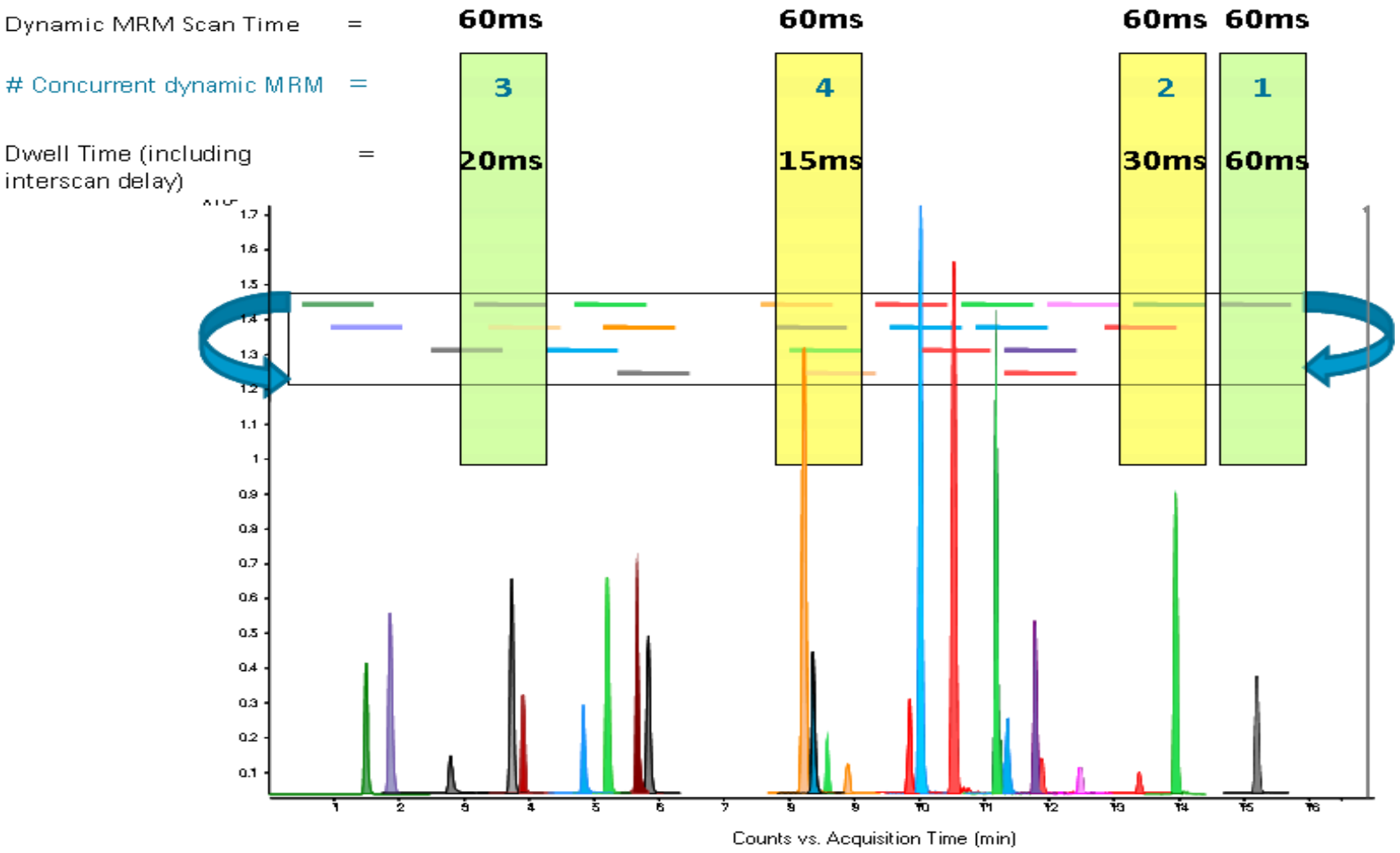


Dynamic MRM: automatically maximizes dwell time

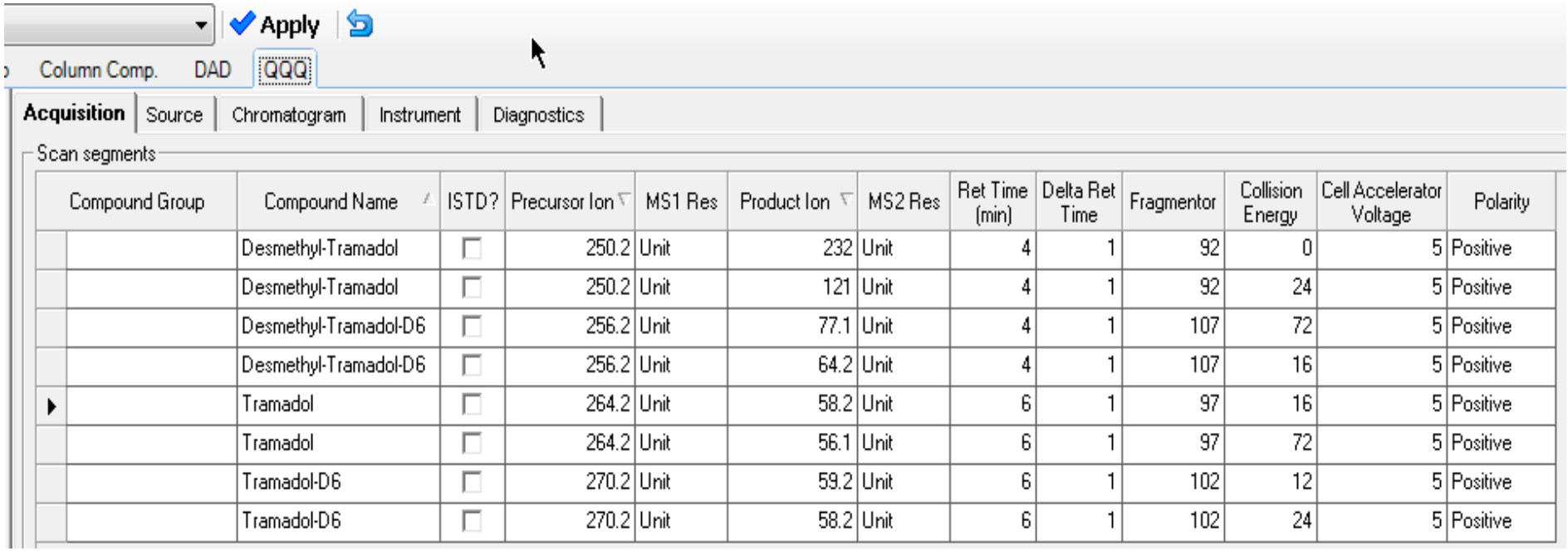


Dynamic MRM for 6400 series QQQ

Monitors transitions only when compound elutes

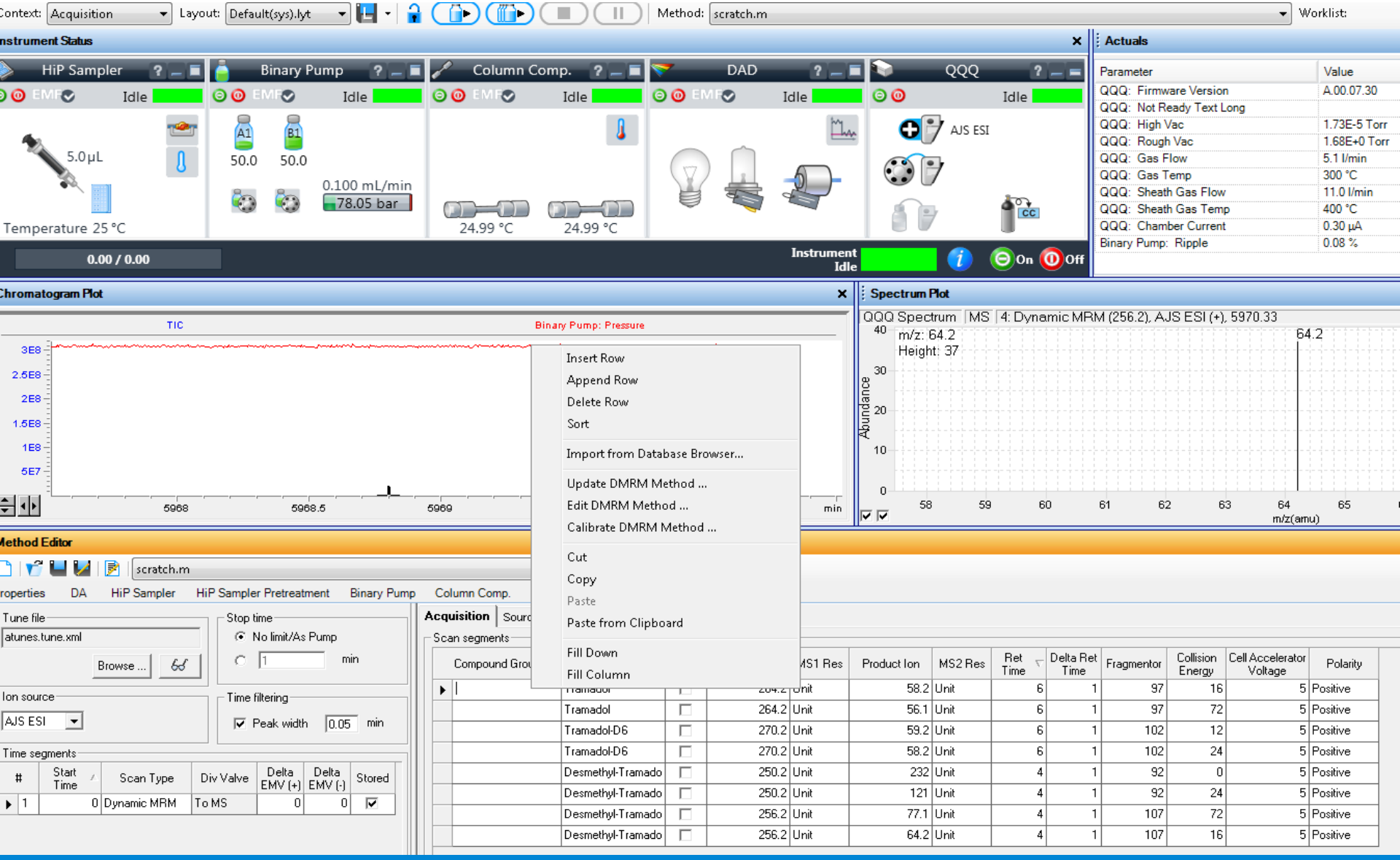


Dynamic MRM: Retention Time and Delta Ret Time



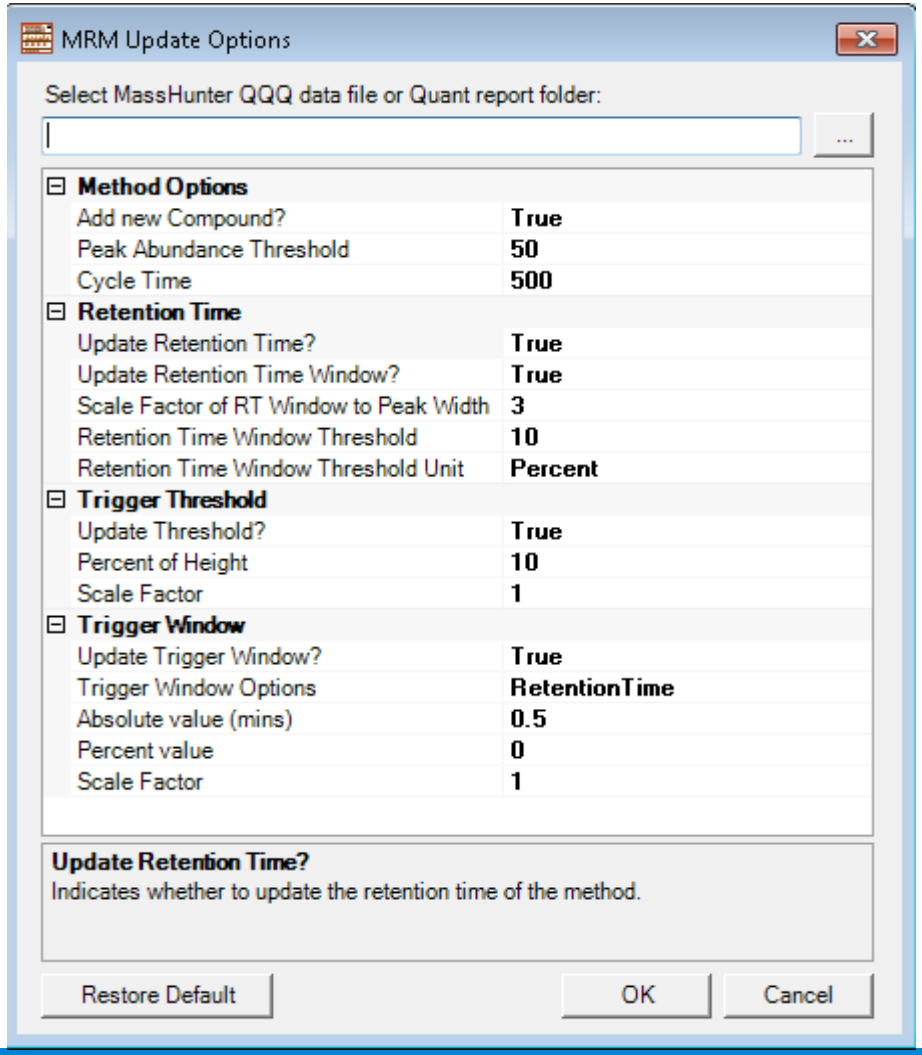
Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Desmethyl-Tramadol	☐	250.2	Unit	232	Unit	4	1	92	0	5	Positive
	Desmethyl-Tramadol	☐	250.2	Unit	121	Unit	4	1	92	24	5	Positive
	Desmethyl-Tramadol-D6	☐	256.2	Unit	77.1	Unit	4	1	107	72	5	Positive
	Desmethyl-Tramadol-D6	☐	256.2	Unit	64.2	Unit	4	1	107	16	5	Positive
	Tramadol	☐	264.2	Unit	58.2	Unit	6	1	97	16	5	Positive
	Tramadol	☐	264.2	Unit	56.1	Unit	6	1	97	72	5	Positive
	Tramadol-D6	☐	270.2	Unit	59.2	Unit	6	1	102	12	5	Positive
	Tramadol-D6	☐	270.2	Unit	58.2	Unit	6	1	102	24	5	Positive

Dynamic MRM – Update Method in Acquisition



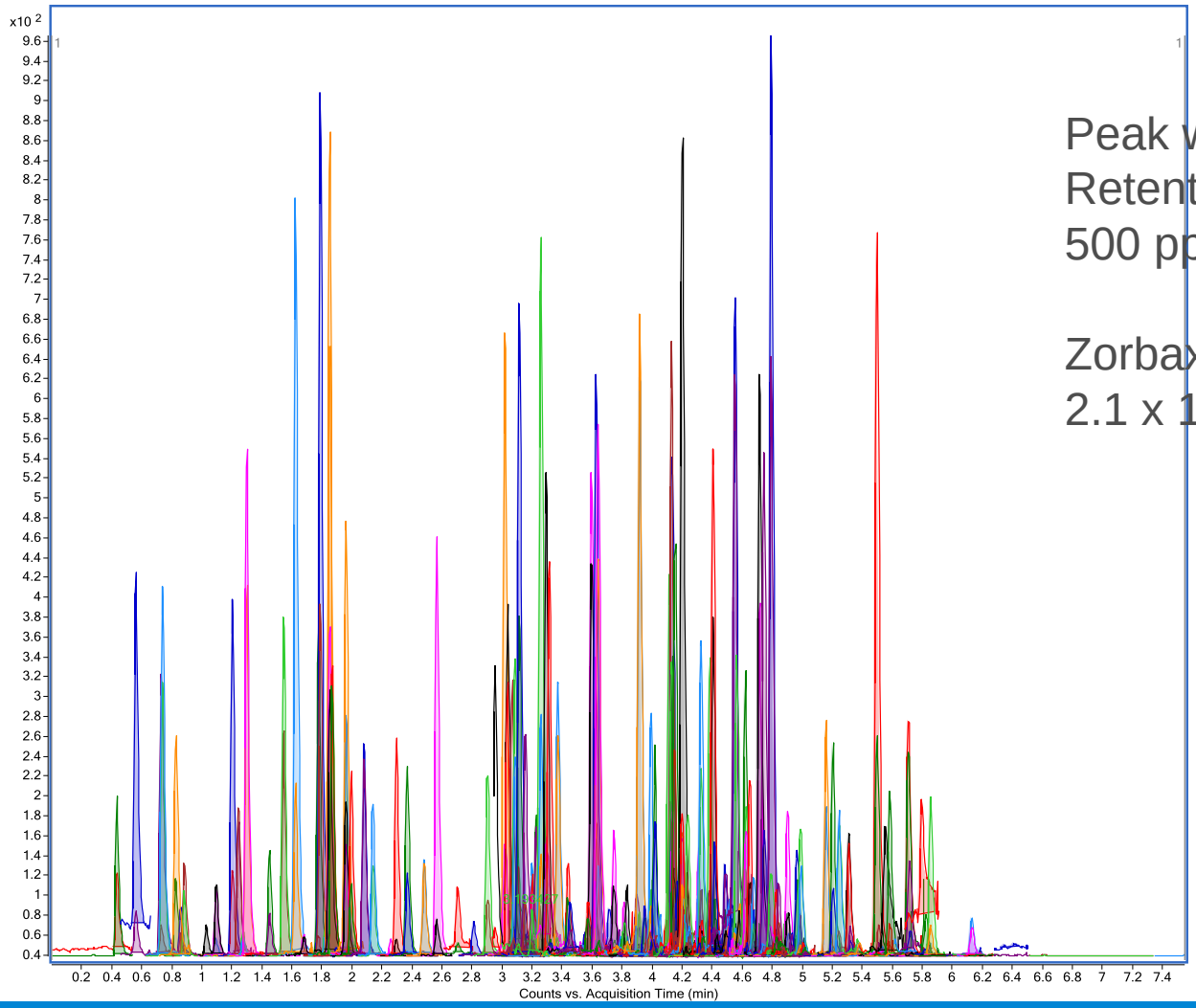
Update RTs, RT windows, add new compounds

Recommend using: Quant report folder



Dynamic MRM Analysis with 6460 QQQ

Eight minute 250 compound pesticide screen



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

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

MassHunter Optimizer

Included as of MassHunter QQQ B.04 acquisition software

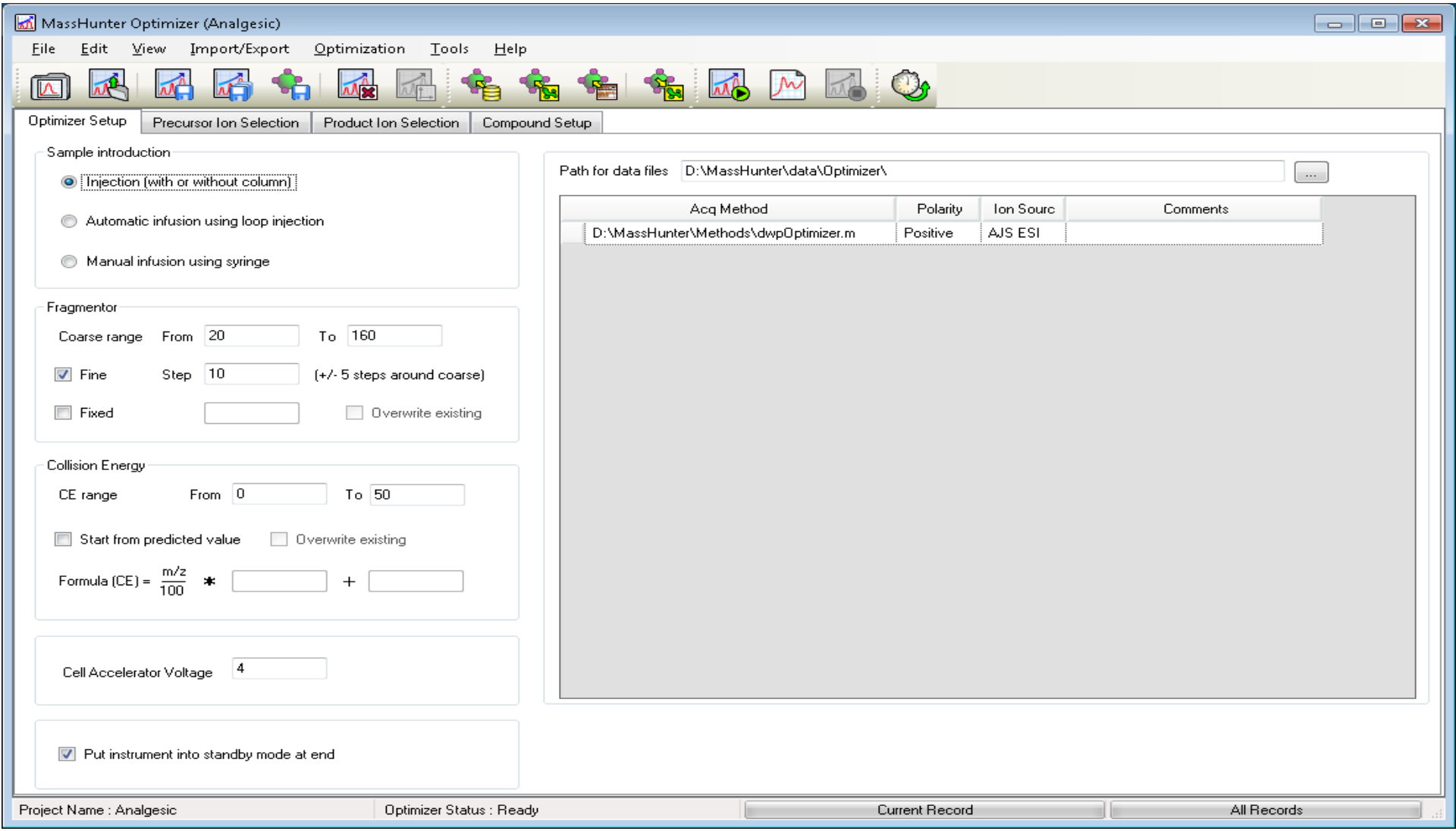
Useful for frequent method development [new compound each week] or for multi-compound methods [e.g. toxicology, environmental applications]

Many toxicology and pesticide transitions are available in new Agilent application kits and from Agilent application chemists.

Can use Flow Injection before LC separation is developed

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

MassHunter Optimizer – method, parameter selection

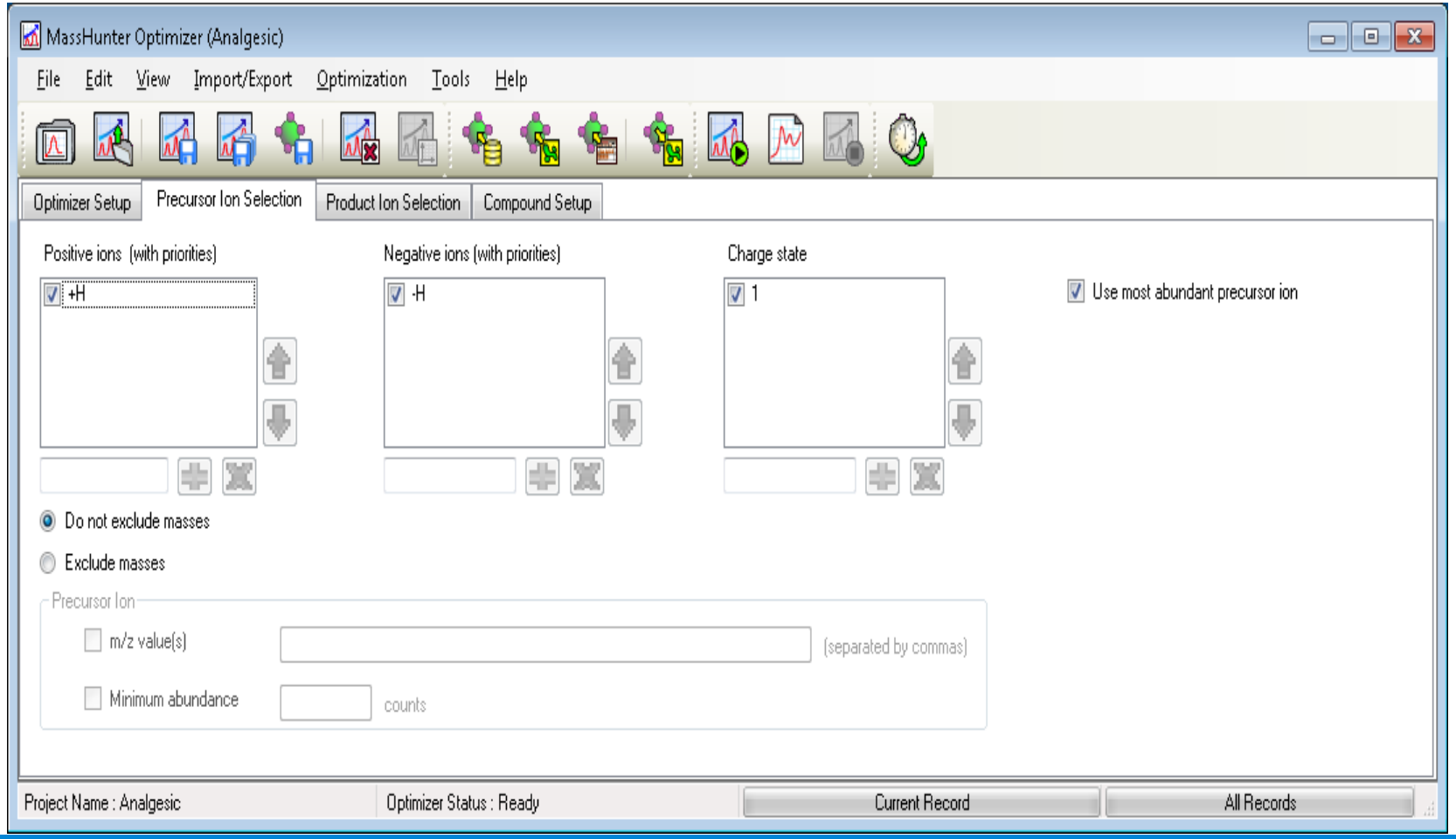


MassHunter Optimizer

Precursor ion selection

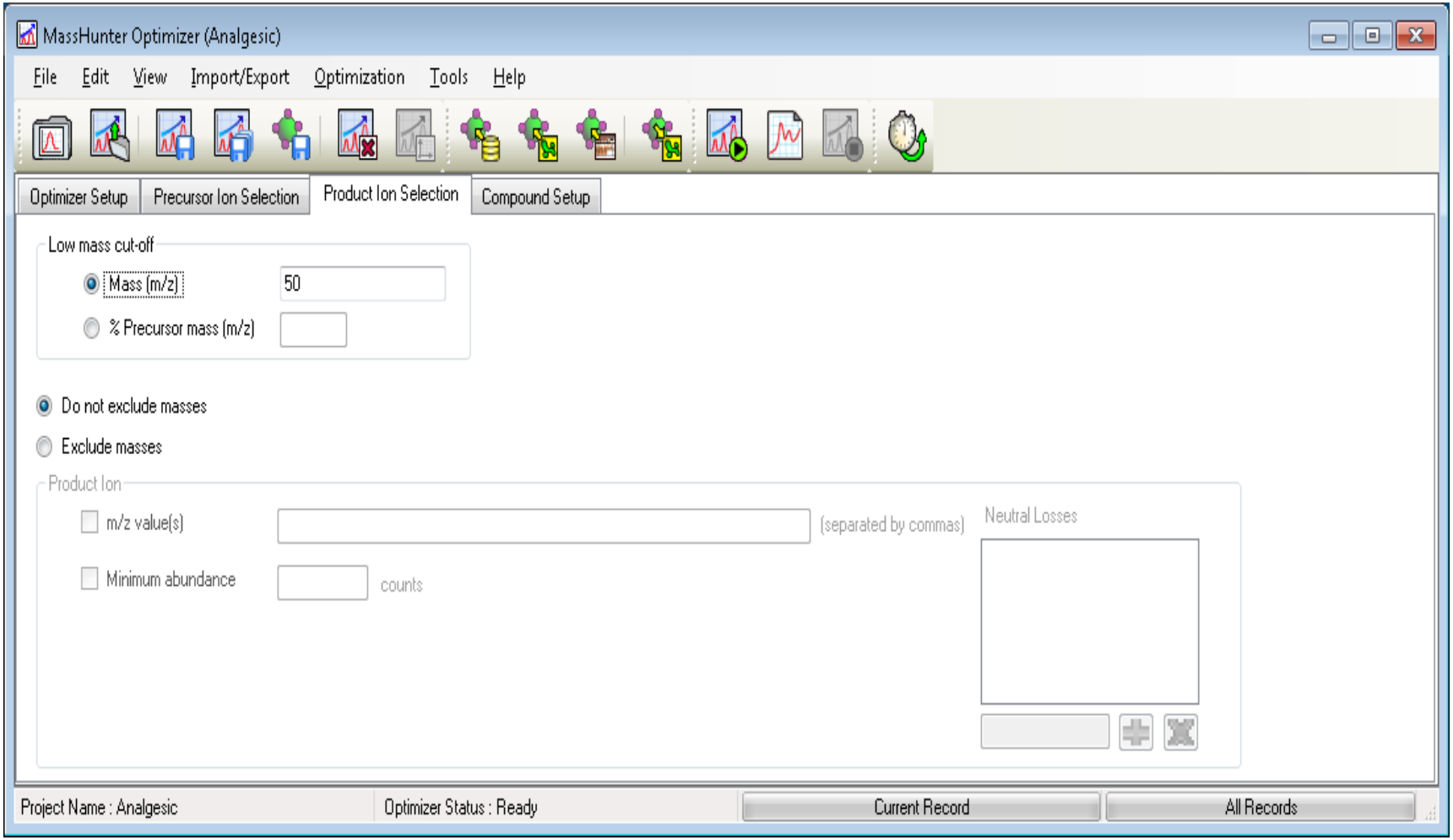
User selectable adducts (H, Na, K, OAc- , HCOO-, etc.)

Can run both positive and negative ion methods in same project

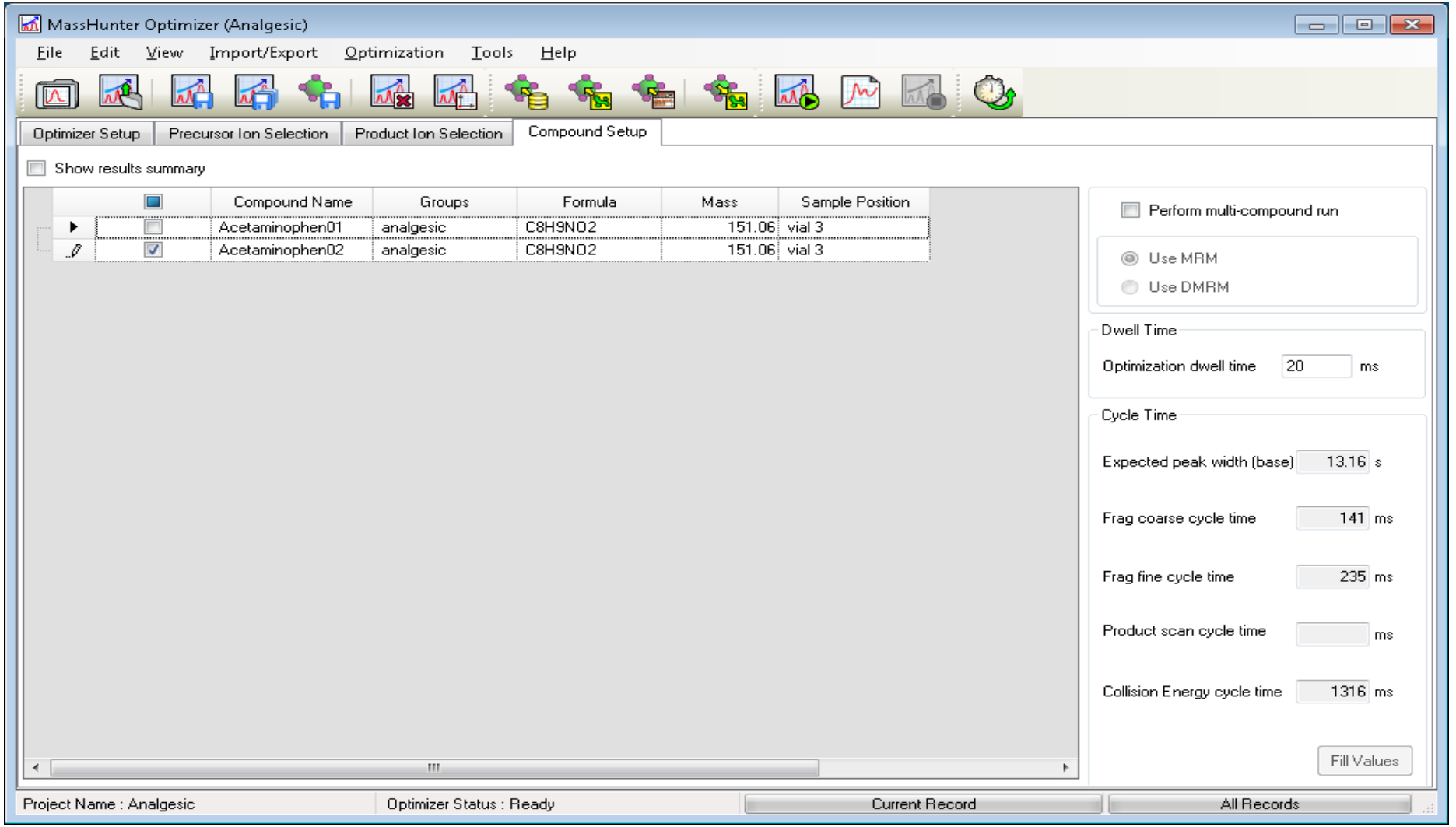


MassHunter Optimizer

Product ion selection: set mass range

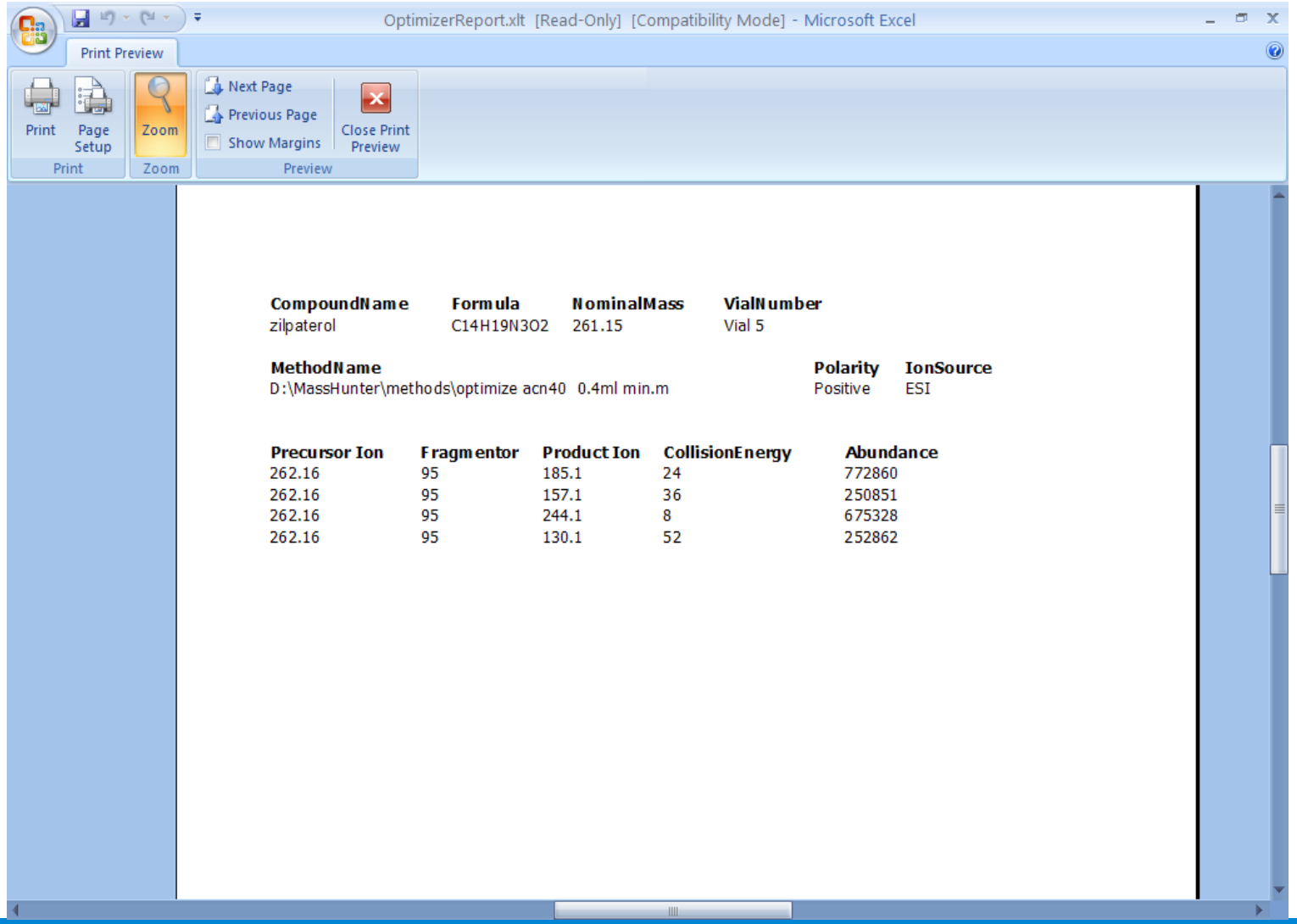


MassHunter Optimizer – input a unique compound name



MassHunter Optimizer results

Results automatically added to MRM database

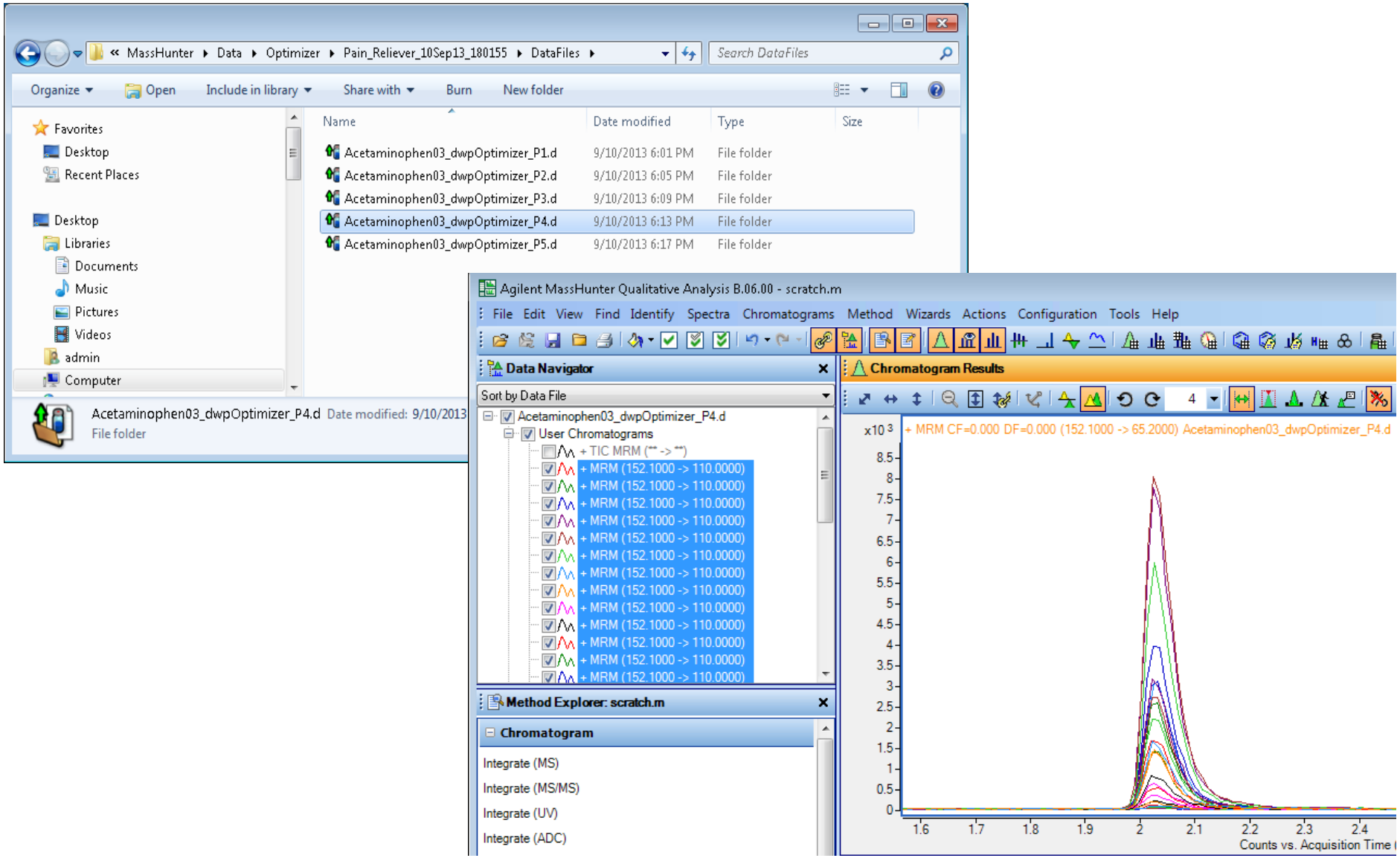


CompoundName	Formula	NominalMass	VialNumber
zilpaterol	C14H19N3O2	261.15	Vial 5

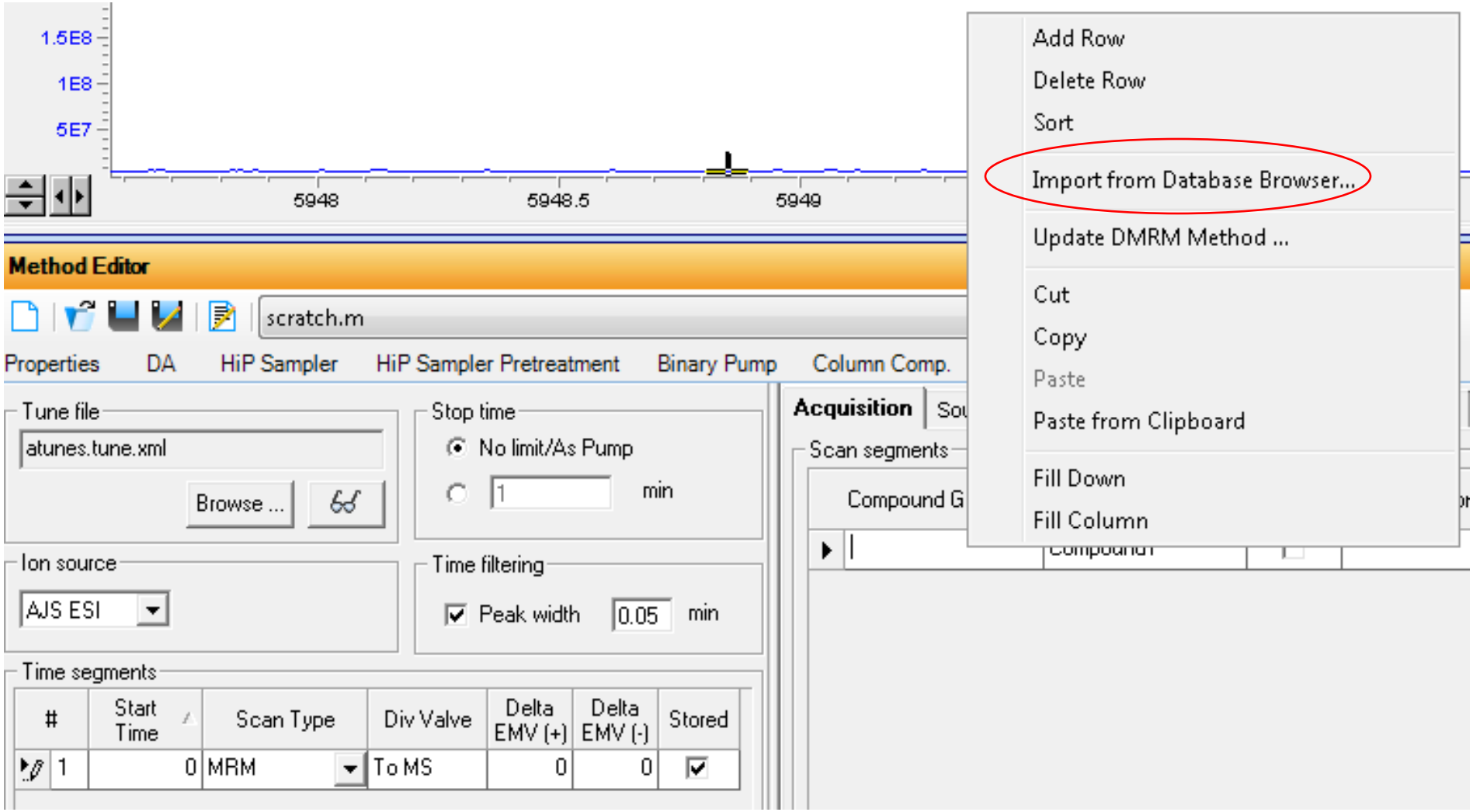
MethodName	Polarity	IonSource
D:\MassHunter\methods\optimize acn40 0.4ml min.m	Positive	ESI

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

MassHunter Optimizer results – inspect data

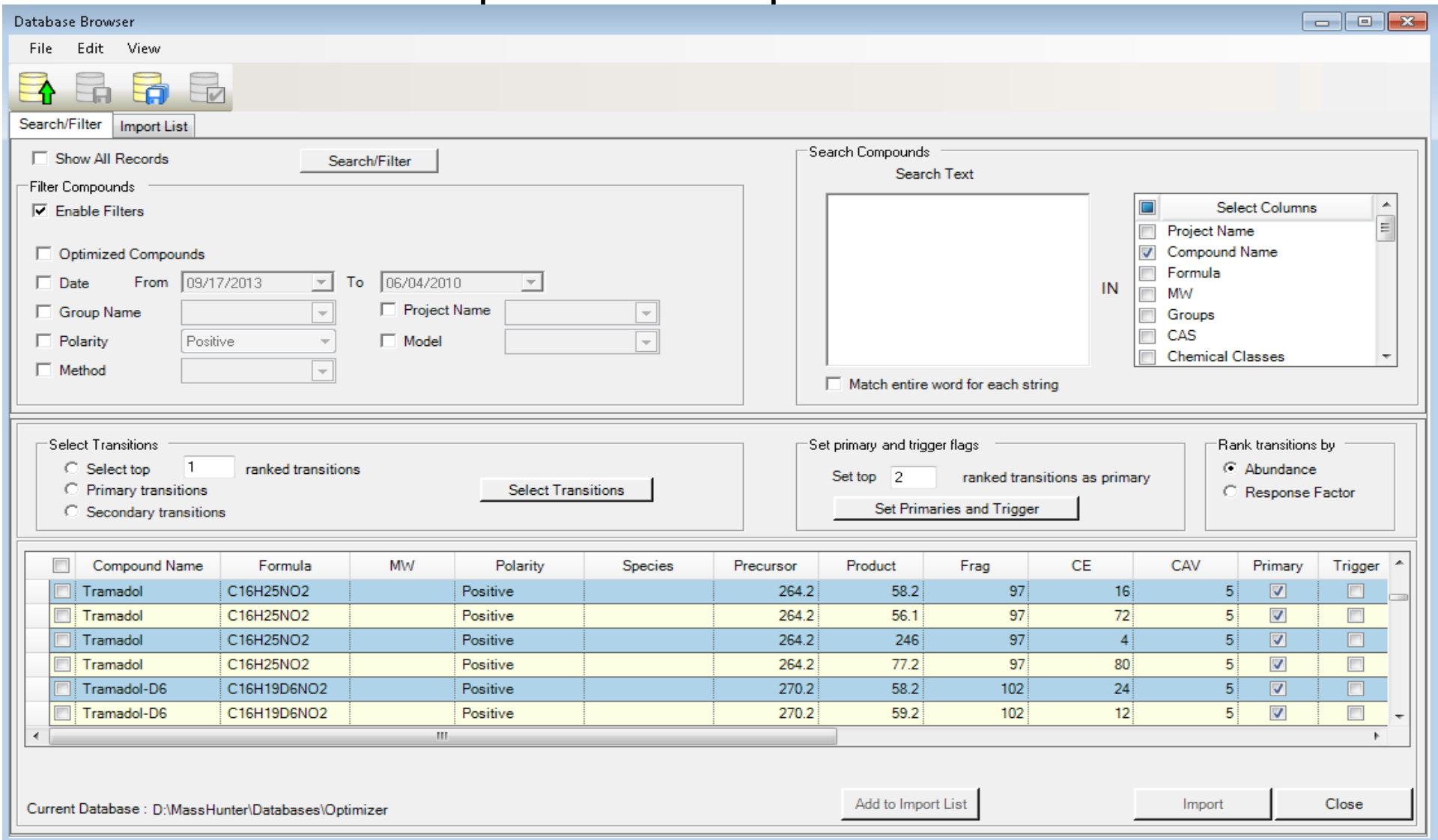


MassHunter Optimizer - import directly into acquisition



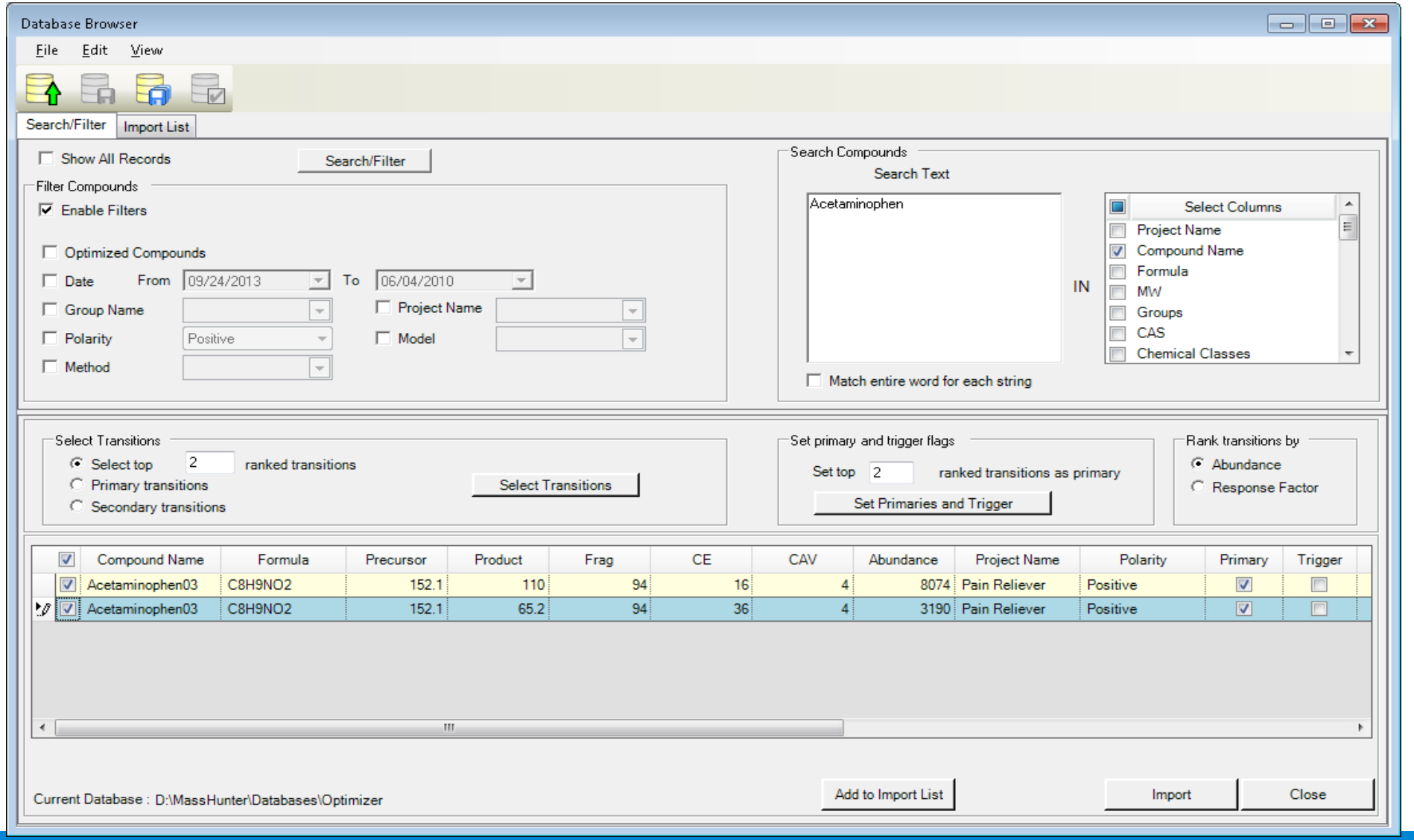
MassHunter Optimizer

Search or filter compounds for import



MassHunter Optimizer

Search or filter compounds for import



MassHunter Acquisition – imported transitions

Column Comp. | DAD | QQQ

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

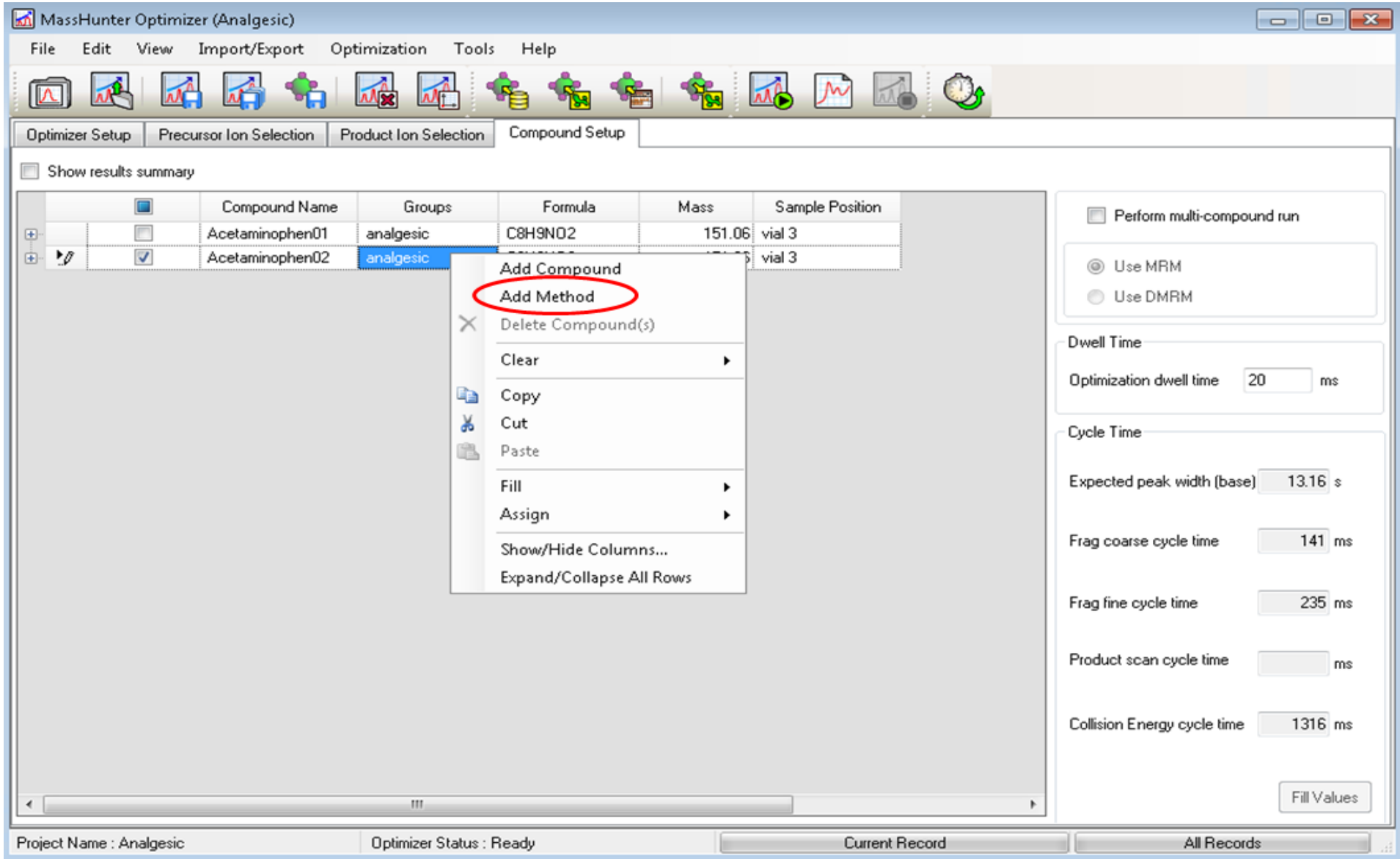
Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Acetaminophen	☐	152.1	Unit	110	Unit	200	94	16	4	Positive
	Acetaminophen	☐	152.1	Unit	65.2	Unit	200	94	36	4	Positive

Acquisition | Source | Chromatogram | Instrument | Diagnostics

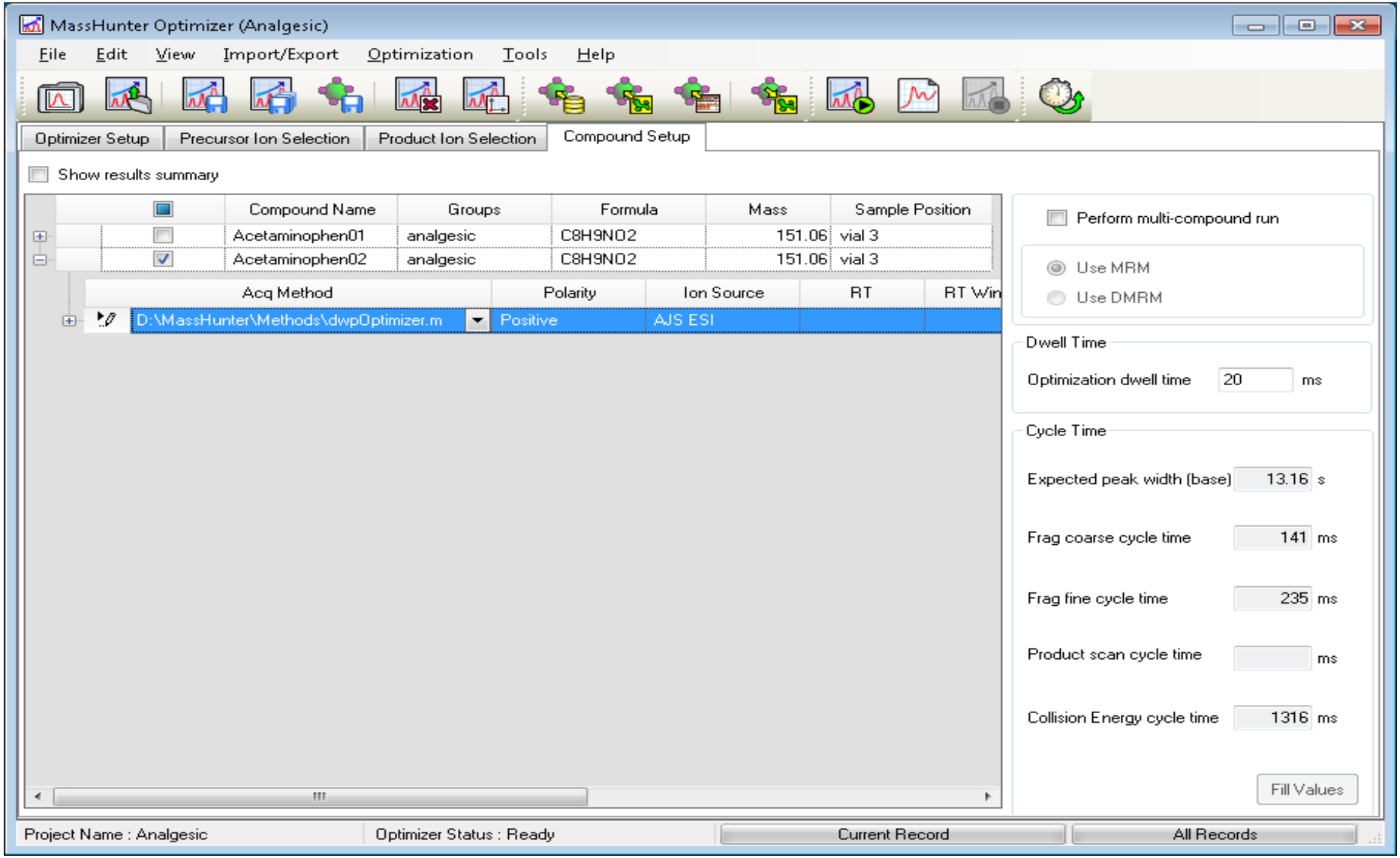
Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Tramadol	☐	264.2	Unit	58.2	Unit	200	97	16	5	Positive
	Tramadol	☐	264.2	Unit	56.1	Unit	200	97	72	5	Positive
	Tramadol-D6	☐	270.2	Unit	58.2	Unit	200	102	24	5	Positive
	Tramadol-D6	☐	270.2	Unit	59.2	Unit	200	102	12	5	Positive
	Desmethyl-Tramadol	☐	250.2	Unit	232	Unit	200	92	0	5	Positive
	Desmethyl-Tramadol	☐	250.2	Unit	121	Unit	200	92	24	5	Positive
	Desmethyl-Tramadol-I	☐	256.2	Unit	64.2	Unit	200	107	16	5	Positive
	Desmethyl-Tramadol-I	☐	256.2	Unit	77.1	Unit	200	107	72	5	Positive

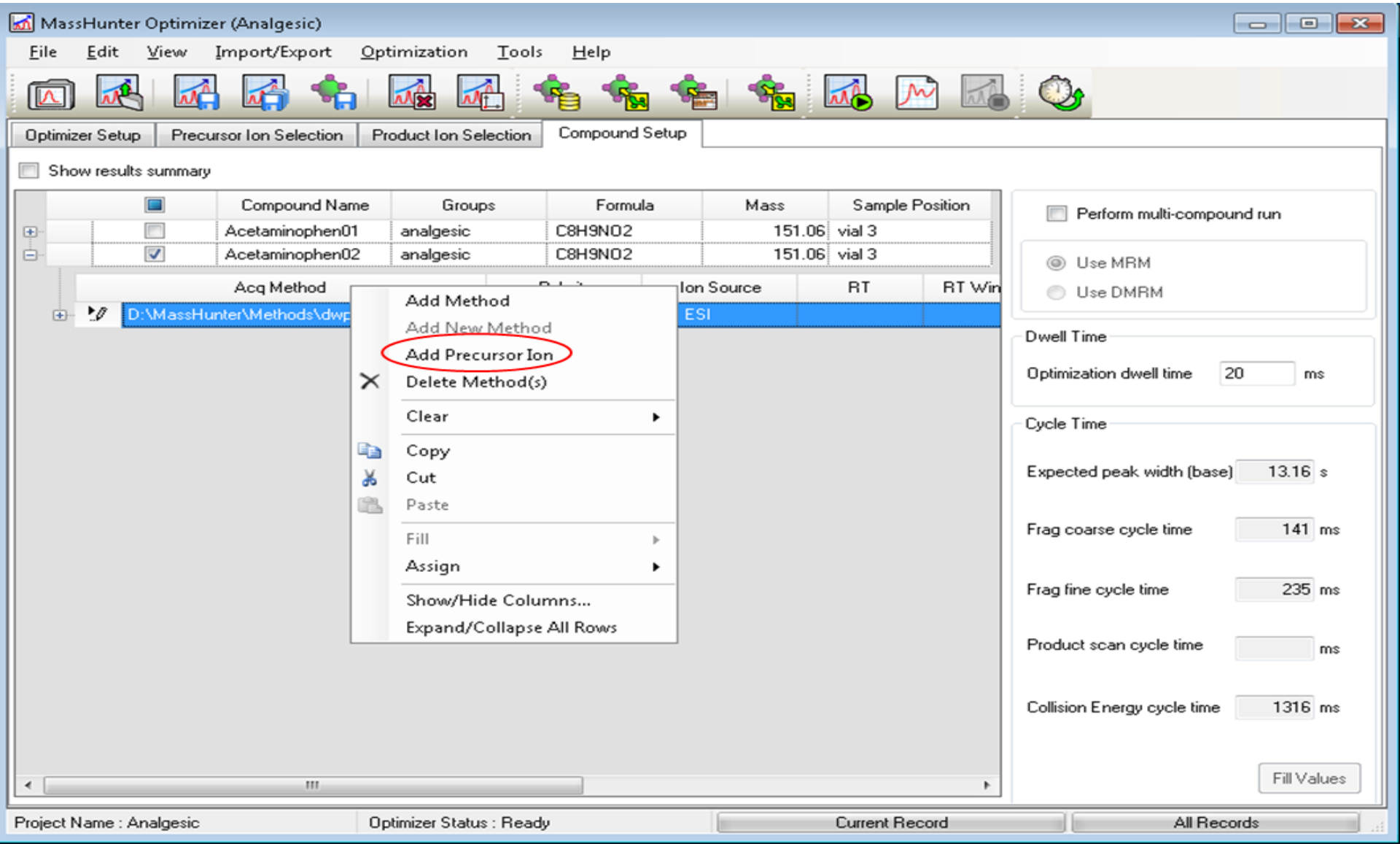
MassHunter Optimizer – input transitions



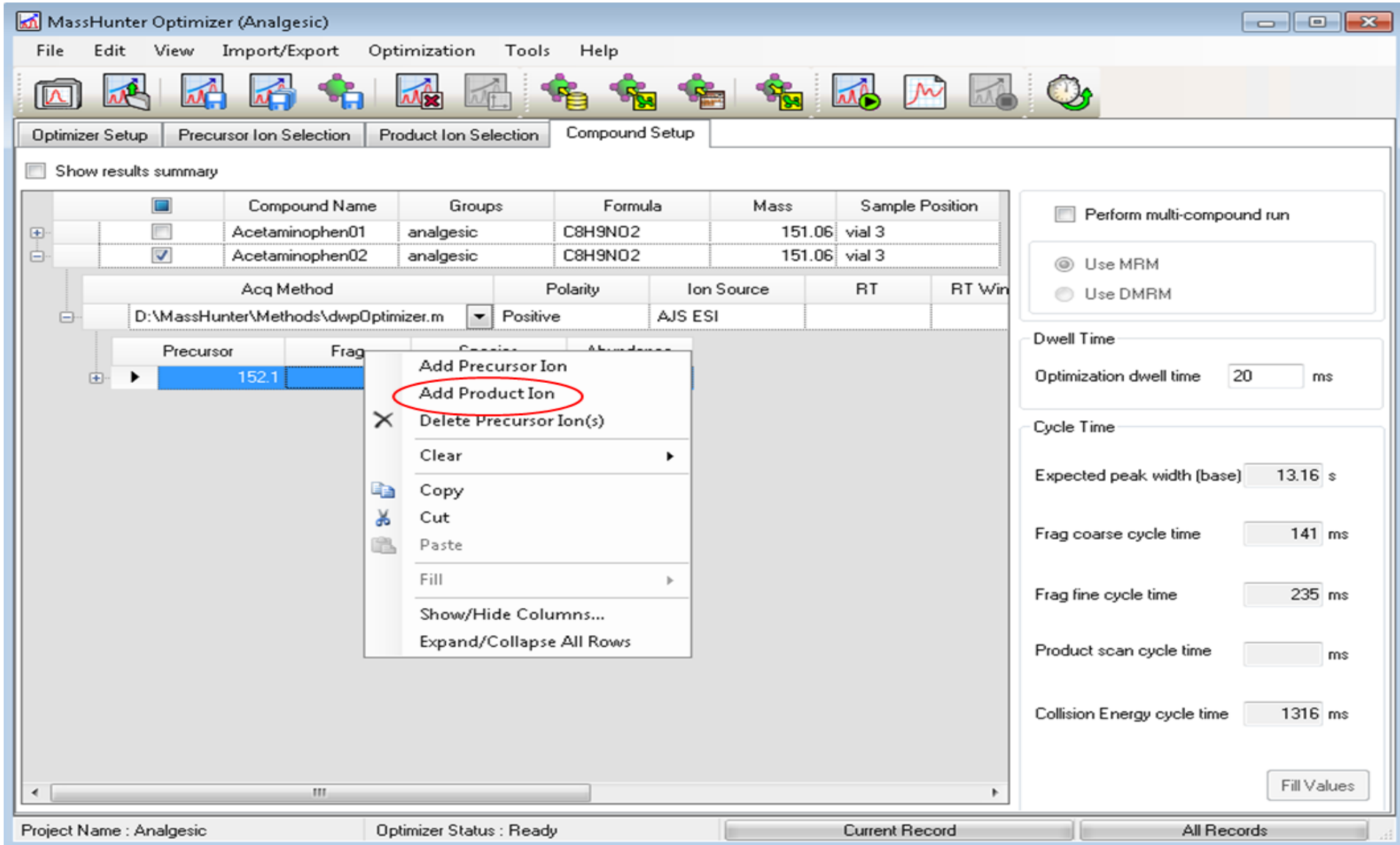
MassHunter Optimizer – input transitions



MassHunter Optimizer – input transitions



MassHunter Optimizer – input transitions



MassHunter Optimizer – input transitions