SCIEX

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Knowledge for Life.™

The Power of Accurate Mass MS/MS Data in Targeted and NON-Targeted (STA) Toxicological Analysis

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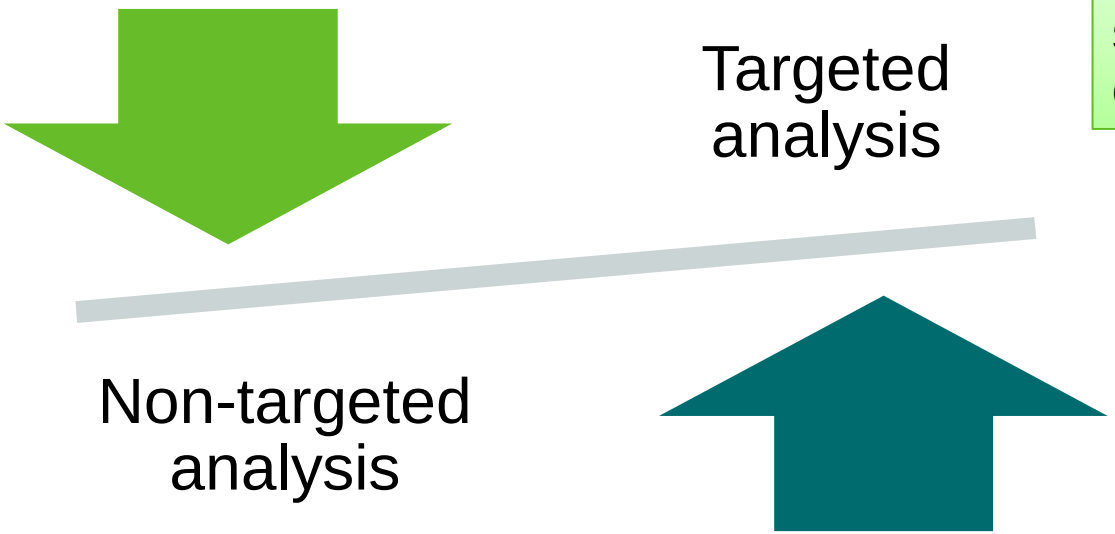
Budapest 28/10/2015

Trending workflows for forensic toxicology testing

Targeted analysis

Routine analyses – Search for known, standard list of compounds

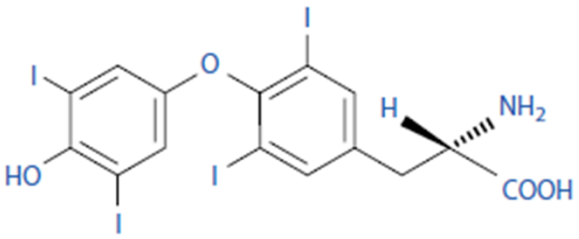
Trending workflows for forensic toxicology testing



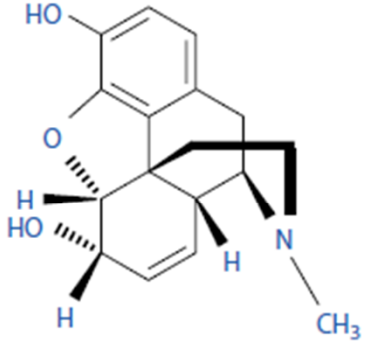
Routine analyses – Search for high risk, standard list of compounds

Surveillance screening – explore for other compounds without pre-existing knowledge

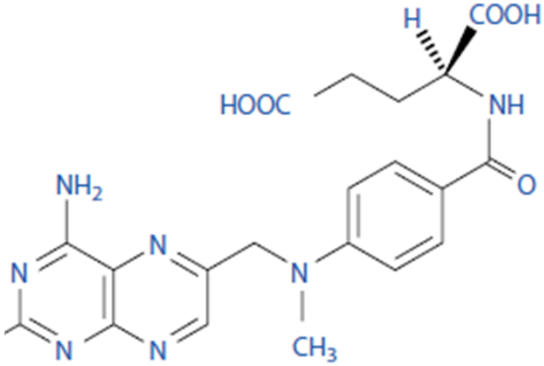
As an example – High-risk compounds commonly targeted drug screening:



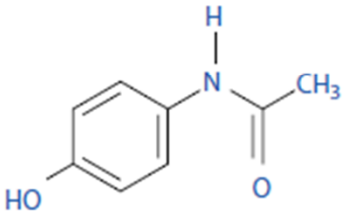
Levothyroxine



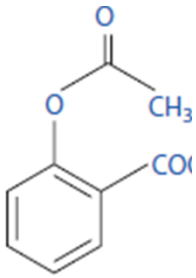
Morphine



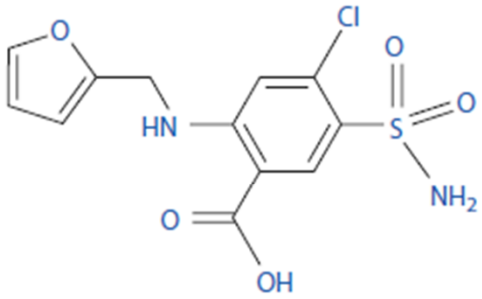
Methotrexate



Paracetamol



Aspirin



Furosemide

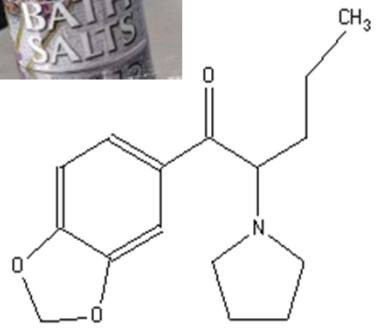
Differing chemical properties:

- **Molecular Weight**
- **Polarity**
- **Solubility**
- **Structure**
- **Stability**
- **Charge**

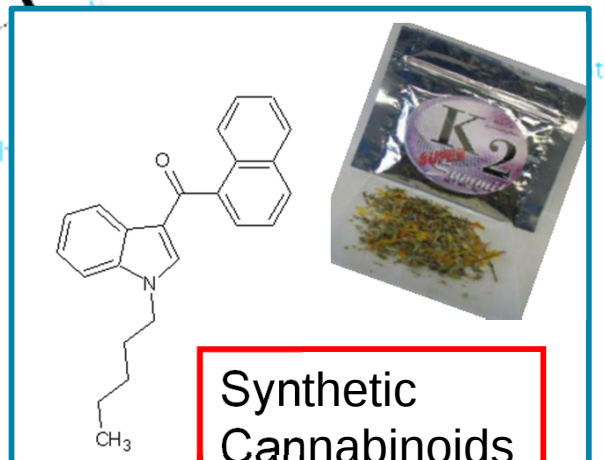
But, to further complicate matters…

Other compounds emerge almost daily

cathinones



MDPV



Synthetic Cannabinoids

JWH-018

Chemical properties of interest:

- **MW**
- **Polarity**
- **Solubility**
- **Structure**
- **Stability**
- **Charge**

But finding all of these potential compounds in complex samples is hard!

- Samples contain a mix of:
  - **Healthy / 'normal' components** – vitamins, proteins, antioxidants, etc.
  - **Known high-risk targeted compounds** – pesticides, drugs of abuse, adulterants, etc.
  - **Unknown compounds** – potential adulterants, metabolites, new synthetic compounds

Accurate mass / high resolution LC-MS/MS hardware is powerful to acquire the necessary data on unknown samples.

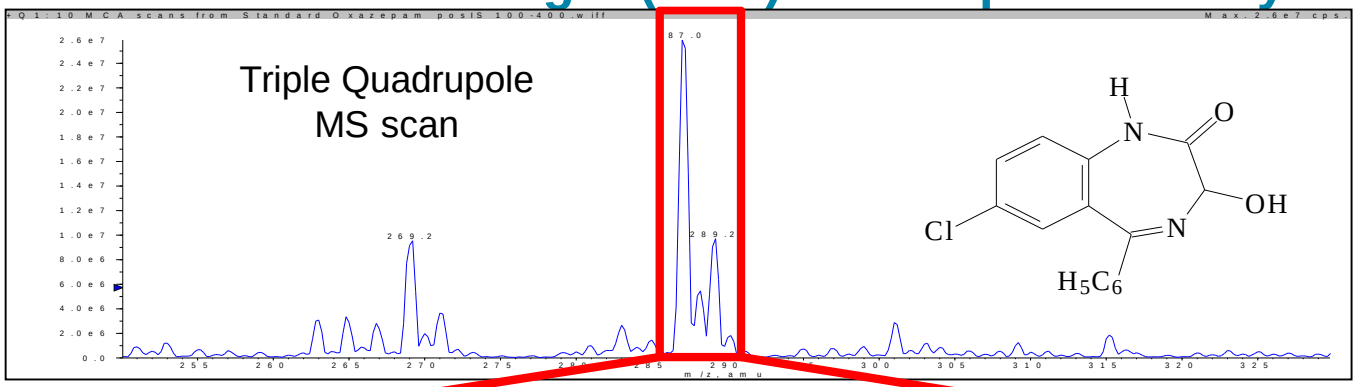
But the data are vast and complex.

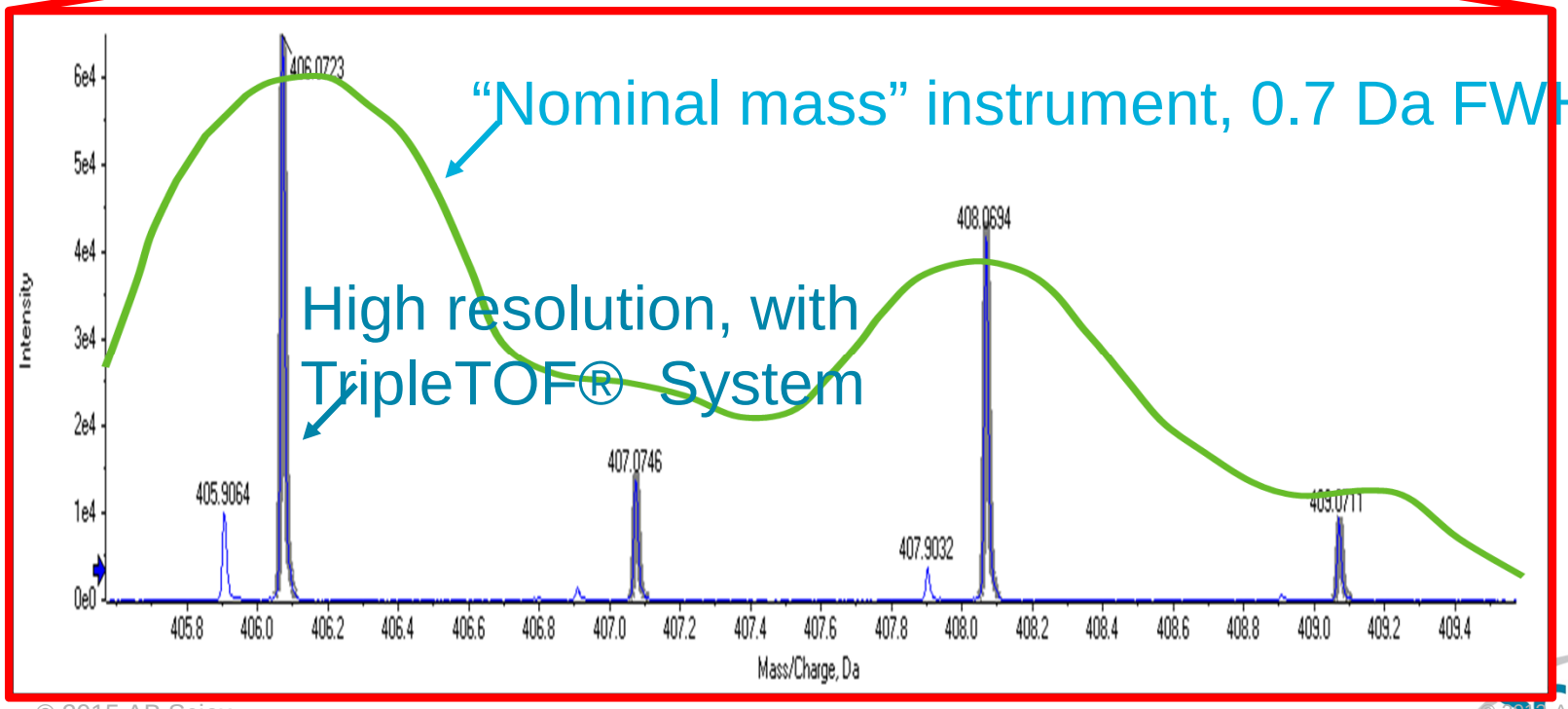
And, turning those data into results and decisions is a big challenge!

Principle of Time-of-Flight (TOF) Mass Spectrometry

- Ions are pulsed and accelerated into TOF analyzer
- Separation of ions (*m/z*) based on time to fly through the flight path on a nanosecond time scale
  - Small *m/z* faster than heavier *m/z*
- Higher resolution with longer flight path (longer TOF tubes, reflector, faster acceleration)

High Resolution Time-of-Flight (TOF) Mass Spectrometry





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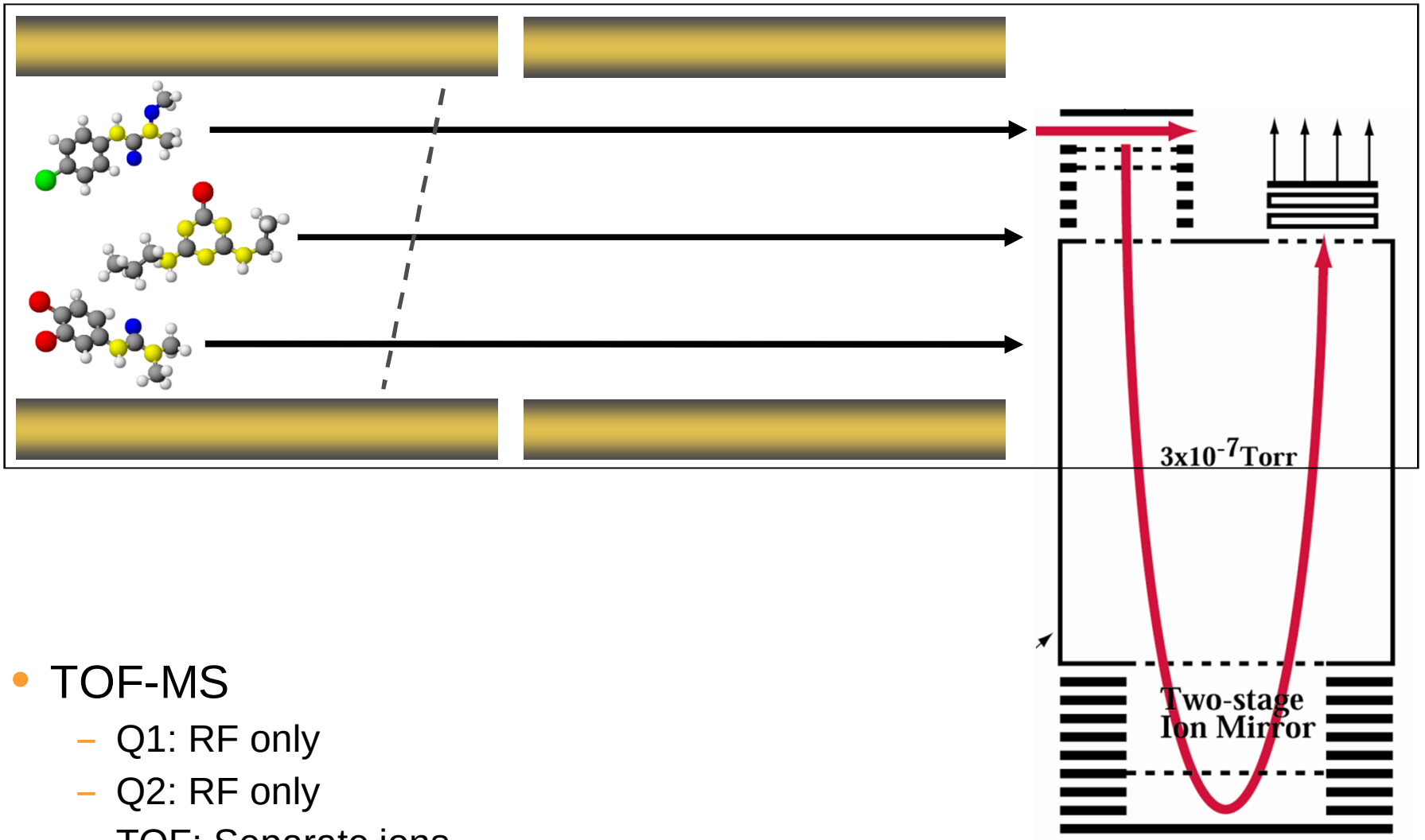
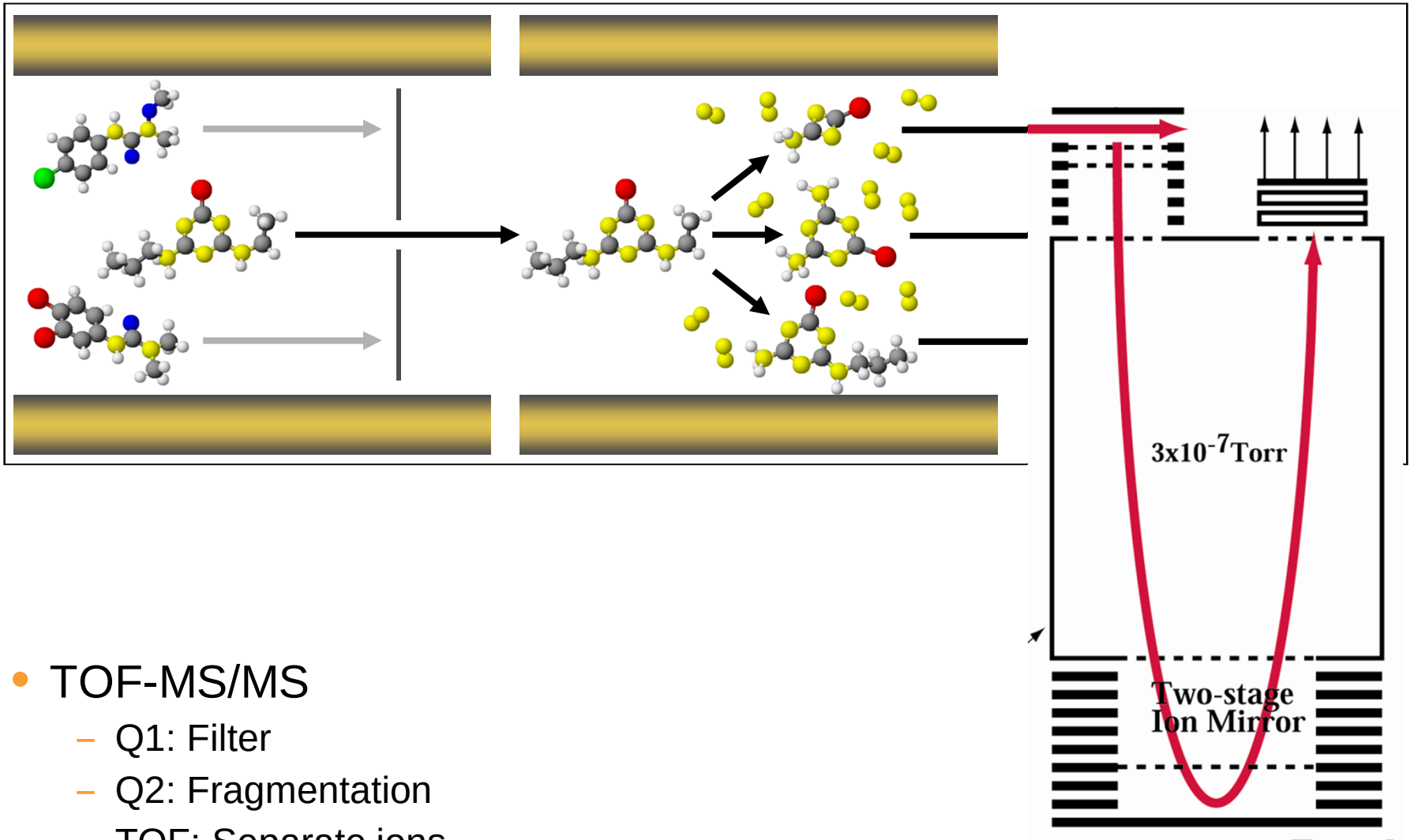
Time-of-Flight MS (TOF-MS)



- TOF-MS
  - Q1: RF only
  - Q2: RF only
  - TOF: Separate ions

Time-of-Flight MS/MS (TOF-MS/MS)



- TOF-MS/MS
  - Q1: Filter
  - Q2: Fragmentation
  - TOF: Separate ions

Non-targeted and Targeted Drug Screening Method Set up

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Accurate Mass Screening Workflow Overview

Injection of diluted sample extracts or direct injection of samples into

AB SCIEX TripleTOF® system

Targeted data acquisition

Non-targeted data acquisition

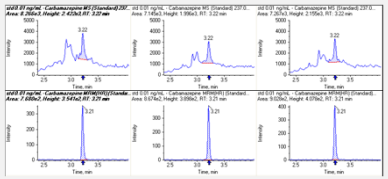
Scheduled MRM^{HR}

TOF-MS-IDA-MS/MS

Targeted processing

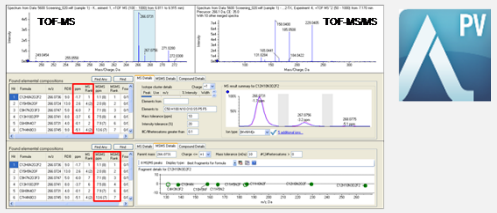
Targeted processing

Non-targeted processing



Increased selectivity using *Scheduled* MRM^{HR} for targeted quantitation

Screening, quantitation, and identification of targeted compounds based on RT and accurate mass MS data and MS/MS library searching



Sample-control-comparison

Identification of unknowns using accurate mass MS, isotope pattern, adducts, and MS/MS data using formula finding, automatic ChemSpider search and Fragmentation Prediction

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What makes MS/MS the preferred technology?

- Reduction of false negative rates
  - Co-eluting compounds
  - Interferences
- Reduction of false positives rates
  - MS/MS Library search

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Reducing False Positive and Negative Using MS2 Library Searching in Screening Method

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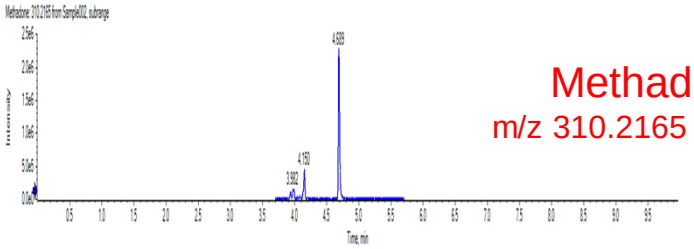
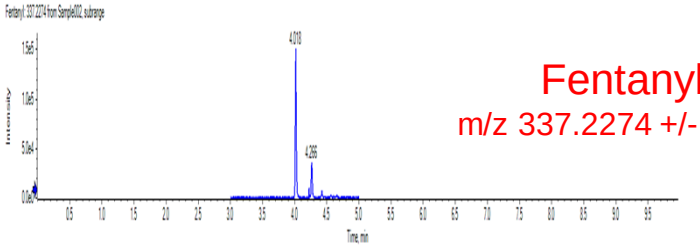
XIC width = 0.010 Da,

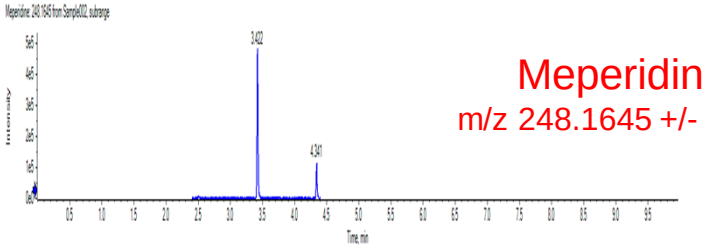
From TOF-MS



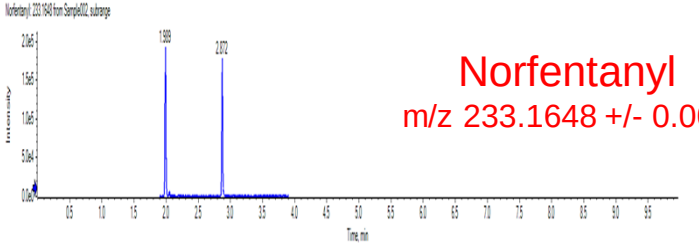
Methadone
m/z 310.2165 +/- 0.005



Fentanyl
m/z 337.2274 +/- 0.005

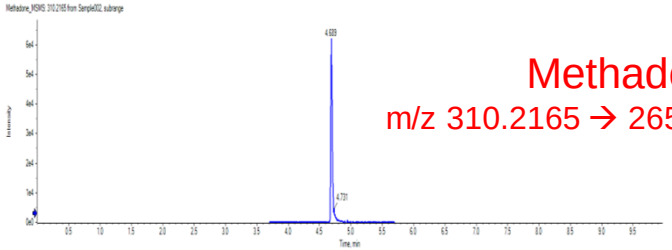


Meperidine
m/z 248.1645 +/- 0.005

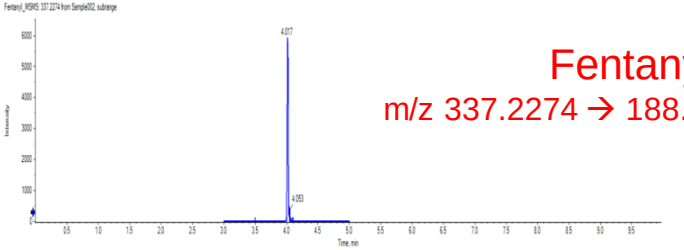


Norfentanyl
m/z 233.1648 +/- 0.005

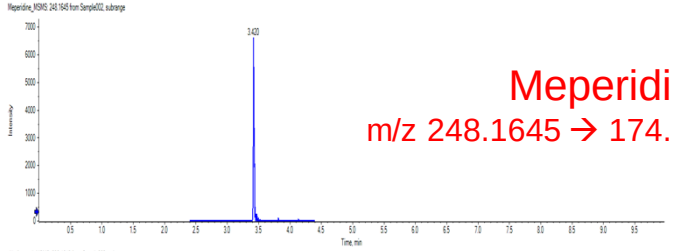
From TOF-MS/MS



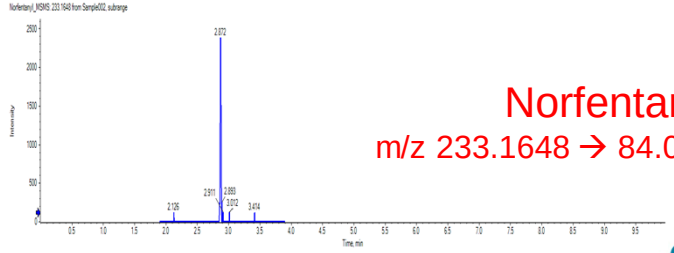
Methadone
m/z 310.2165 → 265.159 +/- 0.005



Fentanyl
m/z 337.2274 → 188.143 +/- 0.005



Meperidine
m/z 248.1645 → 174.128 +/- 0.005

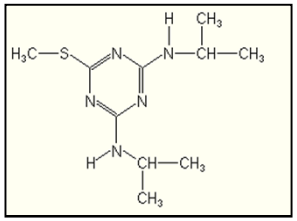


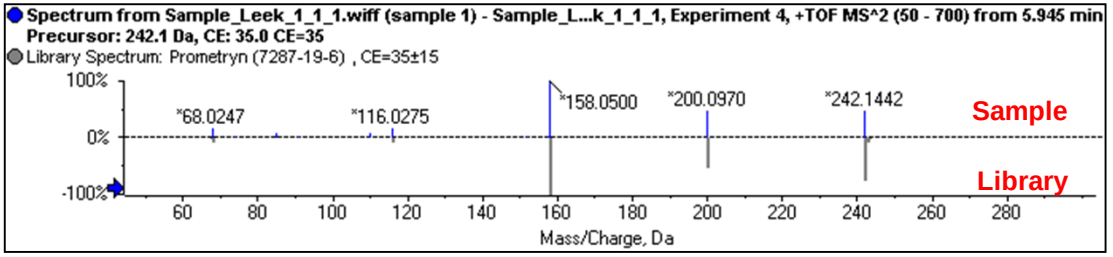
Norfentanyl
m/z 233.1648 → 84.081 +/- 0.005

MS/MS Library Search can reduce false positive

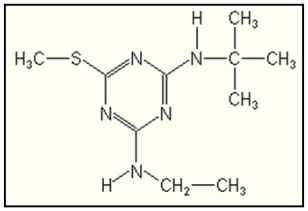
- Structural isomer have same formula and exact mass
- A false positive result can be made if only relying on mass Databases
- A Library match can help achieve the correct answer by using acquired MS/MS data to search against accurate mass MS/MS libraries

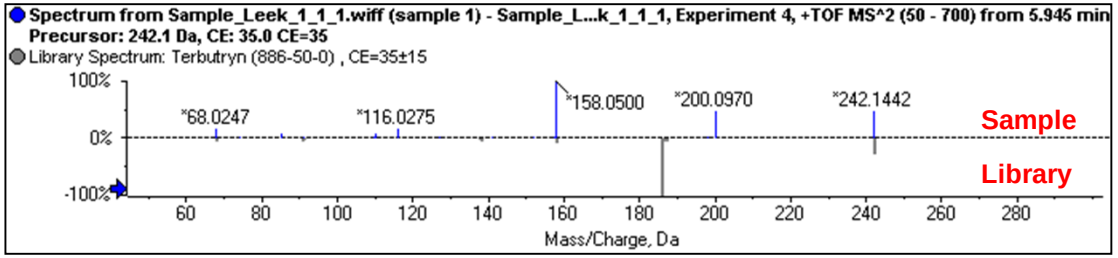
Prometryn





Terbutryn





#	Name	Formula	Isotope	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (ppm)	Found At RT (min)	Intensity	Threshold (ratio of control)	Control Intensity	Library Hit	Purity Score
393	Prometryn	C10H19N5S	0	241.13612	+H	242.14339	0.02	242.14358	0.8	5.96	762861	5	74505	Prometryn	91.4
471	Terbutryn	C10H19N5S	0	241.13612	+H	242.14339	0.02	242.14358	0.8	5.96	762861	5	74505	Prometryn	91.4

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Workflow #1:
Search for what you know

Targeted Screening and Quantitation

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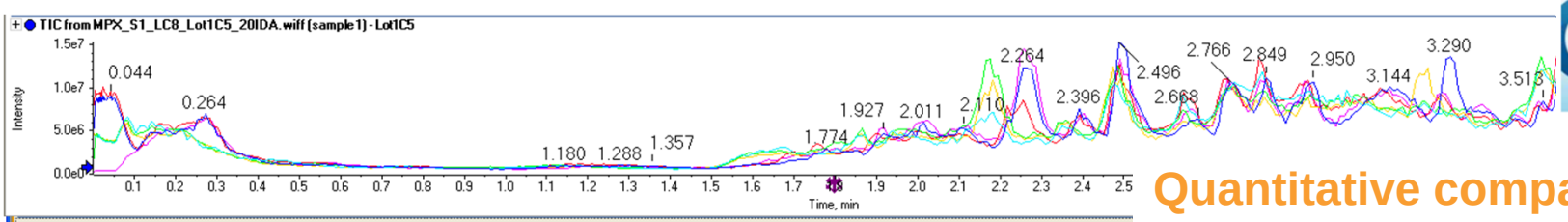
Targeted Screening using MasterView™

1. Define Retention Time and Accurate Mass for Each Compound



Quantitative comparison

MasterView — New Session

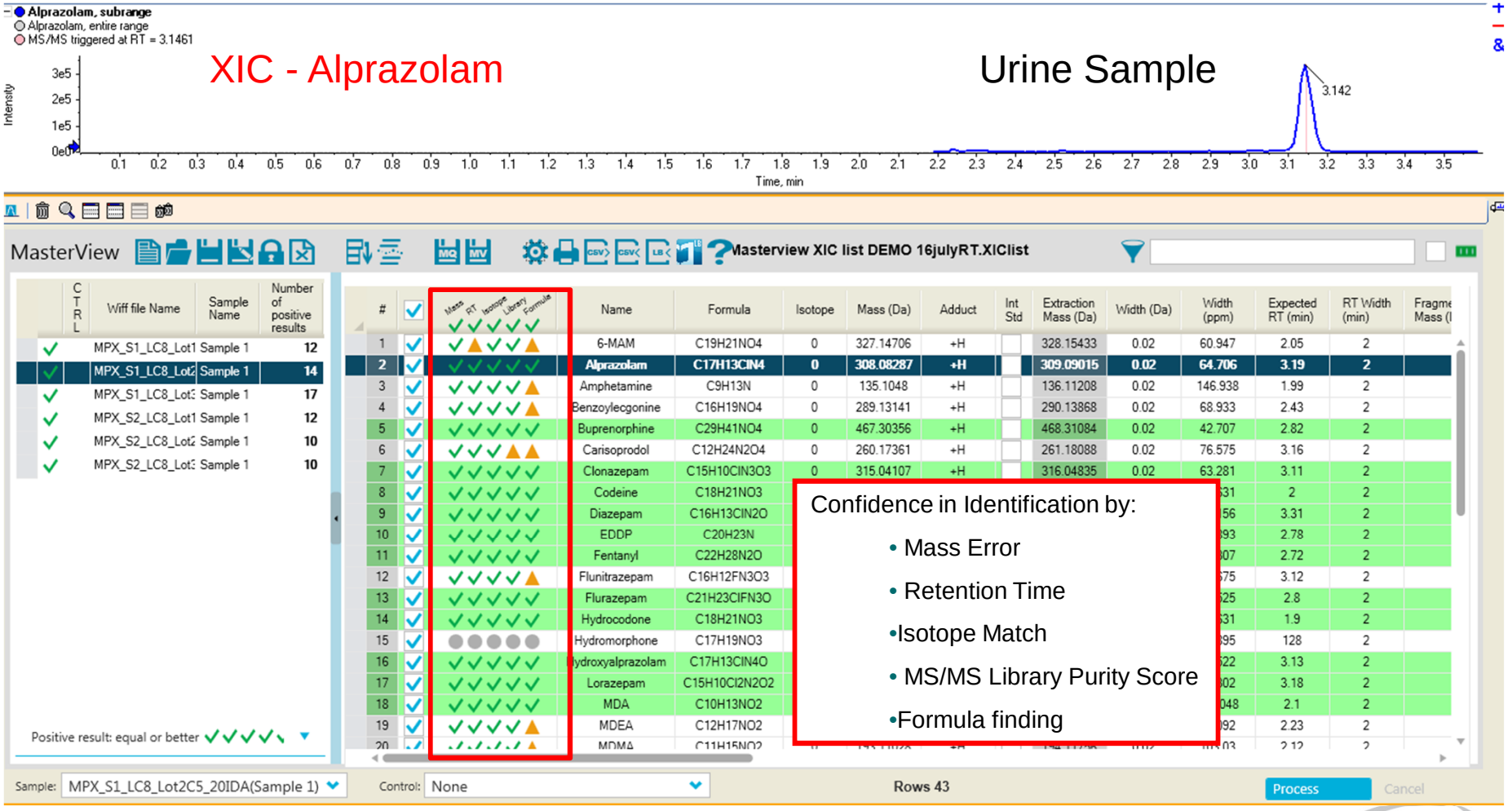
Name	Formula	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Expected RT (min)	RT Width (min)	Known Concentr.	Calculated Concentr.	Threshold (ratio of control)
6-MAM	C19H21NO4	327.14706	+H	328.15433	0.02	2.05	2	10		0.5
Alprazolam	C17H13ClN4	308.08287	+H	309.09015	0.02	3.19	2	10		0.5
Amphetamine	C9H13N	135.1048	+H	136.11208	0.02	1.99	2	10		0.5
Benzoylecgonine	C16H19NO4	289.13141	+H	290.13868	0.02	2.43	2	10		0.5
Buprenorphine	C29H41NO4	467.30356	+H	468.31084	0.02	2.82	2	10		0.5
Carisoprodol	C12H24N2O4	260.17361	+H	261.18088	0.02	3.16	2	10		0.5
Clonazepam	C15H10ClN3O3	315.04107	+H	316.04835	0.02	3.11	2	10		0.5
Codeine	C18H21NO3	299.15214	+H	300.15942	0.02	2	2	10		0.5
Diazepam	C16H13ClN2O	284.07164	+H	285.07892	0.02	3.31	2	10		0.5
EDDP	C20H23N	277.18305	+H	278.19033	0.02	2.78	2	10		0.5
Fentanyl	C22H28N2O	336.22016	+H	337.22744	0.02	2.72	2	10		0.5
Flunitrazepam	C16H12FN3O3	313.08627	+H	314.09355	0.02	3.12	2	10		0.5
Flurazepam	C21H23ClFN3O	387.15137	+H	388.15865	0.02	2.8	2	10		0.5
Hydrocodone	C18H21NO3	299.15214	+H	300.15942	0.02	1.9	2	10		0.5
Hydromorphone	C17H19NO3	285.13649	+H	286.14377	0.02	128	2	10		0.5
Hydroxyalprazolam	C17H13ClN4O	324.07779	+H	325.08507	0.02	3.13	2	10		0.5
Lorazepam	C15H10Cl2N2O2	320.01193	+H	321.01921	0.02	3.18	2	10		0.5

Process Cancel

Screening using extracted ion chromatograms (XIC)

Targeted Screening using MasterView™

3. Result Review using 'Traffic Lights'



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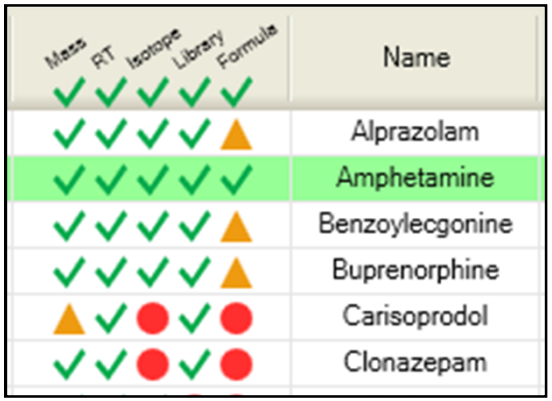
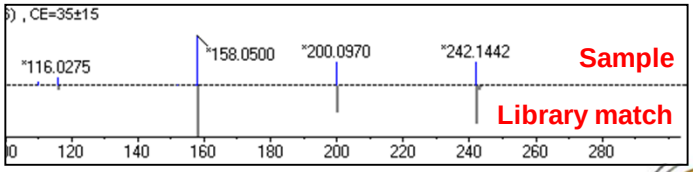
Results review

How you can use the traffic lights in your data review



Positive identification with high confidences

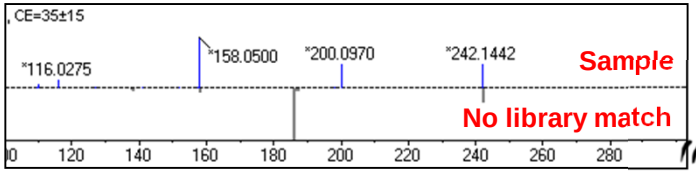
Review – Match



Customizable Review Settings:

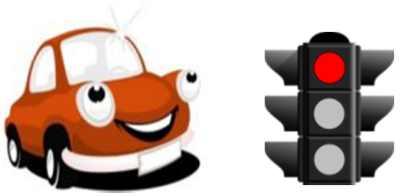
Positive ID with highest confidence

Review – No match



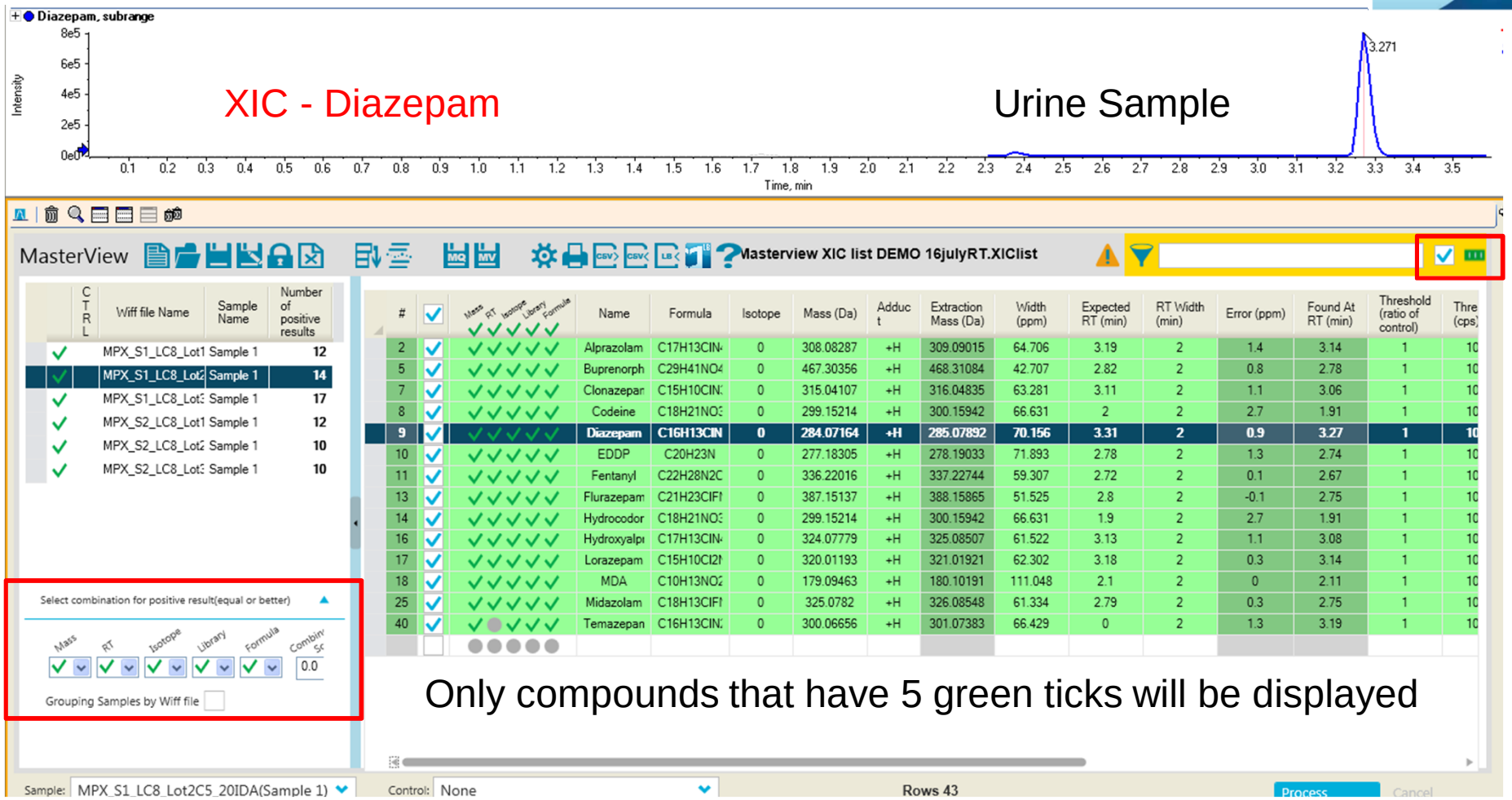
Identified compounds to review

Negative identification



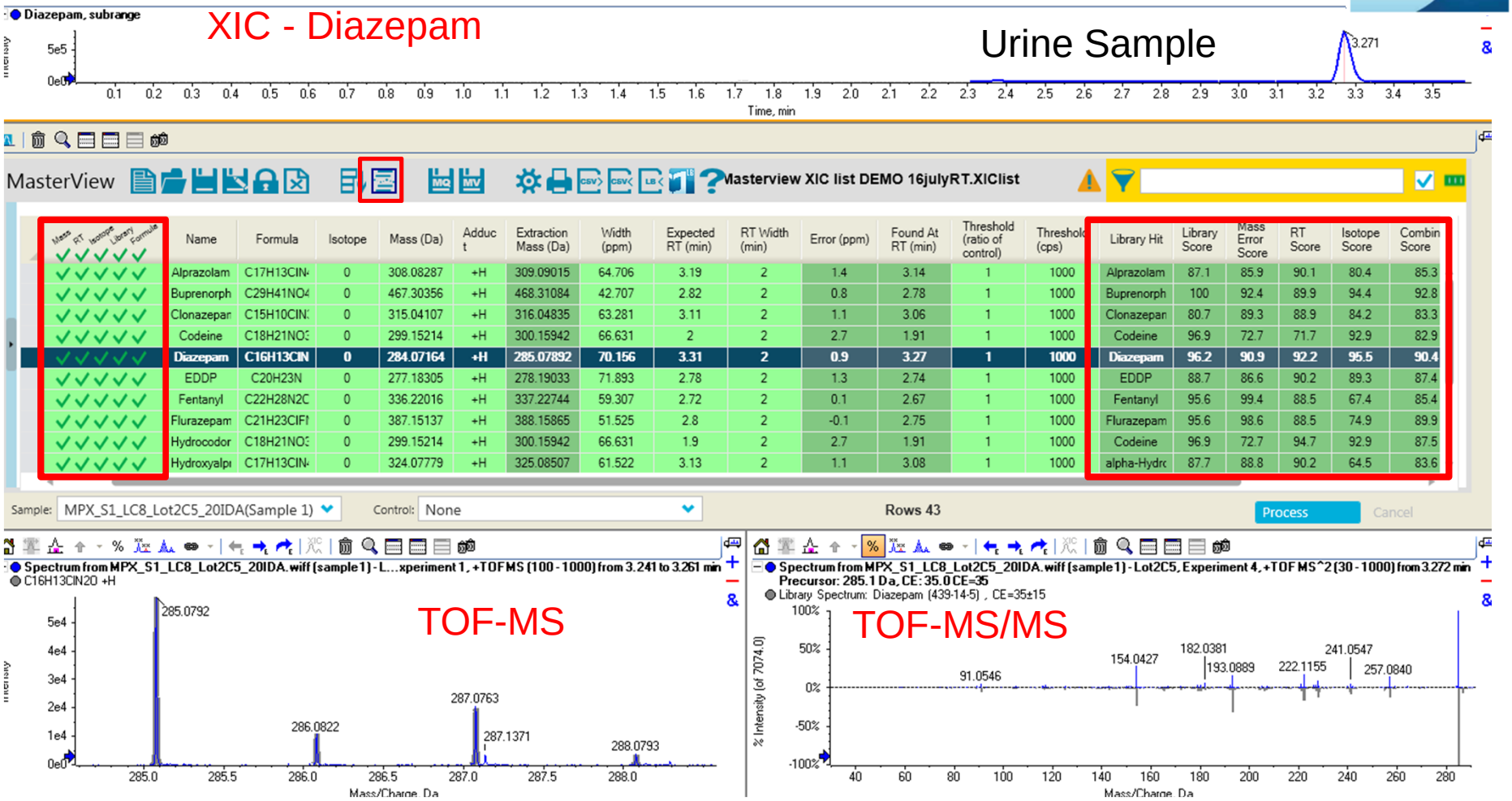
Targeted Screening using MasterView™

4. Result Display (Identified Compounds to Review)



Targeted Screening using MasterView™

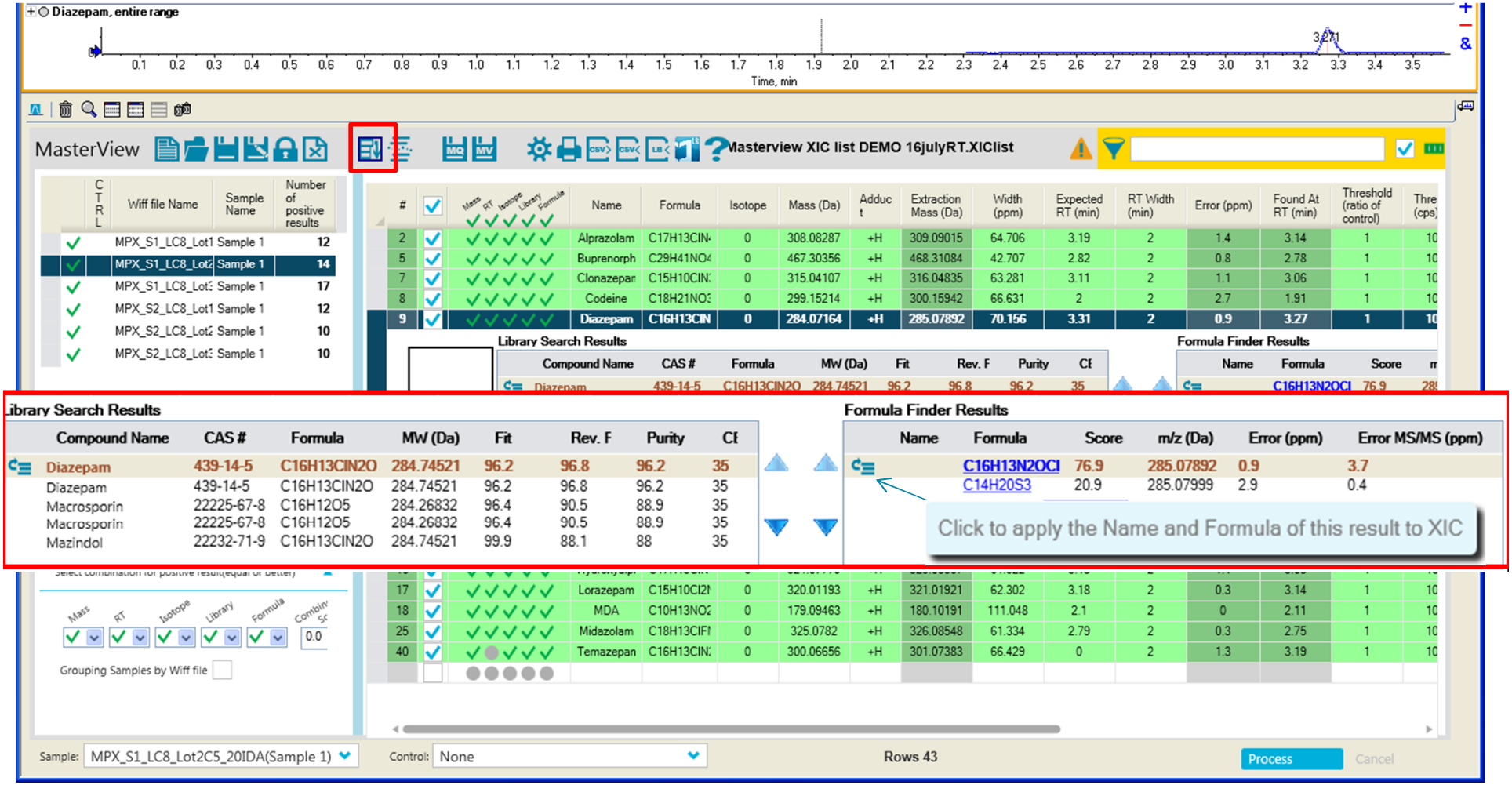
5. Review MS and MS/MS spectra



mirror view of spectra for easy comparison

Targeted Screening using MasterView™

6. Details of Library Search and Formula Finder



automatic formula finding

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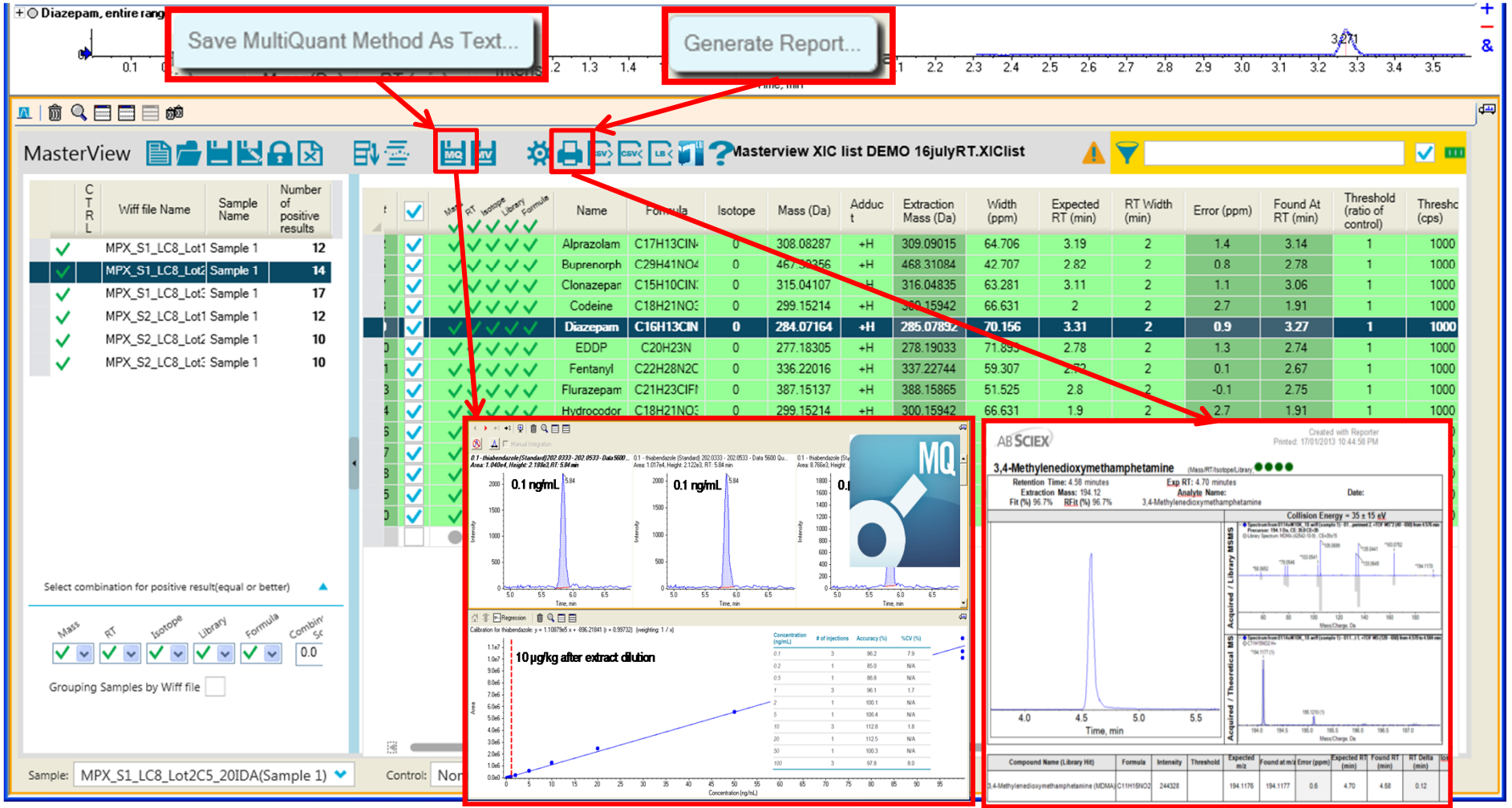
Targeted Screening using MasterView™

7. Quantitation through MultiQuant™ Software and Reporting



Workflow #2:
Remove the ‘healthy’ / ‘normal’ peaks

Reduce unknown peaks to just those that are suspicious

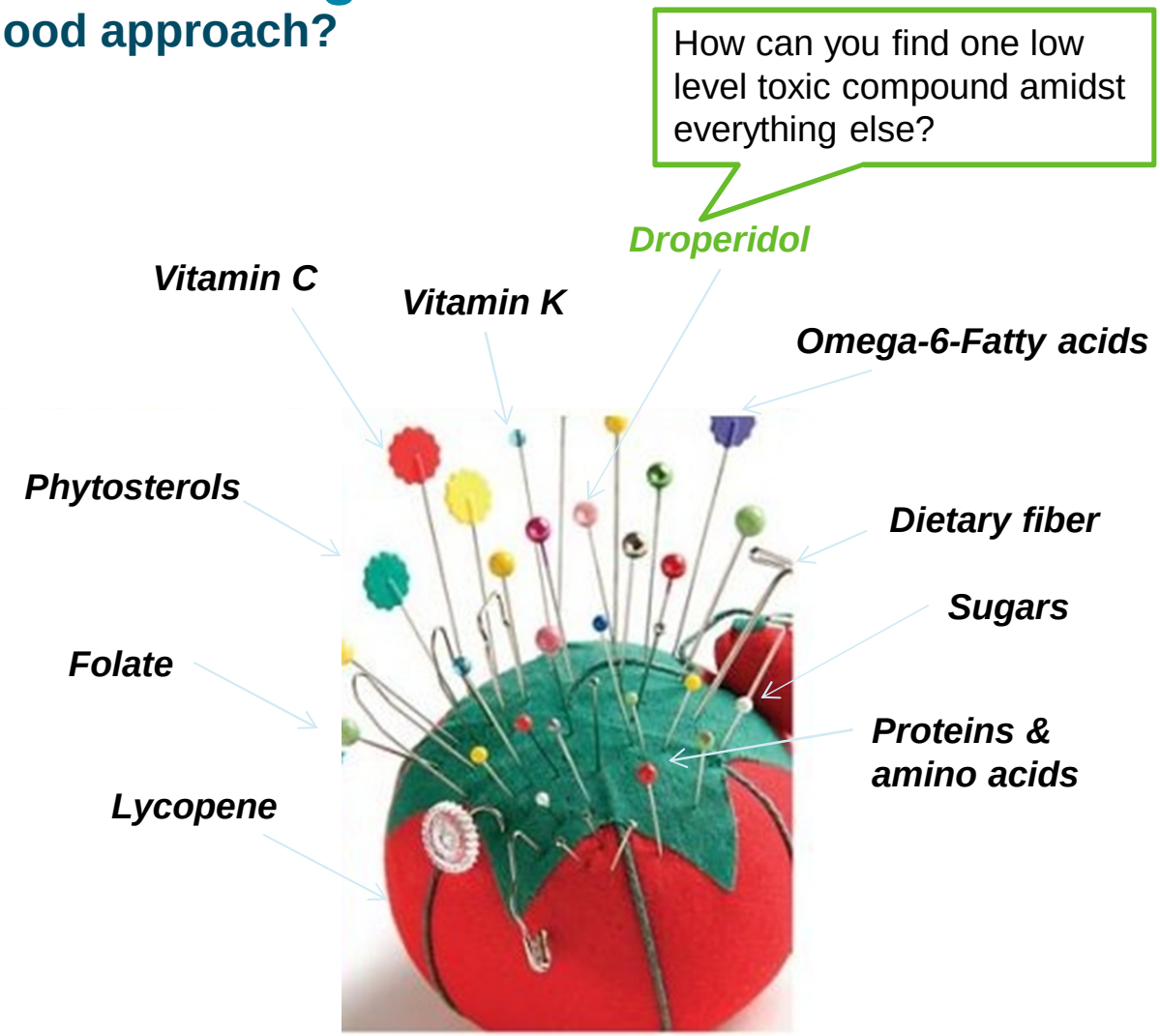
Eliminating the endogenous

Comparative Unknown Screening
What is it and why is this a good approach?

Why do this?

Non-targeted peak finding algorithms will find **thousands** of molecular features in each sample, including:

- Chemical background
- Matrix components
- Unexpected compounds of interest



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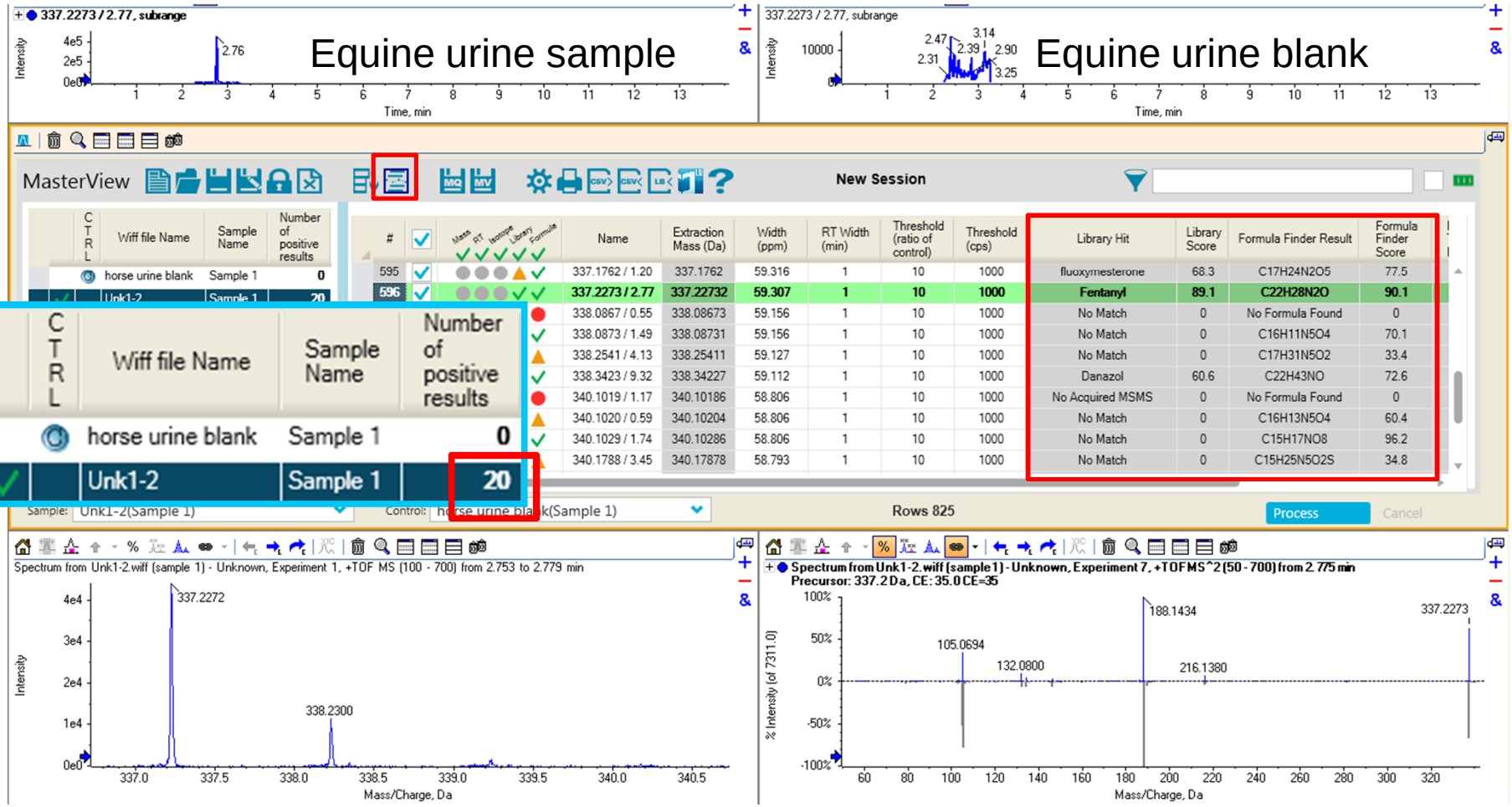
Non-Targeted Screening using MasterView™

1. Automatic Sample-Control-Comparison



825 chromatographic peaks and 20 relevant signals

Workflow #3:
Investigate the unknowns

Evaluate the suspicious offenders-
Simultaneously search for targeted screening and
at the same time identify any unknowns
(unexpected) compounds

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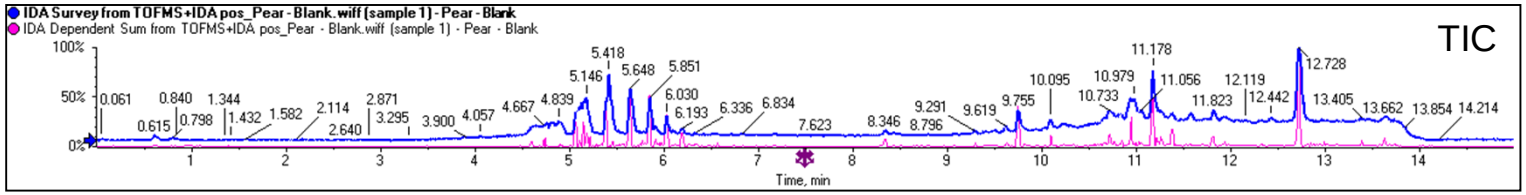
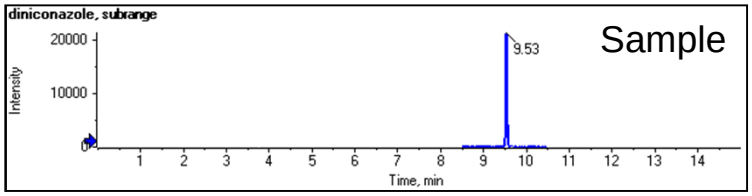
Data Processing Workflow

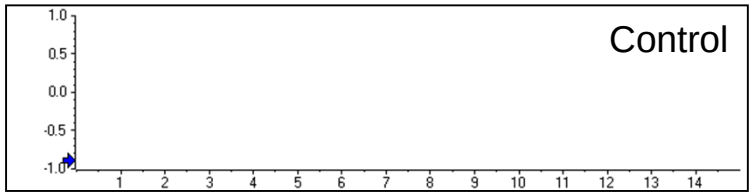
Automatic Non-Target (Unknown) Screening and Identification

TIC of Sample and Control

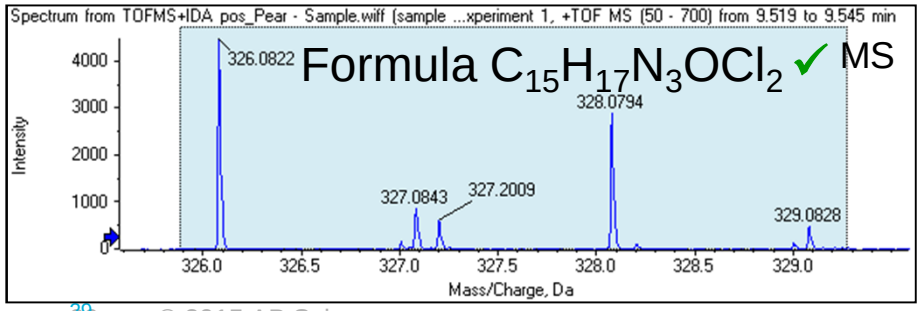


Non-Target XIC Generation and Sample-Control-Comparison

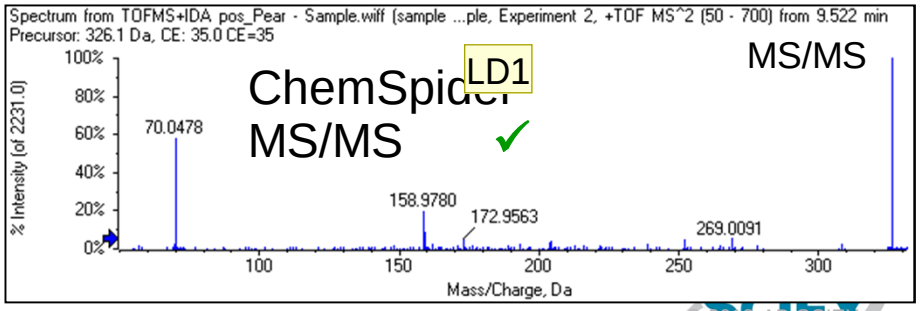




Evaluation of TOF-MS (Formula Finder)



Evaluation of TOF-MS/MS (Search)



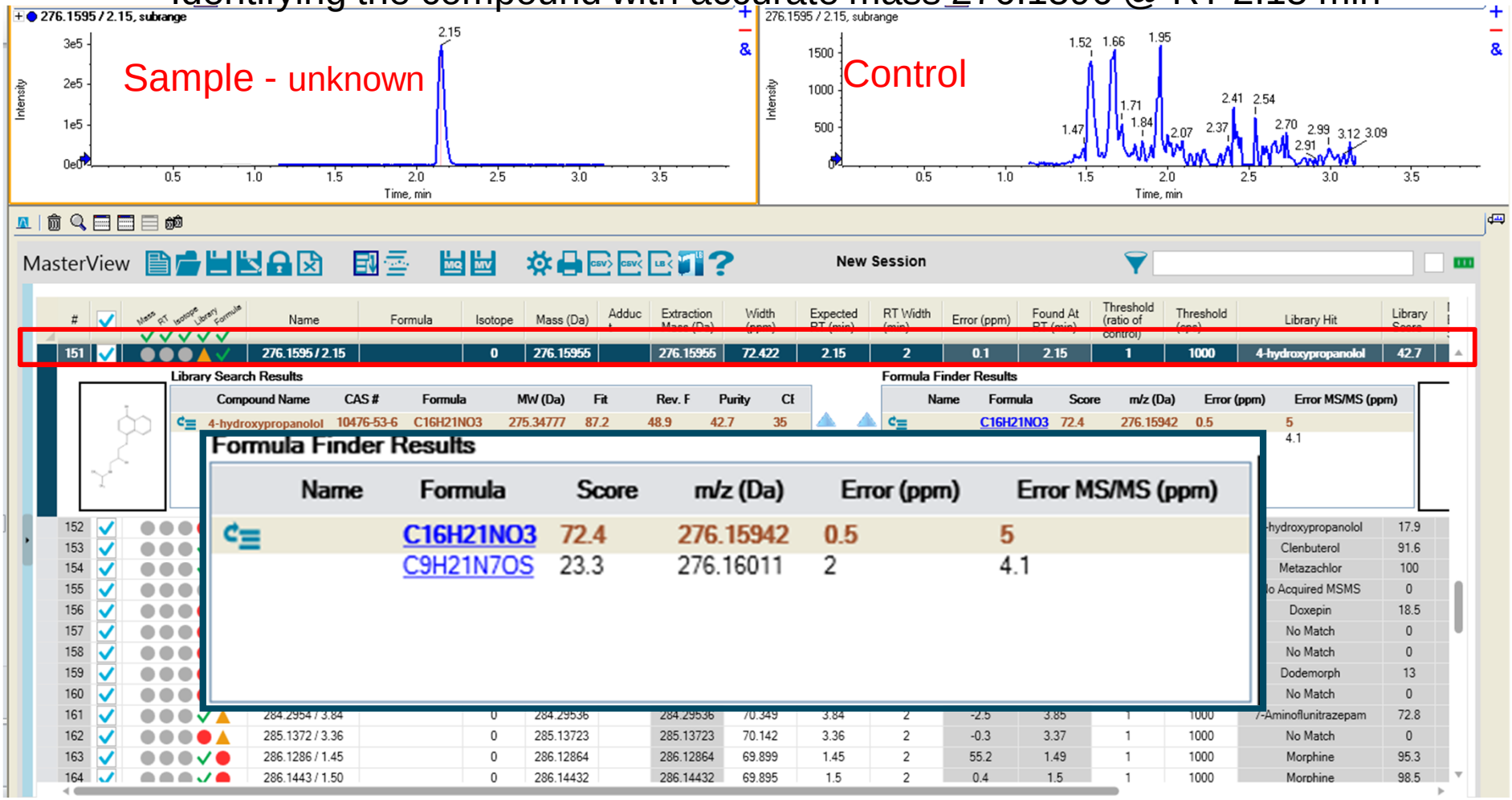
Slide 29

LD1 Please remove logo.
Digregorio, Linda, 8/14/2013

Unknown Structural Elucidations

Unknown (unexpected) non-targeted peaks are automatically added to the original XIC list of targeted compounds

Identifying the compound with accurate mass 276.1596 @ RT 2.15 min



MasterView automatically empirically calculates potential molecular formula for the extracted mass of the unknown compound using both MS and MS/MS data

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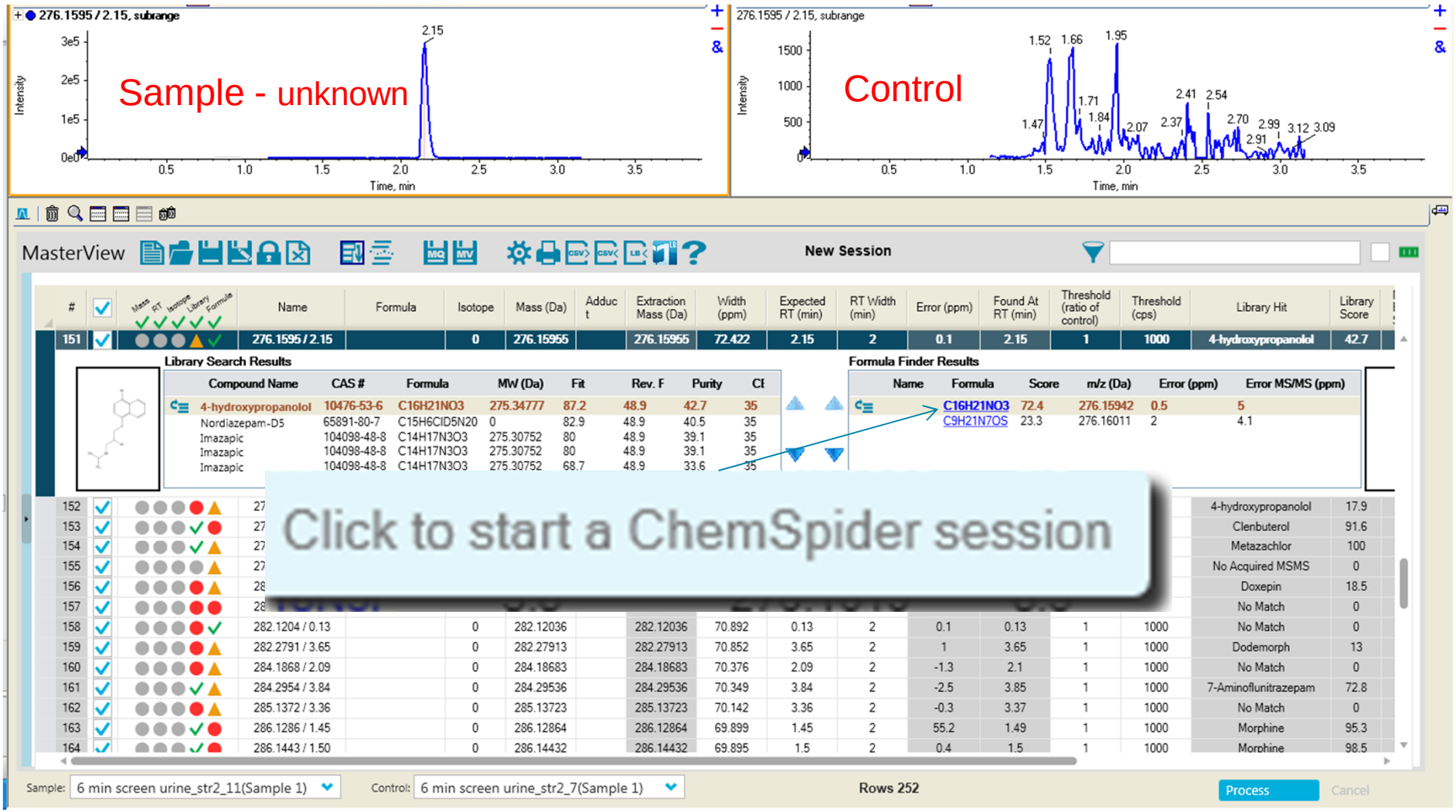
Unknown Structural Elucidations

Identifying the compound with accurate mass 276.1596 @ RT 2.15 min

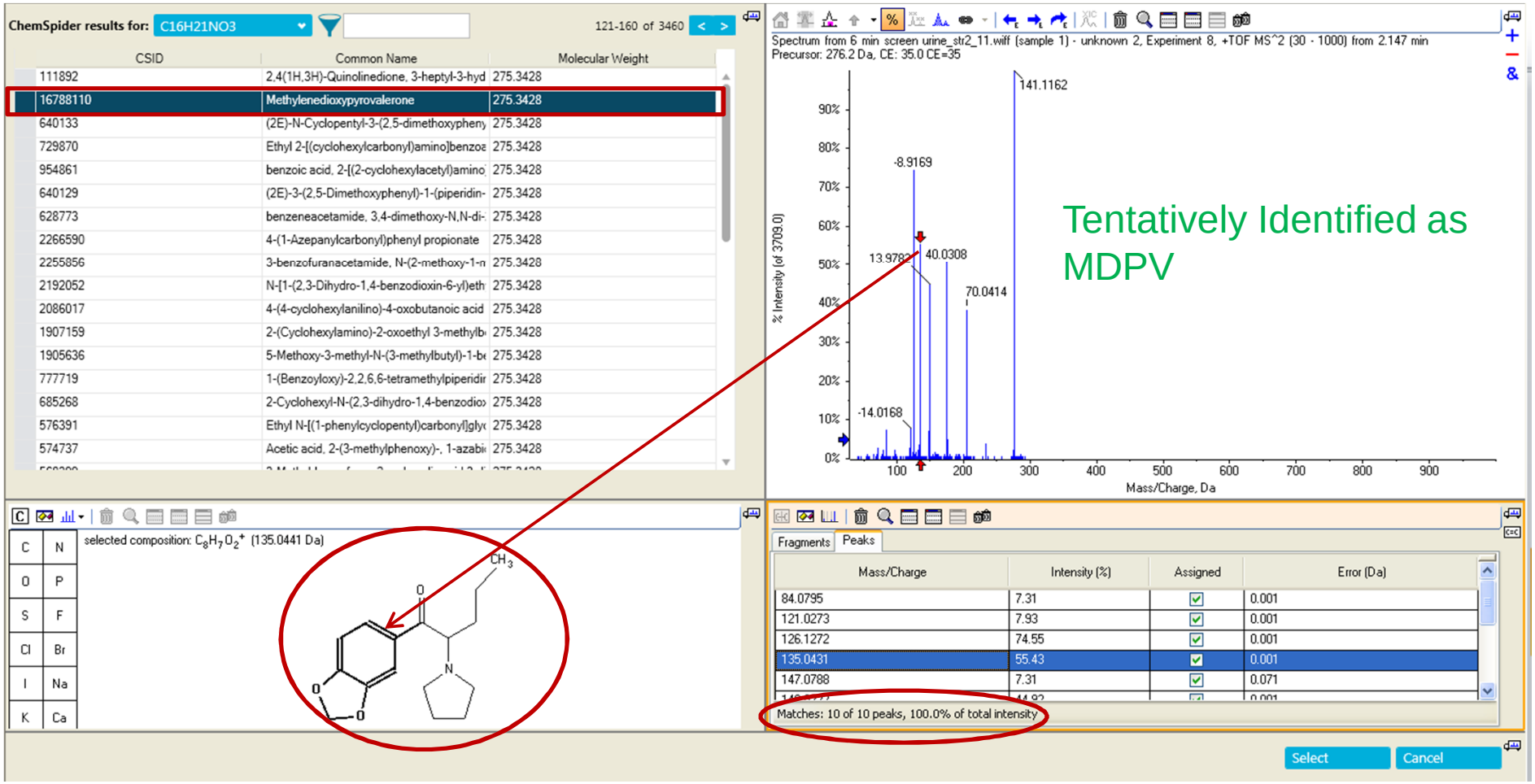


Link to ChemSpider Session to identify potential structures that match the calculated formula

Unknown Structural Elucidations

ChemSpider Search and Automatic MS/MS Interpretation

ChemSpider hits are automatically compared against MS/MS spectrum.



Closest Match – ten of ten peaks matched with 100% explainable MS/MS fragment ions for the MDPV cathinone designer drug structure

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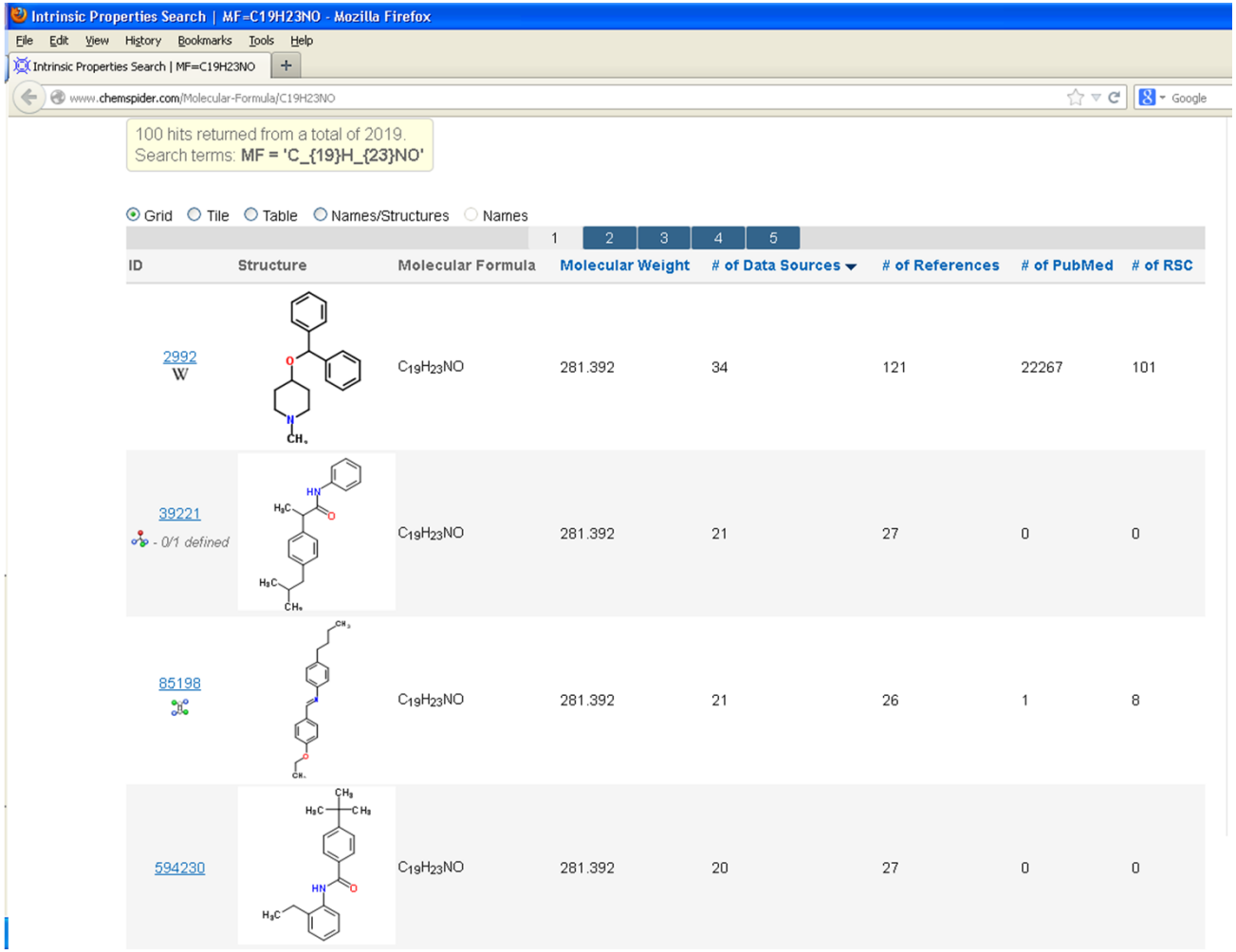
ChemSpider – link

Allows searching based on number of references and data sources



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Data mine as needed

All MS and MS/MS results at your fingertips

There can be infinite ‘unknowns’ in any given sample – including things that are good and things that are potentially harmful.

It can take infinite time in data analysis to assess every possible suspicious peak.

Sometimes we need to look back at old samples.

The right accurate mass LC-MS/MS system will

acquire most of the data you will ever need in just one injection.

Let your hardware do the work for you, and

save time and resources on sample rechecks!

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Knowledge for Life.™

Thanks for listening