An Introduction to Metabolite Identification Strategies

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AB SCIEX Foster City, CA[1]
Elan Pharmaceuticals – South San Francisco, CA[2]

Overview

- Pharma ADME – Challenges and Needs
- The solutions
  - Hardware – fundamental performance characteristics
  - Software and workflow based solution
    - Runtime (IDA) Logic, processing tools for Met ID and quant
- Data examples
  - From Experiments to study IDA logic
  - From Metabolic Stability Screen and ID Studies
- The future… advances in data processing / reporting
  - Structure based approaches and tools
  - Data mining and management

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Pharmaceutical ADME – Challenges and Needs

- A constant need for productivity gains
  - Generation of more useful data at ever increasing rates – fewer resources
  - Managers are looking to eliminate method dev and combine workflows
- Limitations of older MS systems has had interesting consequences
  - Legacy designs had limitations in versatility
  - QqQ / QTRAP in quant labs, accurate mass in qual
    - Causes division of functional departments / workflows
- Modern Chromatography
  - Chromatographic efficiency gains – sub 2 µm and solid core particles
  - Separation = identification = sensitivity = a challenge for mass specs

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The AB SCIEX TripleTOF™ 5600 System

- A Collection of Many Great Attributes
  - ✓ Speed – 10ms per scan
  - ✓ Resolution ~ 35K
  - ✓ Mass accuracy ~ sub 2 ppm MS and MS/MS
  - ✓ Sensitivity – low fg on column (ppt range)
  - ✓ Dynamic Range for both quant and qual
    - ✓ QqQ like performance
  - ✓ Workflow specific solutions
    - ✓ Runtime IDA algorithms

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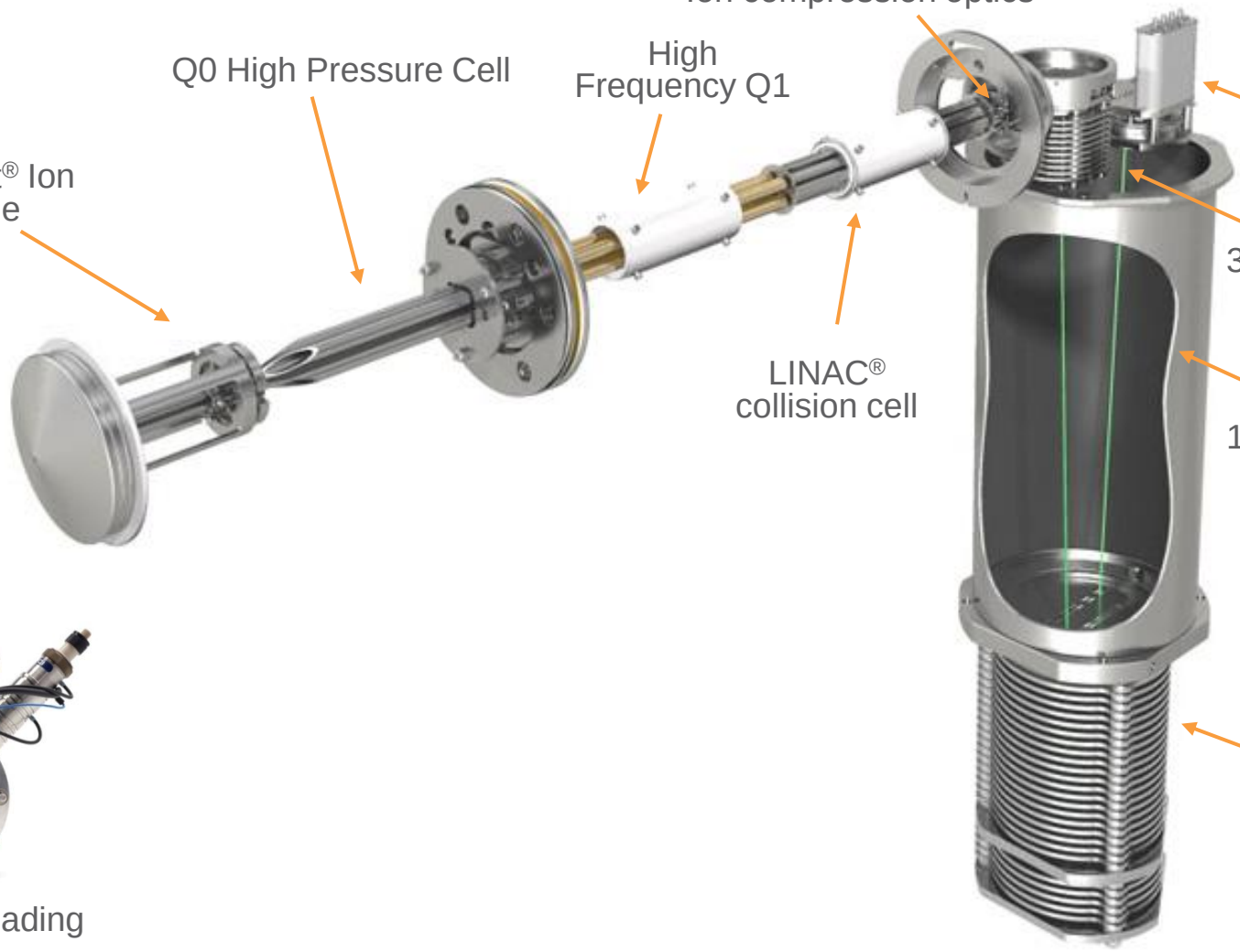
AB SCIEX TripleTOF™ 5600 System

Purpose Built for Quant/Qual Workflows



Industry-Leading Ion Sources

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Customer Goals for Metabolism Screen

- Metabolite ID screen at Elan Pharma
  - General HT screen for metabolites
    - Phase I (Oxides, De-methylations)
    - Phase II (Glucuronides, GSH Conjugates etc)
  - Goals for Screen Setup
    - High throughput – 5-7 minutes per sample
    - Generic method approach
    - UPLC compatible 2 – 3 second wide peaks
    - High resolution and accuracy for structure ID

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Materials and Methods

- Phase I and II (glucuronic acid) timecourse HLM
  - 0,15,30,60 minute timepoints
  - Verapamil, Clozapine, and Imipramine were used as controls
- GSH incubations in HLM
  - 0 and 60 minute time points
  - 5 µM incubation concentration
    - Clozapine, Diclofenac, and Troglitazone as GSH controls
    - After preparation 0.83 µM nominal [C]
- Triple TOF 5600 LCMSMS System
  - Agilent 1290 HPLC
  - Phenomenex Kinetix C-18 2.6µm 2.1 X 100mm
    - 5 minutes gradient
    - 5 µL injection
    - 700 µL/min
    - Simple mobile phases: water:ACN:formic acid
  - CDS calibration with Duo Spray Ion Source
    - Performed hourly

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The Balancing "Quant" and "Qual"

- Sensitivity and linear dynamics – similar to QqQ
- Speed is critical to quant/qual performance
  - 8 to 12 cycles per peak ~ 3 seconds / 10 scan = 300ms
  - Balanced with IDA settings
    - Number of dependant target - How deep you are going to dig? Top 10?
    - How long to accumulate at each scan? e.g. 30ms
- IDA filtration techniques to add some intelligence "selectivity"
  - DBS – Dynamic background subtraction – A must for all IDA workflows
  - MMDF – Multiple Mass Defect Filter
  - NL Filter
  - Isotope pattern filter

    } All use DBS
  - Allows for fewer # dependant targets = short cycle times
  - Improved relevance and quality of aquired MS/MS

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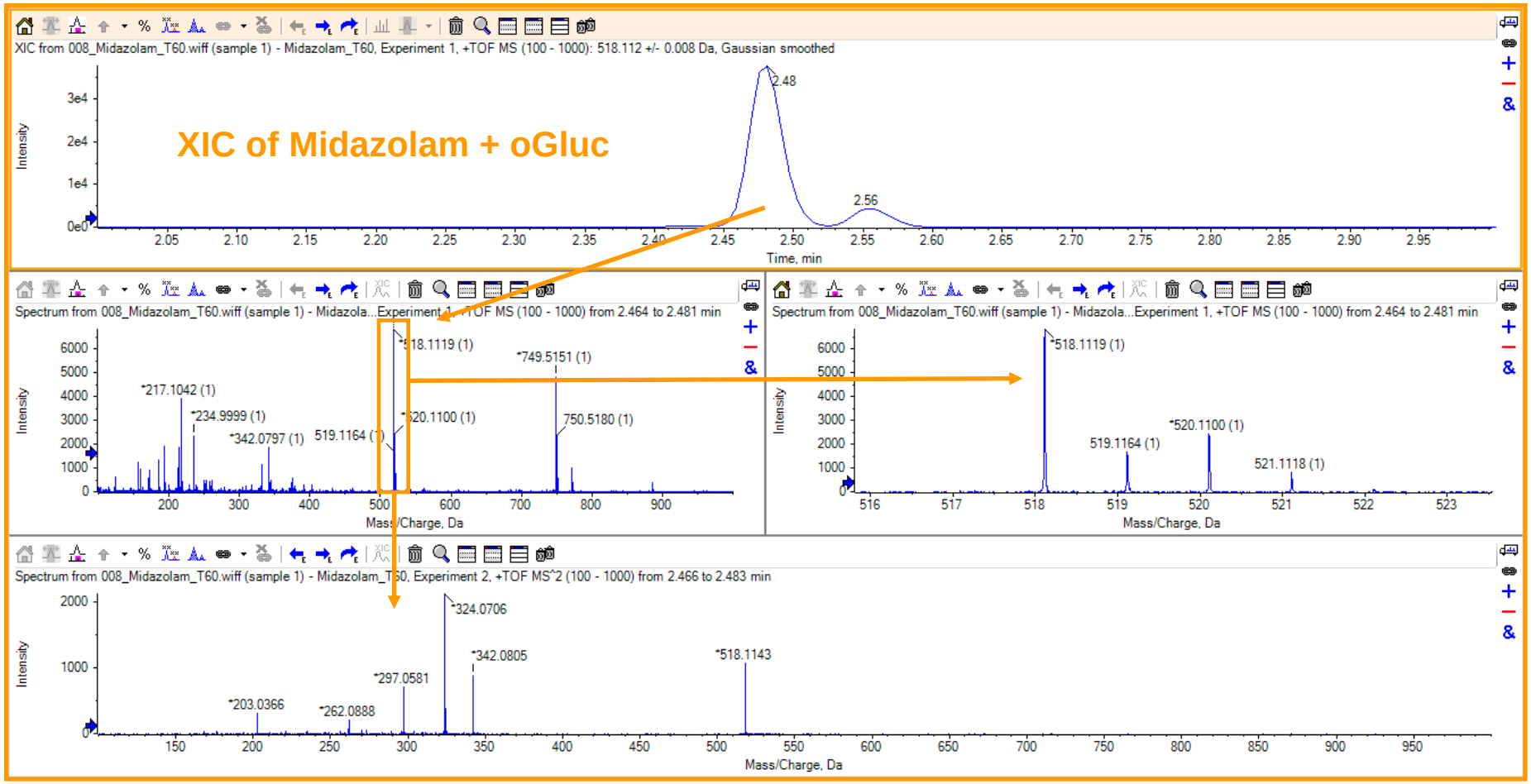
Dynamic Background Subtraction –



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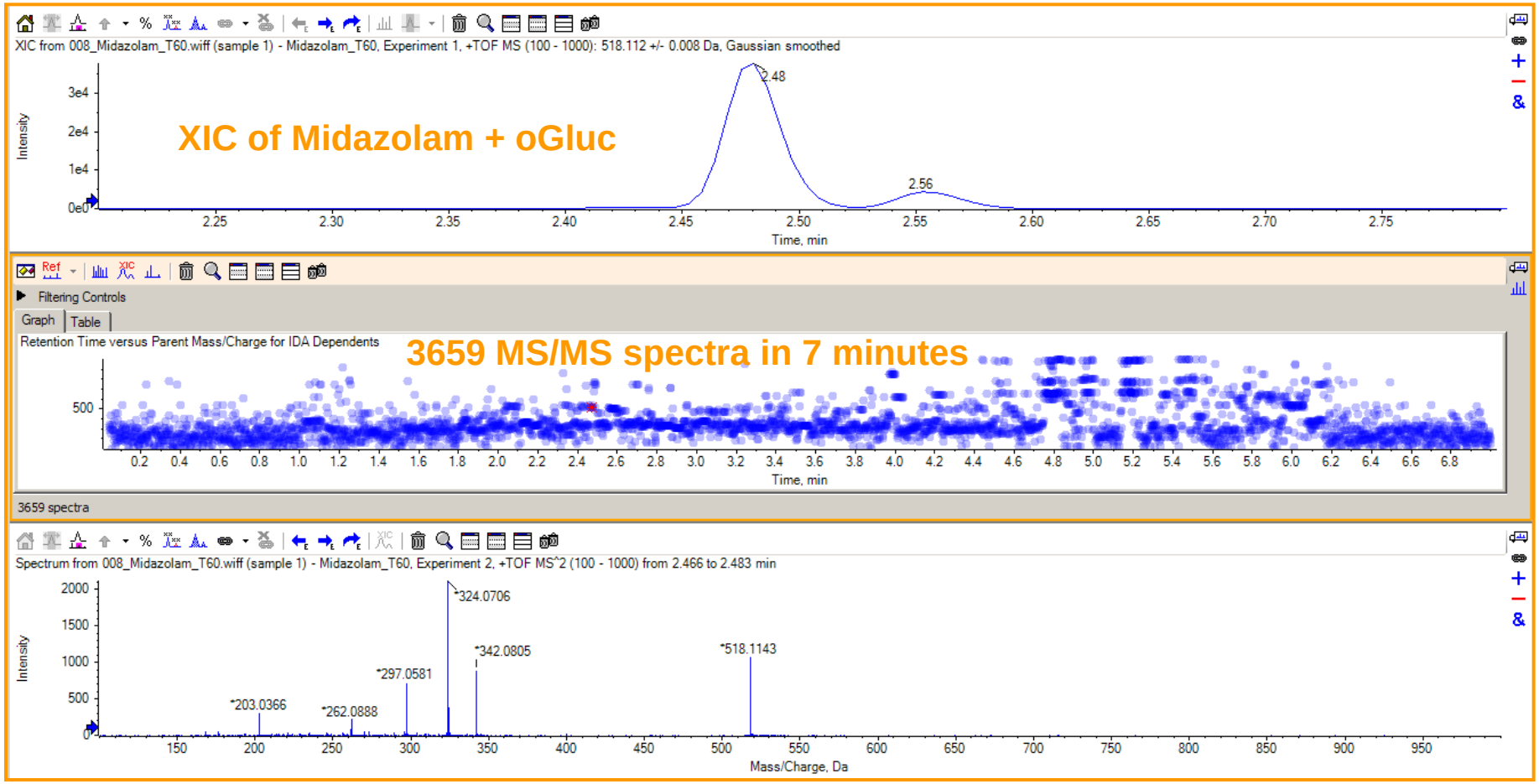
Dynamic Background Subtraction – DBS



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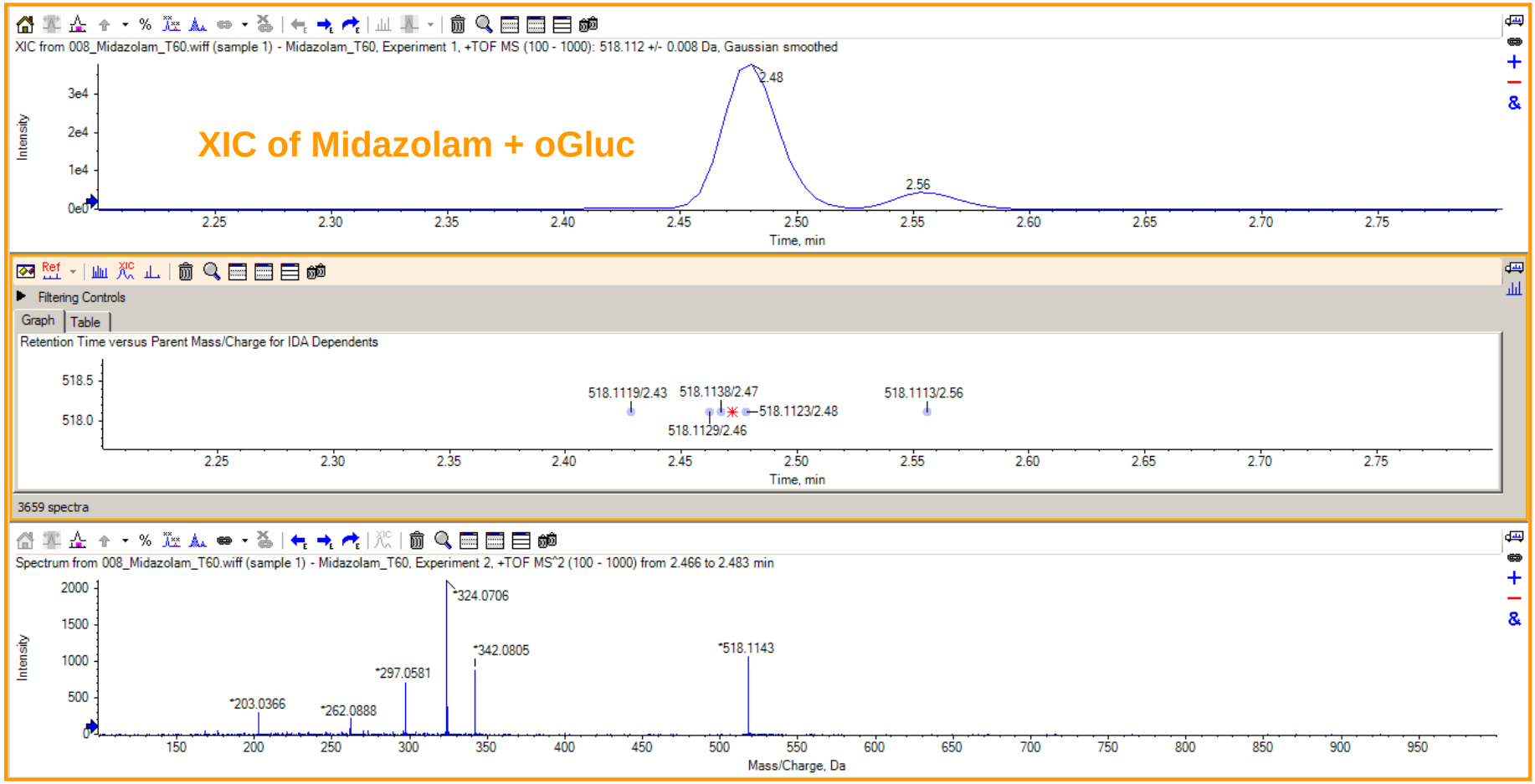
Dynamic Background Subtraction – DBS



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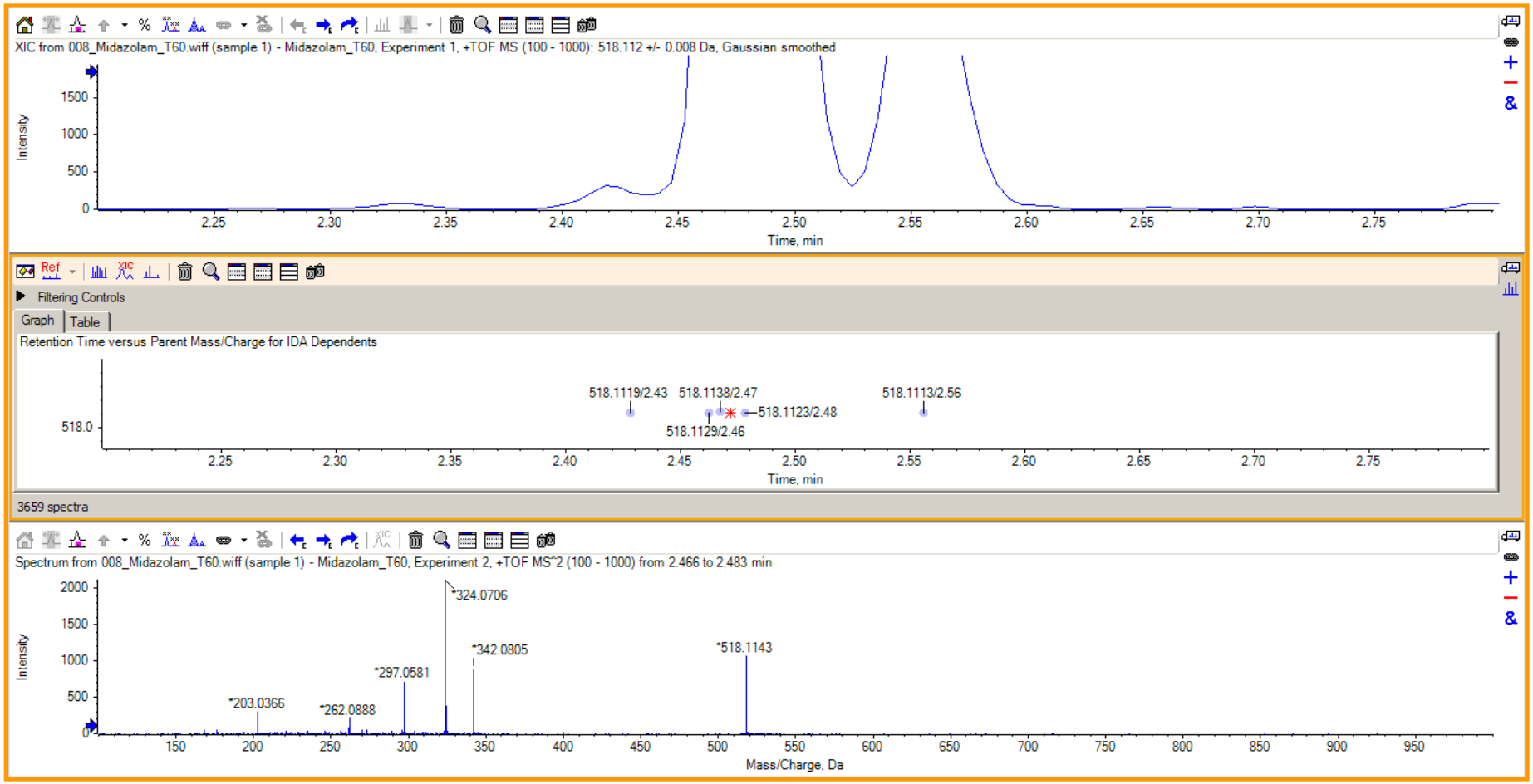

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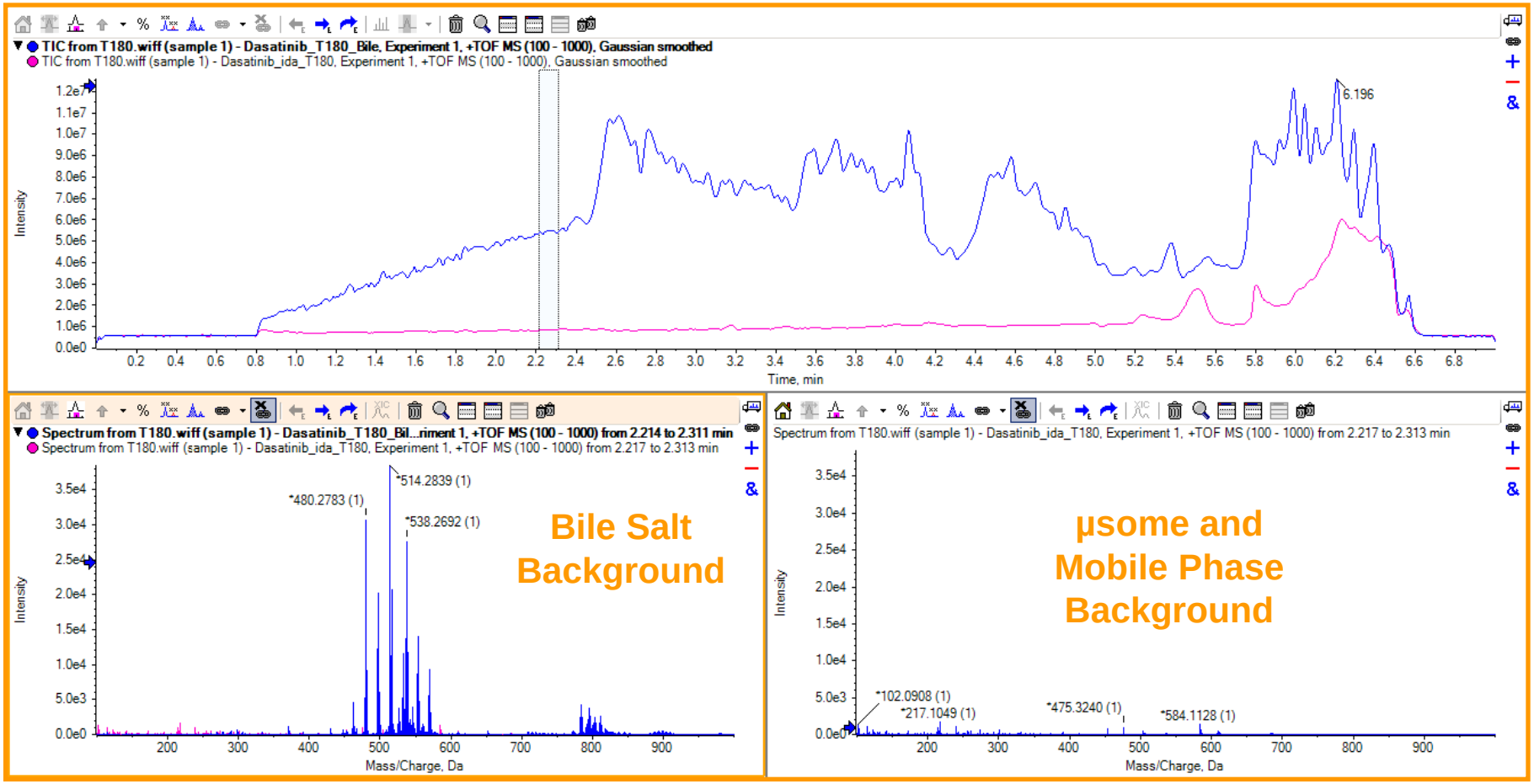
DBS – Bile Example

- Profound impact on IDA efficiency when dealing with high background as with bile samples.



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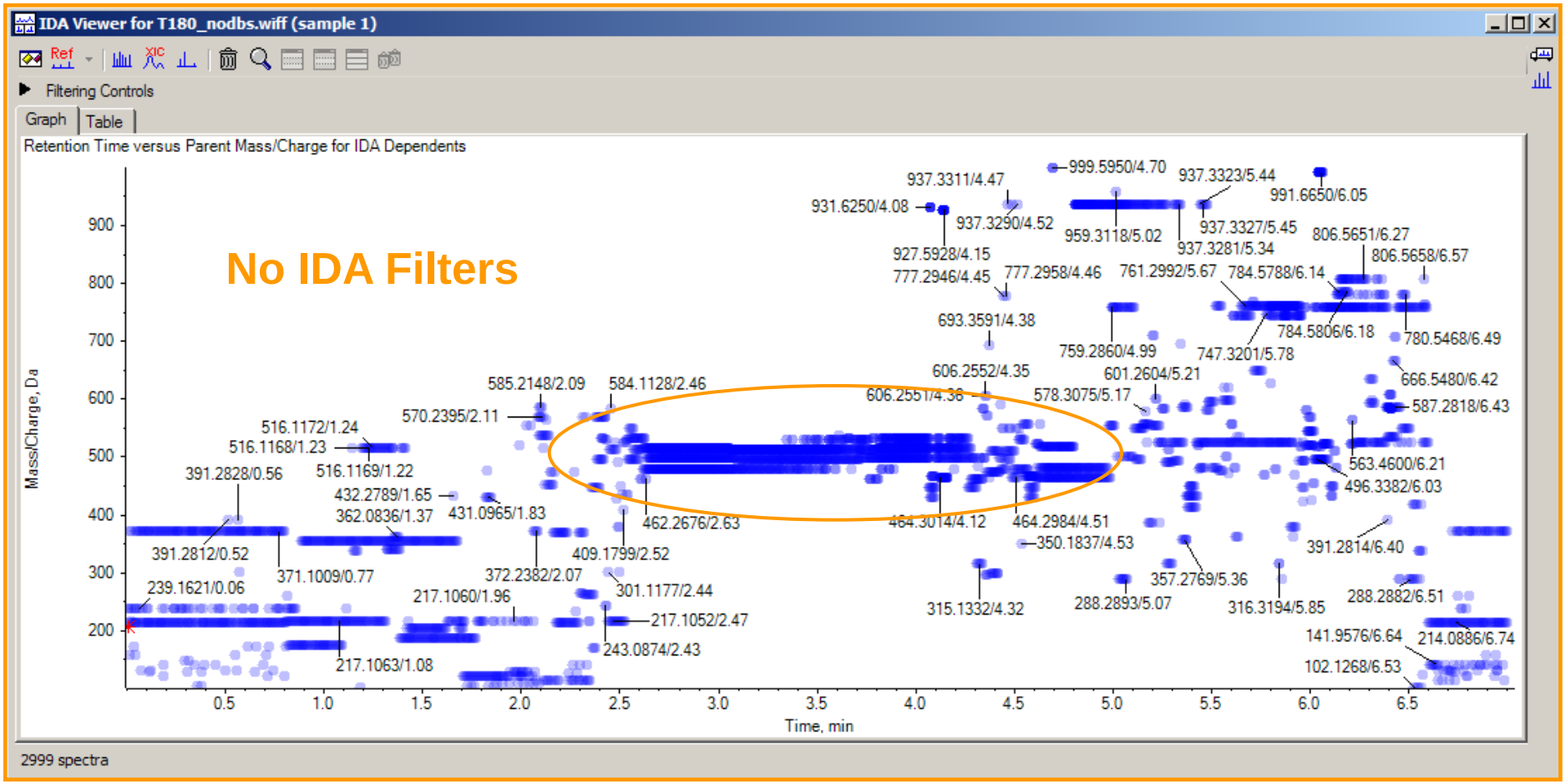

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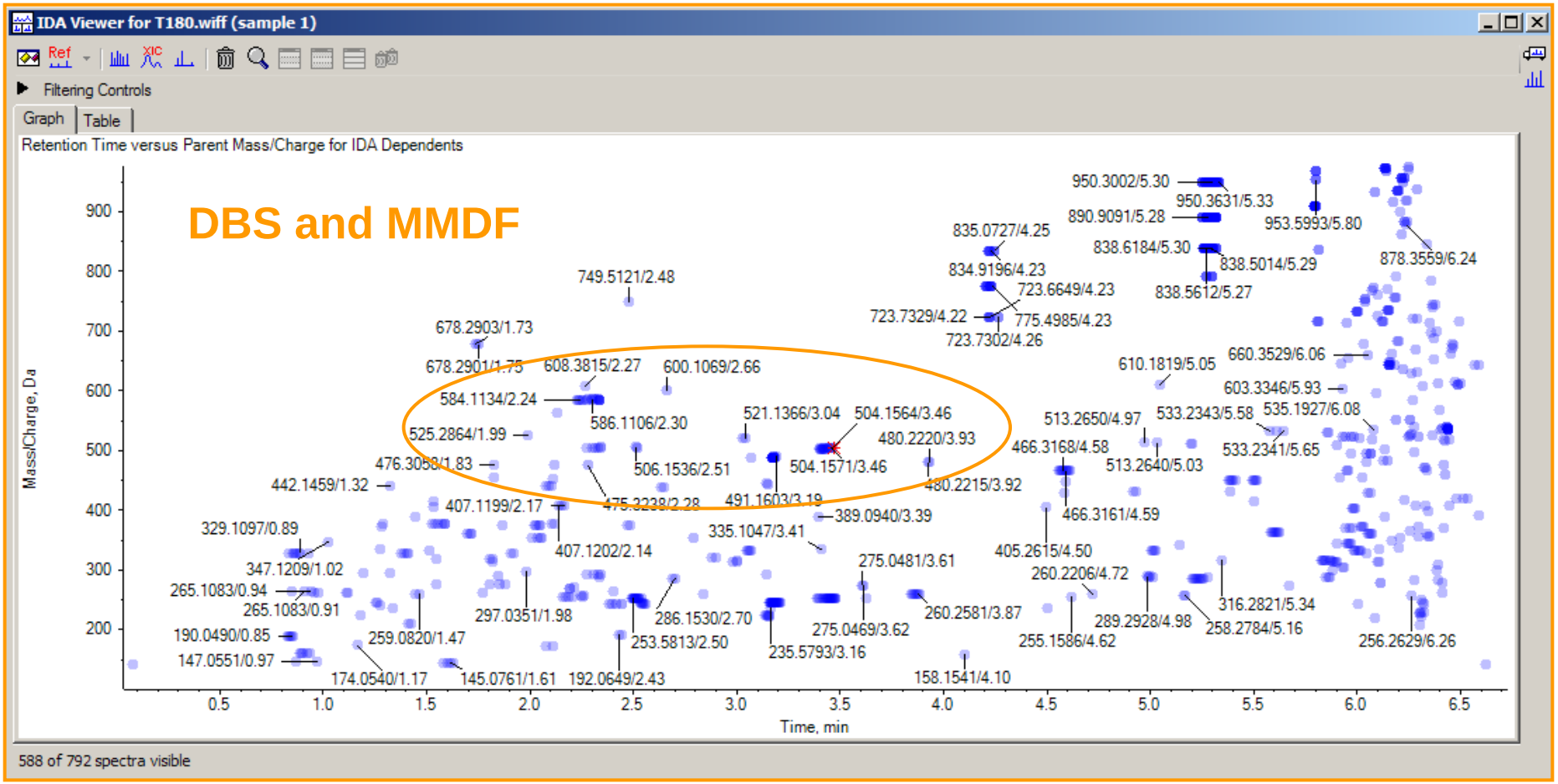
IDA Filters – Bile Example
4X fewer MS/MS spectra – with increased relevance



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General Method Description

- TOF MS with Information Dependant Acquisition (IDA)
  - 50 ms TOF survey with up to 3 dependant MS/MS (50ms each)
  - Cycle time was 0.3 sec
    - Peak widths were 2.5 to 3.5 seconds
  - IDA filters evaluated
    - Dynamic Background Subtraction (DBS) on
    - MMDF on Parent Mass and Parent + GSH
    - NL Filter
    - Isotope pattern filter

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IDA Filters - MMDF, Isotope and NL

- MMDF in non exclusive mode
  - Using the mass defects based on formula
    - Parent
    - Major phase II
    - Predicted cleavages (optional)
    - Easy to implement
  - Useful as a broad general Qual/Quant screen
- Non exclusive mode also allows for simultaneous unpredicted approach
- This is a Runtime Algorithm for IDA target selection
  - PeakView™ sofware also has a post processing version
  - Unique to our Software
- Compatible with DBS

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IDA Filters - MMDF, Isotope and NL (continued)

- Isotope pattern recognition
  - Halogenated species – e.g. Chlorine, Bromine
  - Compounds with heavy atom labels Dueterium 13C, 15N
    - Safer (Non radioactive) and more readily available
    - Provides some selectivity advantages
  - Can be used with heavy GSH

- Accurate NL experiment
  - Relies on Mass selectivity (resolution and accuracy)
    - Triggered by and Accurate of loss rather than physical mass selection as in QqQ.
    - Useful for phase II glucuronide and GSH conjusgates
    - Useful for neutral losses of parent structure that are efficient and conserved through biotransformation.

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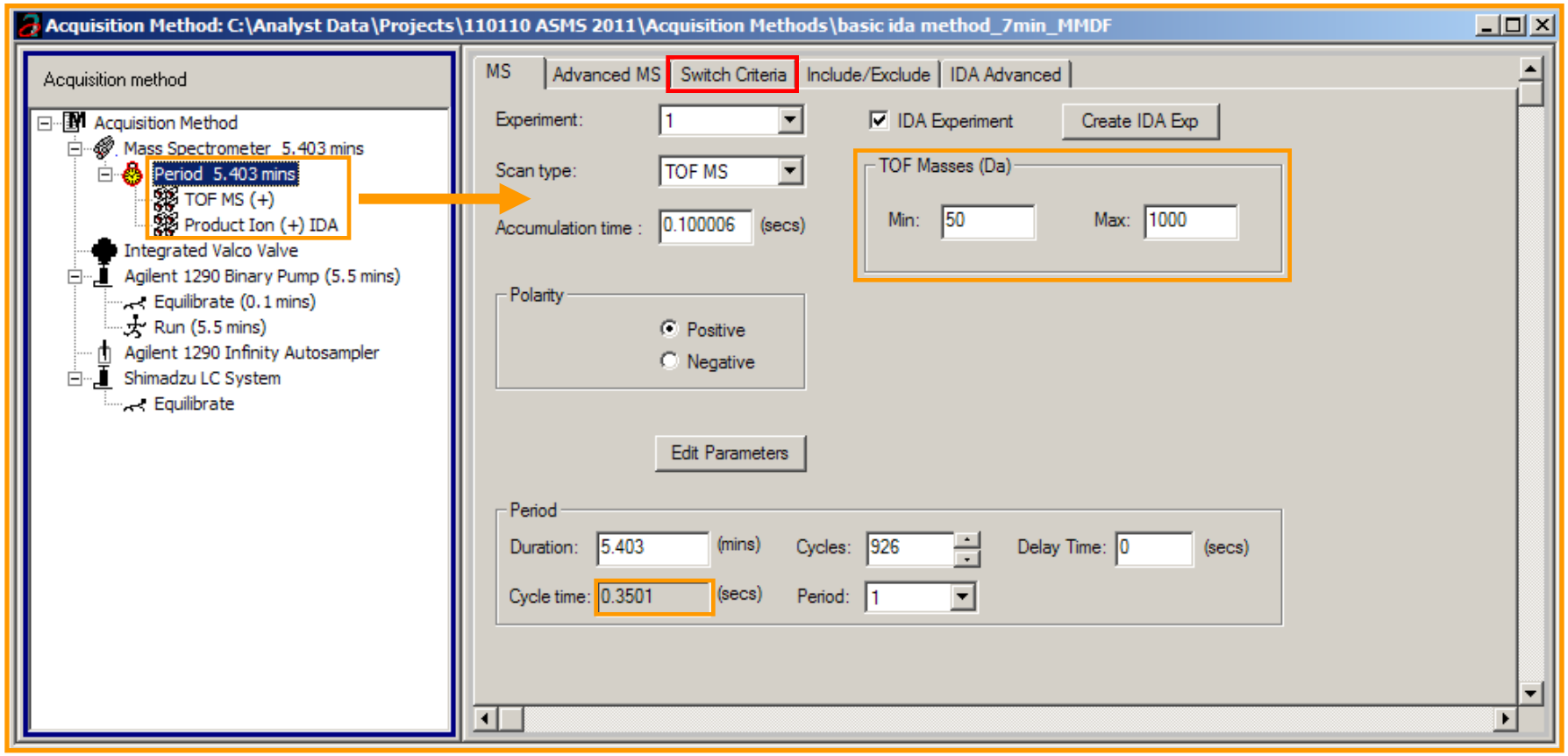
General Method Setup



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Use Switch Criteria Tab to enable MMDF

MS | Advanced MS | Switch Criteria | Include/Exclude | IDA Advanced

Survey -> IDA Experiment

- [ ] For ions greater than: 100 m/z
- [ ] For ions smaller than: 0 m/z
- [ ] With charge state 2 to 4
  Which exceeds 500 cps
- [x] Exclude isotopes within: 4 Da

Mass Tolerance: 50 ( ) ppm (•) mDa

Maximum number of candidate ions to monitor per cycle: 4

Exclude former target ions

(•) Never
( ) Always ( ) For 0 seconds
[ ] After 1 occurence(s)

[x] Mass Defect Filter

[ ] Exclusively — Select the Exclusively check box to trigger a dependent experiment only if the mass defect criteria is met. Clear the check box to prioritize the candidates according to the Switch Criteria.

( ) Peptides

(•) Metabolism

Tolerance: +/- 40 mDa

	Formula	MW (Da)	Width (Da)	Mass Defect (mDa
1	C24H27NO5S	441.1610	100	160.9951
2	C24H27NO5SC10H15N3O6S	746.2292	100	229.1524
3				

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Typical Phase I Modifications and Mass Defects

Modification	Nominal Shift (m/z)	Change in formula	Mass Shift Accurate (m/z)	Mass Defect Shift (mda)
me	14	+CH2	14.0157	15.7
Keto	14	-H2, +O	13.9793	-20.7
OX	16	+O	15.9949	-5.1
Keto OX	30	-H2, +O2	29.9742	-25.8
me OX	30	+CH2, +O	30.0106	10.6
Di OX	32	+O2	31.9898	-10.2
Keto OX Me	44	-H2, +O2, +CH2	43.9898	-10.2
DiKeto OX	44	-H4, +O3	43.9534	-46.6
DiKeto DiOX	60	-H4, +O4	59.9484	-51.6
Di-methyl Di-OX	60	C2H4, +O2	60.0211	21.1
Keto DiOX me	60	-H4, +O3, +CH2	59.9847	-15.3

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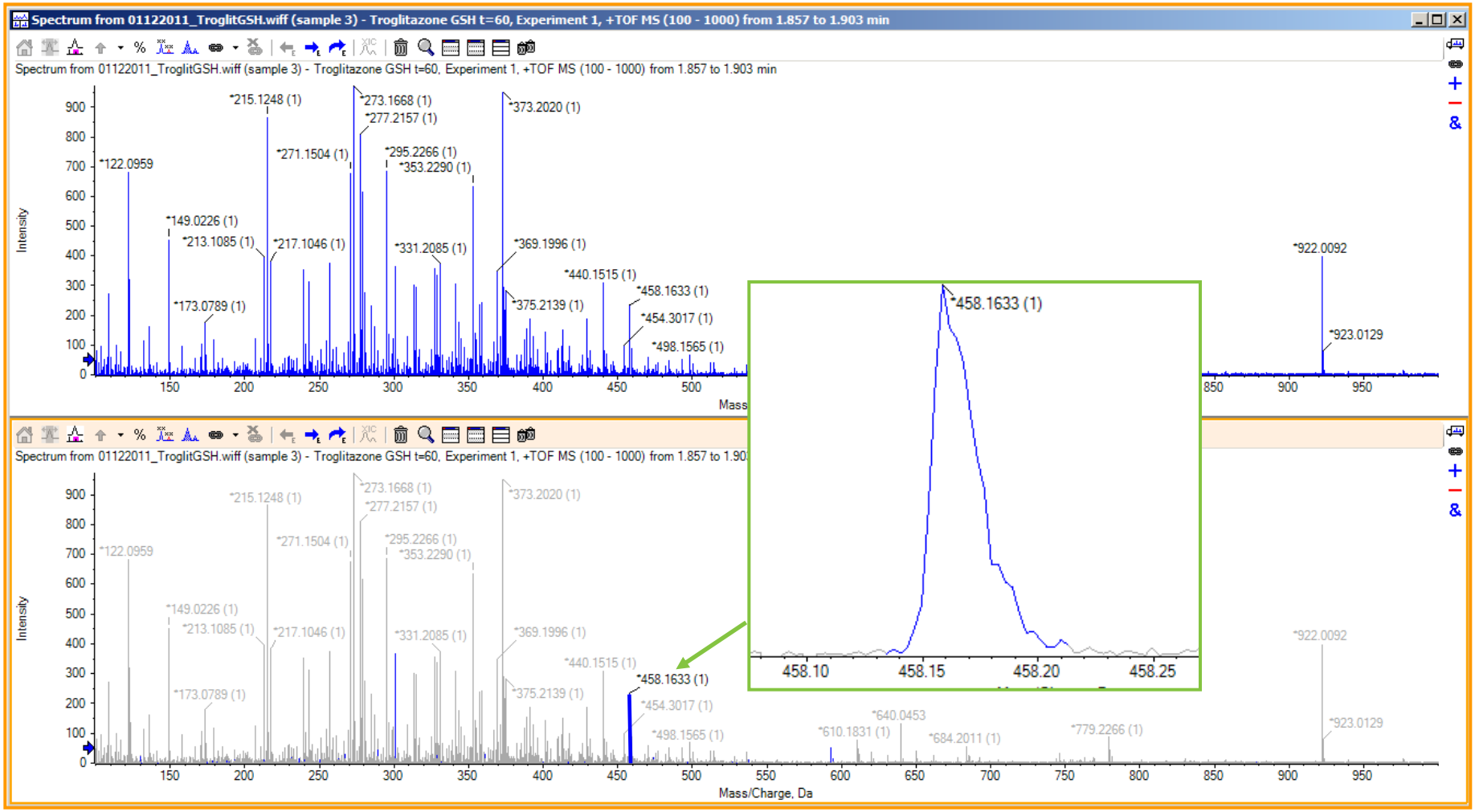
MMDF example an Oxide of Troglitazone



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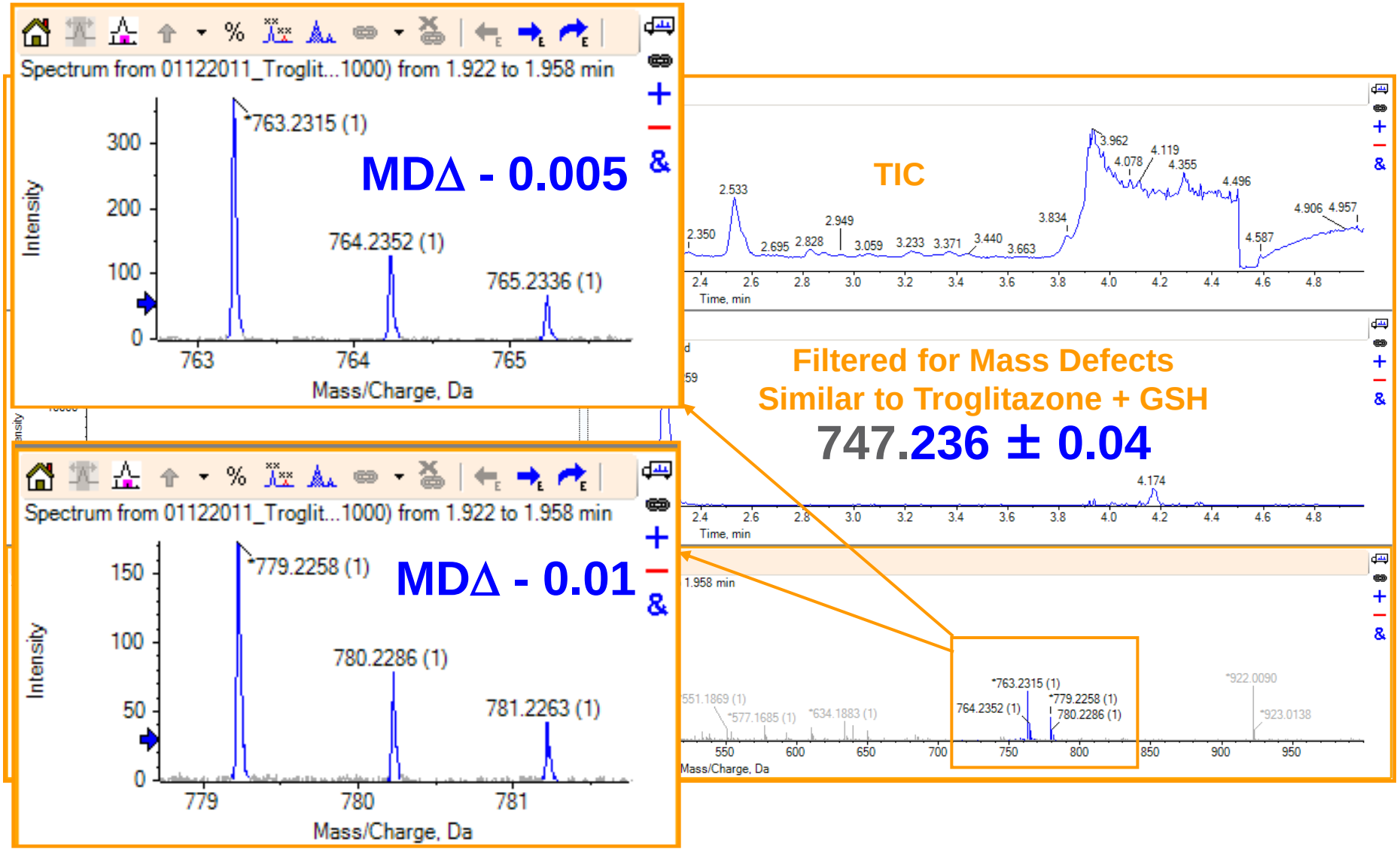
Troglitazone MMDF Explained



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Neutral Loss Mode for parent and GSH

- Acquisition Method
  - Mass Spectrometer 5.019 mins
    - Period 5.019 mins
      - TOF MS (+) — **Low CE ~10 eV**
      - TOF MS (+) — **High CE ~ 35 eV**
      - Product Ion (+) IDA

MS | Advanced MS | Switch Criteria | Include/Exclude | IDA Advanced

☑ Use pattern

○ Match isotopes

○ Mass difference

◉ Neutral loss

	Mass Diff. (Da)	Abundance (%)
1	152.08350	
2	290.19821	
3	176.03210	
4		
5		

Isotope to use for: Most intense

Isotopic calculator

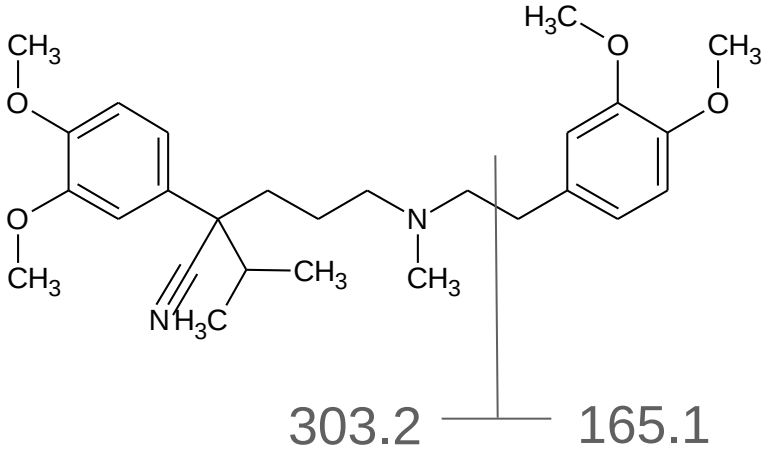
Tolerance: 0.005 | 15

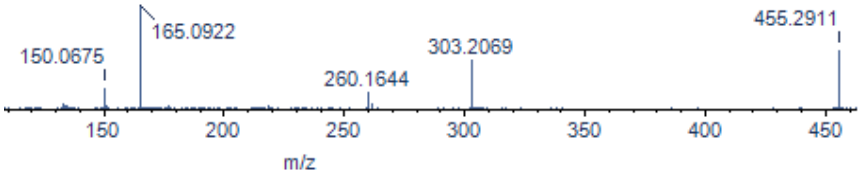
☑ Dynamic background subtract

☐ Rolling collision energy

☐ Adjust CE when using iTRAQ reagent

☐ Infusion IDA





Show only product ions above 15 %

☐	Product Ion	☑	Neutral Loss
☑	150.0675	☑	305.2230
☑	165.0922	☑	290.1982
☑	260.1644	☑	195.1261
☑	303.2069	☑	152.0835

☑ Add product ions, neutral losses from Phase II metabolites

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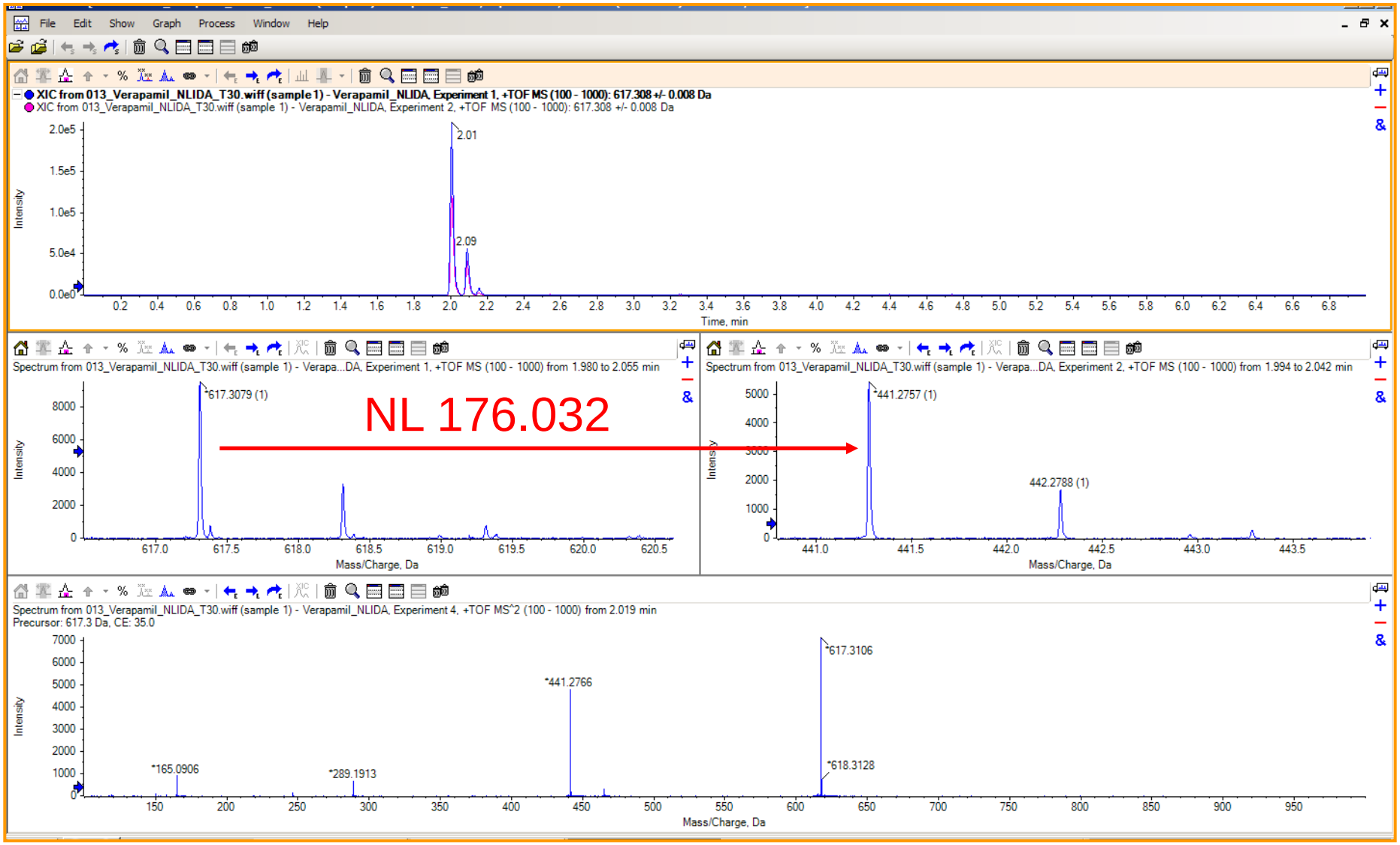
NL Example for Glucuronic Acid



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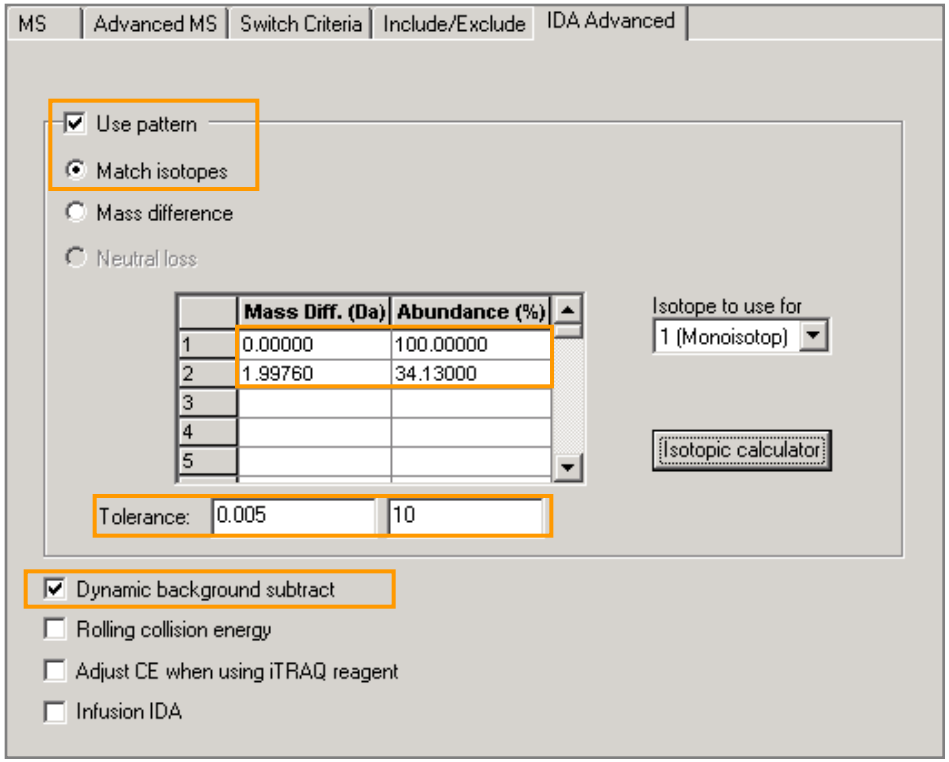
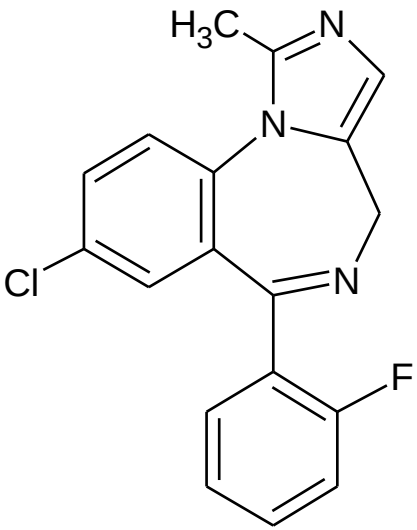
Isotope Pattern for Chlorinated Compounds





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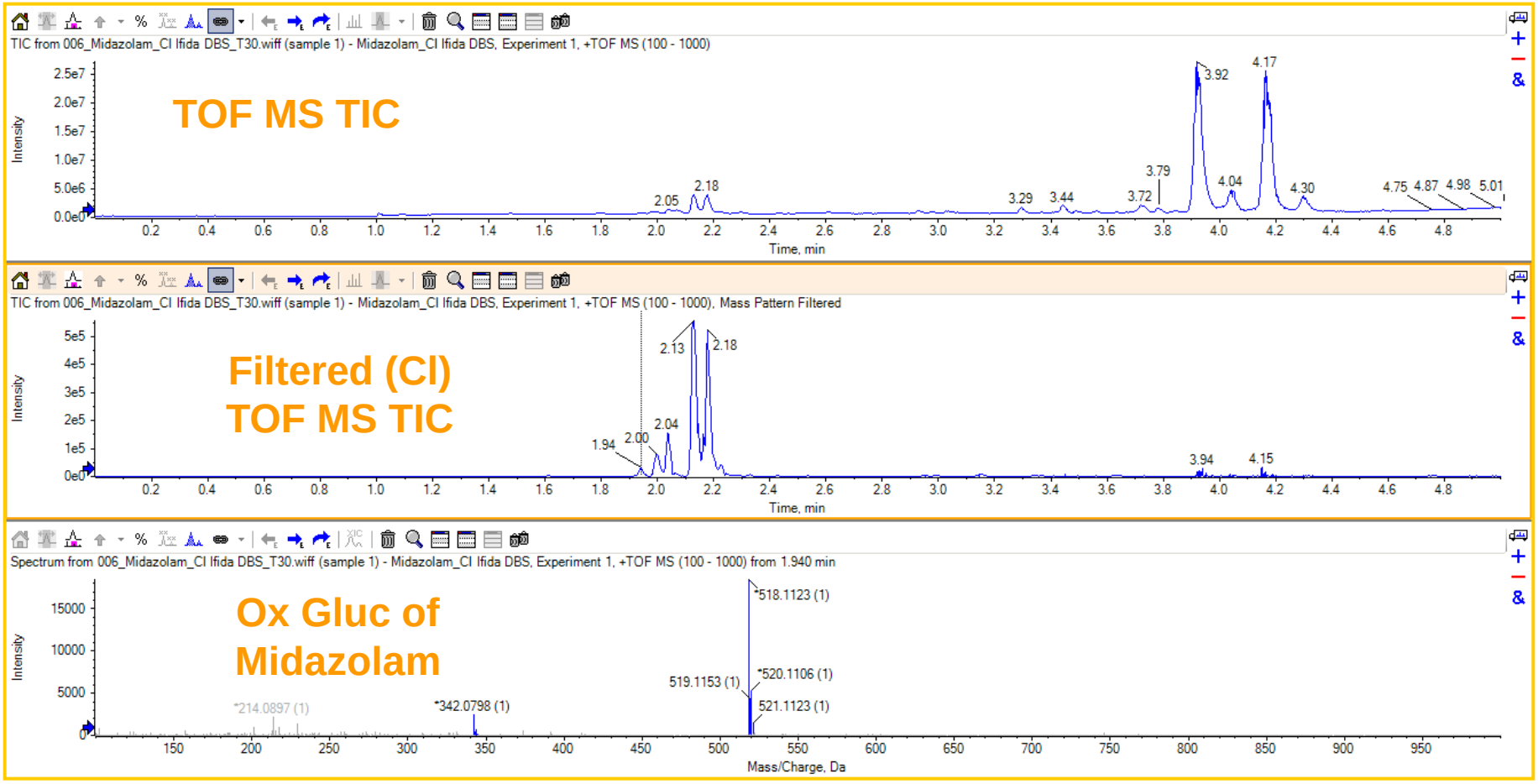
Use of Post Run Isotope Filter with IDA



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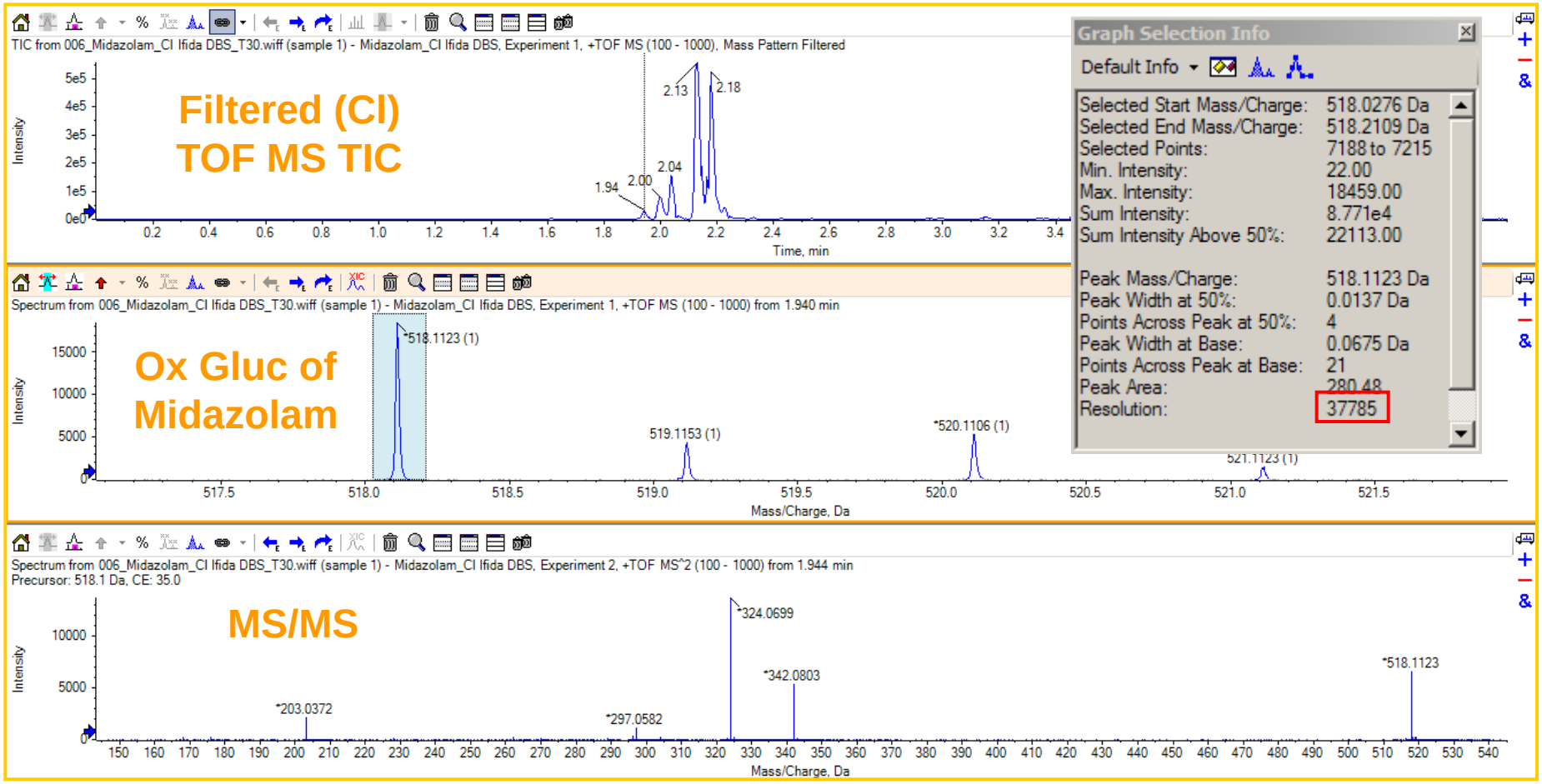
Isotope Filtration Continued



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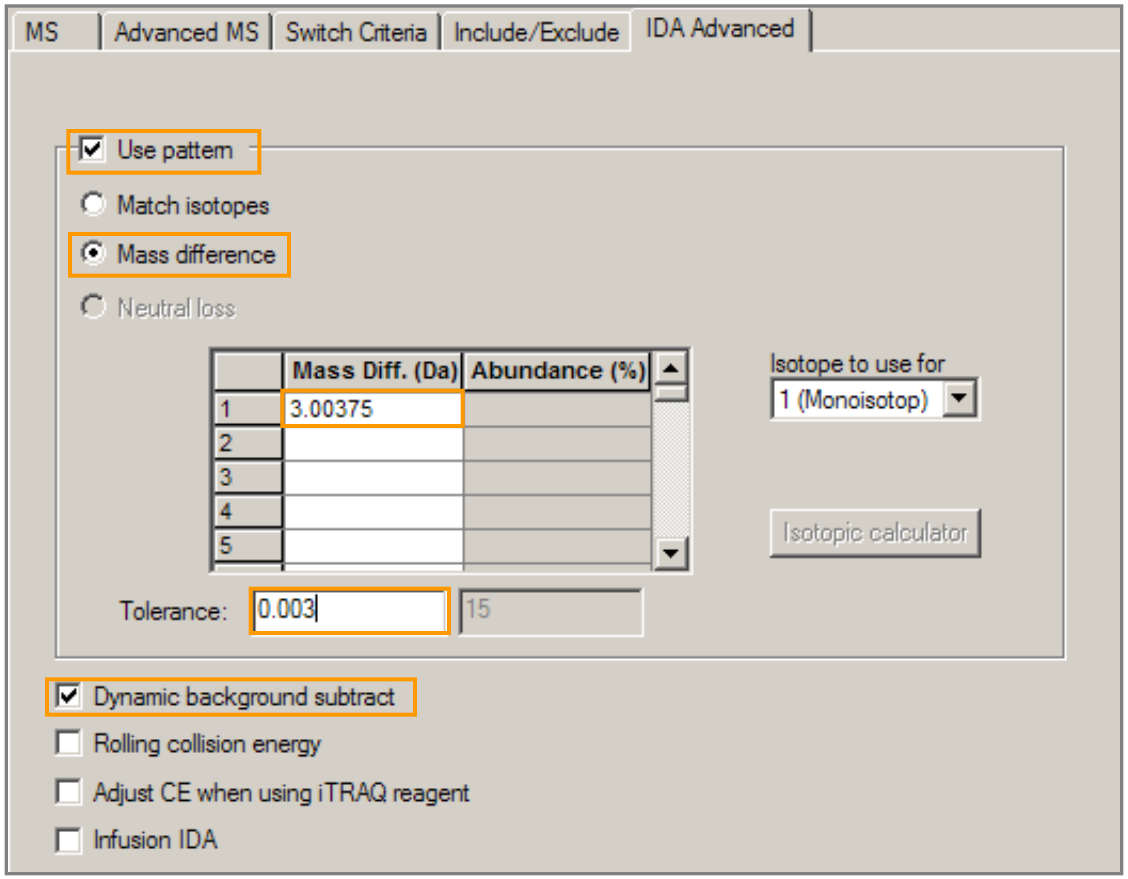
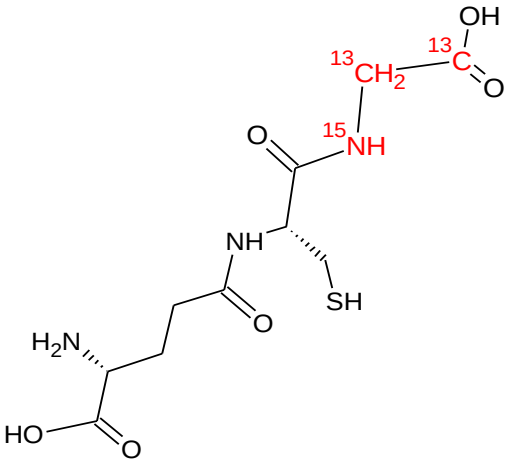
Mass Difference (Similar to Isotope Pattern) For Labeled GSH





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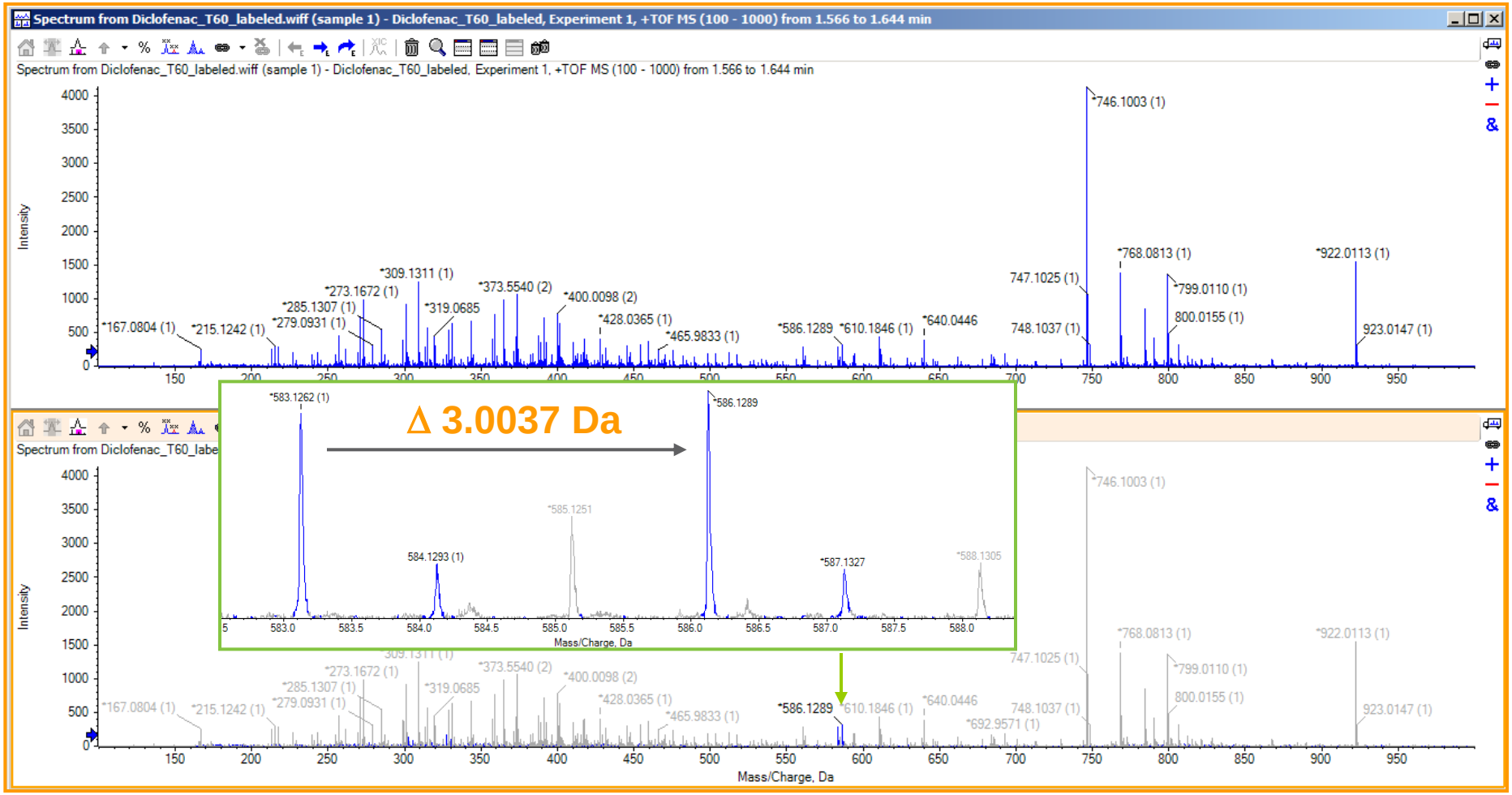
Isotope Example – Heavy/Light GSH



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Batch Processing View –

[blank] - MetabolitePilot™ 1.5 Software

File Edit Setup Help

Workspace: Batch, Results, Correlation

Workflow: Compound Library, Biotrans-formations, Processing Parameters, Create Report

New | Open... | Save | Save As... | How Do I?...

Find Metabolites

	Sample/Controls		Folder	Processing Parameters	Results File
1	MS Sample: 017_Verapamil_T5.wiff Verapamil_T5 MS Controls: 018_Verapamil_T0.wiff Verapamil_T0	Sample... Controls...	Loren's Projects	Verapamil	110522_Verapamil_T5
2	MS Sample: 016_Verapamil_T15.wiff Verapamil_T15 MS Controls: 018_Verapamil_T0.wiff	Sample... Controls...	Loren's Projects	Verapamil	110522_Verapamil_T15
		Sample... Controls...			
4		Sample... Controls...			
5		Sample... Controls...			
6		Sample... Controls...			
7		Sample... Controls...			
8		Sample... Controls...			

Copy Batch Row
Paste Batch Row
Clear Batch Row

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MetabolitePilot™ Software Is used for Sample/Control Comparison – New Structural "Interpretation" Tools

File Edit Setup Help

Workspace

Batch

Results

Correlation

Workflow

Compound Library

Biotrans-formations

Processing Parameters

Create Report

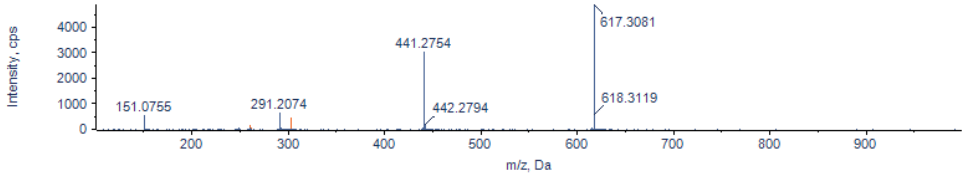
Open... | Save | Save As... | View Processing Parameters... | Show: Interpretation | How Do I?...

Potential Metabolites: 25 of 25 Peaks — Assign ID | Add MS/MS...

	Peak ID	Name	Formula	m/z	ppm	R.T. (min)	Peak Area	% Area	% Score
17	M19	Oxidation	C27H38N2O5	471.2854	0.1	2.40	2.93E+03	1.4	91.4
18	M24	Cyano Conjugate	C28H37N3O4	480.2866	1.9	3.86	3.35E+03	1.6	88.1
19	M12	Di-Oxidation	C27H38N2O6	487.2795	-1.6	2.24	1.72E+02	0.1	88.2
20	M3	Loss of CH2 and CH2+Glucuronide Conjugation	C31H42N2O10	603.2911	-0.2	1.99	2.07E+02	0.1	59.1
21	M9	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3071	0.4	2.08	5.43E+02	0.3	83.7
22	M4	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3074	0.9	2.01	1.86E+03	0.9	86.4
23	M7	Gain of 162.0902	C35H56N2O3S2	617.3806	0.1	2.05	2.02E+02	0.1	37.8
24	M14	Glucuronide Conjugation	C33H46N2O10	631.3220	-0.8	2.32	3.73E+02	0.2	87.0
25	M2	Loss of CH2+Oxidation and Glucuronide Conjugation	C32H44N2O11	633.3016	-0.3	1.92	4.12E+01	0.0	86.1

Interpretation Prepare... | Assign | Apply | Remove — **Selected neutral formula: C32H44N2O10**

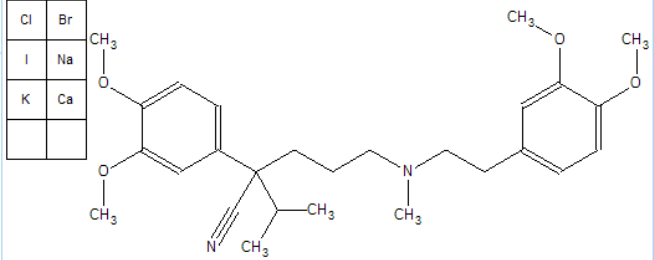
+TOF MS/MS of 617.3071



No structures assigned

Load... | Clear | Save As...

Verapamil, Composition: C27H38N2O4, Mass: 454.2832



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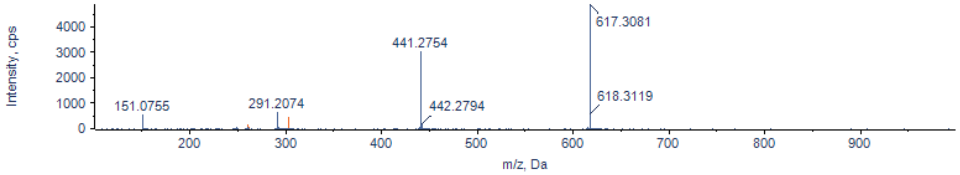
+TOF MS/MS of 617.3071



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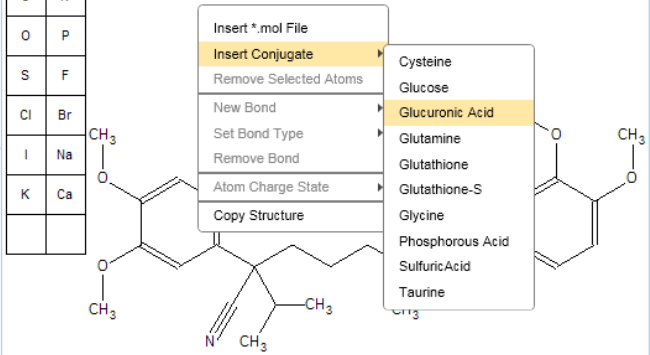

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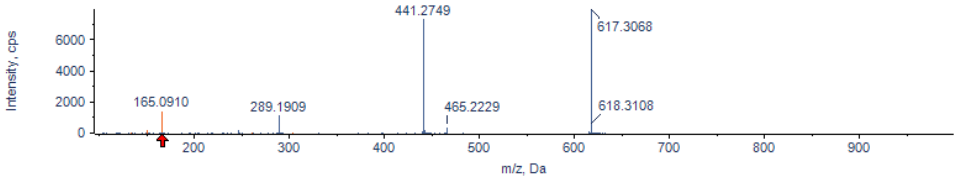
18	M24	Cyano Conjugate	C28H37N3O4	480.2866	1.9	3.86	3.35E+03	1.6	88.1
19	M12	Di-Oxidation	C27H38N2O6	487.2795	-1.6	2.24	1.72E+02	0.1	88.2
20	M3	Loss of CH2 and CH2+Glucuronide Conjugation	C31H42N2O10	603.2911	-0.2	1.99	2.07E+02	0.1	59.1
21	M9	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3071	0.4	2.08	5.43E+02	0.3	83.7
22	M4	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3074	0.9	2.01	1.86E+03	0.9	86.4
23	M7	Gain of 162.0902	C35H56N2O3S2	617.3806	0.1	2.05	2.02E+02	0.1	37.8
24	M14	Glucuronide Conjugation	C33H46N2O10	631.3220	-0.8	2.32	3.73E+02	0.2	87.0
25	M2	Loss of CH2+Oxidation and Glucuronide Conjugation	C32H44N2O11	633.3016	-0.3	1.92	4.12E+01	0.0	86.1

Interpretation Prepare... | Assign | Apply | Remove — **Selected neutral formula: C32H44N2O10**

+TOF MS/MS of 617.3074



Fragments: 18 of 123 Peaks Show: Ion Formula with ppm Error

	Use	Mass (m/z)	Char...	Ion Formula	Error (Da)	RDB	Proposed Structures	Score
6	☑	135.0796	1	C9H11O	-0.0008	5.0	2	27.5
7	☑	150.0671	1	C9H10O2	-0.0004	5.5	3	32.0
8	☑	165.0910	1	C10H13O2	0.0000	5.0	1	38.0
9	☑	219.1372	1	C13H17NO2	0.0118	6.5	5	24.5
10	☑	246.1490	1	C15H20NO2	0.0001	7.0	2	32.5
11	☑	250.1791	1	C15H24NO2	-0.0011	5.0	4	27.5
12	☑	289.1909	1	C17H25N2O2	-0.0002	7.0	17	32.5
13	☑	290.1943	1	C18H28NO2	-0.0172	6.0	1	27.0

Structure Details for C10H12O2

	Use	Broken...	Delta H	Score
1	☑	1	-2	38.0

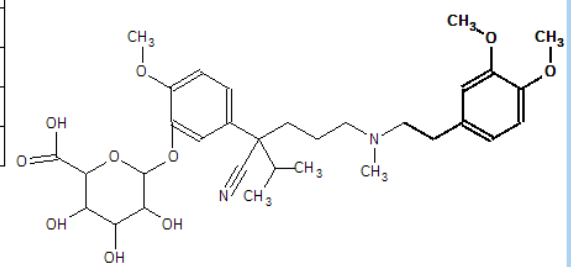
Contained Neutral Losses

	Use	Mass	Formula
1	☑	30.0114	CH2O

Load... | Clear | Save As...

C	N
O	P
S	F
Cl	Br
I	Na
K	Ca

Verapamil_GA, Composition: C32H44N2O10, Mass: 616.2996, Selected: C10H13O2, Mass:



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MetabolitePilot™ Software Is used for Sample/Control Comparison – New Structural "Interpretation" Tools

File Edit Setup Help

Workspace: Batch, Results, Correlation

Workflow: Compound Library, Biotrans-formations, Processing Parameters, Create Report

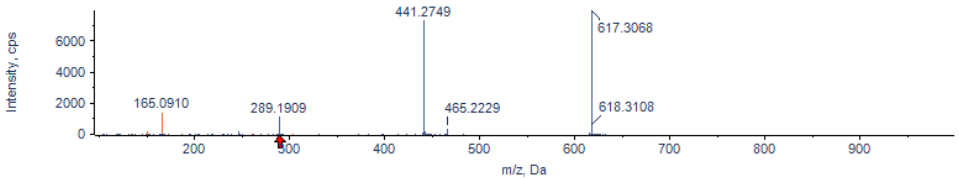
Open... | Save | Save As... | View Processing Parameters... | Show: Interpretation | How Do I?...

Potential Metabolites: 25 of 25 Peaks — Assign ID | Add MS/MS...

	Peak ID	Name	Formula	m/z	ppm	R.T. (min)	Peak Area	% Area	% Score
17	M19	Oxidation	C27H38N2O5	471.2854	0.1	2.40	2.93E+03	1.4	91.4
18	M24	Cyano Conjugate	C28H37N3O4	480.2866	1.9	3.86	3.35E+03	1.6	88.1
19	M12	Di-Oxidation	C27H38N2O6	487.2795	-1.6	2.24	1.72E+02	0.1	88.2
20	M3	Loss of CH2 and CH2+Glucuronide Conjugation	C31H42N2O10	603.2911	-0.2	1.99	2.07E+02	0.1	59.1
21	M9	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3071	0.4	2.08	5.43E+02	0.3	83.7
22	M4	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3074	0.9	2.01	1.86E+03	0.9	86.4
23	M7	Gain of 162.0902	C35H56N2O3S2	617.3806	0.1	2.05	2.02E+02	0.1	37.8
24	M14	Glucuronide Conjugation	C33H46N2O10	631.3220	-0.8	2.32	3.73E+02	0.2	87.0
25	M2	Loss of CH2+Oxidation and Glucuronide Conjugation	C32H44N2O11	633.3016	-0.3	1.92	4.12E+01	0.0	86.1

Interpretation Prepare... | Assign | Apply | Remove — **Selected neutral formula: C32H44N2O10**

+TOF MS/MS of 617.3074



Fragments: 18 of 123 Peaks Show: Ion Formula with ppm Error

	Use	Mass (m/z)	Char...	Ion Formula	Error (Da)	RDB	Proposed Structures	Score
6	☑	135.0796	1	C9H11O	-0.0008	5.0	2	27.5
7	☑	150.0671	1	C9H10O2	-0.0004	5.5	3	32.0
8	☑	165.0910	1	C10H13O2	0.0000	5.0	1	38.0
9	☑	219.1372	1	C13H17NO2	0.0118	6.5	5	24.5
10	☑	246.1490	1	C15H20NO2	0.0001	7.0	2	32.5
11	☑	250.1791	1	C15H24NO2	-0.0011	5.0	4	27.5
12	☑	289.1909	1	C17H25N2O2	-0.0002	7.0	17	32.5
13	☑	290.1943	1	C18H28NO2	-0.0172	6.0	1	27.0

Structure Details for C17H24N2O2

	Use	Broken...	Delta H	Score
1	☑	2	-2	32.5
2	☐	3	-2	26.5
3	☐	3	-2	26.5

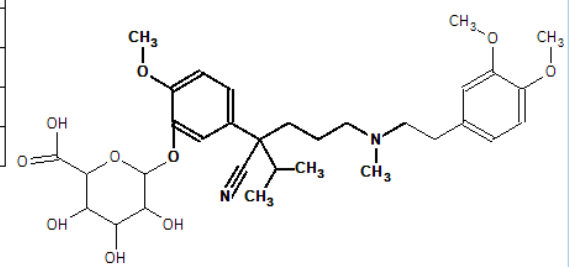
Contained Neutral Losses

	Use	Mass	Formula
1	☑	43.0419	C2H5N

Load... | Clear | Save As...

Verapamil_GA, Composition: C32H44N2O10, Mass: 616.2996, Selected: C17H24N2O2, Ma

C	N
O	P
S	F
Cl	Br
I	Na
K	Ca



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Correlation workspace can be used to view multiple timepoint or species results

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Automatic Metabolite Report Generation

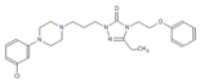
Interpretation – Detailed Report
Nef_FindbyBT_PI_NL.mpr - 04/06/2011

Report Name: «Nefazodone_InterpretationDetailed.docx»
Generated by MetabolitePilot™ 1.5 Software

Compound Information

Compound name:	Nefazodone
Chemical formula:	C25H32N5O2Cl
Adduct:	+H
m/z:	470.2317

Structure



Potential Metabolites

Confirmed	Peak ID	Name	Formula	Assigned	m/z	ppm	R.T. (min)	Peak Area	% Area	% Score
	M11	Loss of Cl→H+Propyl Ketone to Acid	C21H25N5O3		396.1964	-16.7	4.57	3.22E+02	0.9	58.7
√		Parent	C25H32N5O2Cl	√	470.2301	-3.4	5.52	9.64E+02	2.8	90.0
	M8	Loss of C6H4O+Demethylation to Carboxylic Acid	C19H25N5O3Cl		408.1776	-5.2	4.54	9.28E+02	2.7	85.4
	M12	Loss of C6H4O+Demethylation to Carboxylic Acid	C19H25N5O3Cl		408.1784	-3.2	4.65	1.04E+03	3.0	80.4

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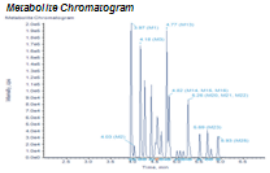
Confirmed	Peak ID	Name	Formula	Assigned	m/z	ppm	R.T. (min)	Peak Area	% Area	% Score
	M6	Loss of C6H4+Oxidation	C19H25N5O3Cl		410.1958	1.0	4.42	2.69E+03	7.7	89.3
	M7	Loss of C6H4+Oxidation	C19H25N5O3Cl		410.1851	-15.3	4.51	4.64E+02	1.3	72.2
	M1	Loss of C6H5Cl+Oxidation	C19H25N5O3		376.2327	-4.3	3.97	5.25E+03	15.0	74.9
	M4	Loss of C6H5Cl+Ketone Formation	C19H27N5O3		374.2180	-1.7	4.27	2.54E+03	7.3	75.6

Metabolite Chromatogram



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Confirmed	Peak ID	Name	Formula	Assigned	m/z	ppm	R.T. (min)	Peak Area	% Area	% Score
	M24	Loss of C10H11N2Cl+Demethylation to Carboxylic Acid	C15H19N3O4		306.1437	-3.7	5.77	4.26E+02	1.2	80.2
	M25	Loss of C10H11N2Cl+Demethylation to Carboxylic Acid	C15H19N3O4		306.1445	-1.1	5.93	1.05E+03	3.0	75.8
	M9	Loss of C6H4+Demethylation to Carboxylic Acid	C19H25N5O4Cl		424.1722	-5.7	4.55	5.76E+02	1.6	72.6
√	M18	Oxidation	C25H32N5O3Cl	√	486.2240	-5.4	5.07	2.87E+02	0.8	83.8
√	M20	Oxidation	C25H32N5O3Cl	√	486.2244	-4.7	5.24	9.22E+02	2.6	84.4
√	M13	Di-Oxidation	C25H32N5O4Cl	√	502.2242	5.3	4.77	4.39E+03	12.5	72.7
√	M16	Di-Oxidation	C25H32N5O4Cl	√	502.2179	-7.3	4.82	1.59E+03	4.8	78.3
	M10	Loss of C6H4	C19H28N5O2Cl		394.1999	-1.4	4.57	1.02E+03	2.9	94.9
	M3	Loss of C6H8Cl	C19H29N5O2		360.2394	0.0	4.18	4.60E+03	13.2	93.3
	M21	Ethyl to Alcohol	C23H28N5O3Cl		458.1942	-2.5	5.26	1.57E+03	4.5	56.1
	M5	Tri-Oxidation	C25H32N5O5Cl	√	518.2134	-5.9	4.27	5.79E+02	1.7	71.3
	M14	Loss of C10H11N2Cl+Di-Oxidation	C15H21N3O4		308.1585	-6.5	4.82	7.61E+02	2.2	70.0
	M19	Loss of C10H11N2Cl+Di-Oxidation	C15H21N3O4		308.1582	-7.4	5.15	3.22E+02	0.9	69.0
	M15	Loss of C6H4+Bis-Ketone Formation	C19H24N5O4Cl		422.1549	-9.6	4.82	2.77E+02	0.8	41.6
	M2	Loss of Cl→H+Di-Oxidation and Ketone Formation	C25H31N5O5		482.2362	-7.4	4.03	4.04E+02	1.2	70.9
	M17	Loss of C10H11N2Cl+Di-Oxidation and Ketone Formation	C15H19N3O5		322.1377	-6.5	5.01	2.90E+02	0.8	46.0
	M22	Loss of C15H19N3O2+Ketone Formation	C10H11N2OCl		211.0617	-7.2	5.29	4.62E+02	1.3	69.2
	M23	Loss of C10H11N2Cl+Oxidation	C15H21N3O3		292.1651	-1.6	5.69	1.14E+03	3.2	86.8

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Mass (m/z):	288.1319	Ion Formula:	C13H18N3O2Cl
Error (mDa):	-16.4	Intensity (cps):	59.5
RDB:	4.0	Score:	30.5

Structure Details

Selected: C13H18N3O2Cl
Mass: 288.1088
Broken Bonds: 2
Delta H: 2
Score: 30.5

Contained Neutral Losses
No contained neutral losses

Mass (m/z):	290.1472	Ion Formula:	C18H20NCl
Error (mDa):	-19.8	Intensity (cps):	67.6
RDB:	7.0	Score:	3.0

Structure Details
No structure

Mass (m/z):	306.1436	Ion Formula:	C18H22NOCl
Error (mDa):	-18.4	Intensity (cps):	19338
RDB:	7.0	Score:	3.0

Structure Details
No structure

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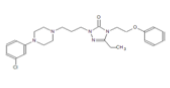
Interpretation Results
– Parent

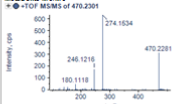
Formula:	C25H32N5O2Cl
ppm:	-3.4
RDB:	12.0

Metabolite Structure



Composition: C25H32N5O2Cl
Mass: 469.2246
Selected neutral formula: C25H32N5O2Cl

Metabolite MS/MS
+ ⊕ +TOF MS/MS of 470.2301



Common productions: 140.0831, 246.1216, 274.1534, 470.2281

Peaks selected for assignment: 125.0829, 140.0831, 140.1070, 154.0994, 160.1118, 209.0918, 218.1232, 246.1216, 274.1534, 470.2281

Assigned: 10 of 10 peaks, score for 10 proposed assignments: 39.5

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Metabolites with MS/MS Data from Various IDA Modes

Peak ID	Name	Formula	m/z	ppm	R.T. (min)	Intensity only	DBS	MMDF	DBS + MMDF	NL DBS
M1	Loss of CH2+Oxidation and Glucuronide Conjugation	C32H44N2O11	633.3013	-0.7	1.62					4.38E+01
M2	Loss of C10H12O2 and CH2+Oxidation	C16H24N2O3	293.1858	-0.6	1.8	2.39E+02	2.47E+02		2.28E+02	
M3	Loss of C17H24N2O2+Desaturation	C10H12O2	165.091	-0.1	1.84	6.61E+02	7.00E+02			
M4	Loss of CH2+Oxidation and Glucuronide Conjugation	C32H44N2O11	633.3016	-0.3	1.92				4.12E+01	1.05E+02
M5	Oxidation	C27H38N2O5	471.2852	-0.3	1.99					8.26E+01
M6	Loss of CH2 and CH2+Glucuronide Conjugation	C31H42N2O10	603.2911	-0.2	1.99	2.07E+02	1.93E+02	1.92E+02	2.07E+02	5.26E+02
M7	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3074	0.9	2.01	1.87E+03	1.82E+03	1.85E+03	1.86E+03	4.62E+03
M8	Loss of C10H12O2 and CH2	C16H24N2O2	277.1916	2	2.02	1.57E+03	1.61E+03		1.49E+03	
M9	Loss of C10H12O2	C17H26N2O2	291.207	1.1	2.05	2.95E+04	2.89E+04	2.82E+04	2.99E+04	
M10	Gain of 162.0902	NA	617.3806	NA	2.05	2.13E+02	2.82E+02	2.21E+02	2.02E+02	
M11	Loss of CH2+Oxidation	C26H36N2O5	457.27	0.7	2.08		6.04E+01	6.29E+01	6.90E+01	
M12	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3071	0.4	2.08	6.04E+02	5.79E+02	5.51E+02	5.43E+02	
M13	Oxidation	C27H38N2O5	471.2852	-0.4	2.1		1.48E+02	1.45E+02	1.57E+02	4.07E+02
M14	Loss of CH2 and CH2+Glucuronide Conjugation	C31H42N2O10	603.2904	-1.4	2.15					6.56E+01
M15	Demethylation and Glucuronide Conjugation	C32H44N2O10	617.3072	0.6	2.16		7.79E+01	6.91E+01		
M16	Loss of CH2+Oxidation	C26H36N2O5	457.2701	0.9	2.18		7.38E+01	6.78E+01	8.35E+01	1.96E+02
M17	Oxidation and Glucuronide Conjugation	C33H46N2O11	647.3202	4.3	2.18					1.11E+02
M18	Di-Oxidation	C27H38N2O6	487.2795	-1.6	2.24	1.75E+02	1.84E+02	1.77E+02	1.72E+02	4.66E+02
M19	Loss of CH2+Oxidation	C26H36N2O5	457.2687	-2.1	2.24		1.22E+02	1.31E+02		3.45E+02
M20	Oxidation	C27H38N2O5	471.2851	-0.5	2.27		1.68E+02	1.61E+02	1.64E+02	4.48E+02
M21	Glucuronide Conjugation	C33H46N2O10	631.322	-0.8	2.32	2.62E+02	2.65E+02	2.66E+02	3.73E+02	9.68E+02
M22	Loss of CH2 and CH2	C25H34N2O4	427.2588	-0.9	2.33	5.66E+02	5.07E+02	5.10E+02	4.72E+02	1.36E+03
M23	Loss of CH2	C26H36N2O4	441.2751	0.7	2.35	1.89E+03	2.10E+03	1.82E+03	1.93E+03	8.93E+03
M24	Loss of CH2O+Ketone Formation	C26H34N2O4	439.259	-0.4	2.36			3.17E+02	3.21E+02	
M25	Loss of CH2+Oxidation	C26H36N2O5	457.2692	-1.1	2.38	9.45E+02	9.30E+02	8.91E+02	9.38E+02	2.24E+03
M26	Oxidation	C27H38N2O5	471.2854	0.1	2.4	3.07E+03	2.84E+03	2.69E+03	2.93E+03	
M27	Loss of CH2	C26H36N2O4	441.2747	-0.2	2.49	4.17E+04	4.18E+04	4.12E+04	4.43E+04	9.66E+04
M28	Loss of CH2O+Ketone Formation	C26H34N2O4	439.2586	-1.3	2.49				2.63E+02	
	Parent	C27H38N2O4	455.2916	2.5	2.51	1.16E+05	1.16E+05	1.18E+05	1.15E+05	2.64E+05
M29	Loss of C11H15NO2+Desaturation	C16H21NO2	260.1645	0.1	2.81		1.67E+02		1.55E+02	
M30	Deaklyated Cyano Conjugate	C19H27N3O2	330.2179	1	3.1	3.08E+03	3.30E+03		3.26E+03	
M31	Cyano Conjugate	C28H37N3O4	480.2866	1.9	3.86	3.44E+03	3.82E+03	3.18E+03	3.35E+03	6.81E+03
					Total Metabolites with MS/MS	**18**	**25**	**21**	**25**	**19**

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Conclusions

- Speed and Run time IDA filters are a critical component of successful Quant/Qual workflows
  - Increase the relevance/Quality and quality of data.
  - Decrease the need for subsequent re analysis
- For generic *in vitro* Quant /Qual
  - Use DBS IDA with short accumulation time 30 to 50ms per MS/MS
  - A totally generic method – no need to change the methods
  - Finds major metabolites and most minor ones too
- For slightly more targeted
  - Workflows using MMDF with DBS is helpful for lower level species
- For *in vivo* matrices (bile) DBS, MMDF, NL and isotope filtration are useful
  - To ignore matrix background and matrix peaks.

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