



Agilent 6400 Series Triple Quadrupole Users Workshop





Agilent 6400 Series Triple Quadrupole Users Workshop

QQQ Method Development and
Optimization

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



Agilent 6400 Series Triple Quadrupole Users Workshop

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Agenda

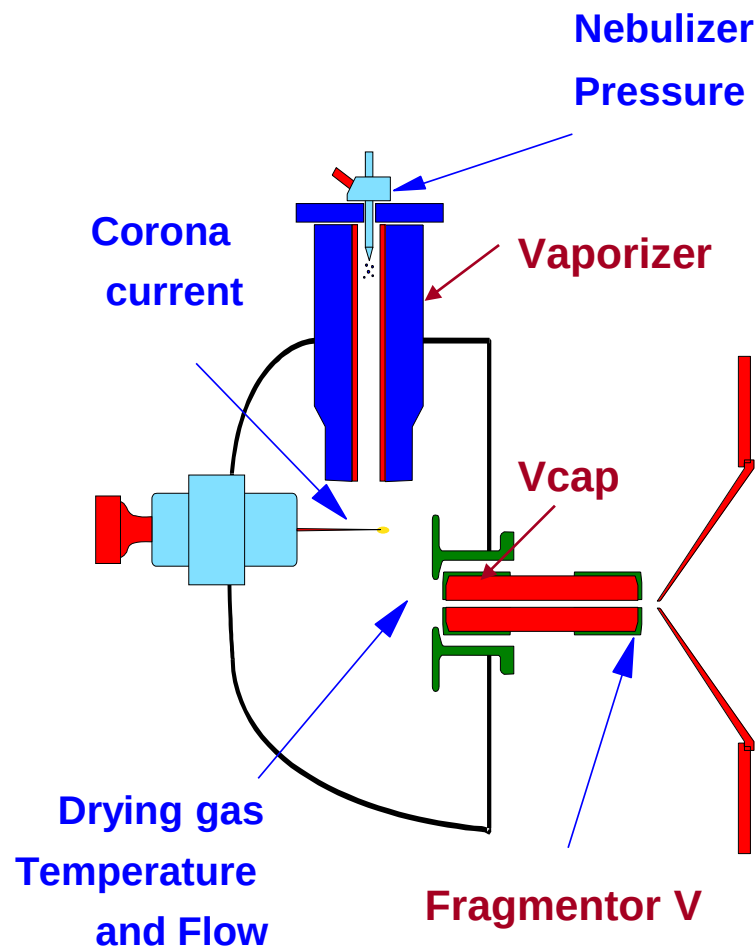
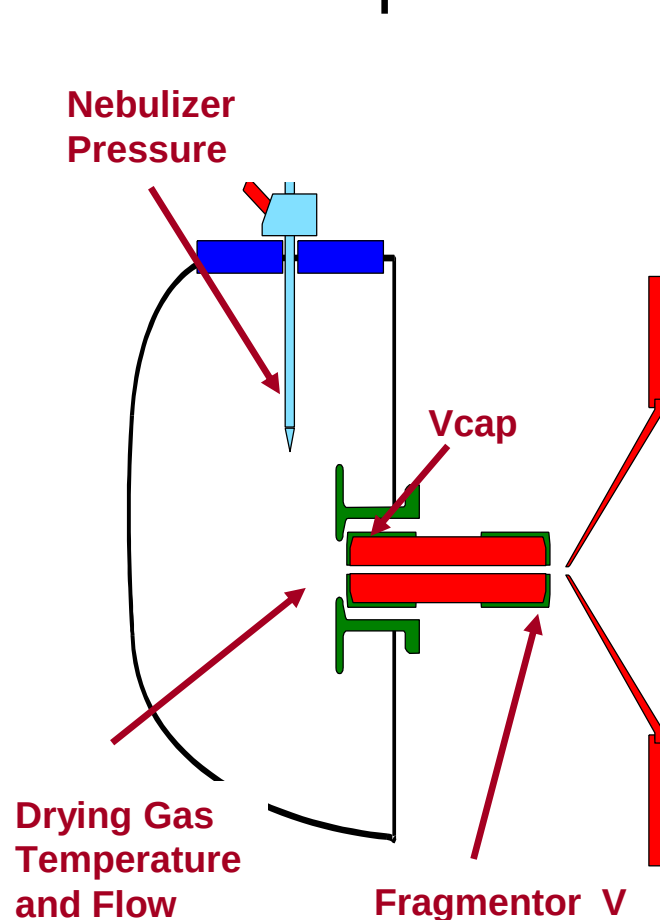
QQQ method development and optimization

- 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.

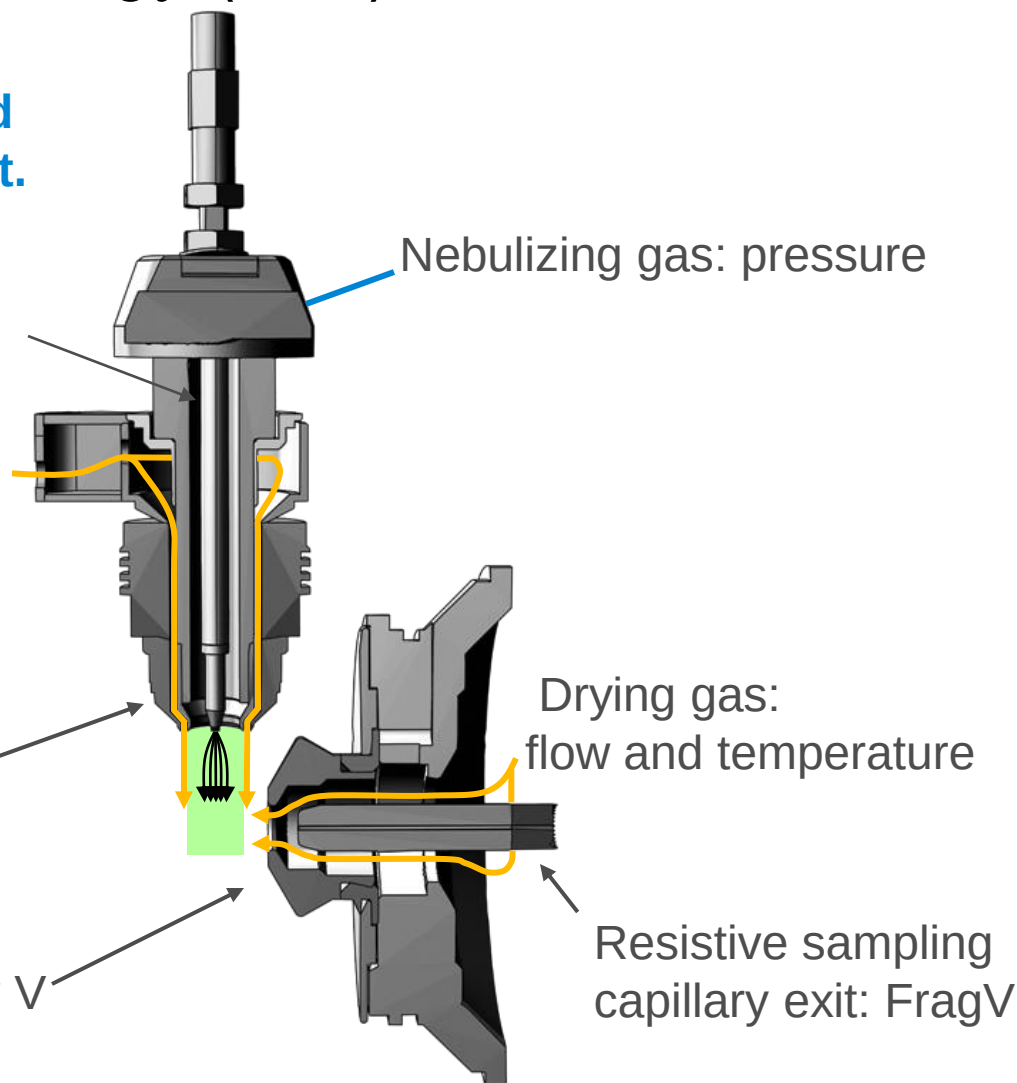
Enhanced efficiency nebulizer

*Sheath gas: flow and temperature

*Nozzle voltage

Resistive sampling capillary entrance: Capillary V

*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: Compound dependent parameters:

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

- 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 $\leq$ 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

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)

Increments and ranges to test

50°C, 225-400°C

2 Lpm, 8 -12 Lpm

5 psi, 25 – 50 psi

500V, 2500-4500V

500V, 0 - 2000V

50°C, 200-350°C

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

Method Editor

Injector Program.m [Apply]

Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump Column Comp. DAD QQQ

☒ Use Injector Program

Function	Parameter
Remote	Set remote line "Start" for 125 ms
Repeat	Repeat 7 time(s)
▶ Draw	Draw default volume from sample with default speed using default offset
Valve	Switch valve to "Mainpass"
Wait	Wait 0.2 min
End Repeat	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

Method Editor

scratch.m

Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump Column Comp. DAD **QQQ**

Acquisition **Source** Chromatogram Instrument Diagnostics

Tune file: atunes.tune.xml

Ion source: AJS ESI

Stop time: ☒ No limit/As Pump ☐ 1 min

Time filtering: ☒ Peak width 0.05 min

Source parameters:

Gas Temp: 300 °C 300 °C

Gas Flow: 5 l/min 5.1 l/min

Nebulizer: 45 psi 45.0 psi

Sheath Gas Temp: 400 °C 400 °C

Sheath Gas Flow: 11 l/min 11.0 l/min

Capillary: Positive 3500 V Negative 3500 V 6772 nA

Nozzle Voltage: 500 V 500 V

Chamber Current: 0.30 µA

Time segments:

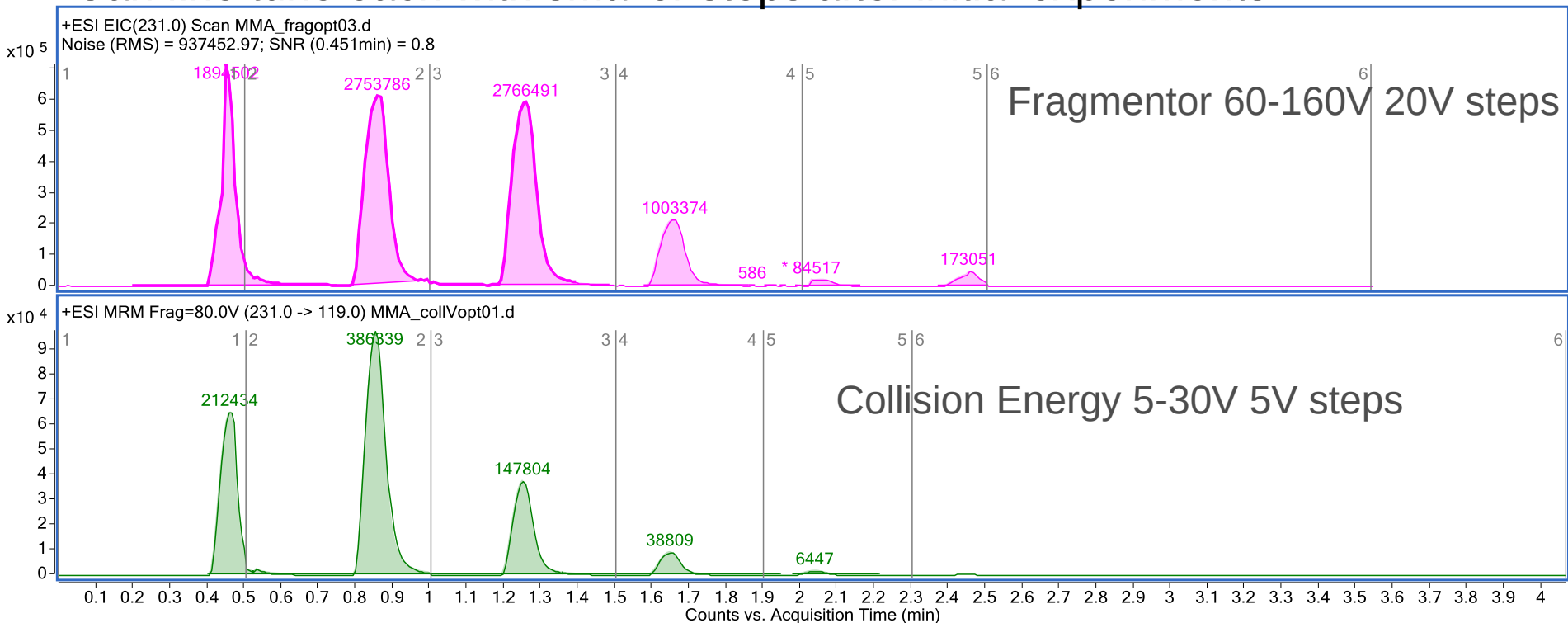
#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	MRM	To MS	0	0	☒
2	0.5	MRM	To MS	0	0	☒
3	1	MRM	To MS	0	0	☒
4	1.5	MRM	To MS	0	0	☒
5	2	MRM	To MS	0	0	☒
6	2.5	MRM	To MS	0	0	☒
7	3	MRM	To MS	0	0	☒

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.

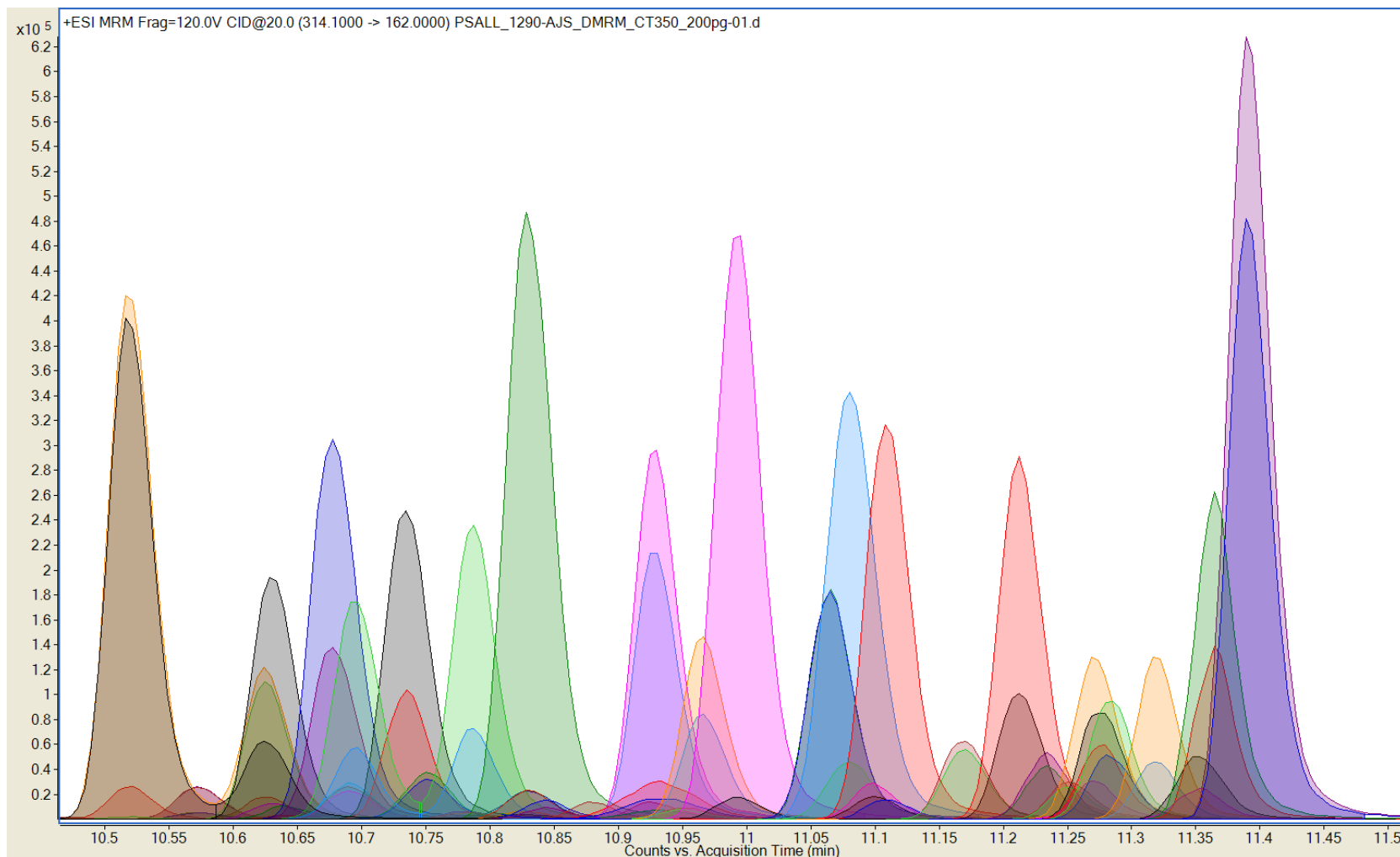
Monitoring many compounds simultaneously lowers dwell time per MRM.

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)

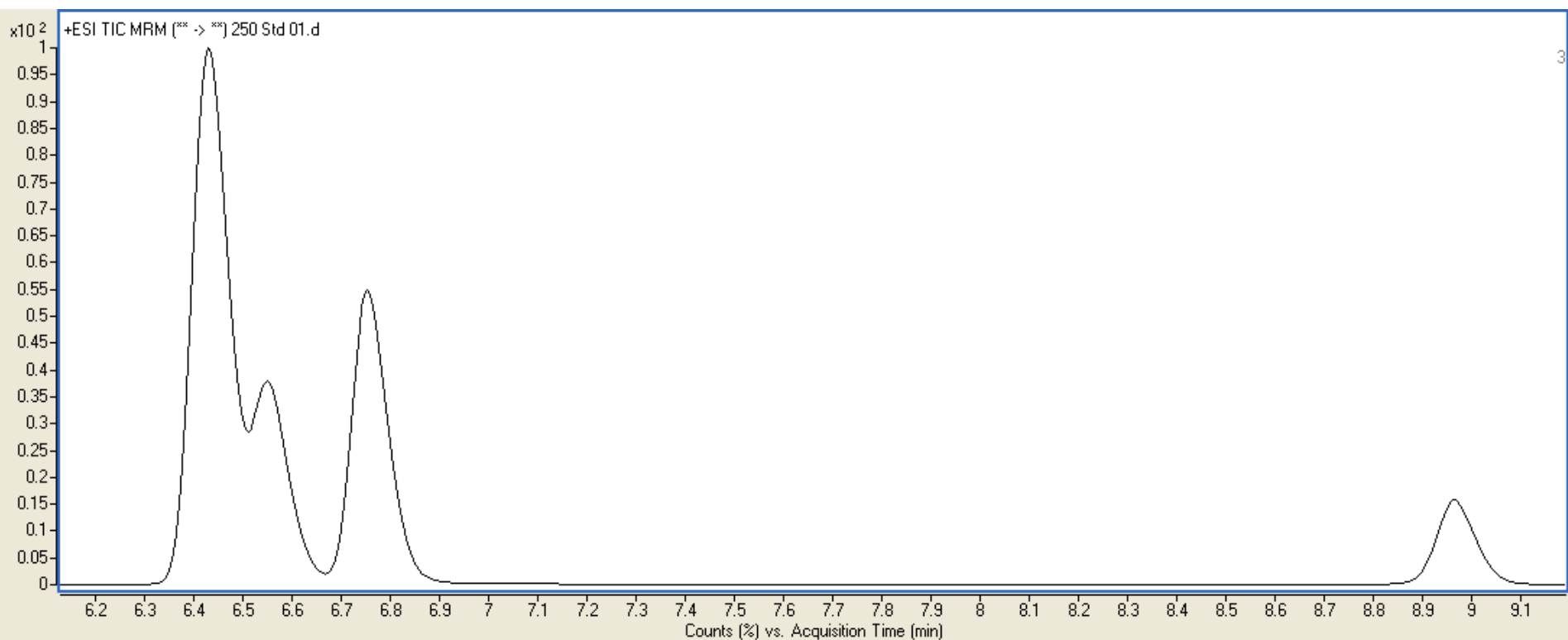
Without Dynamic MRM:

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

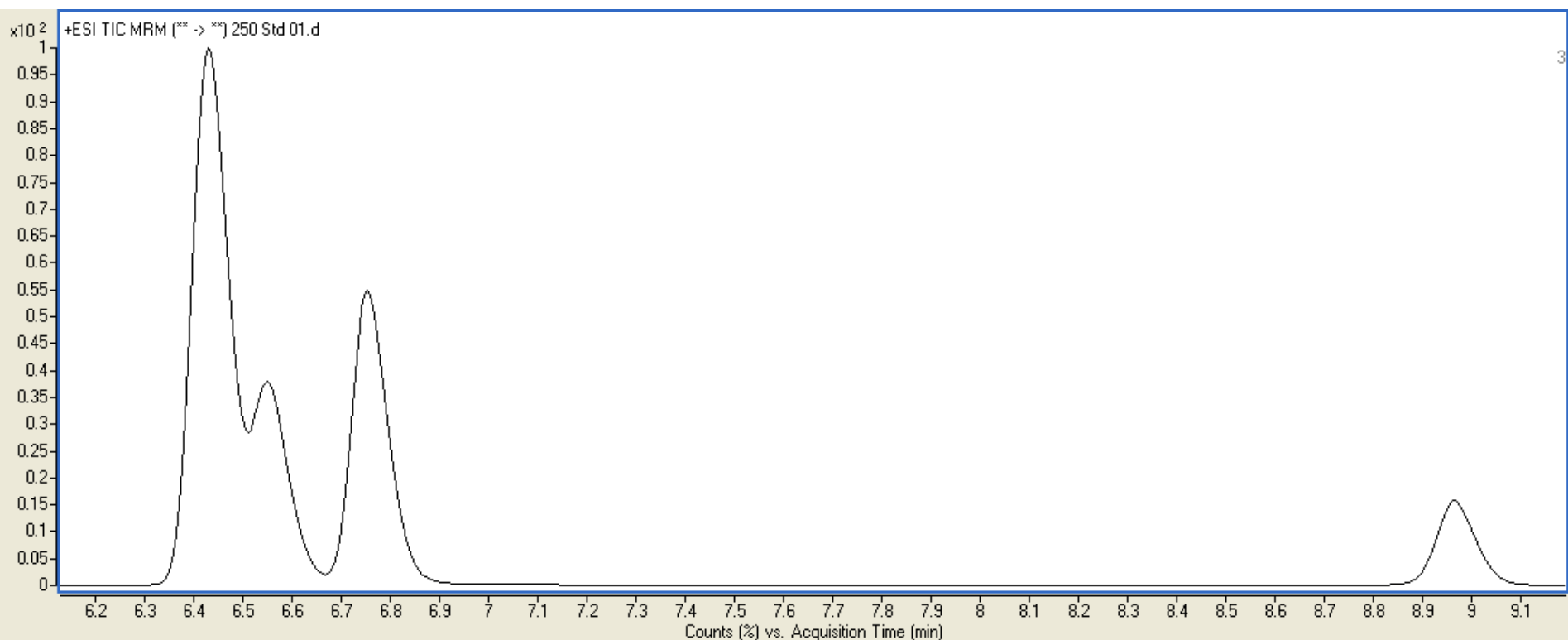
With Dynamic MRM:

- 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

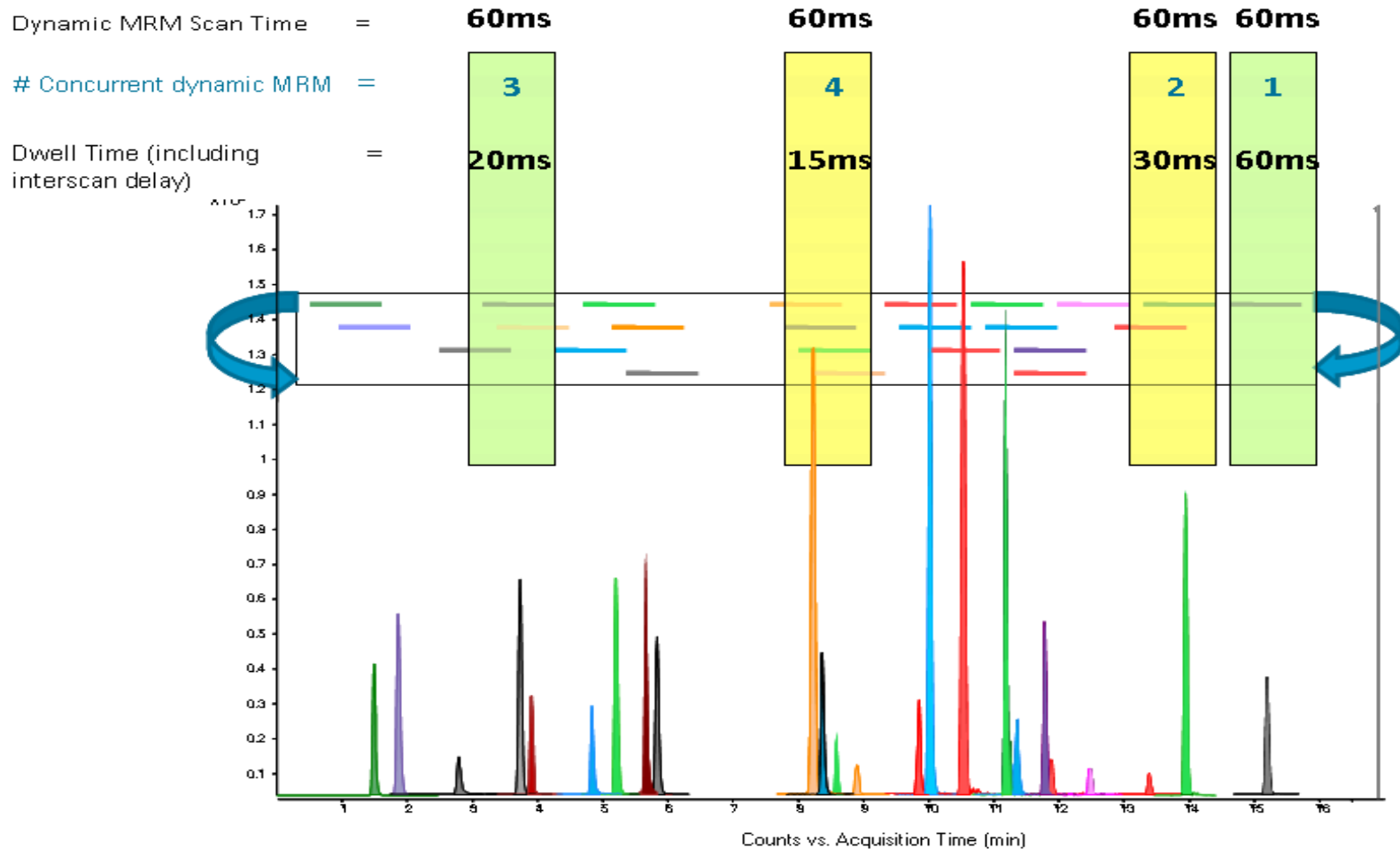


Dynamic MRM: automatically maximizes dwell time



Dynamic MRM for 6400 series QQQ

Monitors transitions only when compound elutes



Dynamic MRM: Retention Time and Delta Ret Time

Column Comp. DAD **QQQ**  

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

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

Context: Acquisition Layout: Default(sys).lyt Method: scratch.m Worklist:

Instrument Status

HiP Sampler: Idle 5.0 μ L Temperature 25 °C

Binary Pump: Idle 50.0 50.0 0.100 mL/min 78.05 bar

Column Comp.: Idle 24.99 °C 24.99 °C

DAD: Idle

QQQ: Idle AJS ESI

Instrument Idle On Off

Actuals

Parameter	Value
QQQ: Firmware Version	A.00.07.30
QQQ: Not Ready Text Long	
QQQ: High Vac	1.73E-5 Torr
QQQ: Rough Vac	1.68E+0 Torr
QQQ: Gas Flow	5.1 l/min
QQQ: Gas Temp	300 °C
QQQ: Sheath Gas Flow	11.0 l/min
QQQ: Sheath Gas Temp	400 °C
QQQ: Chamber Current	0.30 μ A
Binary Pump: Ripple	0.08 %

Chromatogram Plot

TIC Binary Pump: Pressure

Spectrum Plot

QQQ Spectrum MS 4: Dynamic MRM (256.2), AJS ESI (+), 5970.33

m/z: 64.2 Height: 37

Method Editor

scratch.m

Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump Column Comp.

Tune file: atunes.tune.xml

Ion source: AJS ESI

Time segments:

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	Dynamic MRM	To MS	0	0	☒

Acquisition

Scan segments:

Compound Group	MS1 Res	Product Ion	MS2 Res	Ret Time	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Tramadol	204.2	58.2	Unit	6	1	97	16	5	Positive
Tramadol	264.2	56.1	Unit	6	1	97	72	5	Positive
Tramadol-D6	270.2	59.2	Unit	6	1	102	12	5	Positive
Tramadol-D6	270.2	58.2	Unit	6	1	102	24	5	Positive
DesmethyI-Tramado	250.2	232	Unit	4	1	92	0	5	Positive
DesmethyI-Tramado	250.2	121	Unit	4	1	92	24	5	Positive
DesmethyI-Tramado	256.2	77.1	Unit	4	1	107	72	5	Positive
DesmethyI-Tramado	256.2	64.2	Unit	4	1	107	16	5	Positive



Update RTs, RT windows, add new compounds

Recommend using: Quant report folder

MRM Update Options

Select MassHunter QQQ data file or Quant report folder:

...

Method Options	
Add new Compound?	True
Peak Abundance Threshold	50
Cycle Time	500

Retention Time	
Update Retention Time?	True
Update Retention Time Window?	True
Scale Factor of RT Window to Peak Width	3
Retention Time Window Threshold	10
Retention Time Window Threshold Unit	Percent

Trigger Threshold	
Update Threshold?	True
Percent of Height	10
Scale Factor	1

Trigger Window	
Update Trigger Window?	True
Trigger Window Options	RetentionTime
Absolute value (mins)	0.5
Percent value	0
Scale Factor	1

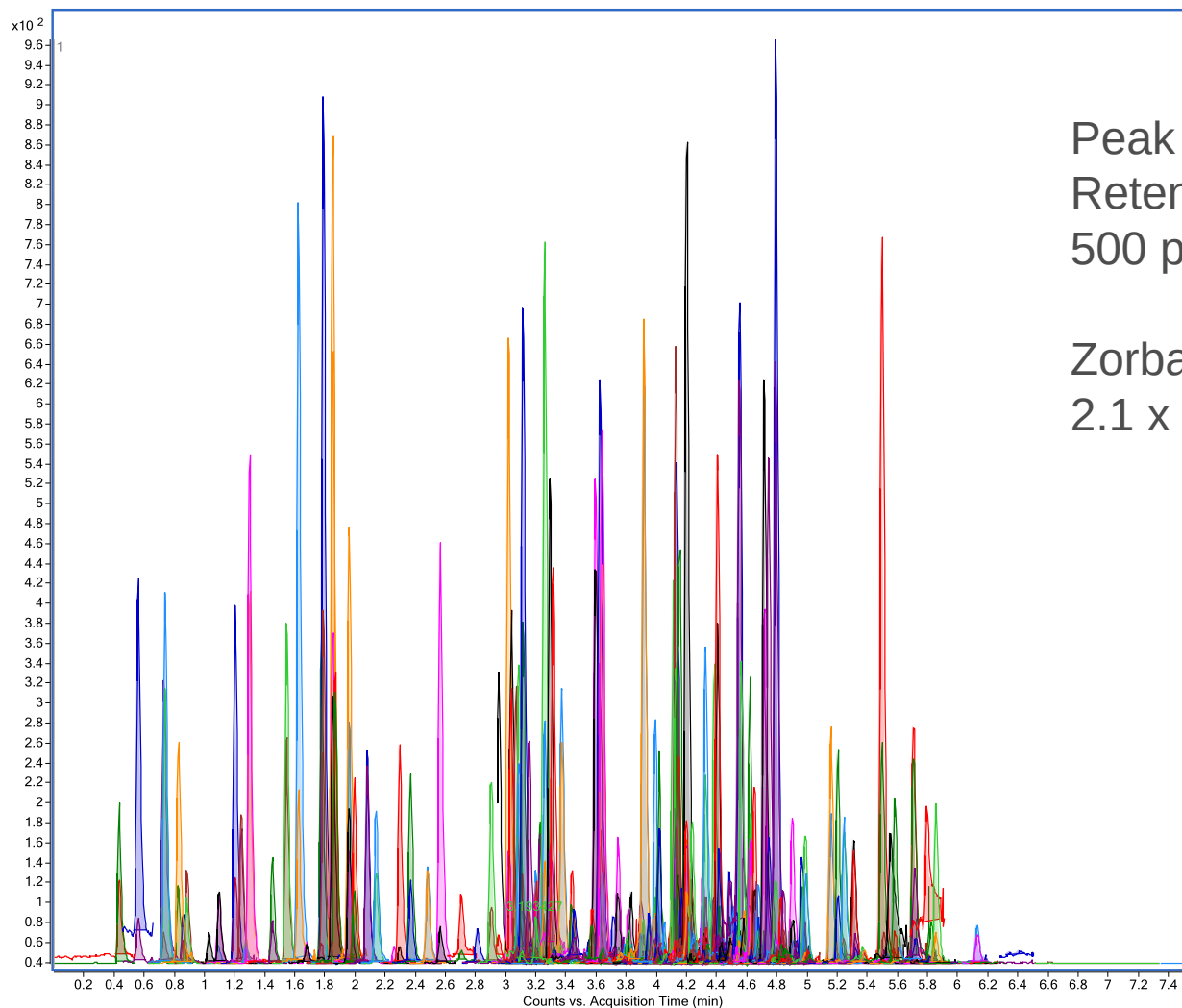
Update Retention Time?
Indicates whether to update the retention time of the method.

Restore Default OK Cancel



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 (1.8 μ m)



MassHunter Optimizer

Included as of MassHunter QQQ B.04 acquisition software

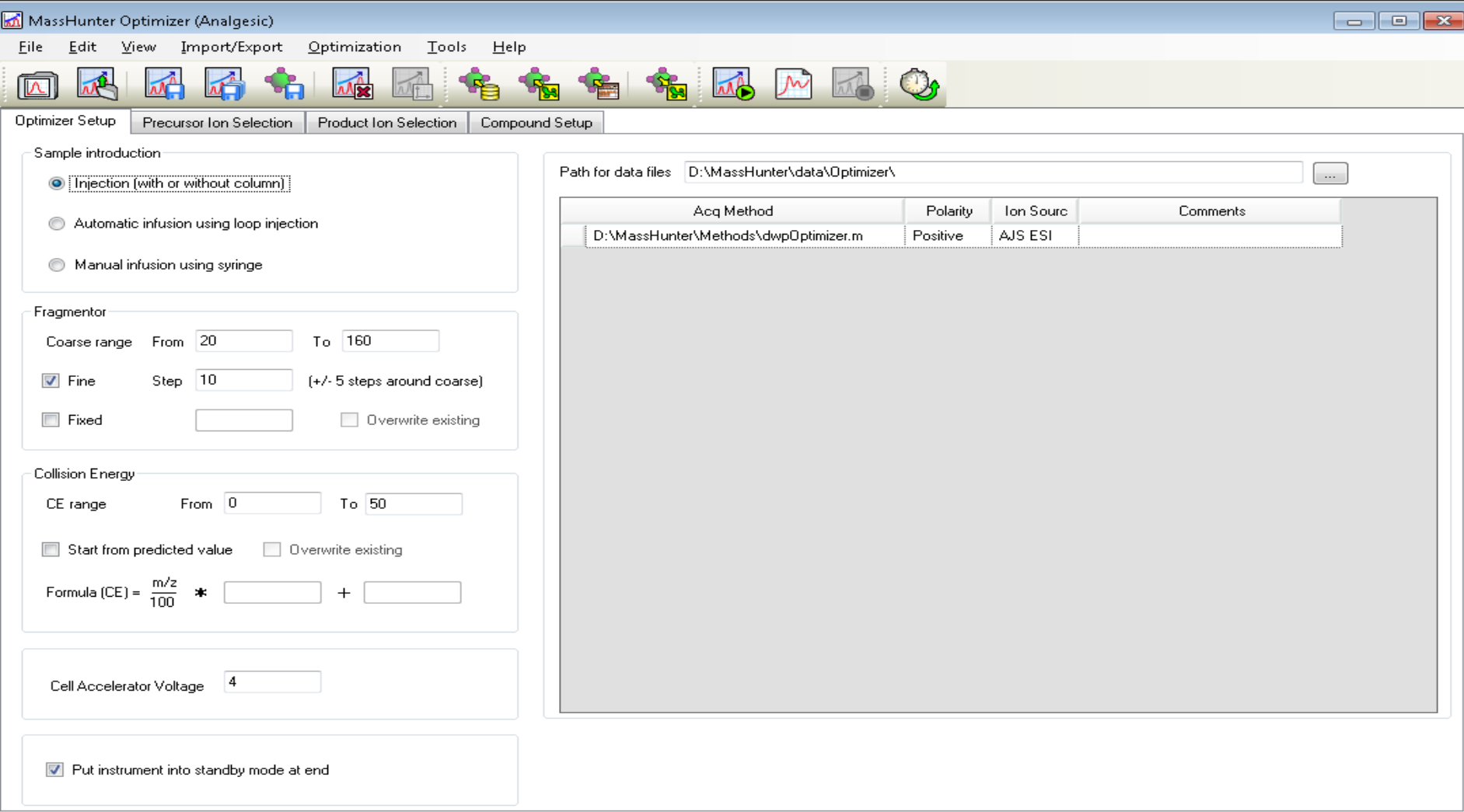
Useful for frequent method development [new compound each week] or for multi-compound methods [e.g. toxicology, environmental applications]

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 (Analgesic)

File Edit View Import/Export Optimization Tools Help

Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup

Sample introduction

- ☒ Injection (with or without column)
- ☐ Automatic infusion using loop injection
- ☐ Manual infusion using syringe

Fragmentor

Coarse range From 20 To 160

☒ Fine Step 10 (+/- 5 steps around coarse)

☐ Fixed ☐ Overwrite existing

Collision Energy

CE range From 0 To 50

☐ Start from predicted value ☐ Overwrite existing

Formula (CE) = $\frac{m/z}{100}$ * +

Cell Accelerator Voltage 4

☒ Put instrument into standby mode at end

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

Acq Method	Polarity	Ion Sourc	Comments
D:\MassHunter\Methods\dlwpOptimizer.m	Positive	AJS ESI	

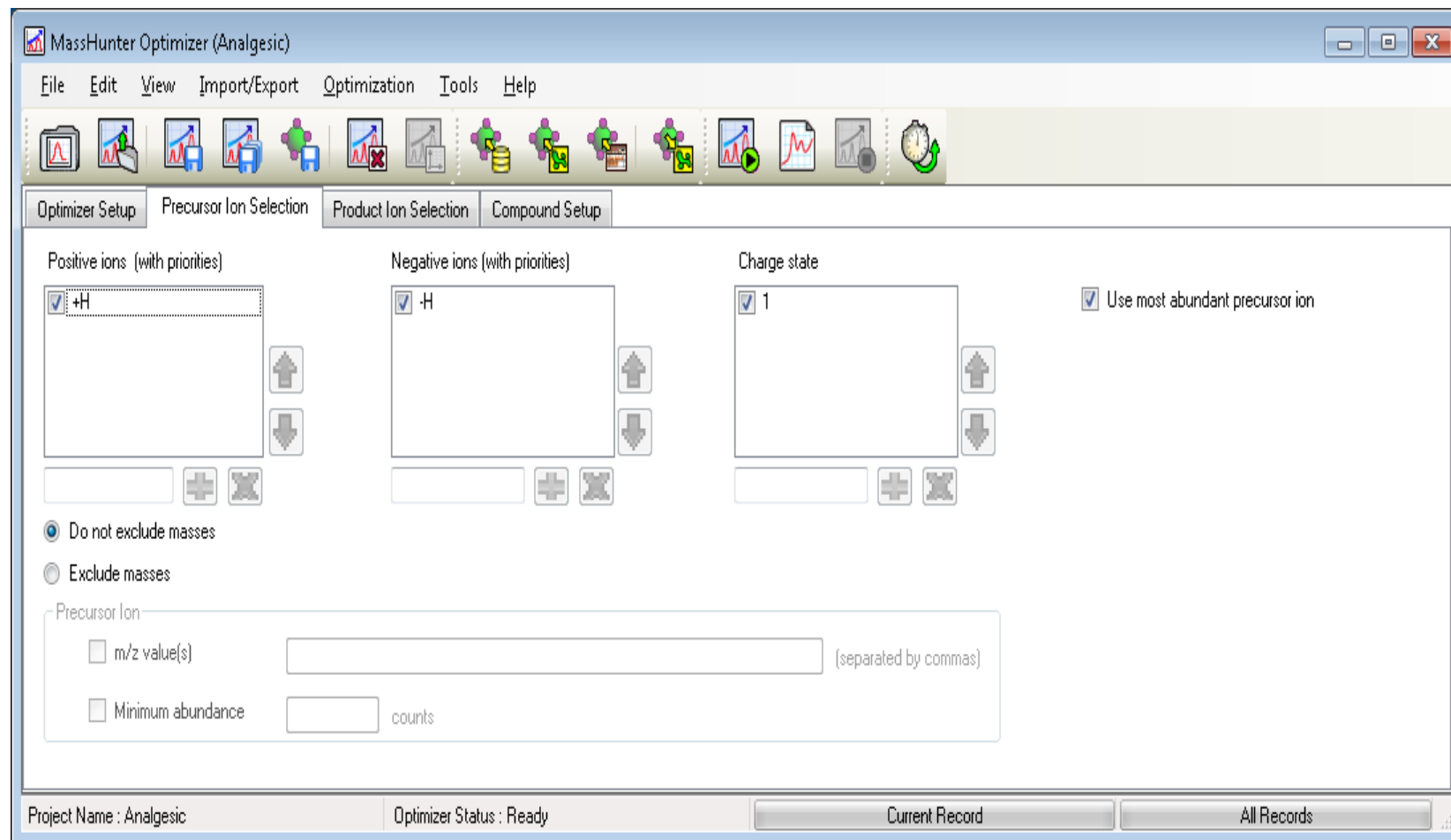
Project Name : Analgesic Optimizer Status : Ready Current Record All Records

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

The screenshot displays the MassHunter Optimizer (Analgesic) software window. The title bar reads "MassHunter Optimizer (Analgesic)". The menu bar includes "File", "Edit", "View", "Import/Export", "Optimization", "Tools", and "Help". The toolbar contains various icons for file operations, optimization, and data analysis. The "Product Ion Selection" tab is active, showing the following settings:

- Low mass cut-off:**
 - ☒ Mass (m/z): 50
 - ☐ % Precursor mass (m/z):
- ☒ Do not exclude masses
- ☐ Exclude masses
- Product Ion:**
 - ☐ m/z value(s): (separated by commas)
 - ☐ Minimum abundance: counts
- Neutral Losses:** (Empty box with + and - buttons)

The status bar at the bottom shows "Project Name : Analgesic", "Optimizer Status : Ready", "Current Record", and "All Records".

MassHunter Optimizer – input a unique compound name

MassHunter Optimizer (Analgesic)

File Edit View Import/Export Optimization Tools Help

Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup

☐ Show results summary

	Compound Name	Groups	Formula	Mass	Sample Position
▶	Acetaminophen01	analgesic	C8H9NO2	151.06	vial 3
▶	Acetaminophen02	analgesic	C8H9NO2	151.06	vial 3

☐ Perform multi-compound run

☒ Use MRM
☐ Use DMRM

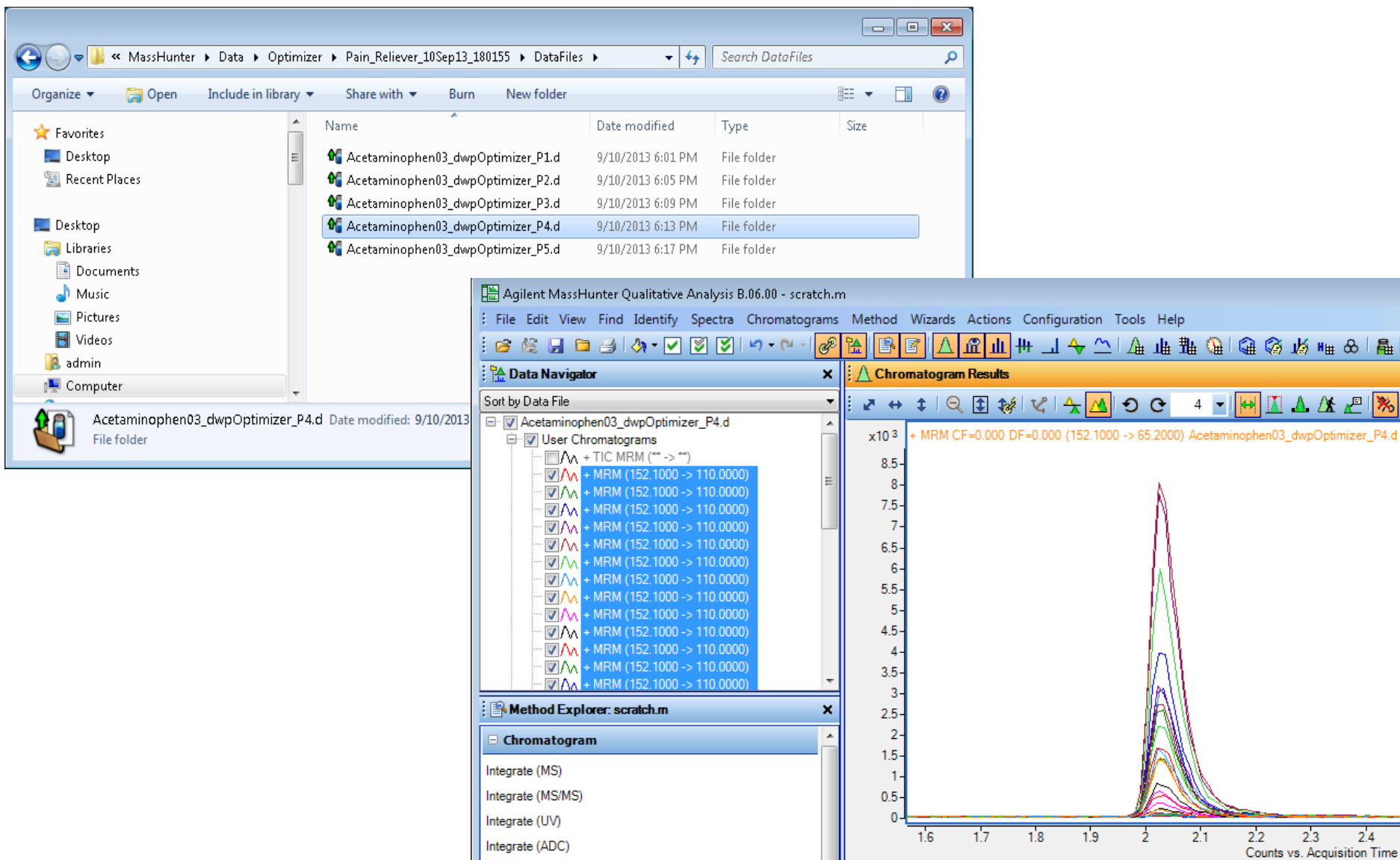
Dwell Time
Optimization dwell time 20 ms

Cycle Time
Expected peak width (base) 13.16 s
Frag coarse cycle time 141 ms
Frag fine cycle time 235 ms
Product scan cycle time ms
Collision Energy cycle time 1316 ms

Fill Values

Project Name : Analgesic Optimizer Status : Ready Current Record All Records

MassHunter Optimizer results – inspect data



MassHunter Optimizer - import directly into acquisition

The screenshot displays the MassHunter Optimizer software interface. At the top, a chromatogram shows a baseline with a single prominent peak at approximately 5948.5 minutes. The y-axis represents intensity, ranging from 5E7 to 1.5E8. Below the chromatogram is the 'Method Editor' section, which includes tabs for 'Properties', 'DA', 'HiP Sampler', 'HiP Sampler Pretreatment', 'Binary Pump', and 'Column Comp.'. The 'Acquisition' tab is currently selected. Within this tab, the 'Scan segments' section is visible, showing a table with one segment defined.

Method Editor

Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump Column Comp.

Tune file: atunes.tune.xml [Browse ...]

Ion source: AJS ESI

Stop time: ☒ No limit/As Pump ☐ 1 min

Time filtering: ☒ Peak width 0.05 min

Acquisition

Scan segments

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	MRM	To MS	0	0	☒

A context menu is open on the right side of the interface, with the option 'Import from Database Browser...' highlighted by a red circle. Other menu options include 'Add Row', 'Delete Row', 'Sort', 'Update DMRM Method ...', 'Cut', 'Copy', 'Paste', 'Paste from Clipboard', 'Fill Down', and 'Fill Column'.



MassHunter Optimizer

Search or filter compounds for import

Database Browser

File Edit View

Search/Filter Import List

☐ Show All Records

Filter Compounds

☒ Enable Filters

☐ Optimized Compounds

☐ Date From 09/17/2013 To 06/04/2010

☐ Group Name

☐ Polarity Positive

☐ Method

☐ Project Name

☐ Model

Search Compounds

Search Text

IN

☐ Match entire word for each string

Select Columns

- ☐ Project Name
- ☒ Compound Name
- ☐ Formula
- ☐ MW
- ☐ Groups
- ☐ CAS
- ☐ Chemical Classes

Select Transitions

☐ Select top 1 ranked transitions

☐ Primary transitions

☐ Secondary transitions

Set primary and trigger flags

Set top 2 ranked transitions as primary

Rank transitions by

☒ Abundance

☐ Response Factor

☐	Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger
☐	Tramadol	C16H25NO2		Positive		264.2	58.2	97	16	5	☒	☐
☐	Tramadol	C16H25NO2		Positive		264.2	56.1	97	72	5	☒	☐
☐	Tramadol	C16H25NO2		Positive		264.2	246	97	4	5	☒	☐
☐	Tramadol	C16H25NO2		Positive		264.2	77.2	97	80	5	☒	☐
☐	Tramadol-D6	C16H19D6NO2		Positive		270.2	58.2	102	24	5	☒	☐
☐	Tramadol-D6	C16H19D6NO2		Positive		270.2	59.2	102	12	5	☒	☐

Current Database : D:\MassHunter\Databases\Optimizer



MassHunter Optimizer

Search or filter compounds for import

Database Browser

File Edit View

Search/Filter Import List

☐ Show All Records Search/Filter

Filter Compounds

☒ Enable Filters

☐ Optimized Compounds

☐ Date From To

☐ Group Name

☐ Polarity

☐ Method

☐ Project Name

☐ Model

Search Compounds

Search Text

Acetaminophen

IN

☐ Match entire word for each string

Select Columns

- ☐ Project Name
- ☒ Compound Name
- ☐ Formula
- ☐ MW
- ☐ Groups
- ☐ CAS
- ☐ Chemical Classes

Select Transitions

☒ Select top ranked transitions

☐ Primary transitions

☐ Secondary transitions

Select Transitions

Set primary and trigger flags

Set top ranked transitions as primary

Set Primaries and Trigger

Rank transitions by

☒ Abundance

☐ Response Factor

☒	Compound Name	Formula	Precursor	Product	Frag	CE	CAV	Abundance	Project Name	Polarity	Primary	Trigger
☒	Acetaminophen03	C8H9NO2	152.1	110	94	16	4	8074	Pain Reliever	Positive	☒	☐
☒	Acetaminophen03	C8H9NO2	152.1	65.2	94	36	4	3190	Pain Reliever	Positive	☒	☐

Current Database : D:\MassHunter\Databases\Optimizer

Add to Import List Import Close



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-H	☐	256.2	Unit	64.2	Unit	200	107	16	5	Positive
	Desmethyl-Tramadol-H	☐	256.2	Unit	77.1	Unit	200	107	72	5	Positive



MassHunter Optimizer – input transitions

MassHunter Optimizer (Analgesic)

File Edit View Import/Export Optimization Tools Help

Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup

☐ Show results summary

		Compound Name	Groups	Formula	Mass	Sample Position
☐	☐	Acetaminophen01	analgesic	C8H9NO2	151.06	vial 3
☒	☒	Acetaminophen02	analgesic			vial 3

Context Menu:

- Add Compound
- Add Method**
- Delete Compound(s)
- Clear
- Copy
- Cut
- Paste
- Fill
- Assign
- Show/Hide Columns...
- Expand/Collapse All Rows

Perform multi-compound run ☐

Use MRM ☒

Use DMRM ☐

Dwell Time

Optimization dwell time ms

Cycle Time

Expected peak width (base) s

Frag coarse cycle time ms

Frag fine cycle time ms

Product scan cycle time ms

Collision Energy cycle time ms

Fill Values

Project Name : Analgesic

Optimizer Status : Ready

Current Record

All Records

MassHunter Optimizer – input transitions

MassHunter Optimizer (Analgesic)

File Edit View Import/Export Optimization Tools Help

Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup

☐ Show results summary

	Compound Name	Groups	Formula	Mass	Sample Position
☐	Acetaminophen01	analgesic	C8H9NO2	151.06	vial 3
☒	Acetaminophen02	analgesic	C8H9NO2	151.06	vial 3

Acq Method	Polarity	Ion Source	RT	RT Win
D:\MassHunter\Methods\dwpmOptimizer.m	Positive	AJS ESI		

☐ Perform multi-compound run

☒ Use MRM
☐ Use DMRM

Dwell Time
Optimization dwell time 20 ms

Cycle Time
Expected peak width (base) 13.16 s
Frag coarse cycle time 141 ms
Frag fine cycle time 235 ms
Product scan cycle time ms
Collision Energy cycle time 1316 ms

Fill Values

Project Name : Analgesic Optimizer Status : Ready Current Record All Records

MassHunter Optimizer – input transitions

MassHunter Optimizer (Analgesic)

File Edit View Import/Export Optimization Tools Help

Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup

☐ Show results summary

	Compound Name	Groups	Formula	Mass	Sample Position
☐	Acetaminophen01	analgesic	C8H9NO2	151.06	vial 3
☒	Acetaminophen02	analgesic	C8H9NO2	151.06	vial 3

Acq Method

D:\MassHunter\Methods\dwpg

- Add Method
- Add New Method
- Add Precursor Ion**
- Delete Method(s)
- Clear
- Copy
- Cut
- Paste
- Fill
- Assign
- Show/Hide Columns...
- Expand/Collapse All Rows

Ion Source RT RT Win

ESI

☐ Perform multi-compound run

☒ Use MRM

☐ Use DMRM

Dwell Time

Optimization dwell time 20 ms

Cycle Time

Expected peak width (base) 13.16 s

Frag coarse cycle time 141 ms

Frag fine cycle time 235 ms

Product scan cycle time ms

Collision Energy cycle time 1316 ms

Fill Values

Project Name : Analgesic

Optimizer Status : Ready

Current Record

All Records



MassHunter Optimizer – input transitions

MassHunter Optimizer (Analgesic)

File Edit View Import/Export Optimization Tools Help

Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup

☐ Show results summary

	Compound Name	Groups	Formula	Mass	Sample Position
☐	Acetaminophen01	analgesic	C8H9NO2	151.06	vial 3
☒	Acetaminophen02	analgesic	C8H9NO2	151.06	vial 3

Acq Method	Polarity	Ion Source	RT	RT Win
D:\MassHunter\Methods\dwpOptimizer.m	Positive	AJS ESI		

Precursor	Frag
152.1	

- Add Precursor Ion
- Add Product Ion**
- Delete Precursor Ion(s)
- Clear
- Copy
- Cut
- Paste
- Fill
- Show/Hide Columns...
- Expand/Collapse All Rows

☐ Perform multi-compound run

☒ Use MRM
☐ Use DMRM

Dwell Time
Optimization dwell time 20 ms

Cycle Time
Expected peak width (base) 13.16 s
Frag coarse cycle time 141 ms
Frag fine cycle time 235 ms
Product scan cycle time ms
Collision Energy cycle time 1316 ms

Fill Values

Project Name : Analgesic Optimizer Status : Ready Current Record All Records

MassHunter Optimizer – input transitions

MassHunter Optimizer (Analgesic)

File Edit View Import/Export Optimization Tools Help

Optimizer Setup Precursor Ion Selection Product Ion Selection Compound Setup

☐ Show results summary

	Compound Name	Groups	Formula	Mass	Sample Position
☐	Acetaminophen01	analgesic	C8H9NO2	151.06	vial 3
☒	Acetaminophen02	analgesic	C8H9NO2	151.06	vial 3

Acq Method	Polarity	Ion Source	RT	RT Win
D:\MassHunter\Methods\dwpOptimizer.m	Positive	AJS ESI		

Precursor	Frag	Spec	Abu
152.1			
	Product	CE	Abu
	110		
	65		

- Add Precursor Ion
- Add Product Ion
- Delete Precursor Ion(s)
- Clear
- Copy
- Cut
- Paste
- Fill
- Show/Hide Columns...
- Expand/Collapse All Rows

☐ Perform multi-compound run

☒ Use MRM

☐ Use DMRM

Dwell Time

Optimization dwell time 20 ms

Cycle Time

Expected peak width (base) 6.58 s

Frag coarse cycle time 141 ms

Frag fine cycle time 235 ms

Product scan cycle time ms

Collision Energy cycle time 658 ms

Fill Values

Project Name : Analgesic Optimizer Status : Ready Current Record All Records