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Chemically Intelligent Metabolite ID Workflows

Dave Heywood
MS Field Marketing

Overview

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- Metabolite Profiling or Metabolite ID?
- Mass Spectrometry and Accurate Mass Measurements
- Metabolite ID Workflow
  - Comprehensive data collection
  - Intelligent data interpretation
  - Tools for interpreting structure and elemental composition
  - Qual/Quan, integrating quantitative measurements
- Gratuitous commercial type slides

What's Metabolic Profiling at Waters?

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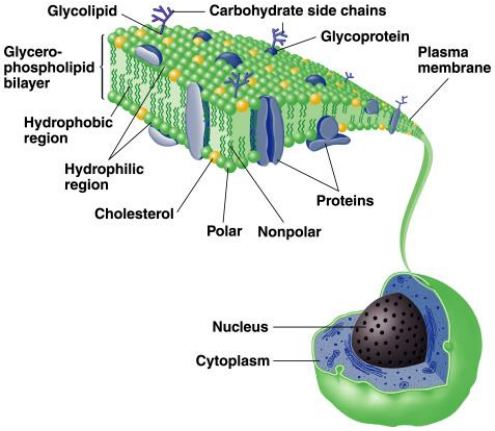
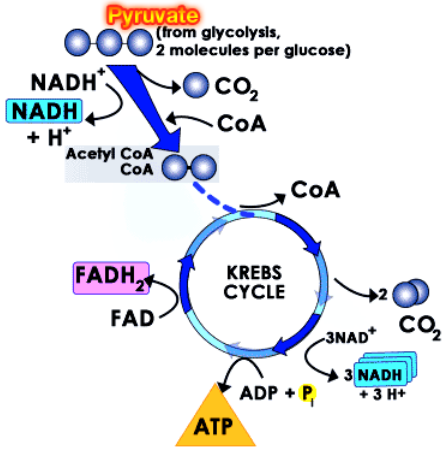
Metabolic Profiling

METABOLITE ID AND DRUG METABOLISM

BIOMARKER ANALYSIS
LIPIDOMICS AND METABOLOMICS

TRADITIONAL MEDICINE





Analytical Challenges in Metabolic Profiling

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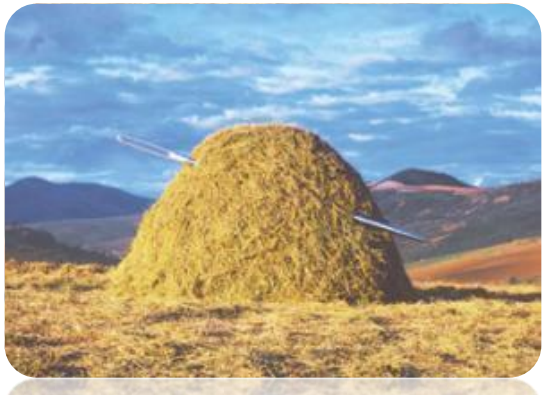
- **Extracting the maximum amount of information**
  - Complex mixtures with wide dynamic range
  - Full structural characterization
- **Providing wide range of experimental options**
  - New ways of extracting more information
- **Increase productivity**
  - Provide consistently high performance for users of all experience levels
  - Provide ability to expand analytical possibilities in the future



Acquity UPLC The Gold Standard in Liquid Chromatography

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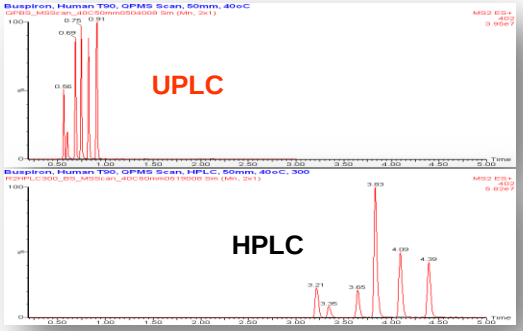
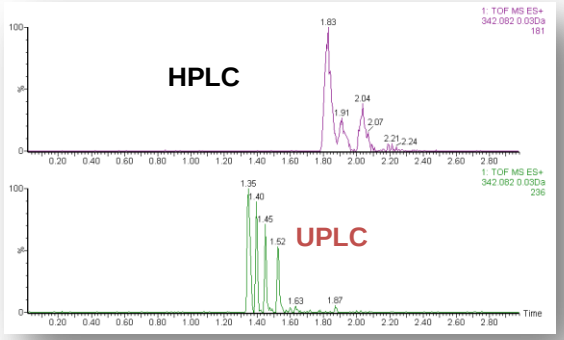
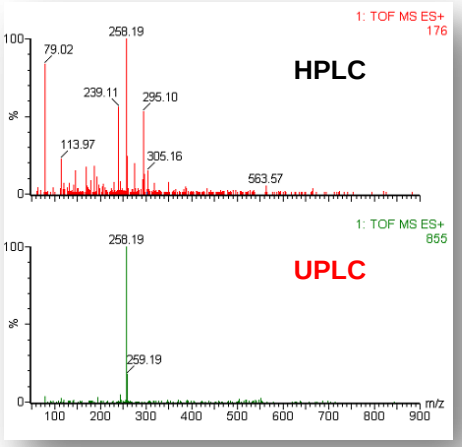


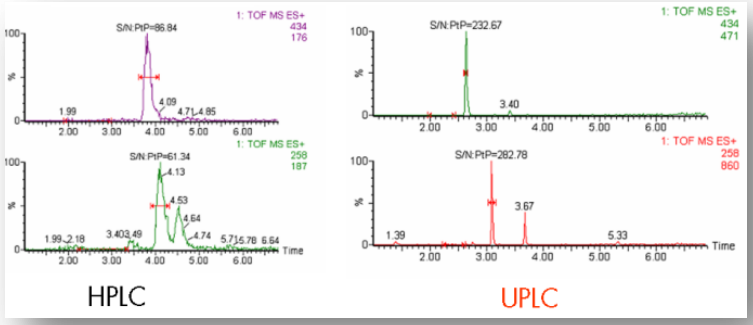
Speed

Resolution



II





Sensitivity

Better MS Data

Tandem Quadrupole MS

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- Highest Sensitivity for targeted analysis
- Accurate quantitative data
- True class specific screening
- Affordable rugged mass spectrometry technology

TQ Schematic

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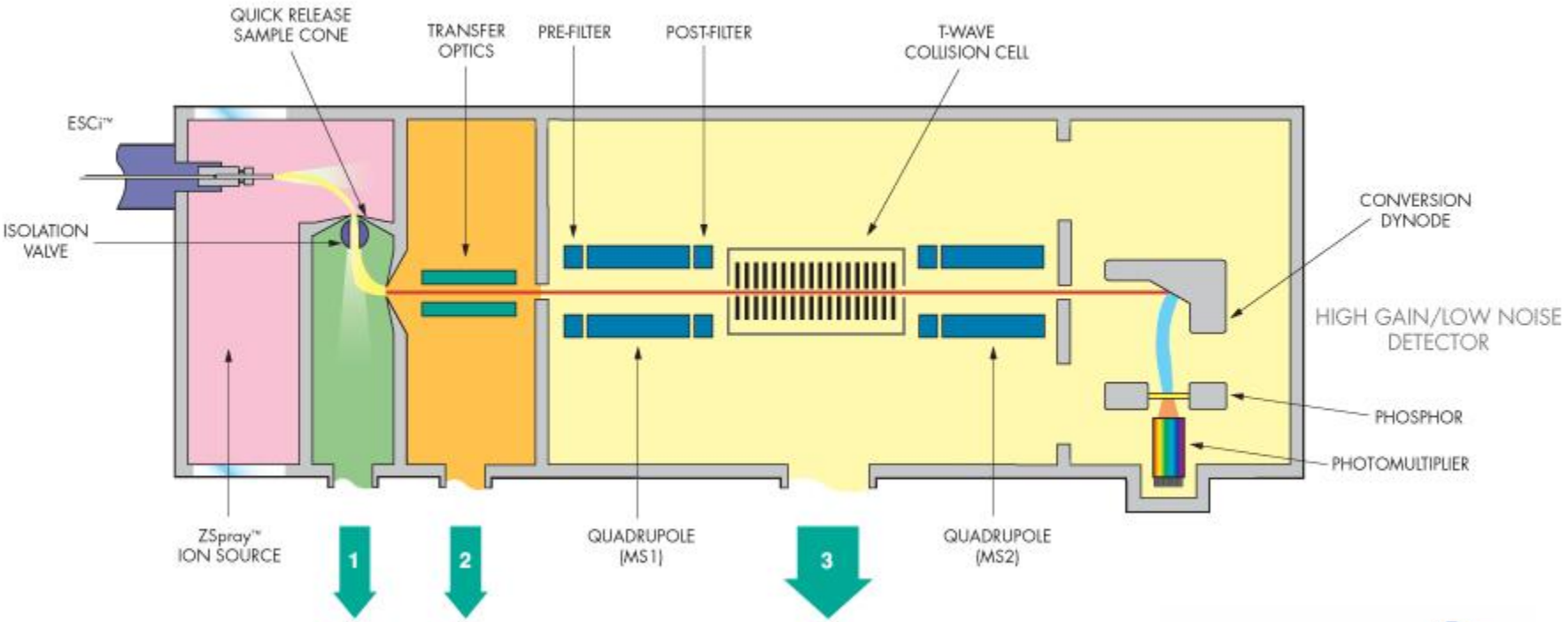

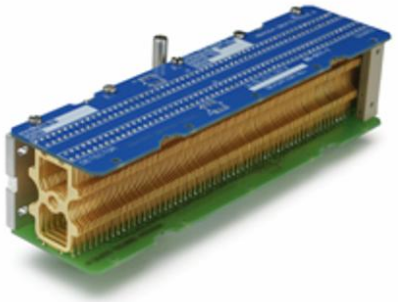

Orthogonal Acceleration Time-Of-Flight

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Xevo G2 QTof

SYNAPT™ G2

- High resolution mass spectra
  - Resolution independent of scan speed
  - MS and MS/MS
- High mass measurement accuracy (Accurate Mass)
  - MS and MS/MS
- High sensitivity full scan data
- MS^E
  - Structural analysis for metabolite localization
- Ideal for qualitative and more recently combined qualitative and quantitative workflows

Xevo QTOF

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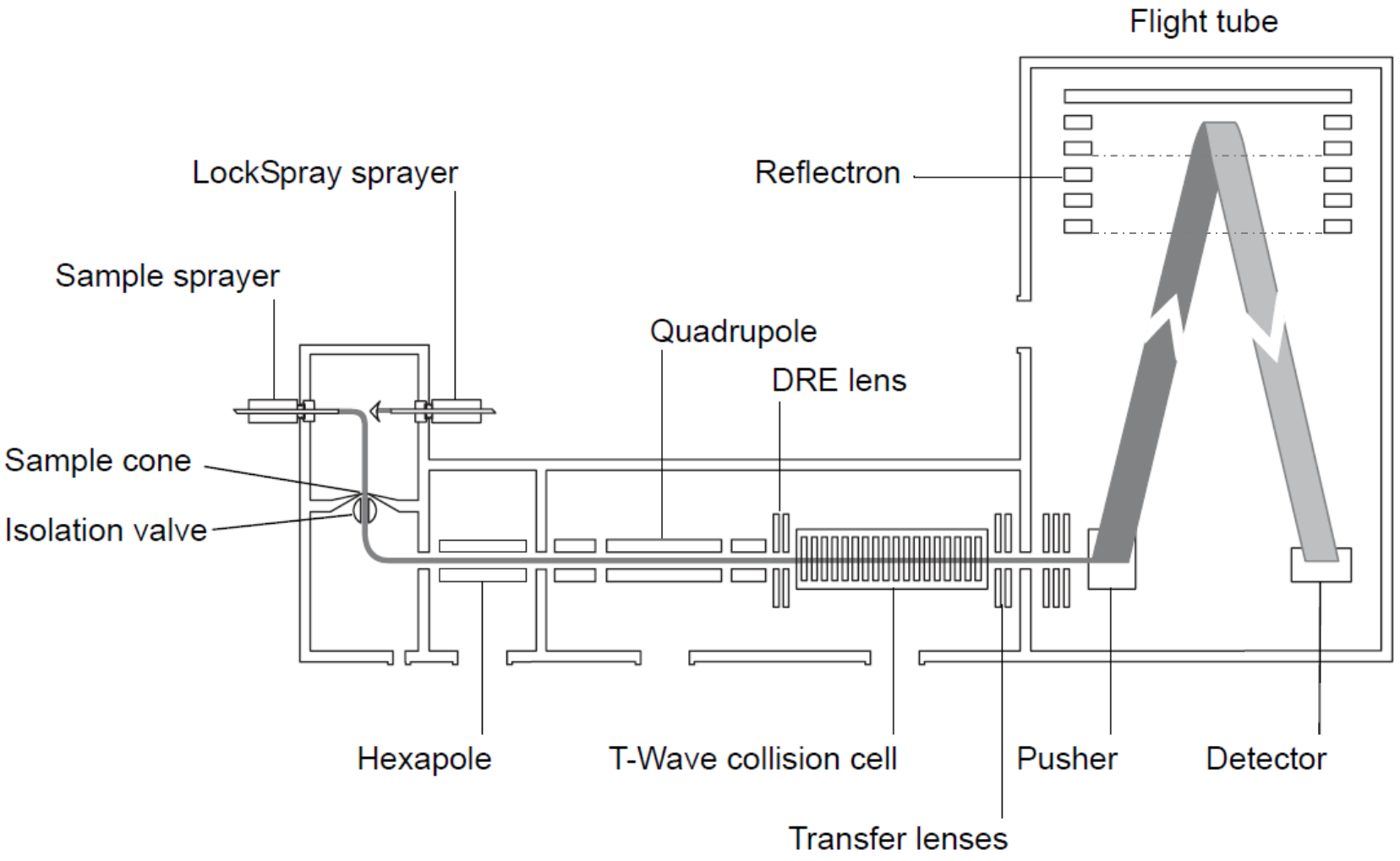

What is Accurate Mass?

The Fundamentals of Accurate Mass

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- carbon has a mass of 12
- hydrogen has a mass of 1
- oxygen has a mass of 16
- nitrogen has a mass of 14

But this is not strictly “Accurate”

The Fundamentals of Exact Mass

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- carbon has a mass of 12.0000
- hydrogen has a mass of 1.0078
- oxygen has a mass of 15.9949
- nitrogen has a mass of 14.0031

- It is possible to have combinations of atoms which have the same nominal (or integer) mass but different accurate mass
- If such compounds can be mass measured with sufficient accuracy it is possible to determine elemental composition

Simple Examples

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- CO = 27.9949
- N_2 = 28.0061
- C_2H_4 = 28.0313

- These elemental combinations have the same nominal mass but different accurate masses
- A nominal mass measurement cannot distinguish these
- If any compounds differ in their elemental compositions by substitution of any of these elements, then the exact mass measurement will show this

Mass Measurement Accuracy

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- The accuracy of the measurement is quoted as the difference (error) between the measured mass and the calculated mass
- The accuracy is measured in
  - milliDaltons (1mDa = 0.001 mass units)
  - ppm = parts per million = $\Delta m/m \times 10^6$

Example:

True' mass = 400.0000

Measured mass = 400.0020

Difference = 0.0020 (2 mDa)

$$\text{ppm error} = \frac{0.002}{400} \times 10^6 = 5 \text{ ppm}$$

When Exact Mass Makes a Difference
(assuming elements $C_{50}H_{100}N_5O_5S_2$)

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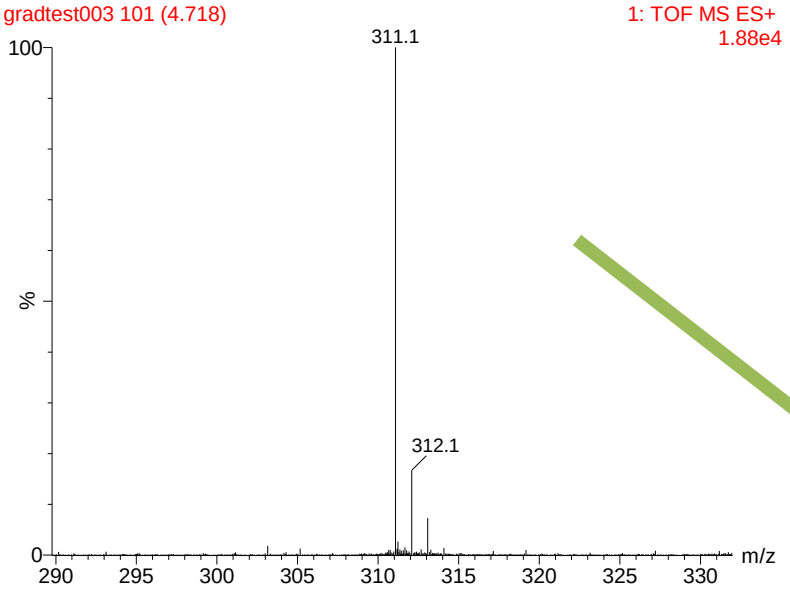
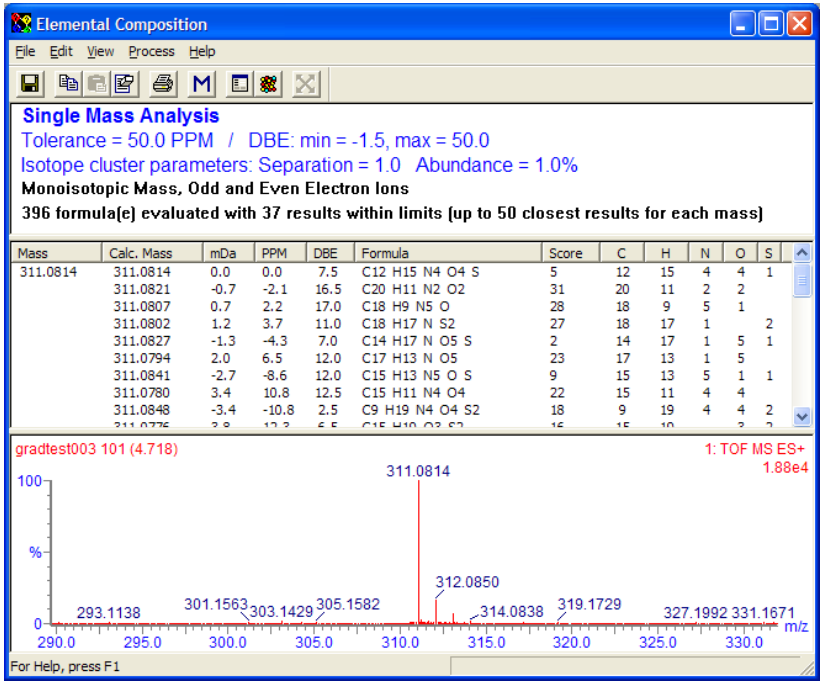


Nominal mass measured spectrum (ie quadrupole or ion trap data)

~50ppm tolerance @ m/z 311.0814

37 possible results



When Exact Mass Makes a Difference
(assuming elements $C_{50}H_{100}N_5O_5S_2$)

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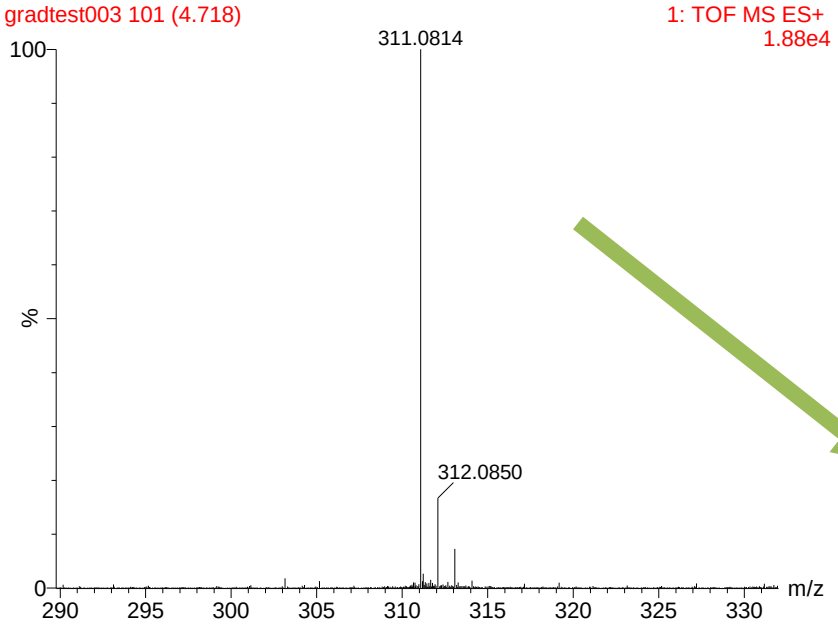
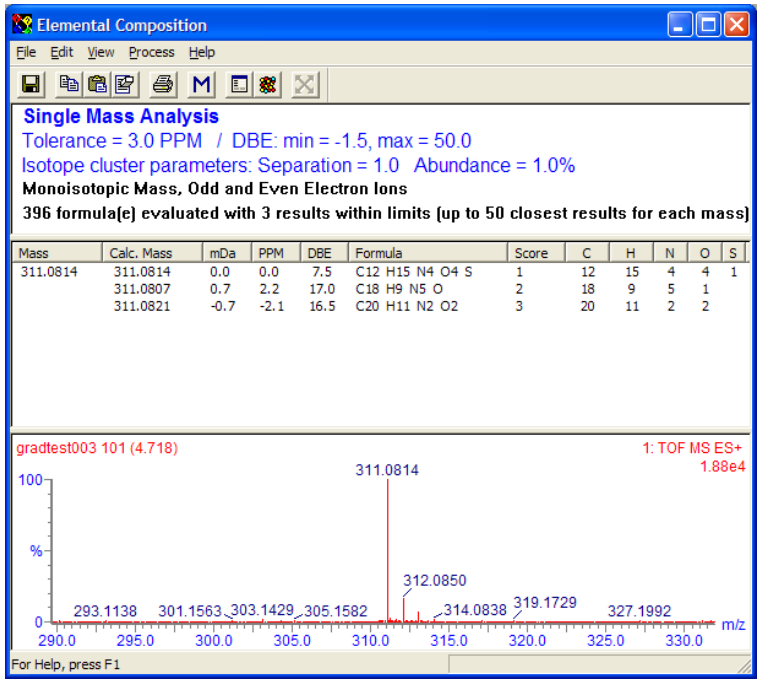


Exact Mass Measured Spectrum

3ppm tolerance @ m/z 311.0814

3 Possible Results



Exact mass for Selectivity
Isobaric metabolites of Rabeprazole

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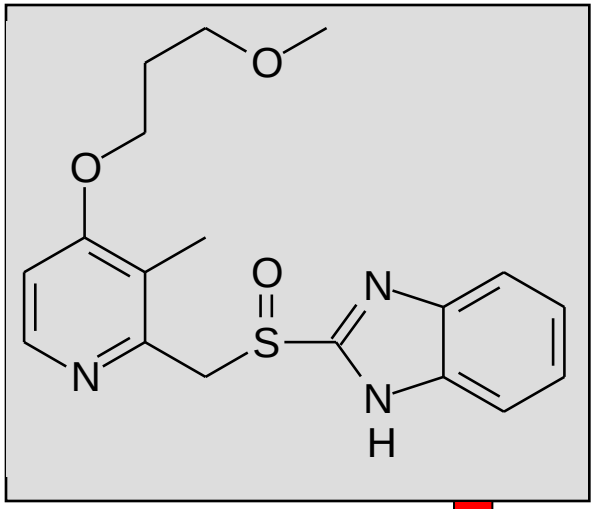
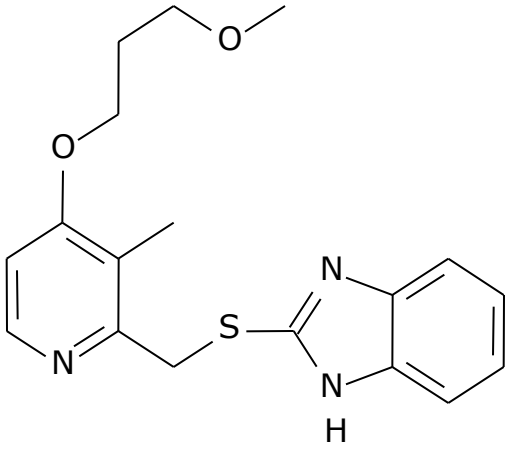
Loss of O =
-16 mass shift



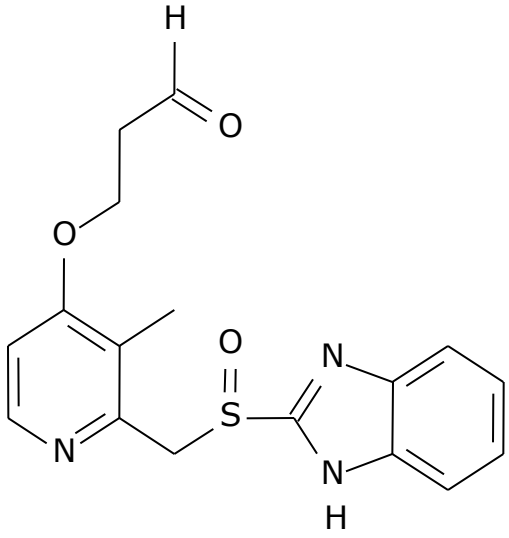
Loss of CH4 =
-16 mass shift



Sulphide

[M+H]+ 344.1433

Difference = 36 mDa



Aldehyde

[M+H]+ 344.1069

What are the Benefits of Exact Mass?

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- Measurement of mass to 4 decimal places
- High confidence in confirming expected compounds
  - Distinguishes them from compounds of similar mass
- Compound identification
  - Prediction of elemental composition
- Patent submission and publication
  - ACS require better than 5ppm mass accuracy for publication

What instruments are used for exact mass?

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- Magnetic sector mass spectrometers
  - these have traditionally been used for exact mass measurement
  - required skilled operator to get good results
- Orthogonal time-of-flight (oa-TOF) mass spectrometers
  - routine operation with good mass accuracy (<1ppm)
  - Accurate isotope ratio measurements
- Ion Cyclotron Resonance mass spectrometers (FTICR, Orbitrap)
  - generally expensive and more difficult to operate
  - good mass accuracy (<1ppm)

Advanced Elemental Composition Calculations

Elemental Composition Analysis

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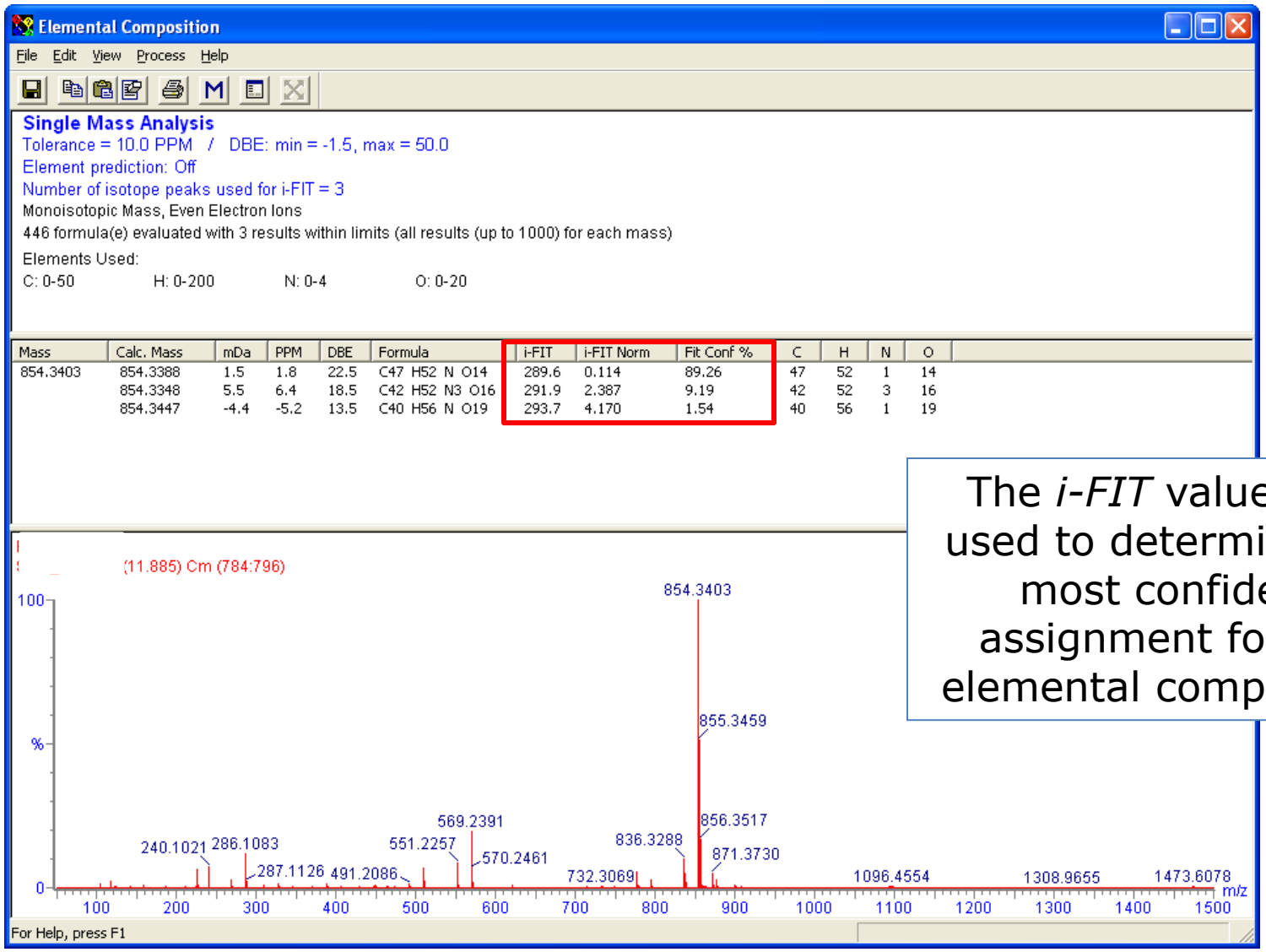
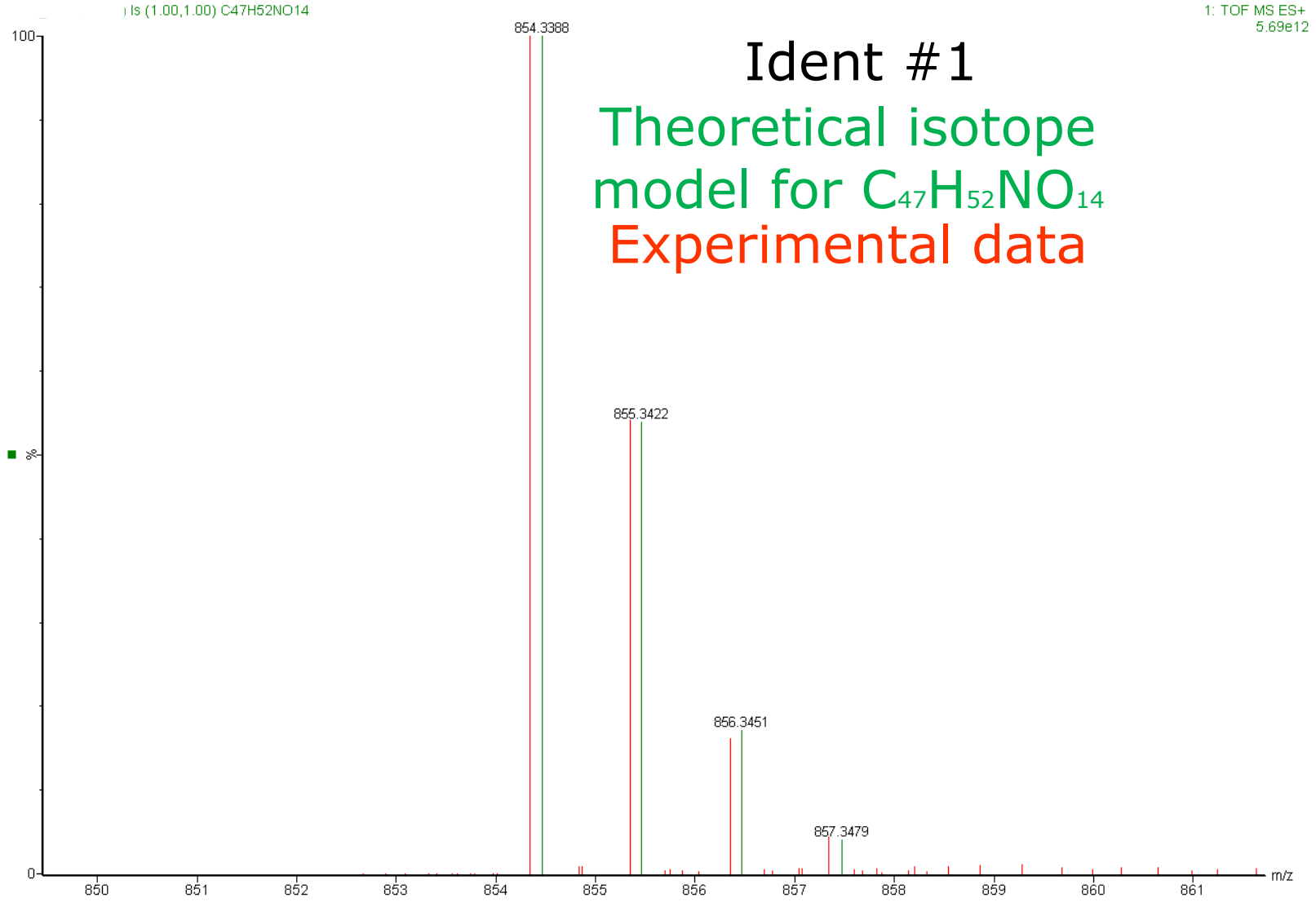


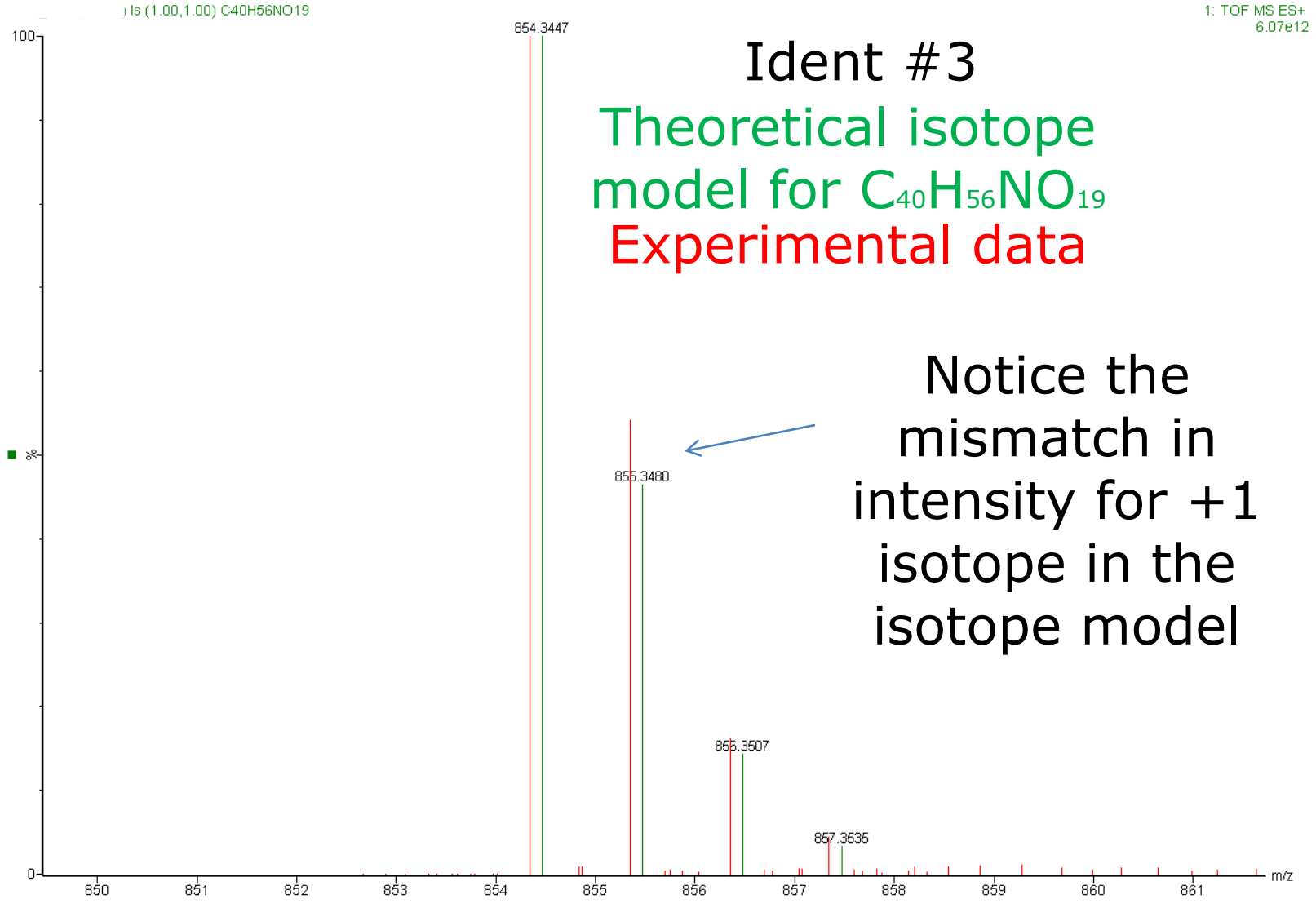
The *i-FIT* values are used to determine the most confident assignment for the elemental composition



Ident #1

Theoretical isotope model for $C_{47}H_{52}NO_{14}$

Experimental data



Ident #3

Theoretical isotope model for $C_{40}H_{56}NO_{19}$

Experimental data

Notice the mismatch in intensity for +1 isotope in the isotope model

Exact Mass Measurement
Elemental Composition – Isotopic Fit

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	without isotope abundance information					2% isotopic abundance accuracy	5% isotopic abundance accuracy
molecular mass [Da]	10 ppm	5 ppm	3 ppm	1 ppm	0.1 ppm	3 ppm	5 ppm
150	2	1	1	1	1	1	1
200	3	2	2	1	1	1	1
300	24	11	7	2	1	1	6
400	78	37	23	7	1	2	13
500	266	115	64	21	2	3	33
600	505	257	155	50	5	4	36
700	1046	538	321	108	10	10	97
800	1964	973	599	200	20	13	111
900	3447	1712	1045	345	32	18	196

***BMC Bioinformatics* 2006, 7:234 doi:10.1186/1471-2105-7-234**

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Metabolite ID and Drug Metabolism

Challenges of R&D in the Pharmaceutical Industry

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- Pharmaceutical R&D is a long, costly and risky activity. On average it takes 12 years to develop and market a NME

The price of innovation: new estimates of drug development costs

Joseph A. DiMasi [a,*], Ronald W. Hansen [b], Henry G. Grabowski [c]

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[b] *William E. Simon Graduate School of Business Administration, University of Rochester, Rochester, NY, USA*

[c] *Department of Economics, Duke University, Durham, NC, USA*

Received 17 January 2002; received in revised form 24 May 2002; accepted 28 October 2002

Abstract

The research and development costs of 68 randomly selected new drugs were obtained from a survey of 10 pharmaceutical firms. These data were used to estimate the average pre-tax cost of new drug development. The costs of compounds abandoned during testing were linked to the costs of compounds that obtained marketing approval. The estimated average out-of-pocket cost per new drug is US$ 403 million (2000 dollars). Capitalizing out-of-pocket costs to the point of marketing approval at a real discount rate of 11% yields a total pre-approval cost estimate of US$ 802 million (2000 dollars). When compared to the results of an earlier study with a similar methodology, total capitalized costs were shown to have increased at an annual rate of 7.4% above general price inflation.

Working with the Pharmaceutical Industry

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For a decade we have partnered with the pharmaceutical industry to bring meaningful impact

"In terms of efficiency for metabolite identification studies, the accurate mass LC/MS^E approach has provided significant gains...

...typical savings using the approach outlined in this paper have been in the range of 13 hours per molecule. As a result the capacity for conducting preliminary metabolite identification experiments has increased by almost an order of magnitude."

P. R. TILLER, *et al.*
Rapid Commun. Mass Spectrom.
2008; 22: 1053–1061

"UPLC with QTof and MetaboLynx XS provides an empowering platform for our metabolism scientists...With this complete workflow, we can routinely see more metabolites in a single run, and present a more definitive metabolic pathway picture for our clients in less time."

DR. DAVID JOHNSON,
DIRECTOR OF DMPK,
MICROCONSTANTS

The 9 biggest pharmaceutical companies worldwide* use Waters Metabolite Identification System Solution

*ranked by global prescription drug sales

Drug Metabolism Workflow to Maximize Productivity

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Start with the most complete dataset
Single injection UPLC-MS^E acquisition and automated fragment ion alignment

Only look at what's of interest
Chemically intelligent Mass Defect Filters and peak by peak control comparison

Identify all common *and* parent specific metabolites
Software prediction of Phase I dealkylations and common biotransformations

Automatically assign the site of metabolism
MassFragment Metabolite Localization automatically compares the parent and metabolite MS/MS spectrum to assign the site of biotransformation

Report all metabolites, reactive intermediates, inhibition assays with their rates and routes of formation
Quantitative and qualitative data reporting

A Typical Metabolite ID Experiment

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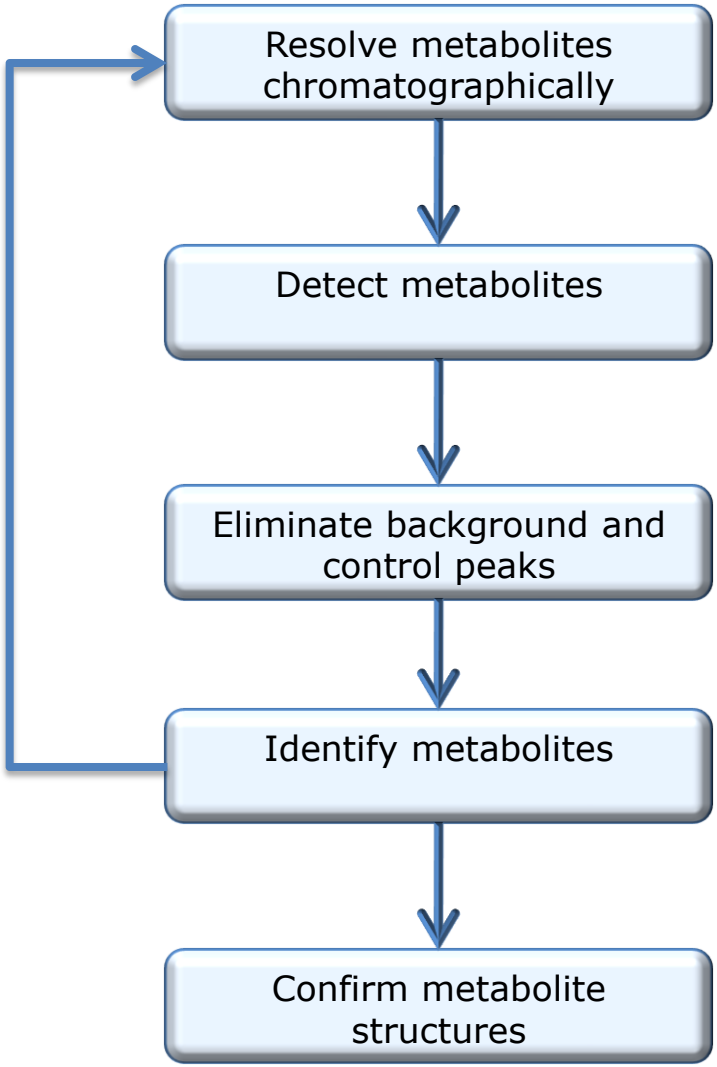

Full Scan MS
Targeted MS/MS or DDA
Precursor Ion Scan – QQQ
Neutral Loss Scan - QQQ

Analyte minus Control (Exact Mass?)
MDF if Exact Mass data

Combine data from multiple data files (instruments?)

Our Metabolite Identification WorkFlow

For in-vitro/in-vivo samples

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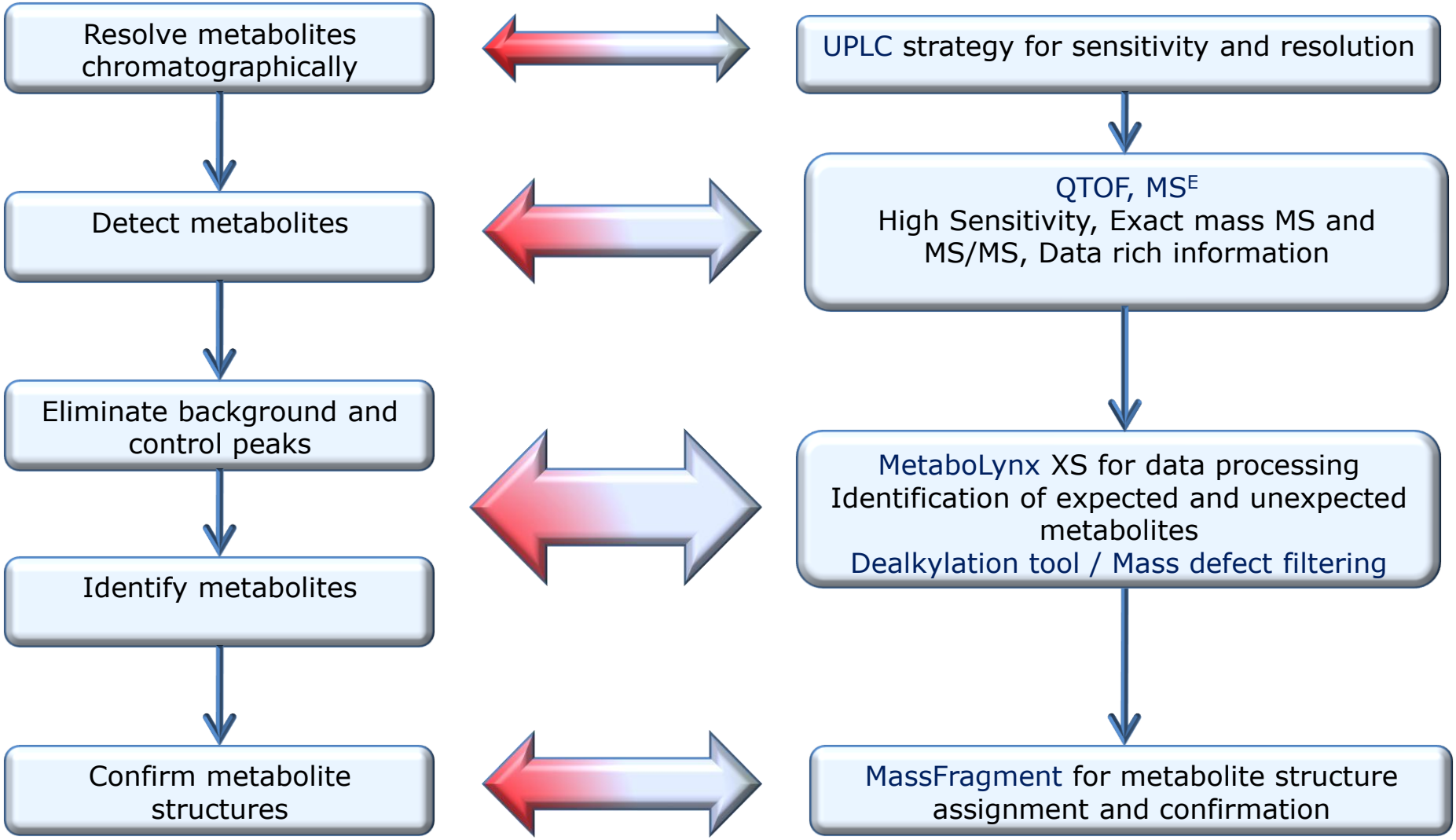

Metabolite ID Workflow to Maximize Productivity

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Analyze

ACQUITY UPLC
Maximum chromatographic resolution,sensitivity, and speed for MS-based studies

QTOF with MS^E
Exact mass analysis with data-rich information

Interpret

Chemically Intelligent data processing
Identification of expected & unexpected metabolites
Dealkylation / Mass Defect Filter tools

Tools for structural elucidation
Elecomp^E and MassFragment for comprehensive structural elucidation

GOAL = Rapid Confident Metabolite Identifications

Collect MS/MS of Parent Compound

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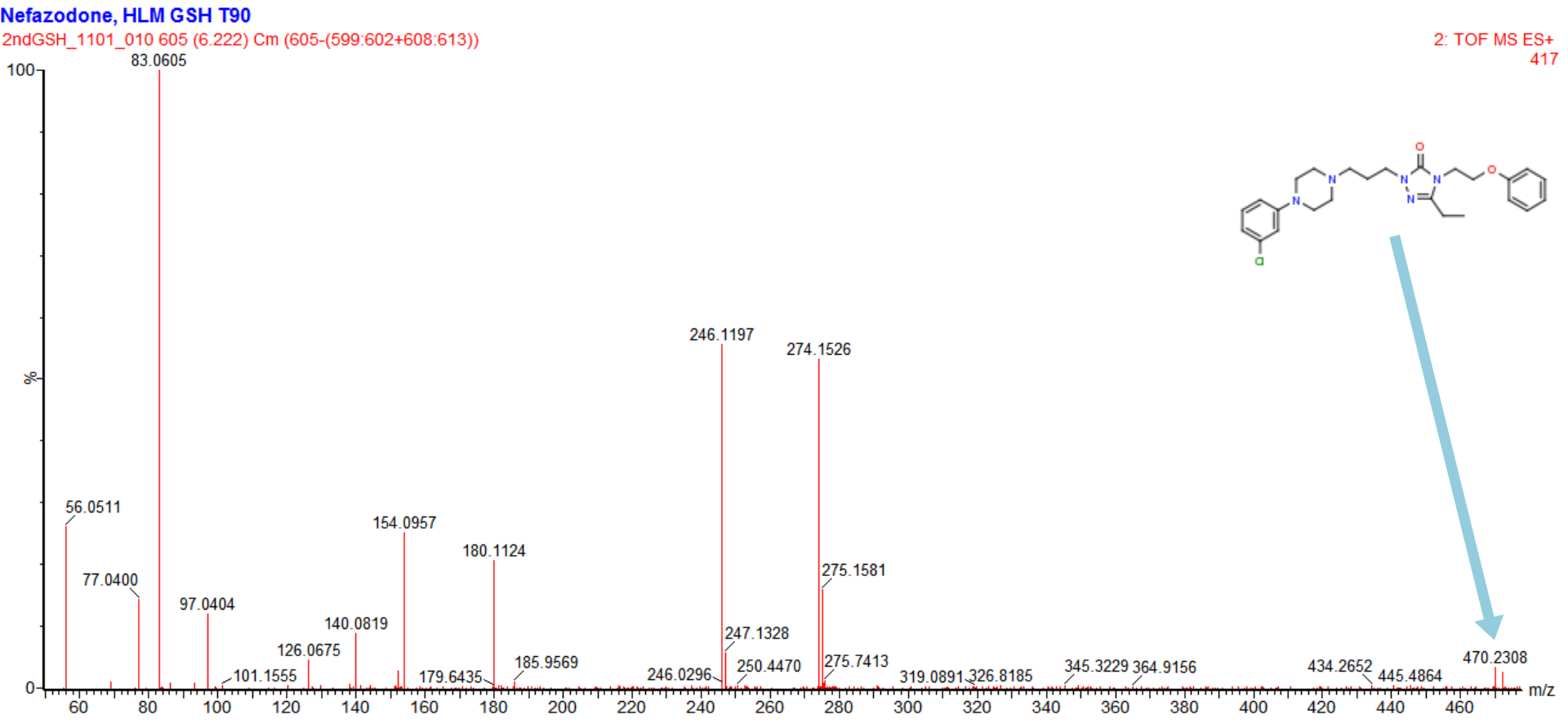

Confirmation of structures → Structure elucidation step

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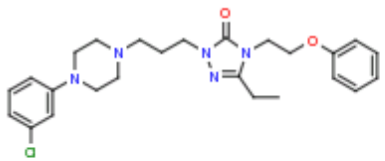
- The goal is to provide the most likely structure for the fragment ions generated and reduce the bottleneck in the processing and rationalization of structural fragment assignment

- This software algorithm assigns structures by taking fragment ion spectra of the drug or compound and using it to automatically calculate fragments based on a series of novel chemically intelligent algorithms

- This approach is based on systematic bond disconnection for the precursor structure instead of the usual 'rule based approach'

Submission

Structure	
Product ion(s) (Da)	55.9926 5 56.0237 2 56.0511 109 69.0095 4 77.0400 59 83.0605 417 83.7390 1 mode: +ve neutral -ve *n* 100 top ions (raw only) % 2 Int. cutoff (raw only) +/- 0.01 0.1 0.01 Filter no-structure results:
DBE	0 to 50
Electron count	odd: even: both:
Maximum H deficit	6
Fragment number of bonds	one: (fastest) two: three: four: (fast)
Scoring method	use SMARTS: use scoring function:
Scoring function parameters	phenyl: 8 aromatic: 6 multiple: 4 ring: 2 single: 1
General parameters	hetero modifier: 0.5 H-penalty: 0 max score: 16
Output order by	mass: intensity:

Submit

Systematic Bond Disconnection and Exact Mass - MassFragment

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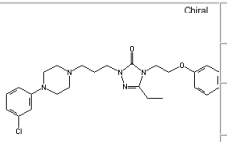
Report

Input:

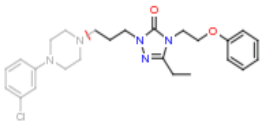
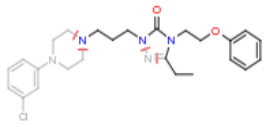
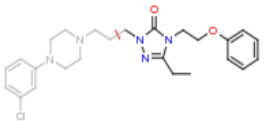
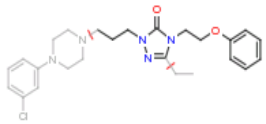


ID (job)	18
Mass (Da)	720.3128
Formula	$C_{37}H_{48}N_6O_5S_2$
DBE	17

Experiment:

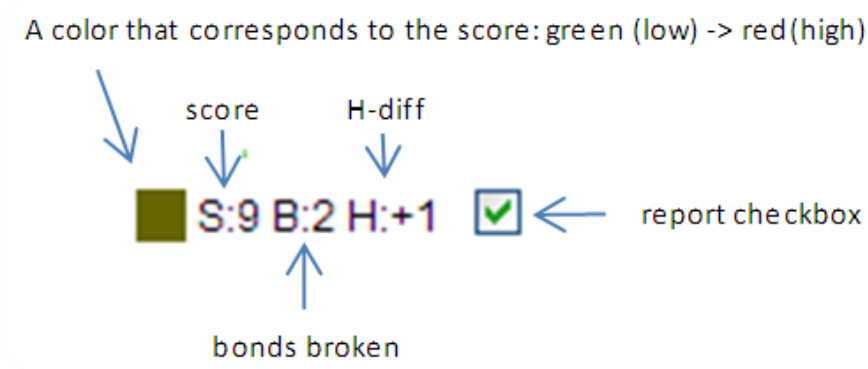
Product ion(s) (Da)	171.0973 197.0782 227.2023 268.1506 296.1432 426.1859 507.2465 533.2224 721.3160 +/- 0.01 in positive mode, structure filter on
DBE	0 to 50
Electron count	both
Maximum H deficit	6
Fragment number of bonds	4
Scoring	aromatic: 6, multiple: 4, ring: 2, phenyl: 8, other: 1 H-deficit: 0, hetero modifier: 0.5, max score: 16
Order:	intensity
Plot:	show ○ hide ◉

Results:

Daughter (Da)	Mass (Da)	Mass error (mDa)	Formula	DBE	ΔFormula	Structure(s), score & H-deficit
274.1545	274.1556	-1.1	$C_{15}H_{20}N_3O_2$	7.5	$C_{10}H_{13}N_2Cl$	 S:0.5 B:1 H:0 ☐  S:6 B:4 H:0 ☐
246.1247	246.1243	+0.4	$C_{13}H_{16}N_3O_2$	7.5	$C_{12}H_{17}N_2Cl$	 S:1 B:1 H:0 ☐  S:1.5 B:2 H:+1 ☐

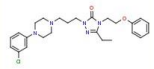
Results

Nomenclature explanation



Report

Input:

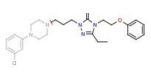
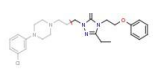
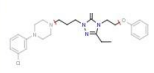
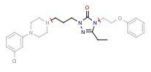
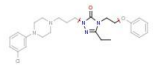


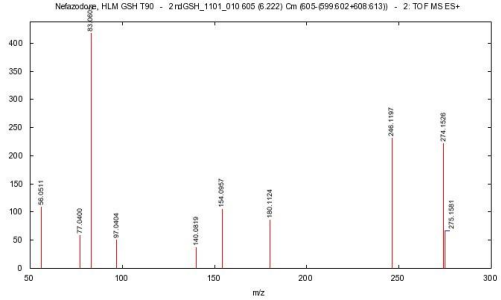
ID (job)	38
Mass (Da)	469.2245
Formula	$C_{25}H_{32}N_5O_2Cl$
DBE	12

Experiment:

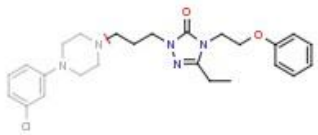
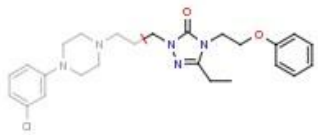
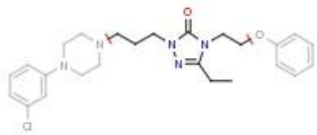
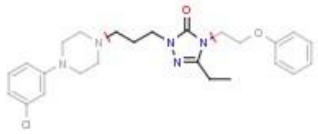
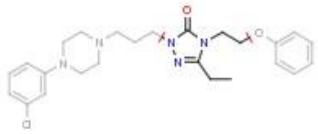
Product ion(s) (Da)	140.0819 154.0957 180.1124 246.1197 274.1526 275.1581 56.0511 77.0400 83.0605 97.0404 +/- 0.01 in positive mode, structure filter 1
DBE	0 to 50
Electron count	both
Maximum H deficit	6
Fragment number of bonds	4
Scoring	aromatic: 6, multiple: 4, ring: 2, phenyl: 8, other: 1 H-deficit: 0, hetero modifier: 0.5, max score: 16
Order:	mass
Plot:	show ◉ hide ○
Files:	DMX CSV

Results:

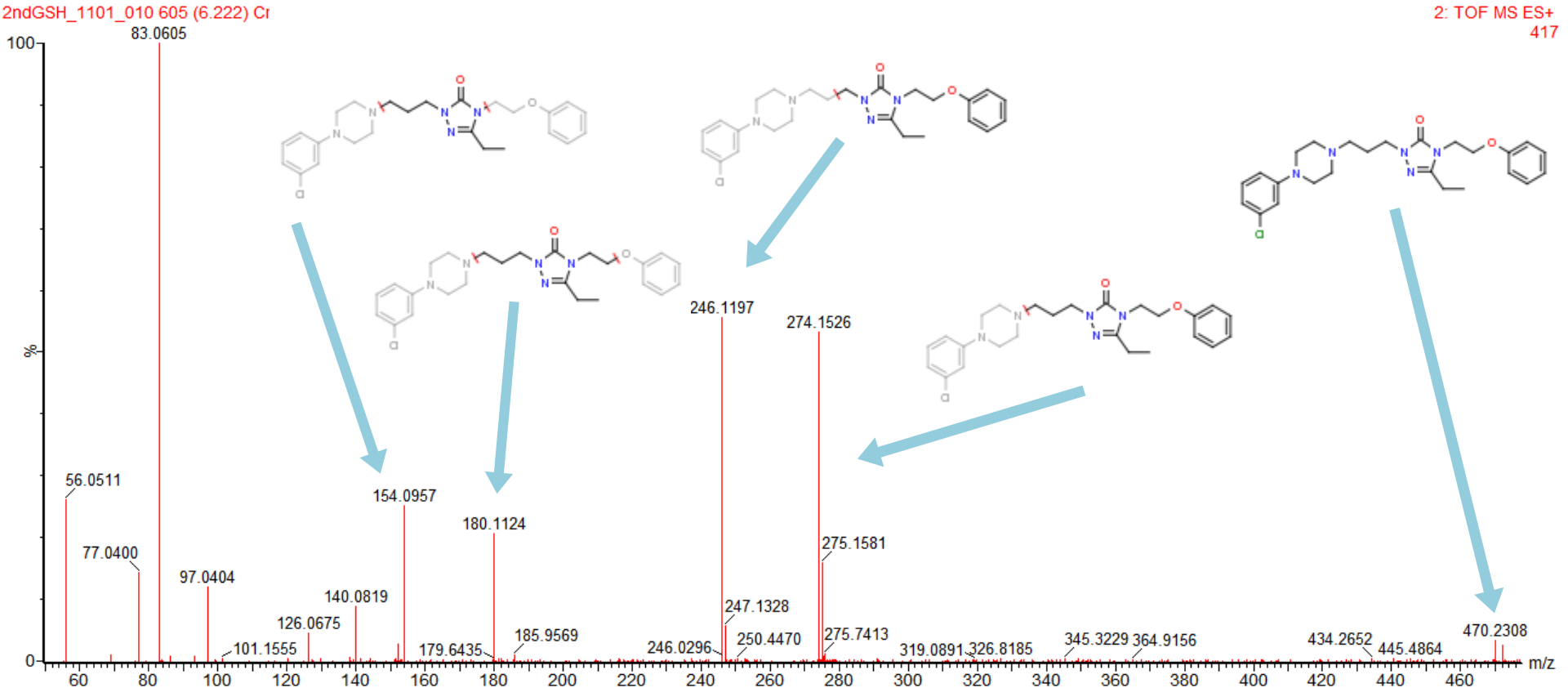
274.1526 ¬+ (+0H)	246.1197 ¬+ (+0H)	180.1124 ¬+ (-1H)
		
274.1556 (-3.0.mDa) (S:0.5, B:1) $C_{15}H_{20}N_3O_2$ (-$C_{10}H_{13}N_2Cl$)	246.1243 (-4.6.mDa) (S:1.0, B:1) $C_{13}H_{16}N_3O_2$ (-$C_{12}H_{17}N_2Cl$)	180.1137 (-1.3.mDa) (S:1.0, B:2) $C_9H_{14}N_3O$ (-$C_{16}H_{19}N_2OCl$)
154.0957 ¬+ (+1H)	**140.0819** ¬+ (+1H)	
		
154.0980 (-2.3.mDa) (S:1.0, B:2) $C_7H_{12}N_3O$ (-$C_{18}H_{21}N_2OCl$)	140.0824 (-0.5.mDa) (S:1.0, B:2) $C_6H_{10}N_3O$ (-$C_{19}H_{23}N_2OCl$)	



Results:

274.1526 ¬+ (+0H)	246.1197 ¬+ (+0H)	180.1124 ¬+ (-1H)
		
274.1556 (-3.0.mDa) (S:0.5, B:1) $C_{15}H_{20}N_3O_2$ (-$C_{10}H_{13}N_2Cl$)	246.1243 (-4.6.mDa) (S:1.0, B:1) $C_{13}H_{16}N_3O_2$ (-$C_{12}H_{17}N_2Cl$)	180.1137 (-1.3.mDa) (S:1.0, B:2) $C_9H_{14}N_3O$ (-$C_{16}H_{19}N_2OCl$)
154.0957 ¬+ (+1H)	**140.0819** ¬+ (+1H)	
		
154.0980 (-2.3.mDa) (S:1.0, B:2) $C_7H_{12}N_3O$ (-$C_{18}H_{21}N_2OCl$)	140.0824 (-0.5.mDa) (S:1.0, B:2) $C_6H_{10}N_3O$ (-$C_{19}H_{23}N_2OCl$)	

Nefazodone, HLM GSH T90



Acquiring a Comprehensive MS and MS/MS Data Set

Conventional Data Directed LC-MS/MS

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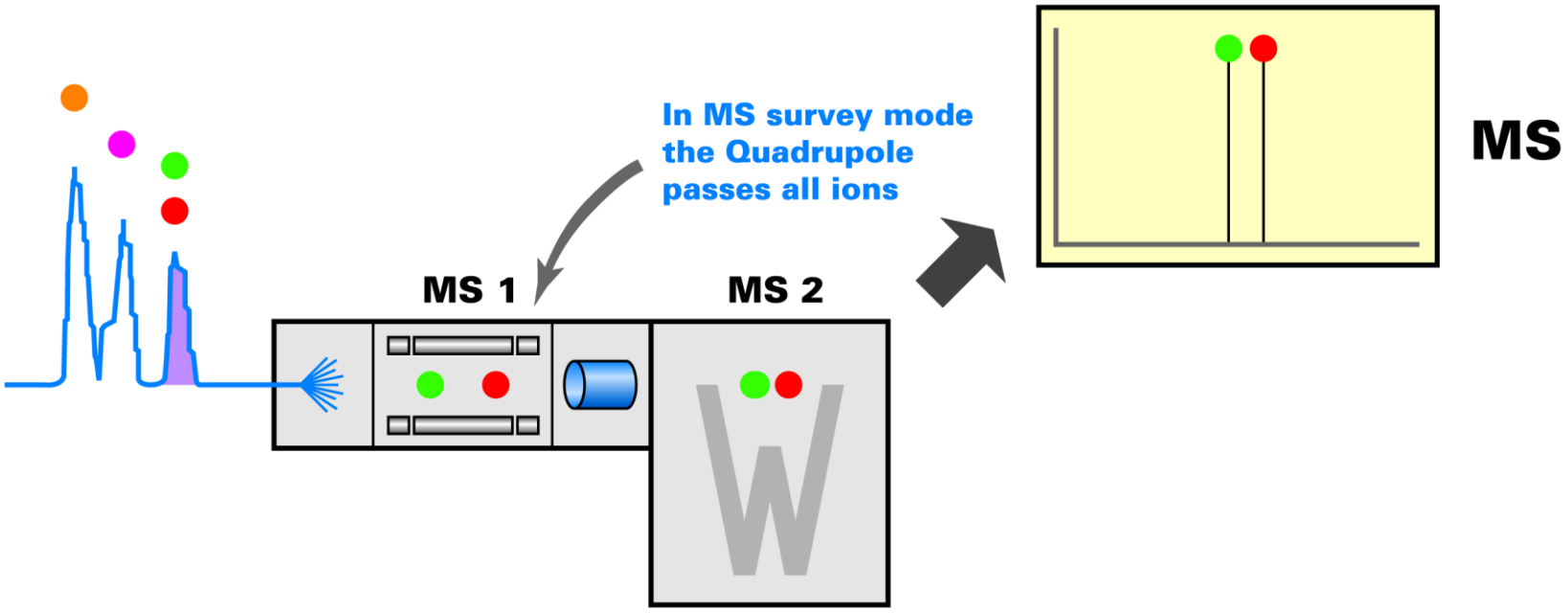

Precursor Survey Scan

Conventional Data Directed LC-MS/MS

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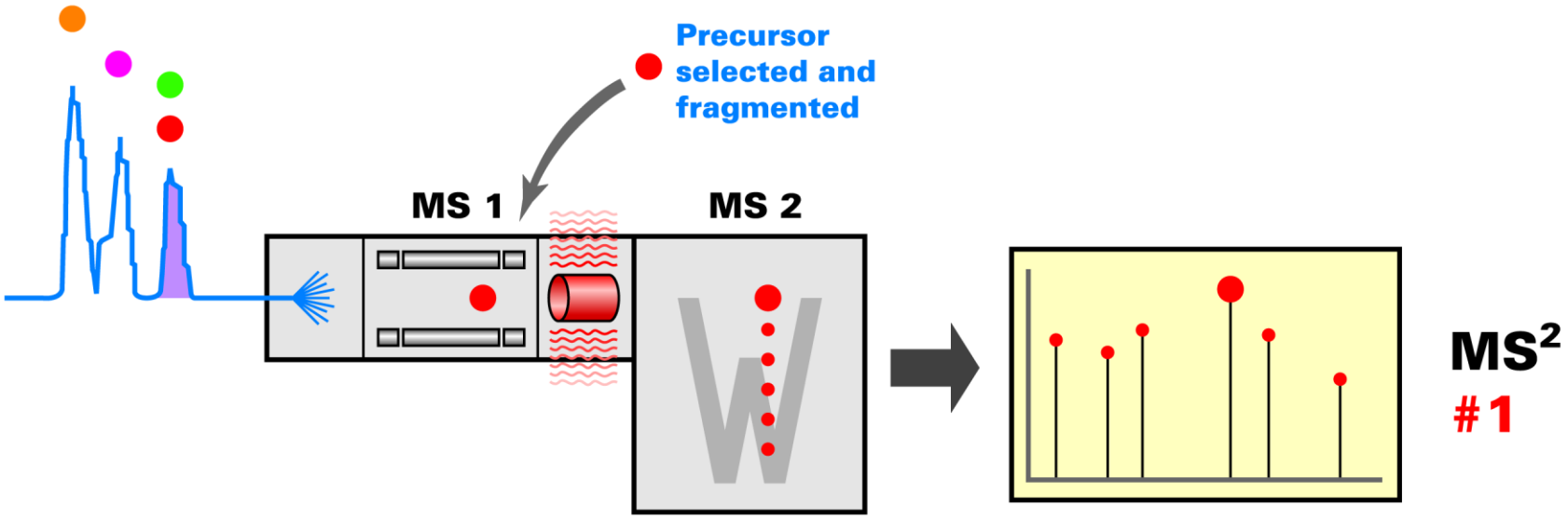

Product Ion Scan

Precursor Ion Selection

Which one(s)?

Ion Transmission Window +/- 2 Da

Product Ion Spectrum
Typically very fast (.1-1 second)

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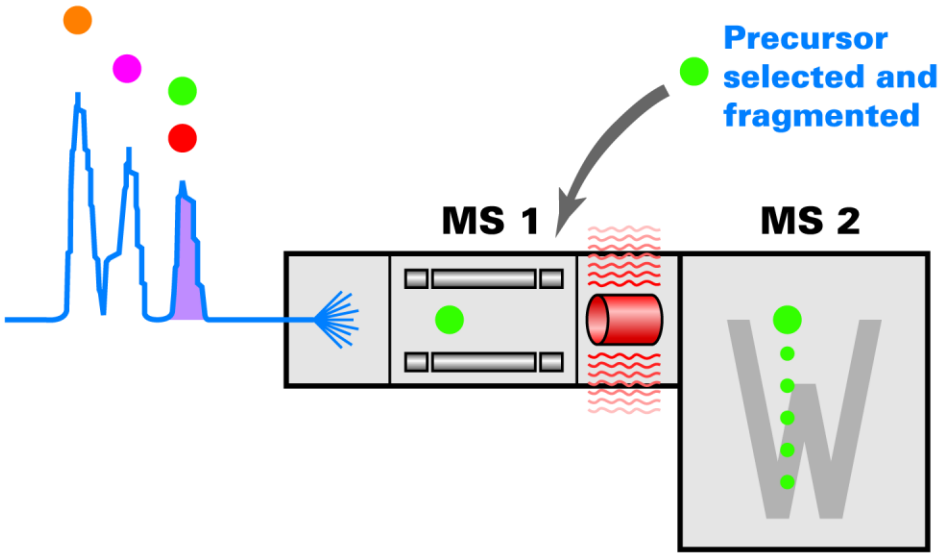

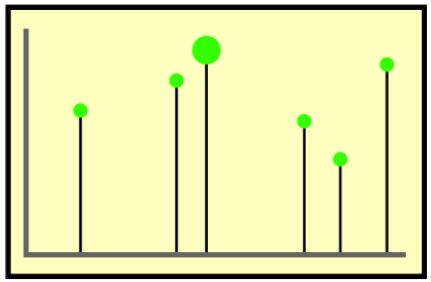

MS^2
#2

Next Product Ion Scan….

Data Directed Analysis LC-MS/MS

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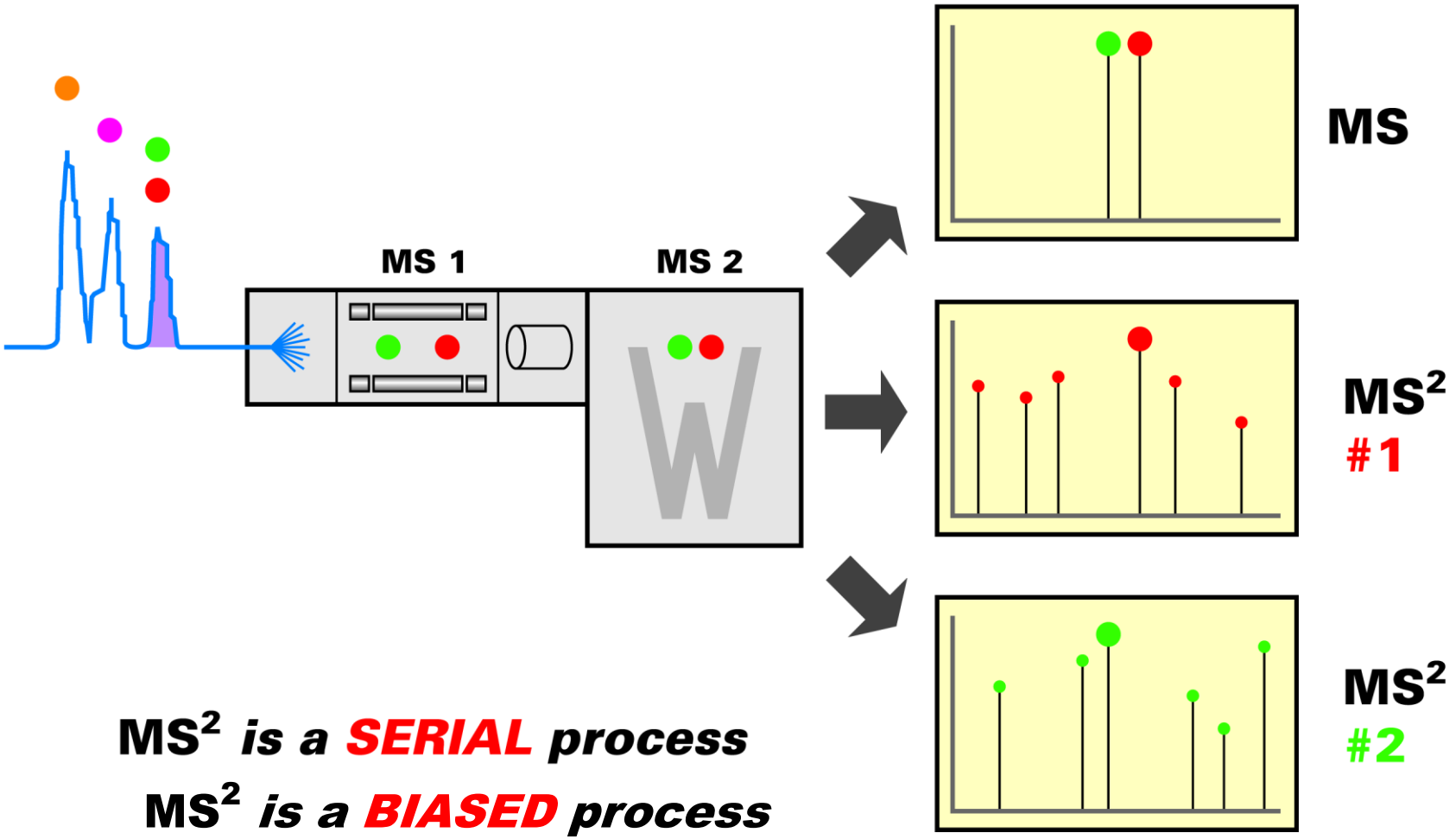

MS² *is a SERIAL process*

MS² *is a BIASED process*

MS² *is a DISCONTINUOUS process*

Alternate Scanning LC-MS (LC-MS^E)

...*monitor precursors*

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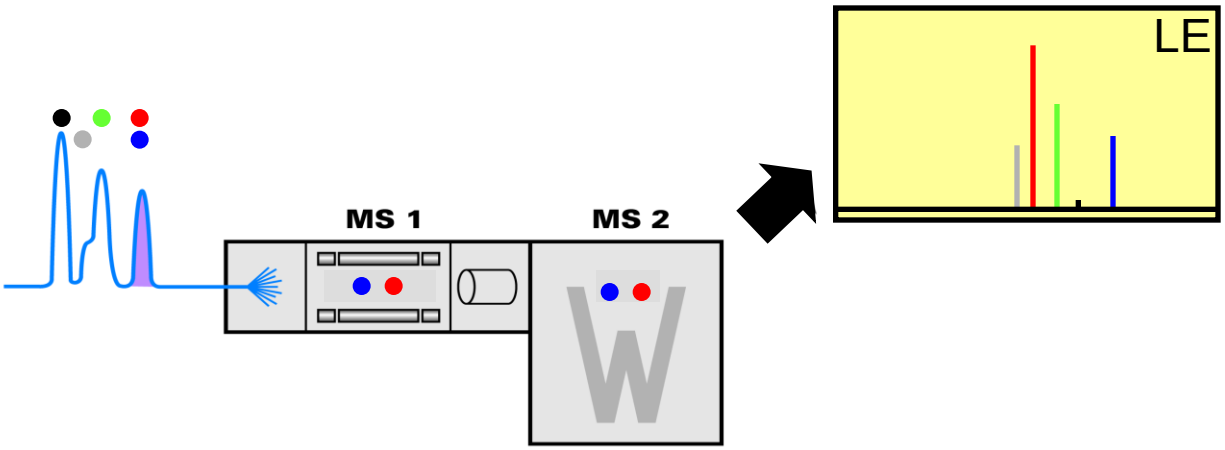

Alternate Scanning LC-MS (LC-MS^E)

…monitor fragments (no pre-selection)

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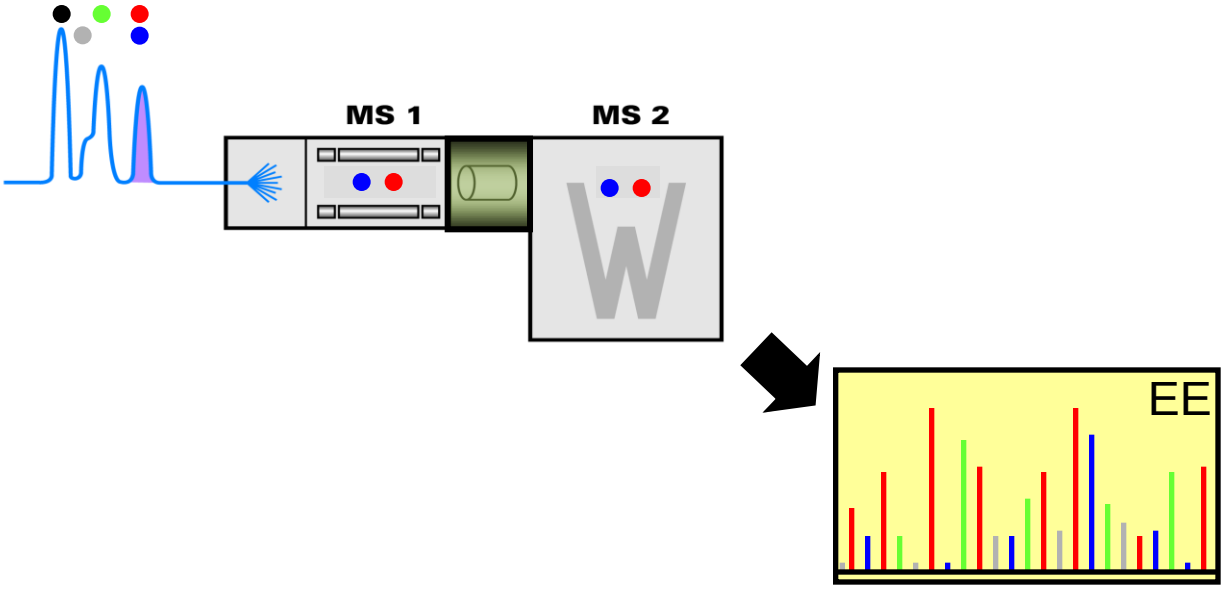

Alternate Scanning LC-MS (LC-MS^E)

...precursor and fragment profiles

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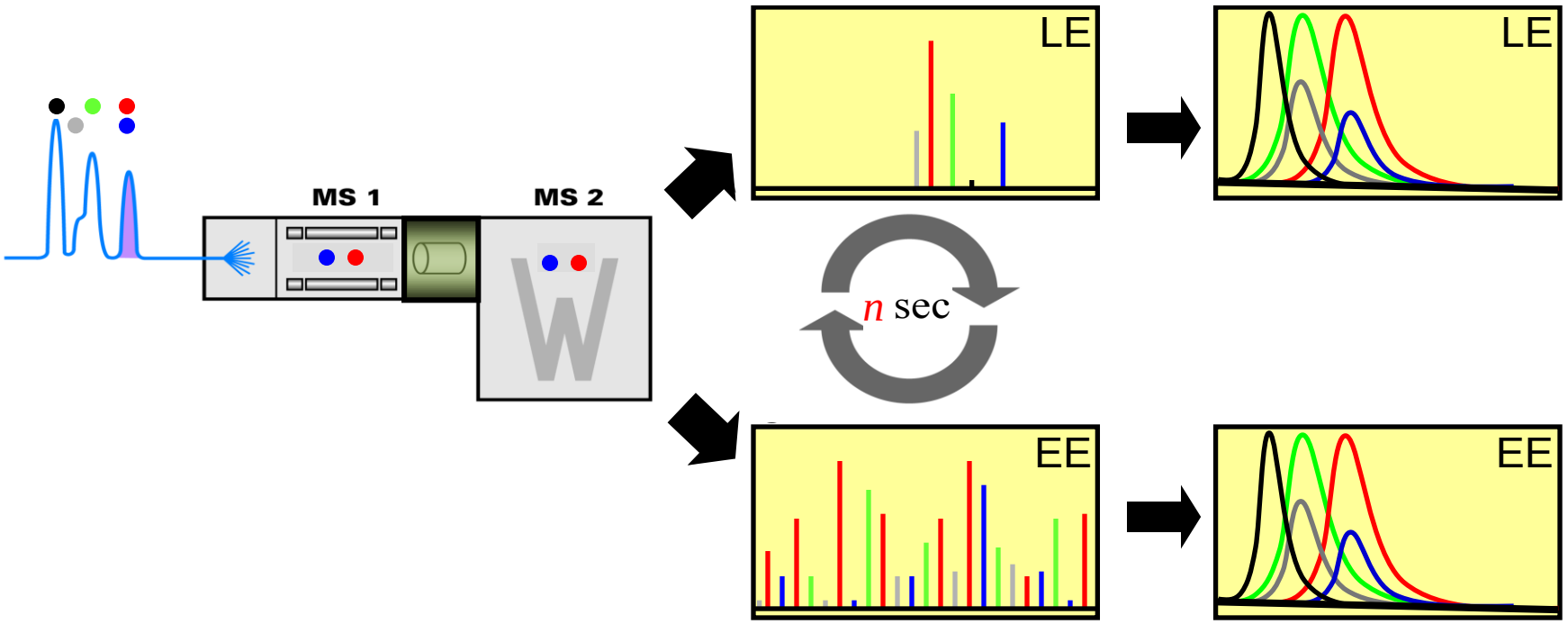
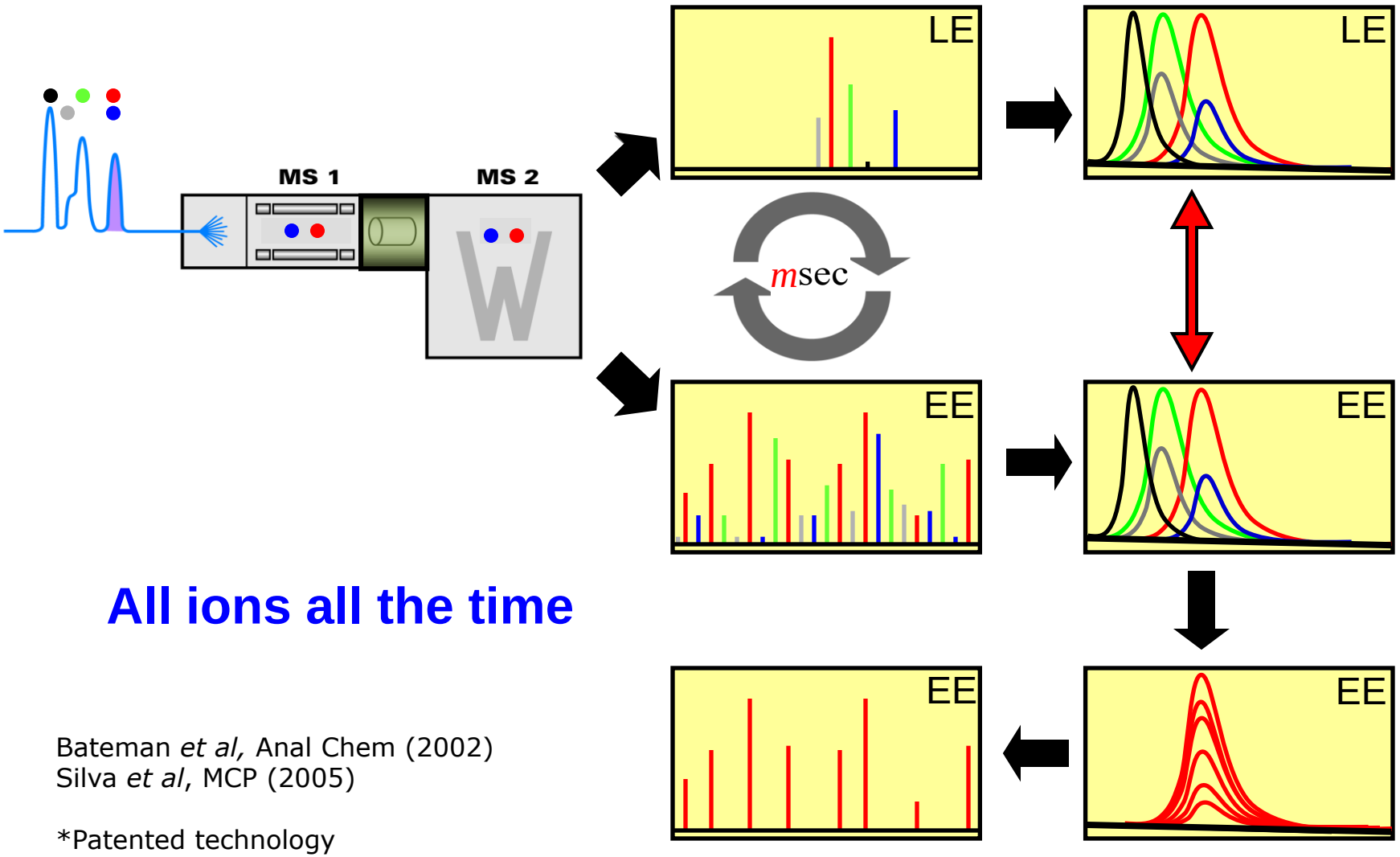


Alternate Scanning LC-MS (LC-MS^E)

…time resolved mass measurements



All ions all the time

Bateman *et al,* Anal Chem (2002)
Silva *et al*, MCP (2005)

*Patented technology

Metabolite ID Workflow to Maximize Productivity

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Analyze

ACQUITY UPLC
Maximum chromatographic resolution,sensitivity, and speed for MS-based studies

QTOF with MS^E
Exact mass analysis with data-rich information

Interpret

Chemically Intelligent data processing
Identification of expected & unexpected metabolitesDealkylation / Mass Defect Filter tools

Tools for structural elucidation
Elecomp^E and MassFragment for comprehensive structural elucidation

GOAL = Rapid Confident Metabolite Identifications

Mass Defect Filters

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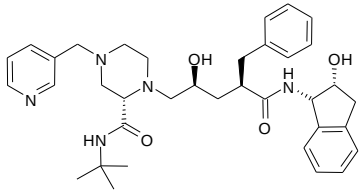
- Widely applied to chromatographic data sets by Zhang et al, Bateman et al.
  - Integral part of many LC-based metabolite ID workflows
- MetaboLynx XS chemically intelligent MDF that incorporates novel, structure-based dealkylations

Indinavir



$C_{36}H_{48}N_5O_4$

MH+ = 614. **3706**

Significance of exact mass filtering

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Biotransformation	Nominal mass	Accurate mass	Decimal Place shift
+O	+16	+ 15.9949	- 0.0051
+O_2	+32	+ 31.9898	- 0.0102
-H_2	-2	- 2.0157	- 0.0157
-CH_2	-14	- 14.0157	- 0.0157
-Cl+O	-18	- 17.9662	+0.0338
+C_2H_2O	+42	+ 42.0106	+0.0106
+SO_3	+80	+ 79.9568	- 0.0432
+$C_6H_8O_6$	+176	+176.0321	+0.0321
+$C_6H_8O_7$	+192	+192.0270	+0.0270
+$C_2H_5NO_2S$	+107	+107.0042	+0.0042
+$C_{10}H_{15}N_3O_6S$	+305	+305.0682	+0.0682

Phase1 Metabolism < 0.04

Phase2 Metabolism < 0.07

*The mass shift may be larger if a compound undergoes O-Dealkylation or N-Dealkylation

Mass defect filtering for full scan MS

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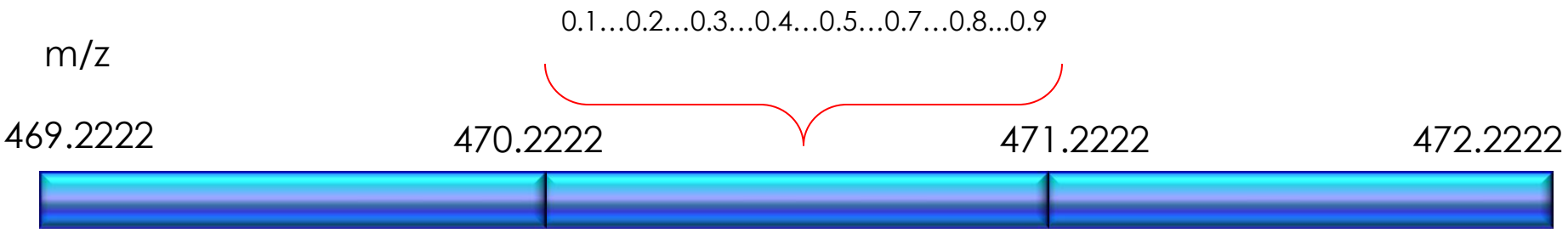
Compound A : m/z = 471.2222

TIC : No Filter



TIC : Filter 70 mDa

470.1872 – 470.2572

Mass Defect Filtering
Filtering out false positives

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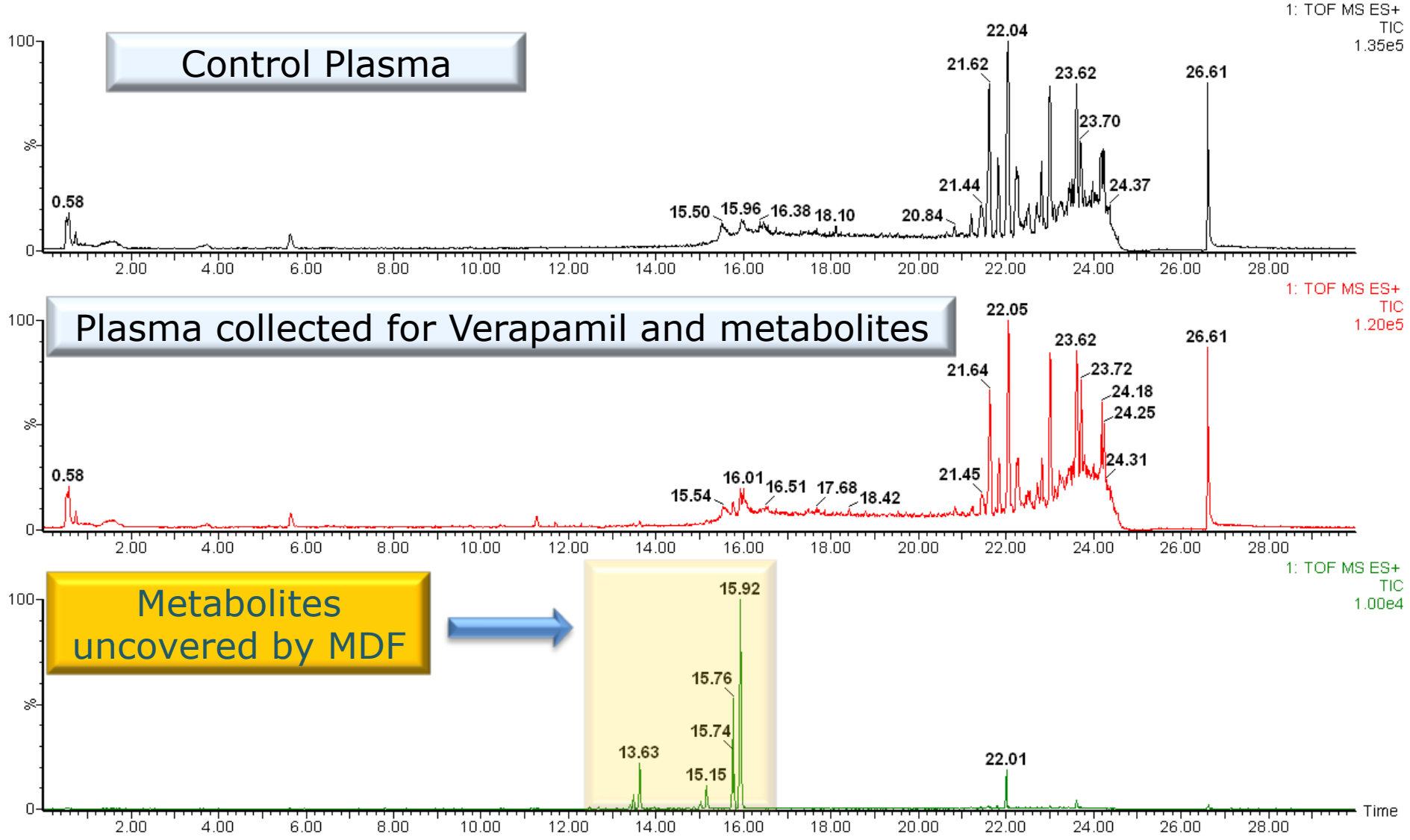

Risks involved in setting up the MDF

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- It is not a linear relationship with mass!
- Fixed linear MDF difficult to automate because risk of metabolic cleavages
- Depends whether S, Cl or Br present
- We can miss important metabolites if filters are not set-up correctly → false negatives
- The C-Heteroatom tool is key to provide the 'correct MDF's

Dealkylated metabolites → Linear Mass Defect for Verapamil

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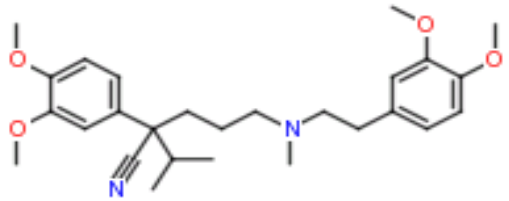
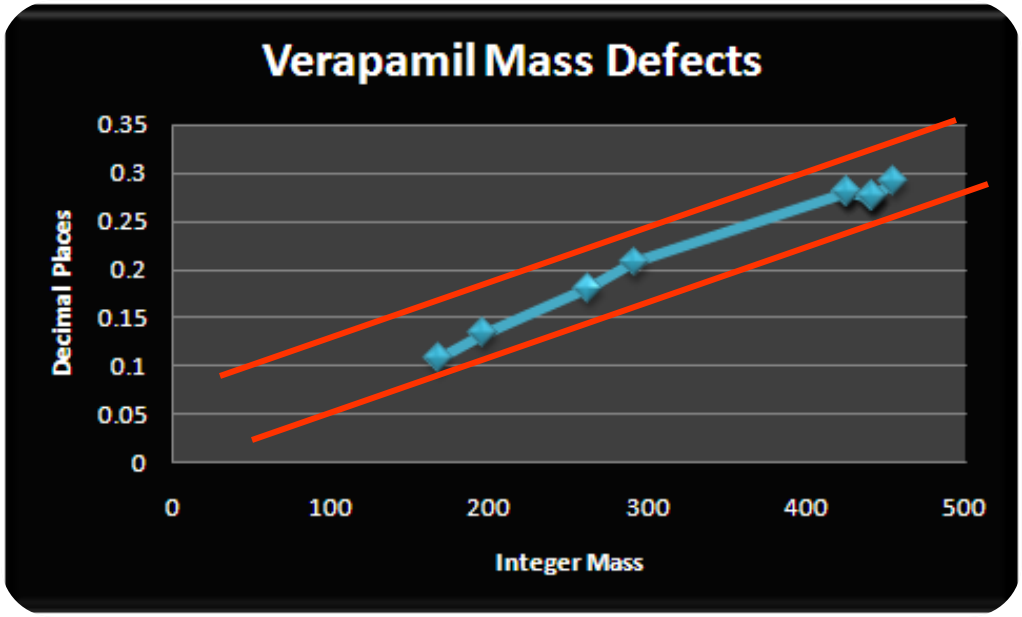


ID (job)	155
Mass (Da)	454.2832
Formula	$C_{27}H_{38}N_2O_4$
DBE	10

-	none
R_12: -C17H24N2O2	167.0994
R_11: -C16H21NO2	196.1260
R_10: -C11H15NO2	262.1729
R_9: -C10H12O2	291.1994
R_8: -CH2O	425.2726
R_7: -CH2O	425.2726
R_6: -CH2O	425.2726
R_5: -CH2O	425.2726
R_4: -CH2	441.2675
R_3: -CH2	441.2675
R_2: -CH2	441.2675
R_1: -CH2	441.2675
R_0: -CH2	441.2675
none	455.2832

Linear Relationship integer mass vs. decimal places



Dealkylated metabolites → Non-Linear Mass Defect for Glyburide

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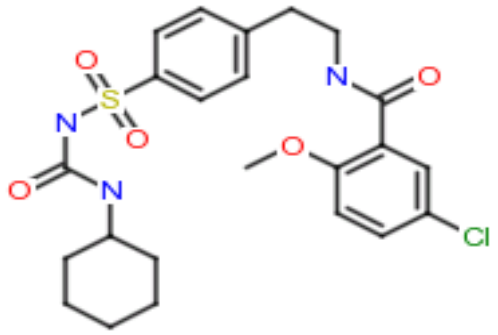
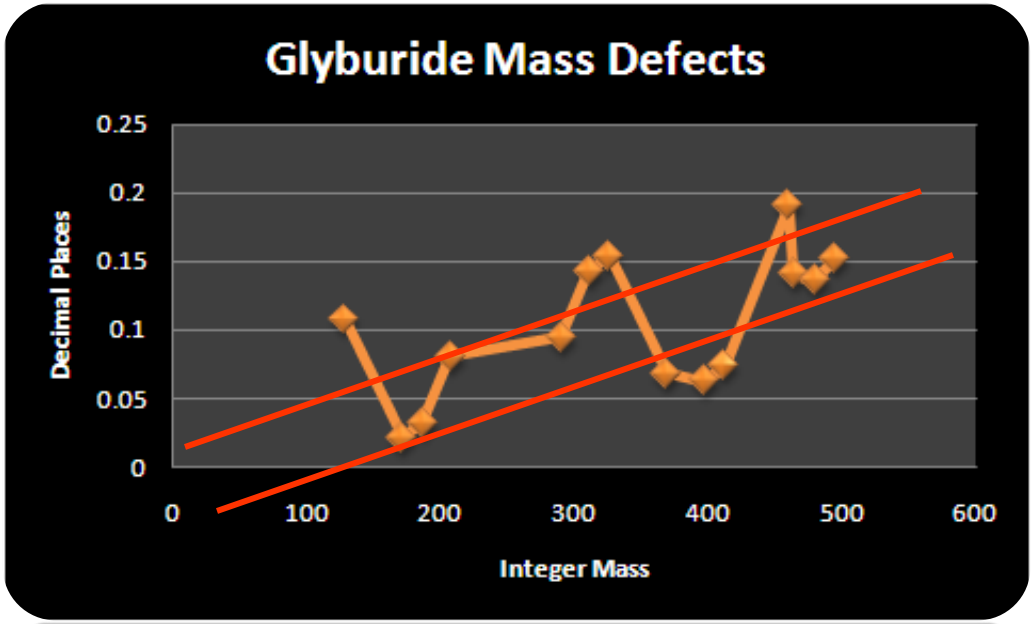


ID (job)	156
Mass (Da)	493.1438
Formula	$C_{23}H_{28}N_3O_5SCl$
DBE	11

-	none
R_12: -C16H15N2O4SCl	128.0997
R_11: -C15H21N3O3S	171.0134
R_10: -C15H20N2O3S	186.0243
R_9: -C16H14NO2Cl	207.0725
R_8: -C7H12N2O3S	290.0869
R_7: -C8H6NO2Cl	311.1351
R_6: -C8H5O2Cl	326.1460
R_5: -C7H11NO	369.0597
R_4: -C6H11N	397.0546
R_3: -C6H10	412.0655
R_2: -Cl+H	460.1827
R_1: -CH2O	464.1332
R_0: -CH2	480.1281
none	494.1438

Non-Linear Relationship integer mass vs. decimal places



Linear Fixed MDF vs. Intelligent MDF

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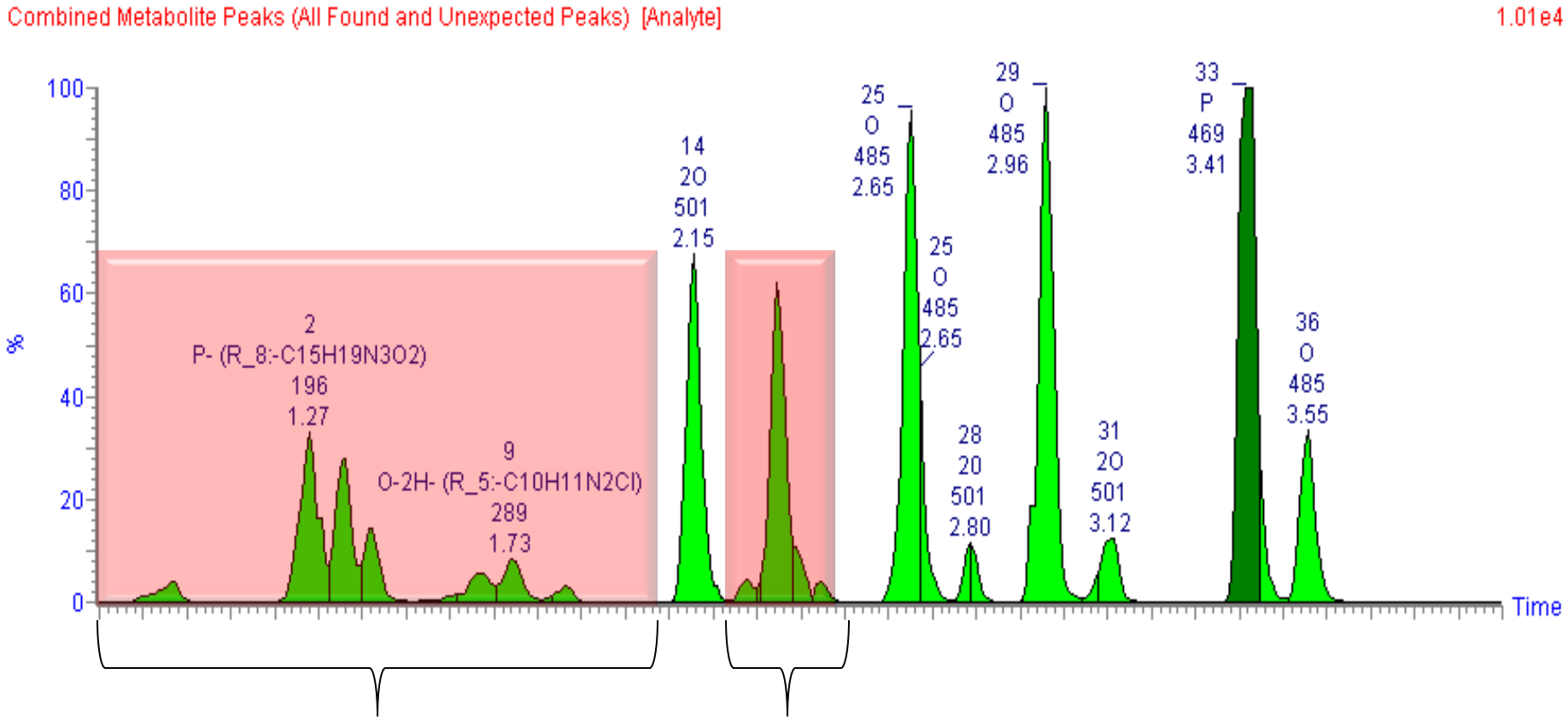

Metabolic cleavages detected by Intelligent MDF

Parent compound fragment ion characterization with Metabolynx, MS^E & MassFragment

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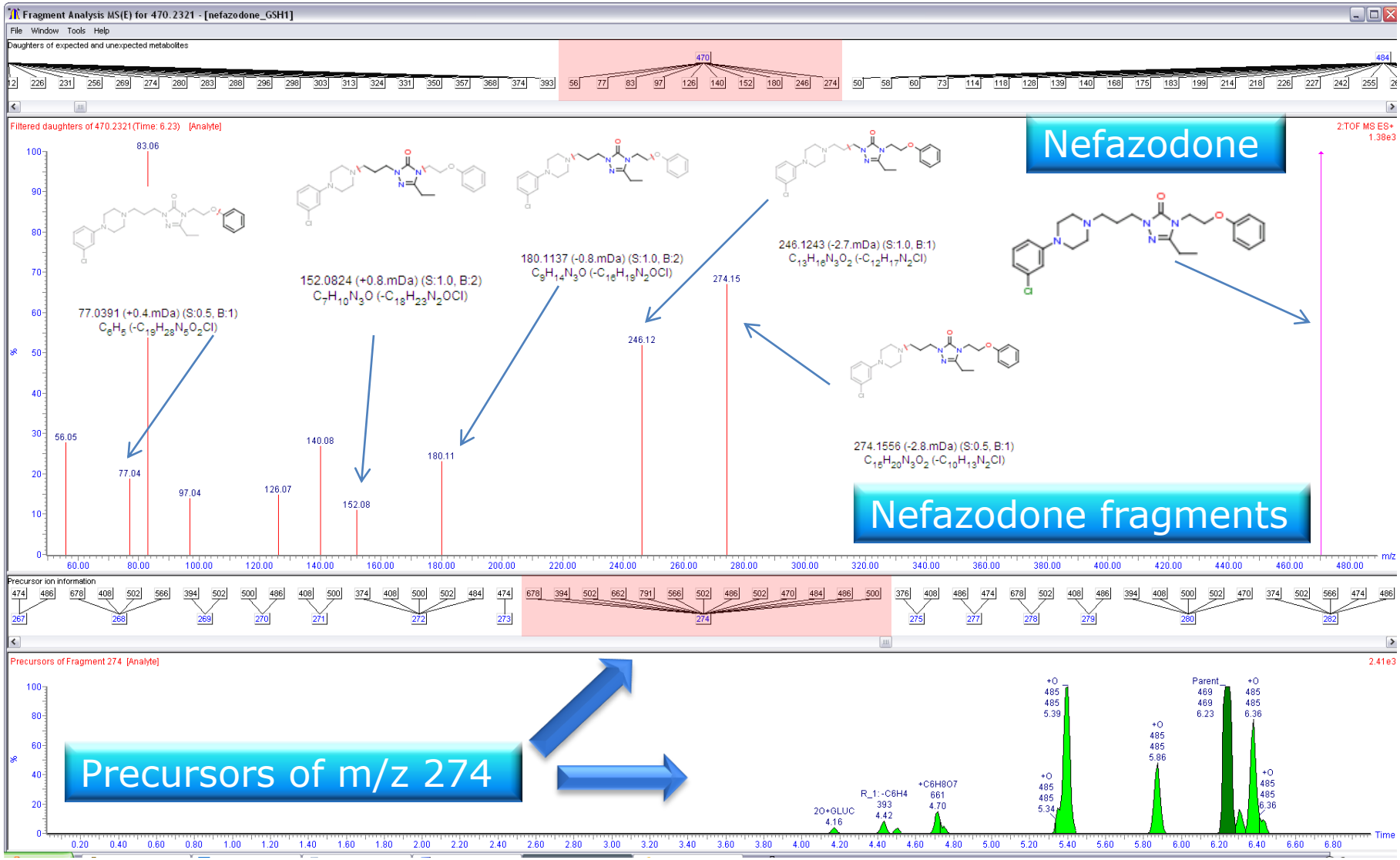
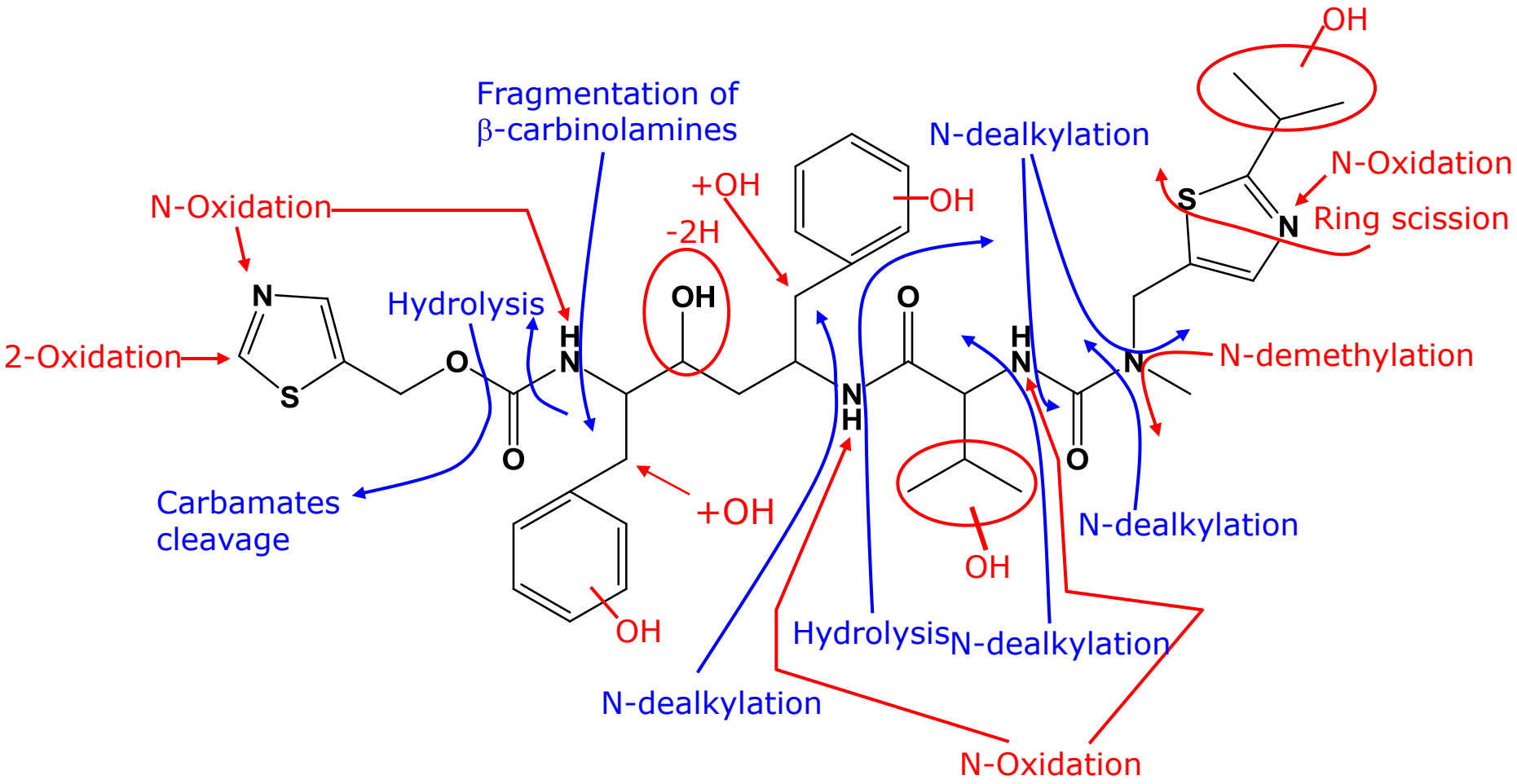


Dealkylations and other Biotransformations



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IsoCount Metabolite Localization

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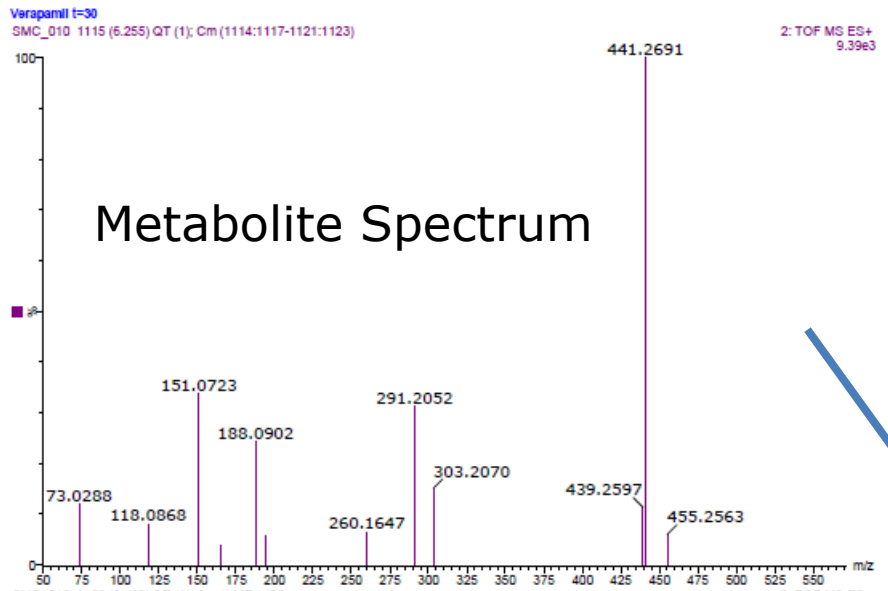
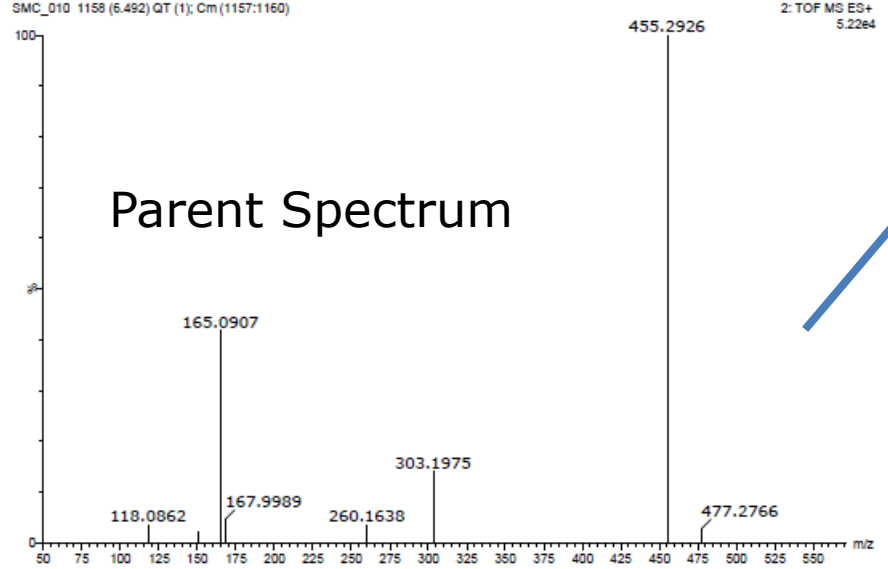
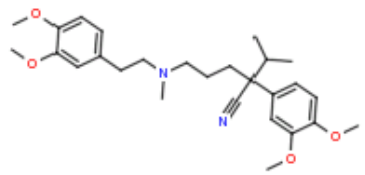


Metabolite Spectrum



Parent Spectrum

IsoCount - Submission

Structure	
Biotransform	-90.0468 --- "-C7-H6" -78.9105 --- "-Br+H" -67.9984 --- "-C-F3+H" -67.9222 --- "-Cl2+H2" -61.9156 --- "-Br+H+O"
Metabolite ion(s) (Da)	73.0288 1119 118.0868 748 151.0723 3177 188.0902 2285
Parent ion(s) (Da)	118.0862 1776 150.0663 1109 165.0907 21823 167.9989 2328
Mode	positive negative
Maximum H deficit	4
Mass Tolerance (mDa)	10
Fragment number of bonds	one: (fastest) two:
Scoring method	use SMARTS:
General parameters	hetero modifier: 0.5 H-penalty: 0
Result parameters	show all show results for plausible biotransforms Order fragments by mass intensity

Submit

Spectral Peak Match Mirror Plot

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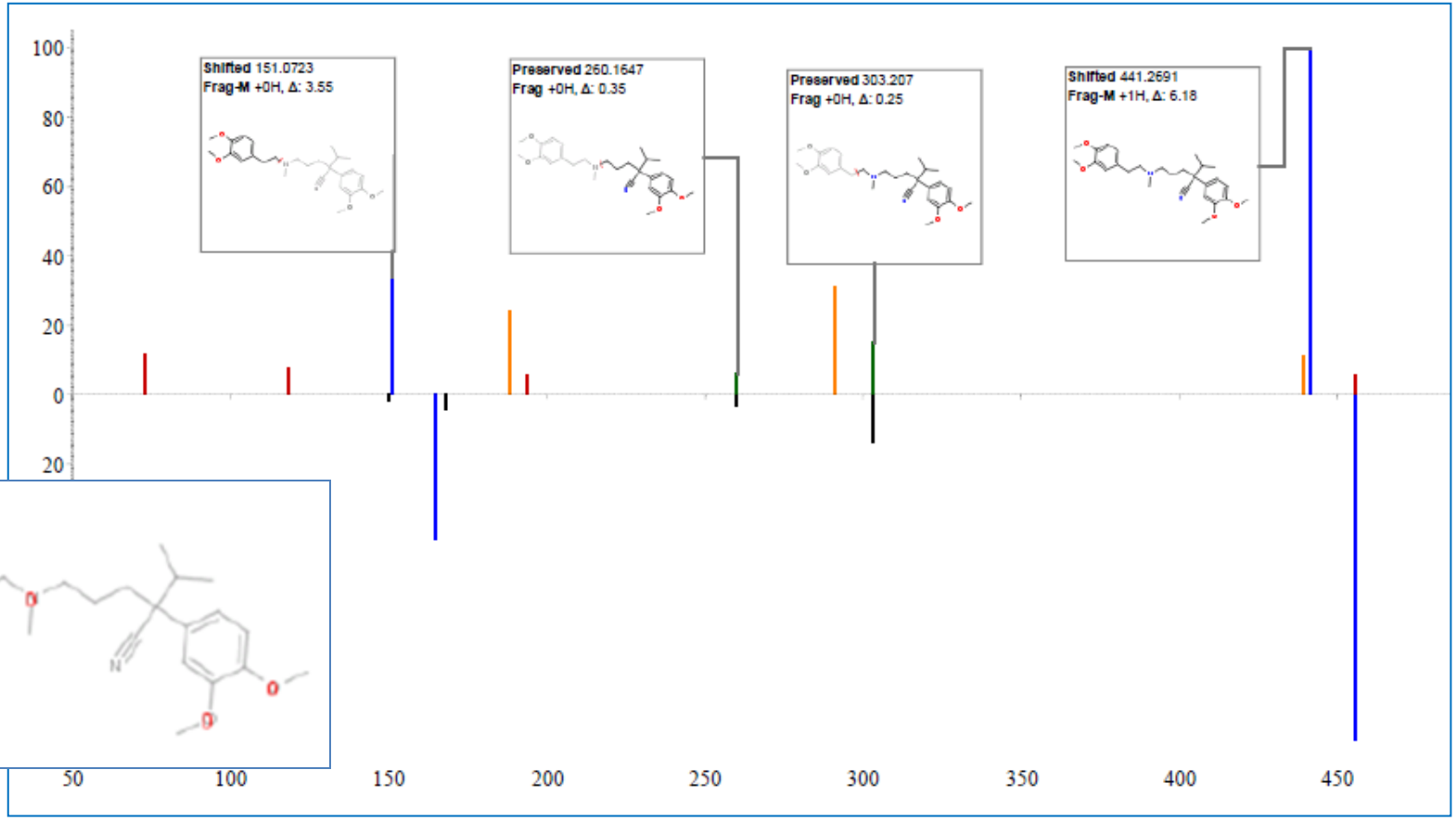
Metabolite Spectrum

Preserved
Shifted
New
Unmatched



Parent Spectrum

Viewing Quantitative Data

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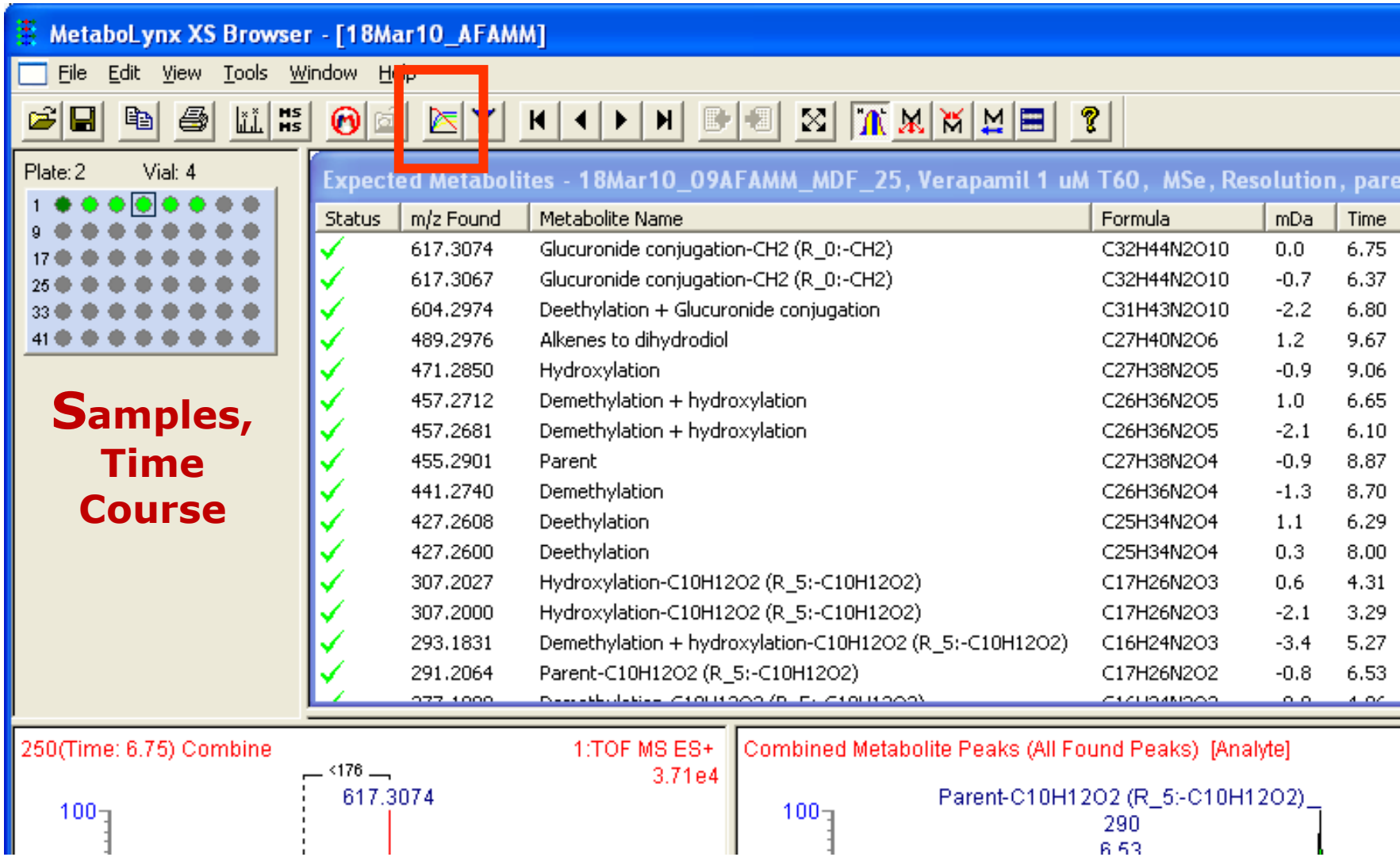

Accessible Quantitative Analysis

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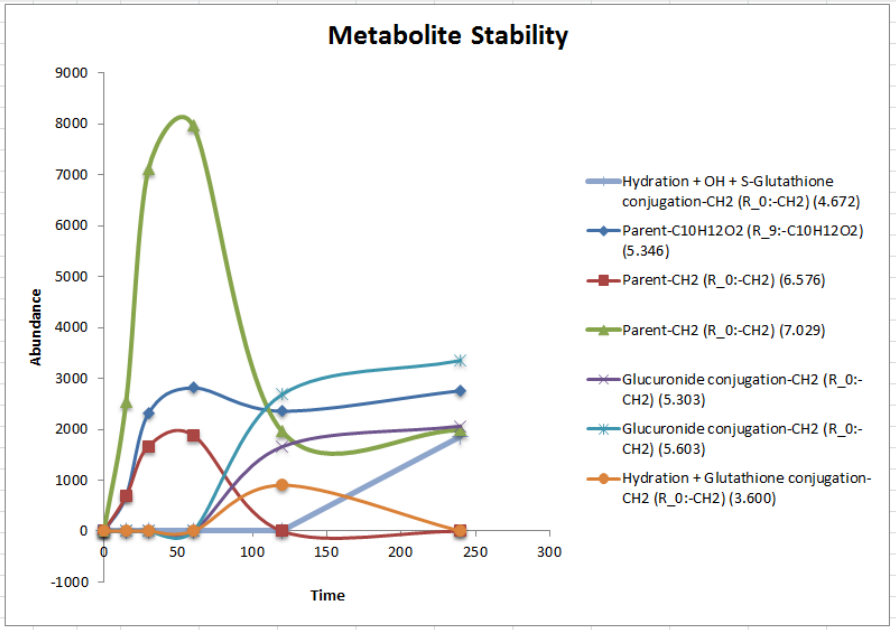
	A	B	C	D	E	F	G
1	Metabolite Stability Analysis						
2	Exported Sun Sep 19 13:03:43 2010						
3							
4	C27H38N2O4 half life 0.00mins						
5							
6	Sample count:	6					
7	Metabolite count:	7					
8							
9	Time	0	15	30	60	120	240
10	Parent-C10H12O2 (R_9:-C10H12O2) (5.346)	0	694.2	2310.4	2814.3	2357.4	2760.1
11	Parent-CH2 (R_0:-CH2) (6.576)	0	686.7	1669.8	1887.3	0	0
12	Parent-CH2 (R_0:-CH2) (7.029)	0	2549.1	7105.7	7960.6	1957.2	1988.2
13	Glucuronide conjugation-CH2 (R_0:-CH2) (5.303)	0	0	0	0	1658	2062.7
14	Glucuronide conjugation-CH2 (R_0:-CH2) (5.603)	0	0	0	0	2686	3350.6
15	Hydration + Glutathione conjugation-CH2 (R_0:-CH2) (3.600)	0	0	0	0	902.5	0
16	Hydration + OH + S-Glutathione conjugation-CH2 (R_0:-CH2) (4.672)	0	0	0	0	0	1851.8



Metabolite Stability

Ready

110%

Generates snapshot of metabolism for directing future studies

Waters

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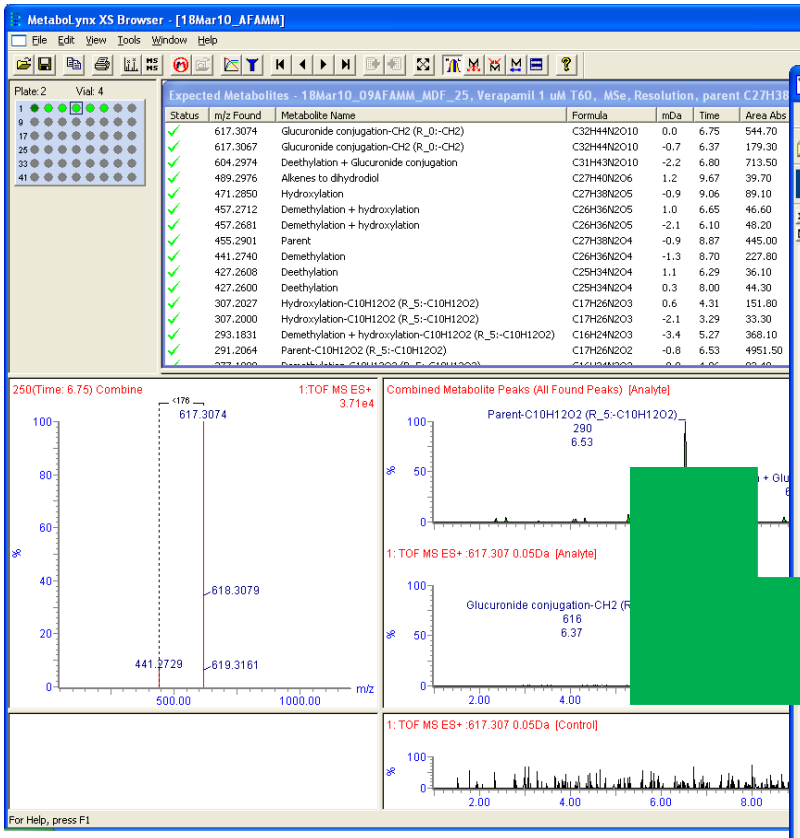

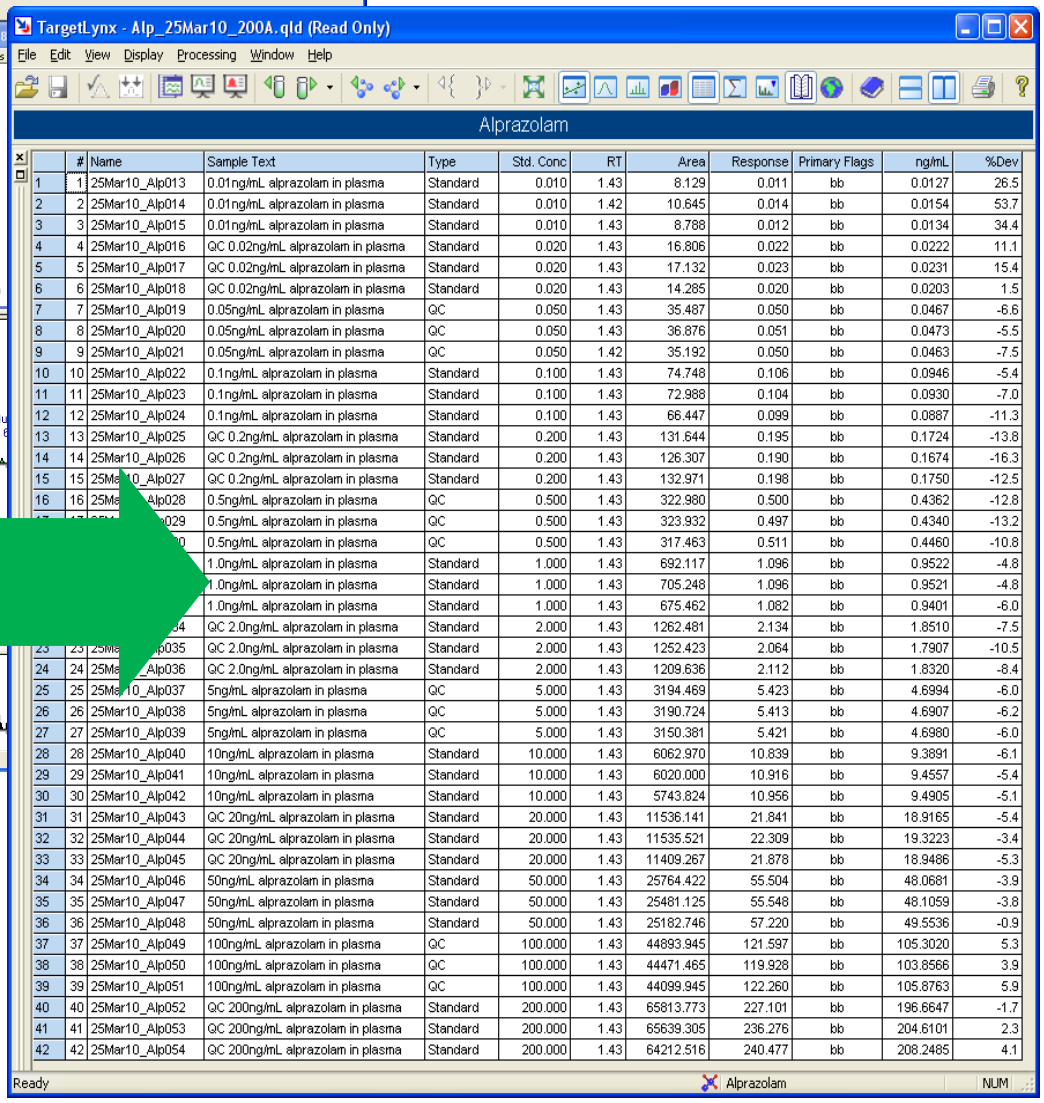

	#	Name	Sample Text	Type	Std. Conc	RT	Area	Response	Primary Flags	ng/mL	%Dev
1	1	25Mar10_Alp013	0.01ng/mL alprazolam in plasma	Standard	0.010	1.43	8.129	0.011	bb	0.0127	26.5
2	2	25Mar10_Alp014	0.01ng/mL alprazolam in plasma	Standard	0.010	1.42	10.645	0.014	bb	0.0154	53.7
3	3	25Mar10_Alp015	0.01ng/mL alprazolam in plasma	Standard	0.010	1.43	8.788	0.012	bb	0.0134	34.4
4	4	25Mar10_Alp016	QC 0.02ng/mL alprazolam in plasma	Standard	0.020	1.43	16.806	0.022	bb	0.0222	11.1
5	5	25Mar10_Alp017	QC 0.02ng/mL alprazolam in plasma	Standard	0.020	1.43	17.132	0.023	bb	0.0231	15.4
6	6	25Mar10_Alp018	QC 0.02ng/mL alprazolam in plasma	Standard	0.020	1.43	14.285	0.020	bb	0.0203	1.5
7	7	25Mar10_Alp019	0.05ng/mL alprazolam in plasma	QC	0.050	1.43	35.487	0.050	bb	0.0467	-6.6
8	8	25Mar10_Alp020	0.05ng/mL alprazolam in plasma	QC	0.050	1.43	36.876	0.051	bb	0.0473	-5.5
9	9	25Mar10_Alp021	0.05ng/mL alprazolam in plasma	QC	0.050	1.42	35.192	0.050	bb	0.0463	-7.5
10	10	25Mar10_Alp022	0.1ng/mL alprazolam in plasma	Standard	0.100	1.43	74.748	0.106	bb	0.0946	-5.4
11	11	25Mar10_Alp023	0.1ng/mL alprazolam in plasma	Standard	0.100	1.43	72.988	0.104	bb	0.0930	-7.0
12	12	25Mar10_Alp024	0.1ng/mL alprazolam in plasma	Standard	0.100	1.43	66.447	0.099	bb	0.0887	-11.3
13	13	25Mar10_Alp025	QC 0.2ng/mL alprazolam in plasma	Standard	0.200	1.43	131.644	0.195	bb	0.1724	-13.8
14	14	25Mar10_Alp026	QC 0.2ng/mL alprazolam in plasma	Standard	0.200	1.43	126.307	0.190	bb	0.1674	-16.3
15	15	25Ma_Alp027	QC 0.2ng/mL alprazolam in plasma	Standard	0.200	1.43	132.971	0.198	bb	0.1750	-12.5
16	16	25Ma_Alp028	0.5ng/mL alprazolam in plasma	QC	0.500	1.43	322.980	0.500	bb	0.4362	-12.8
		_Alp029	0.5ng/mL alprazolam in plasma	QC	0.500	1.43	323.932	0.497	bb	0.4340	-13.2
			0.5ng/mL alprazolam in plasma	QC	0.500	1.43	317.463	0.511	bb	0.4460	-10.8
			1.0ng/mL alprazolam in plasma	Standard	1.000	1.43	692.117	1.096	bb	0.9522	-4.8
			1.0ng/mL alprazolam in plasma	Standard	1.000	1.43	705.248	1.096	bb	0.9521	-4.8
			1.0ng/mL alprazolam in plasma	Standard	1.000	1.43	675.462	1.082	bb	0.9401	-6.0
			QC 2.0ng/mL alprazolam in plasma	Standard	2.000	1.43	1262.481	2.134	bb	1.8510	-7.5
23	23	25Ma_Alp035	QC 2.0ng/mL alprazolam in plasma	Standard	2.000	1.43	1252.423	2.064	bb	1.7907	-10.5
24	24	25Ma_Alp036	QC 2.0ng/mL alprazolam in plasma	Standard	2.000	1.43	1209.636	2.112	bb	1.8320	-8.4
25	25	25Mar10_Alp037	5ng/mL alprazolam in plasma	QC	5.000	1.43	3194.469	5.423	bb	4.6994	-6.0
26	26	25Mar10_Alp038	5ng/mL alprazolam in plasma	QC	5.000	1.43	3190.724	5.413	bb	4.6907	-6.2
27	27	25Mar10_Alp039	5ng/mL alprazolam in plasma	QC	5.000	1.43	3150.381	5.421	bb	4.6980	-6.0
28	28	25Mar10_Alp040	10ng/mL alprazolam in plasma	Standard	10.000	1.43	6062.970	10.839	bb	9.3891	-6.1
29	29	25Mar10_Alp041	10ng/mL alprazolam in plasma	Standard	10.000	1.43	6020.000	10.916	bb	9.4557	-5.4
30	30	25Mar10_Alp042	10ng/mL alprazolam in plasma	Standard	10.000	1.43	5743.824	10.956	bb	9.4905	-5.1
31	31	25Mar10_Alp043	QC 20ng/mL alprazolam in plasma	Standard	20.000	1.43	11536.141	21.841	bb	18.9165	-5.4
32	32	25Mar10_Alp044	QC 20ng/mL alprazolam in plasma	Standard	20.000	1.43	11535.521	22.309	bb	19.3223	-3.4
33	33	25Mar10_Alp045	QC 20ng/mL alprazolam in plasma	Standard	20.000	1.43	11409.267	21.878	bb	18.9486	-5.3
34	34	25Mar10_Alp046	50ng/mL alprazolam in plasma	Standard	50.000	1.43	25764.422	55.504	bb	48.0681	-3.9
35	35	25Mar10_Alp047	50ng/mL alprazolam in plasma	Standard	50.000	1.43	25481.125	55.548	bb	48.1059	-3.8
36	36	25Mar10_Alp048	50ng/mL alprazolam in plasma	Standard	50.000	1.43	25182.746	57.220	bb	49.5536	-0.9
37	37	25Mar10_Alp049	100ng/mL alprazolam in plasma	QC	100.000	1.43	44893.945	121.597	bb	105.3020	5.3
38	38	25Mar10_Alp050	100ng/mL alprazolam in plasma	QC	100.000	1.43	44471.465	119.928	bb	103.8566	3.9
39	39	25Mar10_Alp051	100ng/mL alprazolam in plasma	QC	100.000	1.43	44099.945	122.260	bb	105.8763	5.9
40	40	25Mar10_Alp052	QC 200ng/mL alprazolam in plasma	Standard	200.000	1.43	65813.773	227.101	bb	196.6647	-1.7
41	41	25Mar10_Alp053	QC 200ng/mL alprazolam in plasma	Standard	200.000	1.43	65639.305	236.276	bb	204.6101	2.3
42	42	25Mar10_Alp054	QC 200ng/mL alprazolam in plasma	Standard	200.000	1.43	64212.516	240.477	bb	208.2485	4.1

Alprazolam MS^E Data Quantitation Curve

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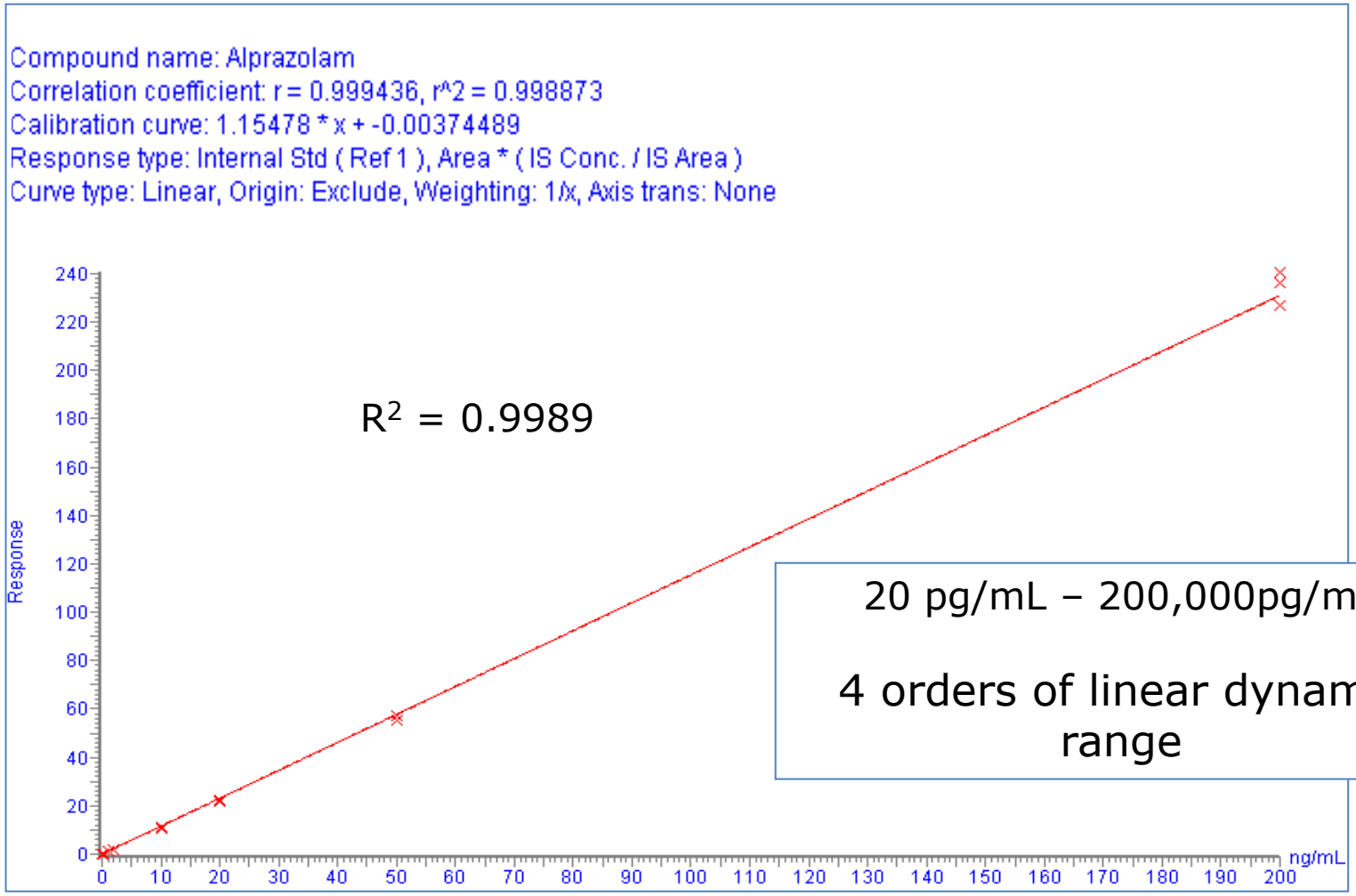

20 pg/mL – 200,000pg/mL

4 orders of linear dynamic range

Additional Capabilities

Application System Solutions

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MetaboLynx™ XS MarkerLynx™ XS BiopharmaLynx™ i-FIT™ ChromaLynx™
TargetLynx™ MassFragment™ OpenLynx™ ProteinLynx Global SERVER™

'Game-Changing'

...accesses the widest range of compounds & applications

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Multimode source
ESI – Electrospray Ionization
APCi – Atmospheric Pressure Chemical Ionization
ESCi® – Dual ESI and APCi

Dual mode source
APPI – Atmospheric Pressure Photo Ionization
APCi – Atmospheric Pressure Chemical Ionization

TRIZAIC™ Source with nanoTile Technology. Plug & Play nanoFlow

nanoFlow™ ESI

MALDI nanoFlow ESI APCi ESCi APPI TRIZAIC ASAP APGC

MALDI – Matrix Assisted Laser Desorption Ionization

ASAP – Atmospheric Pressure Solids Analysis Probe

APGC – Atmospheric Pressure Gas Chromatography

New Xevo source options

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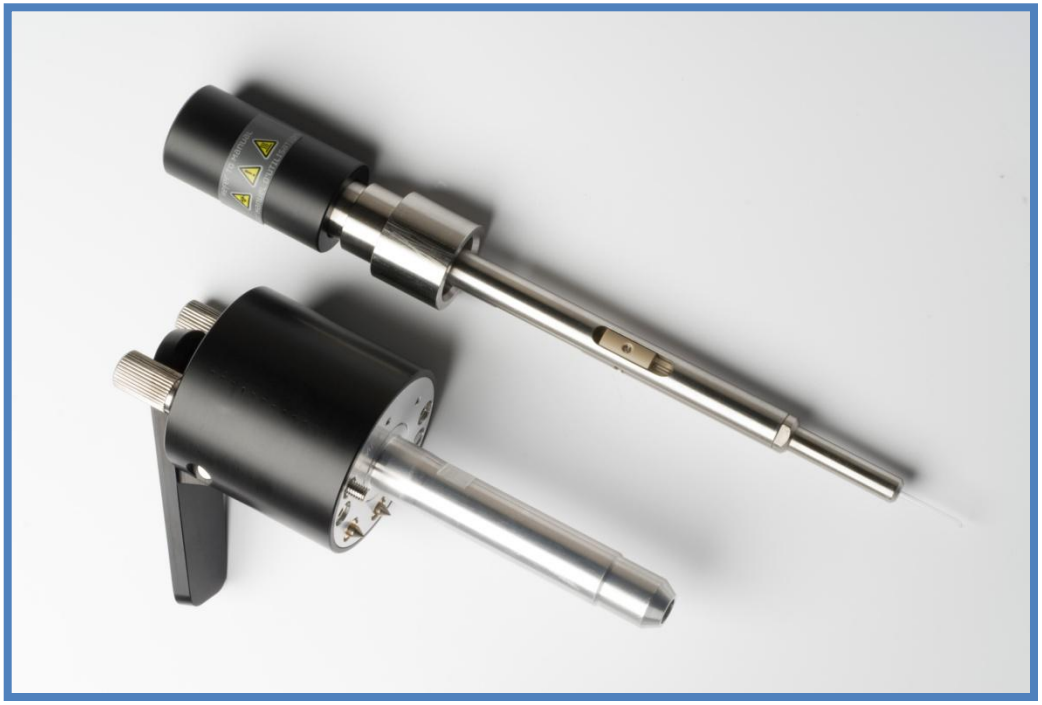
- ASAP
  - Atmospheric pressure solids analysis probe
- Direct sample analysis
  - Fast analysis
  - No sample prep
  - No chromatography
  - Solids and liquids



Waters ASAP Metabolites in Urine

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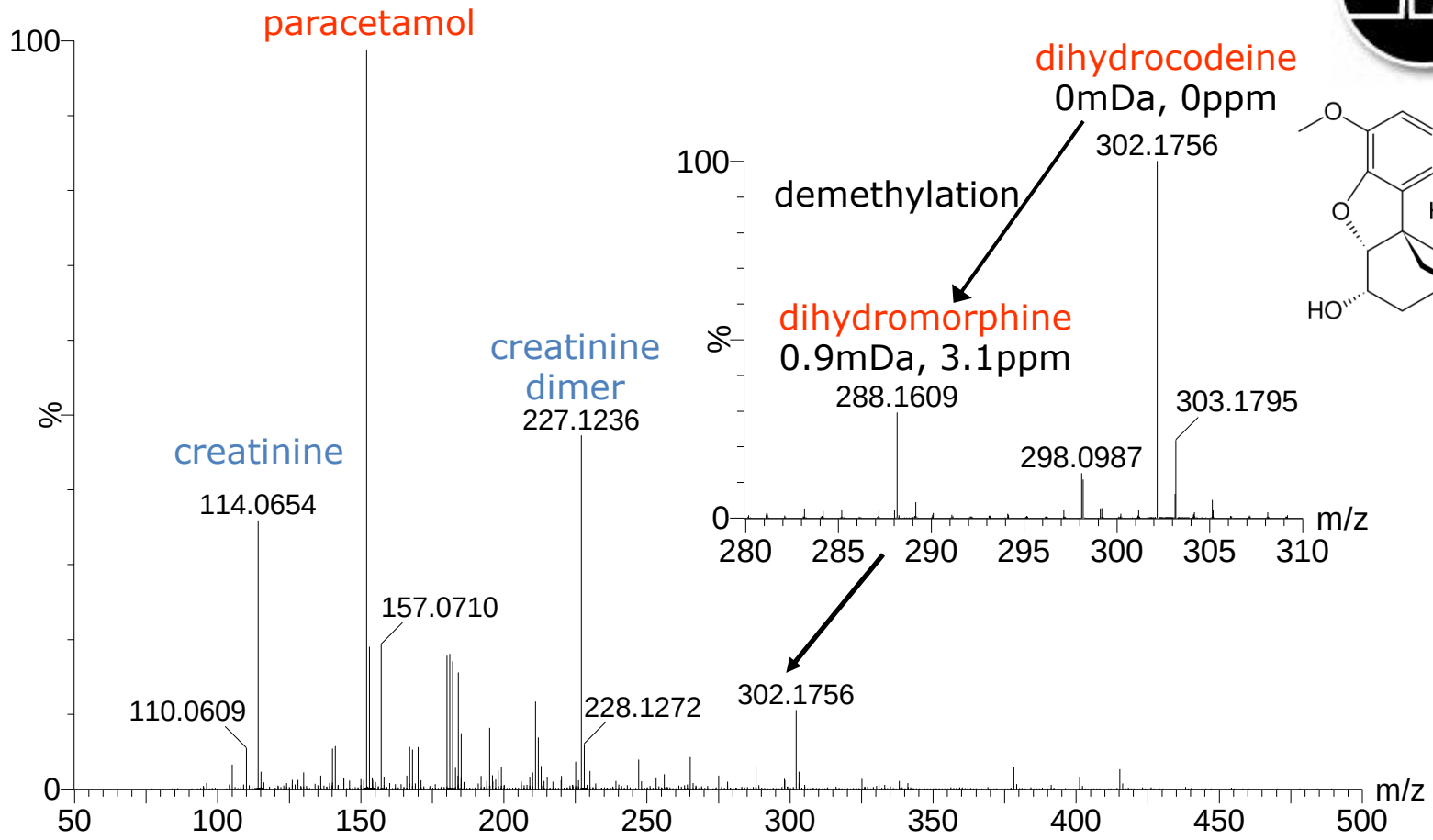
Urine sample from patient after dosing with paracetamol (1000mg) and dihydrocodeine (30mg)



Sample capillary loaded with 1µL neat urine

New Universal source options

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- APGC
  - Atmospheric pressure GC interface
- Extend compound coverage
  - LC & GC on one instrument
  - Very simple to exchange ion sources
  - Clean APCI type spectra

Conclusions

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- Xevo G2 QTOF or Synapt G2 collect sensitive accurate mass data on ALL precursors and products eliminating extra analysis on the same sample
- MetaboLynx XS offers proven, intelligent (structure driven) interpretation of data in an Metabolite ID workflow
- Advanced structural interpretation and advanced elemental composition calculations allow for conclusive metabolite confirmation

Rapid, Confident, Metabolite Identifications

Innovation. Productivity. Effective Decision Making

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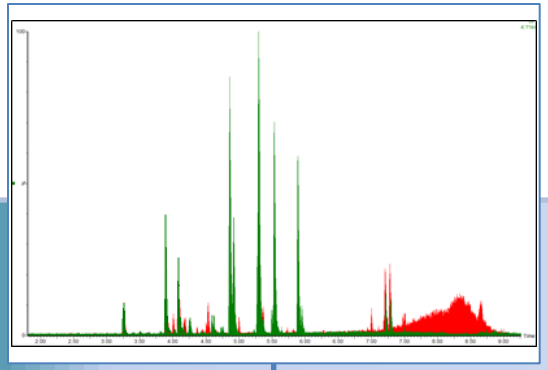

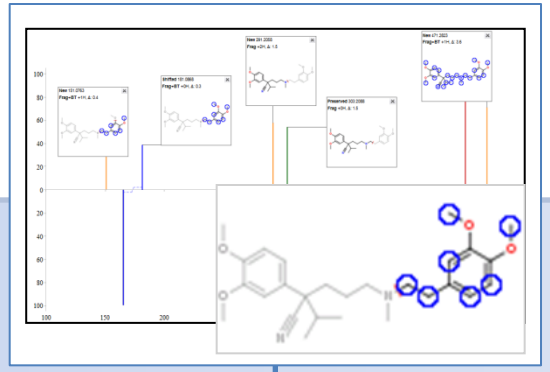

Isolate | **Identify** | **Localize** | **Report**