



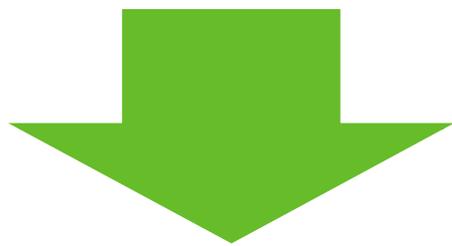
Answers for Science.
Knowledge for Life.™



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

Alexandre Paccou
Sr Manager Support Clinical & Forensic EMEA
Budapest 28/10/2015

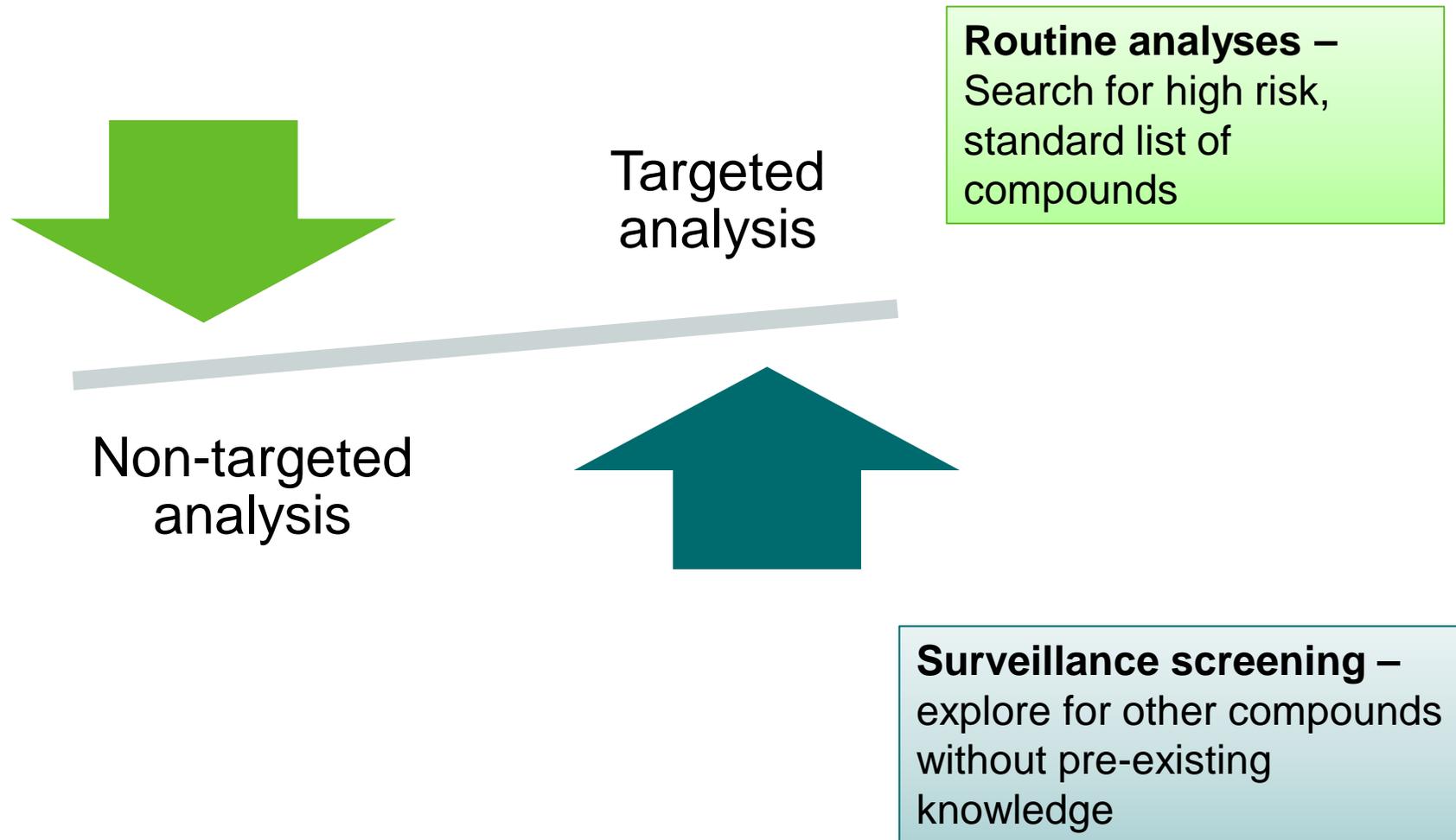
Trending workflows for forensic toxicology testing



Targeted
analysis

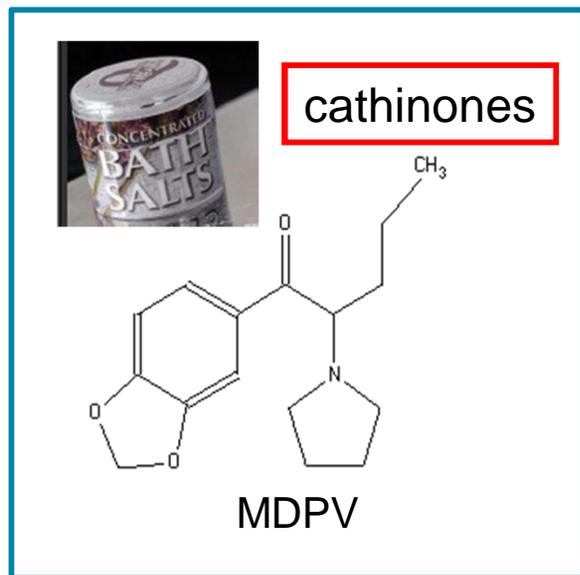
Routine analyses –
Search for known,
standard list of
compounds

Trending workflows for forensic toxicology testing



But, to further complicate matters...

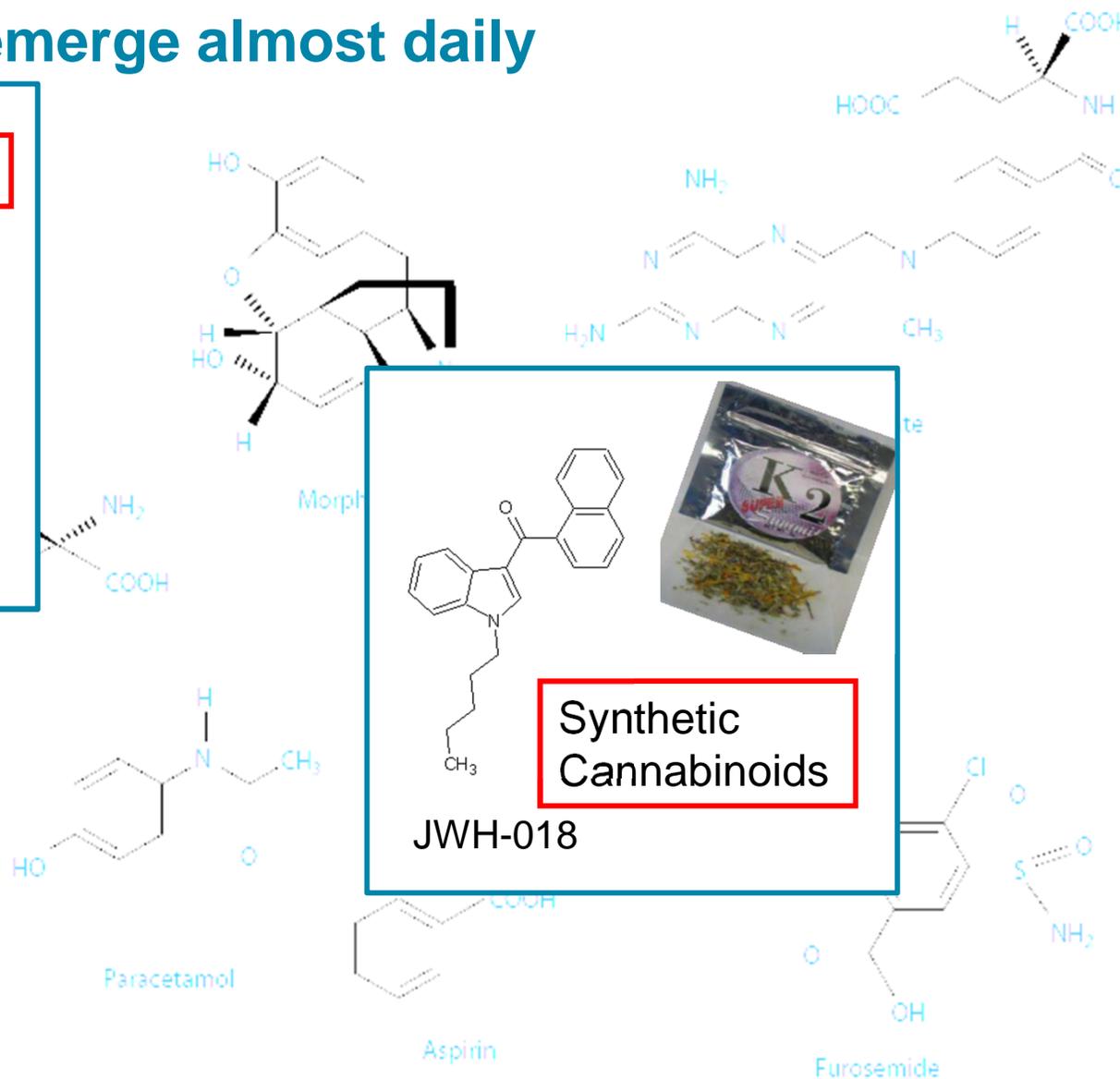
Other compounds emerge almost daily



Levothyroxine

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

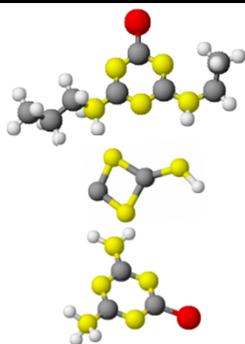
Accurate mass / high resolution LC-MS/MS hardware is powerful to acquire the necessary data on unknown samples.

But the data are vast and complex.

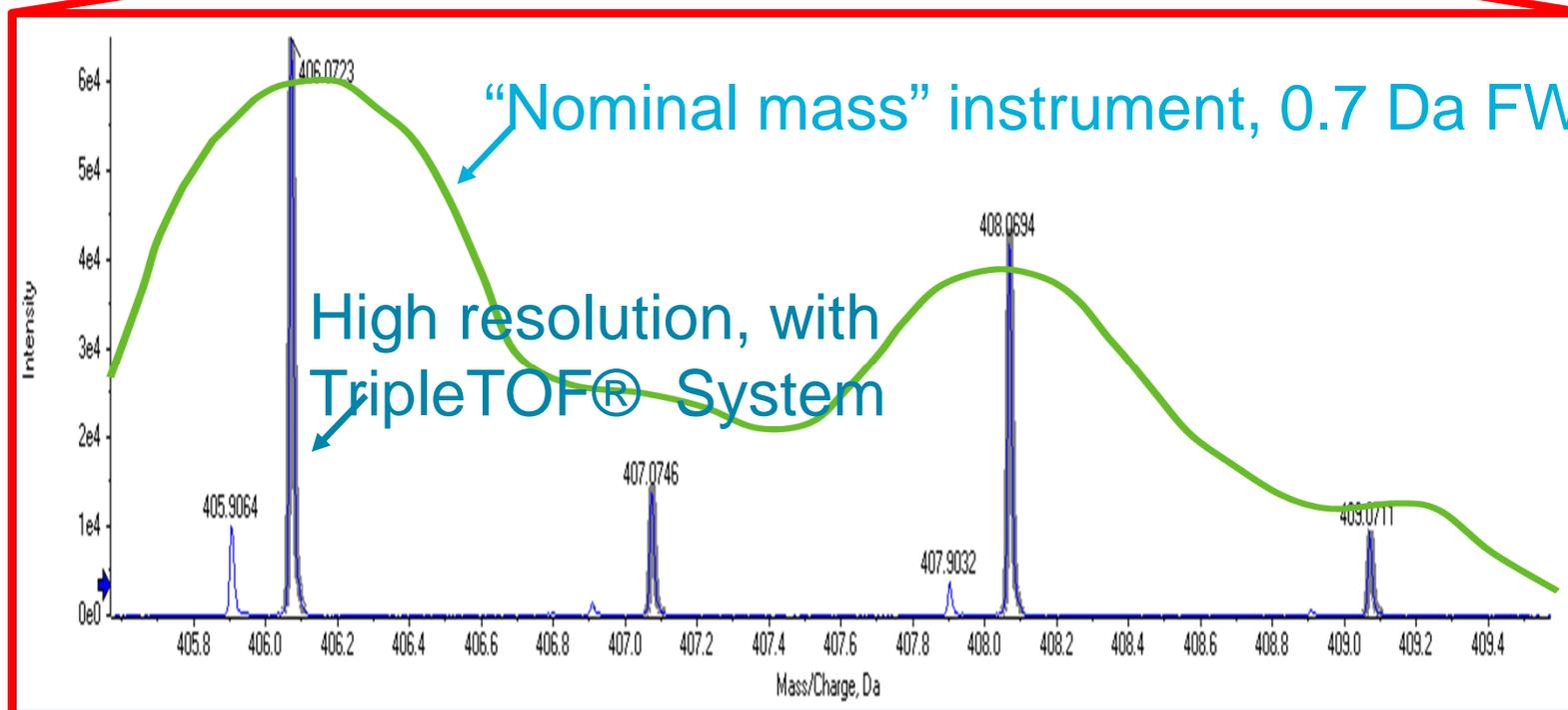
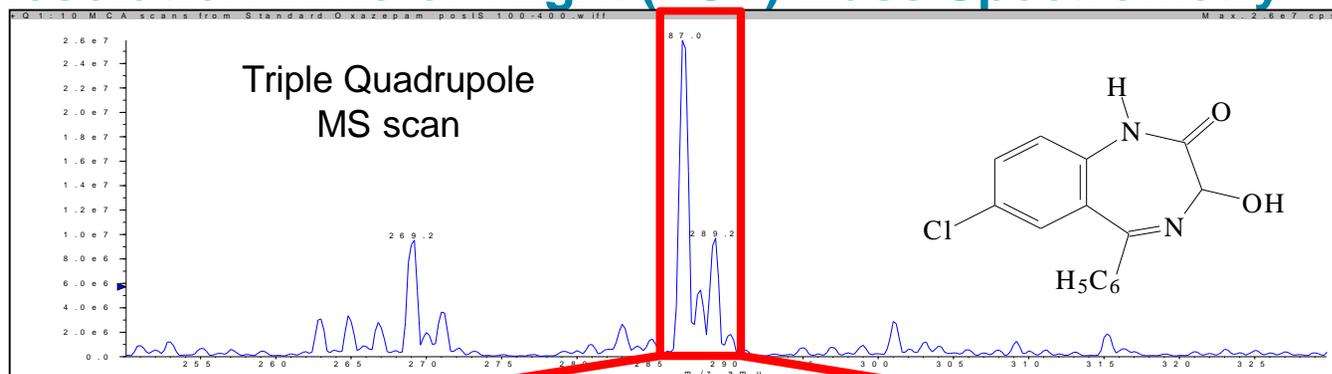
And, turning those data into results and decisions is a big challenge!

Principle of Time of Flight (TOF) mass spectrometry

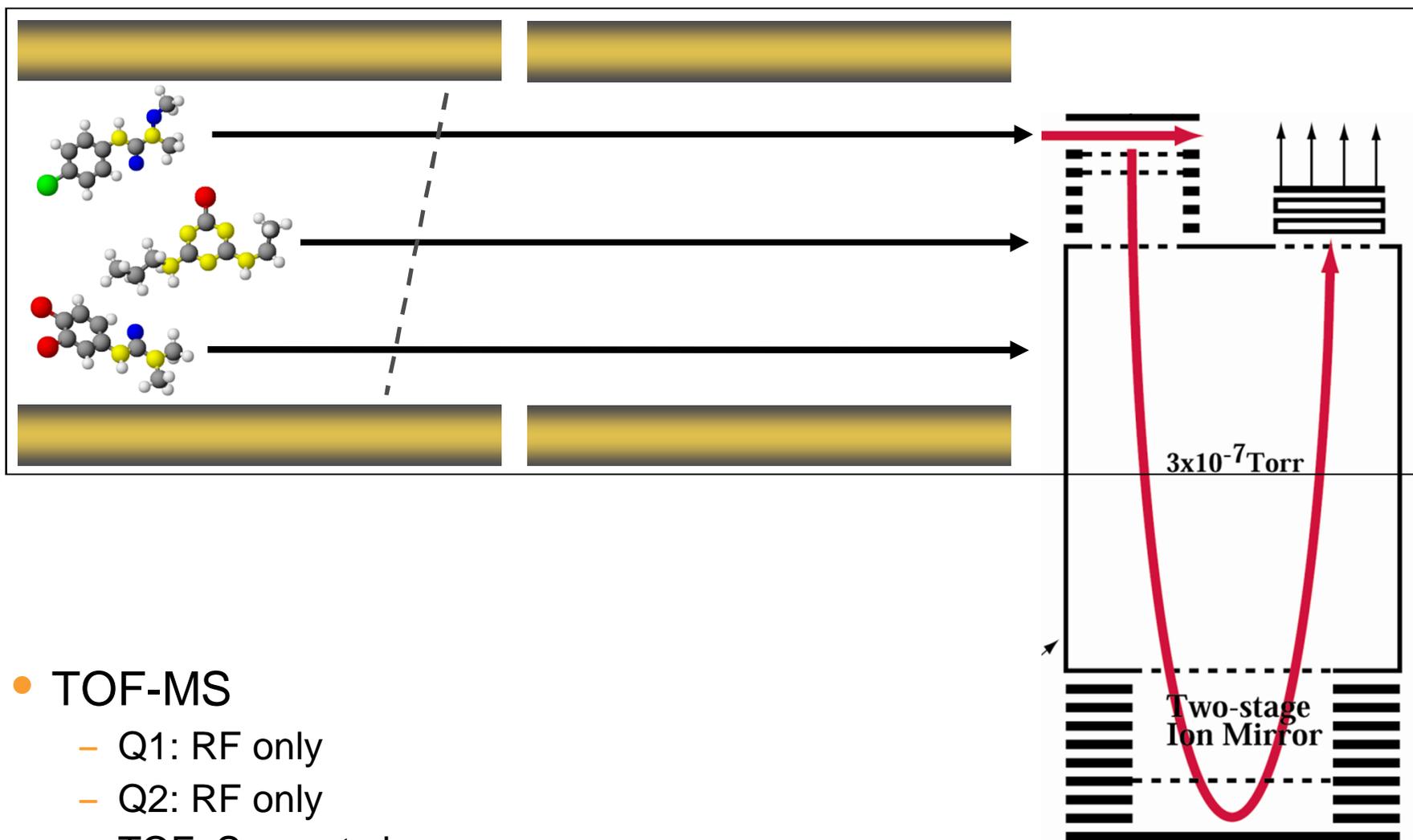
- Ions are pulsed and accelerated into TOF analyzer
- Separation of ions (m/z) based on time to fly through the flight path on a nanosecond time scale
 - Small m/z faster than heavier m/z
- Higher resolution with longer flight path (longer TOF tubes, reflector, faster acceleration)



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

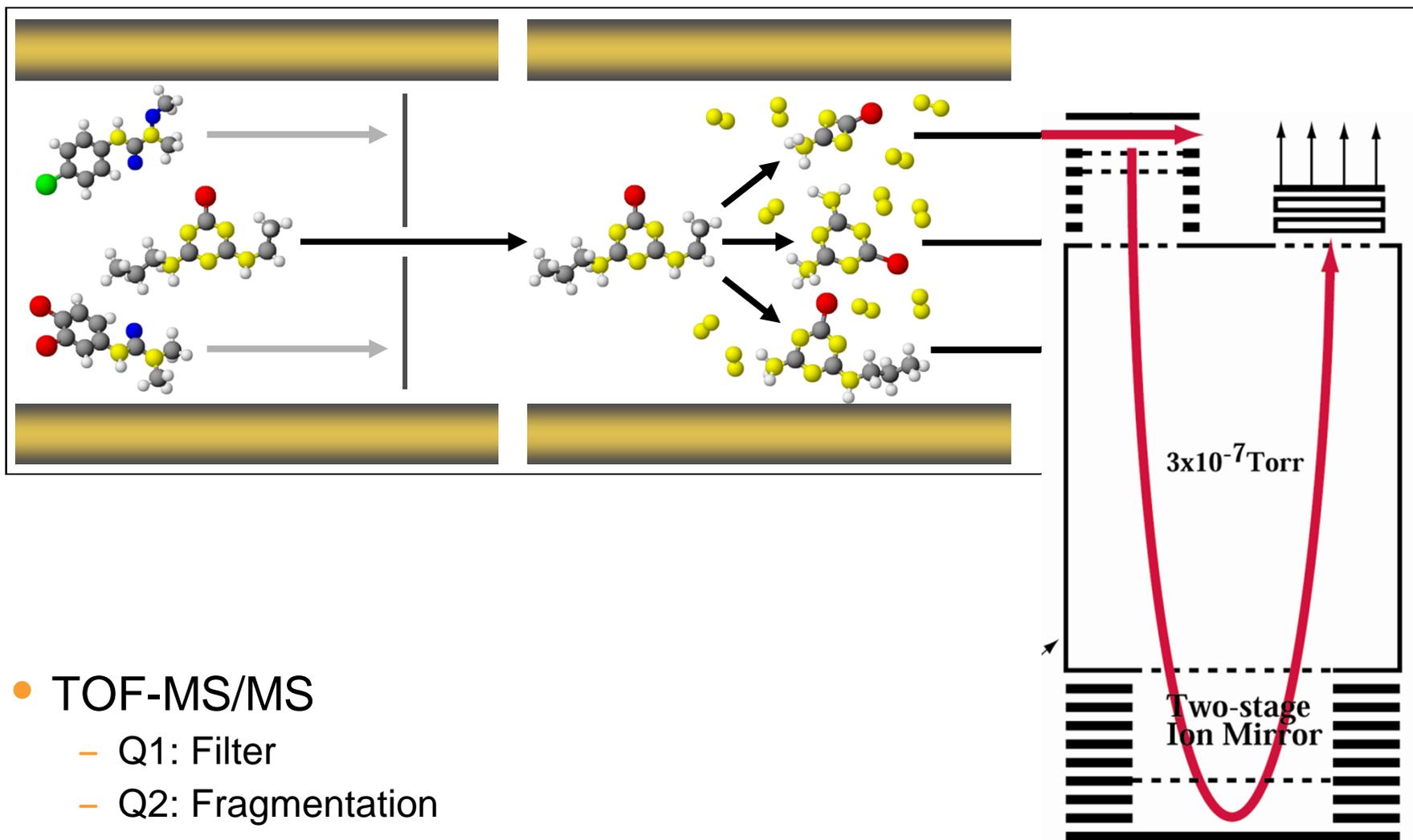


Time-of-Flight MS (TOF-MS)



- 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

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

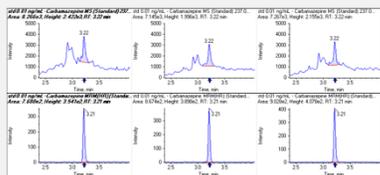
Scheduled MRM^{HR}

TOF-MS-IDA-MS/MS

Targeted processing

Targeted processing

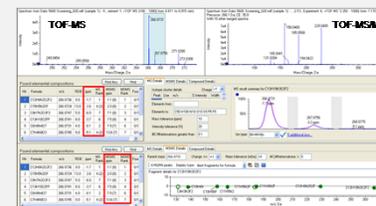
Non-targeted processing



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

What makes MS/MS the preferred technology?

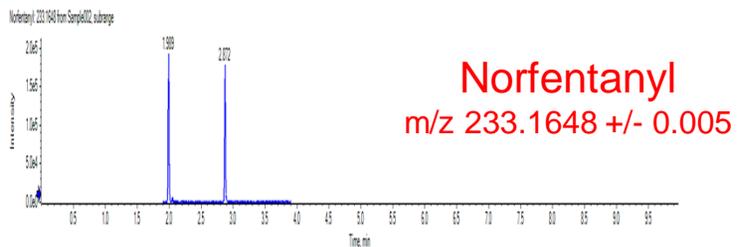
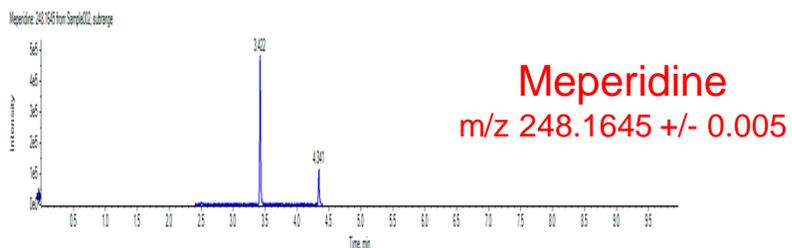
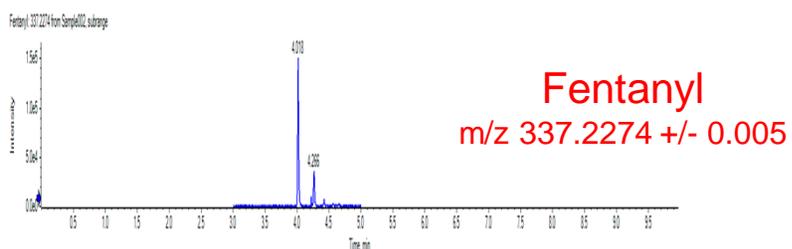
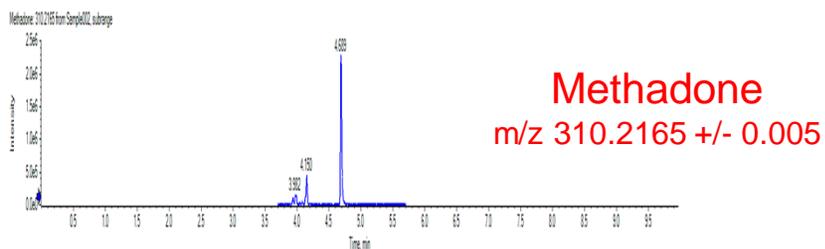
- Reduction of false negative rates
 - Co-eluting compounds
 - Interferences
- Reduction of false positives rates
 - MS/MS Library search



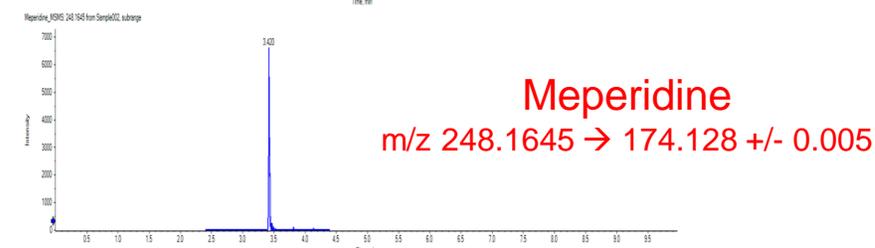
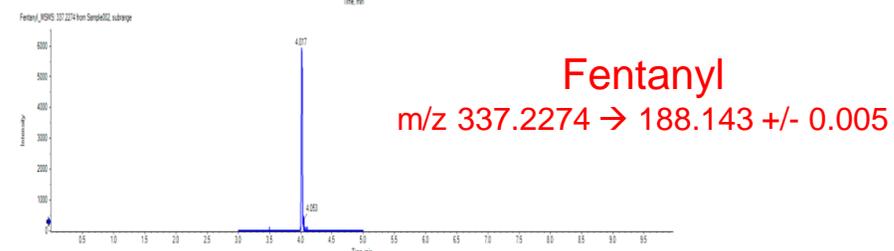
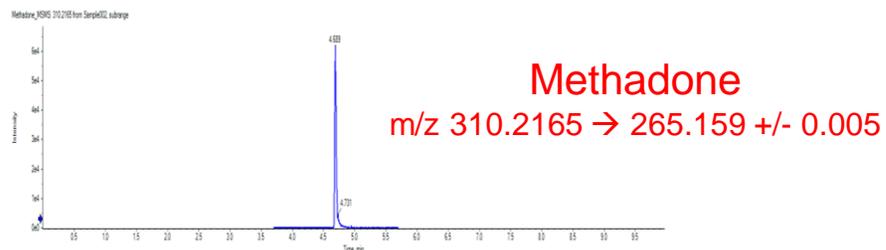
Reducing False Positive and Negative Using MS2 Library Searching in Screening Method

XIC width = 0.010 Da,

From TOF-MS



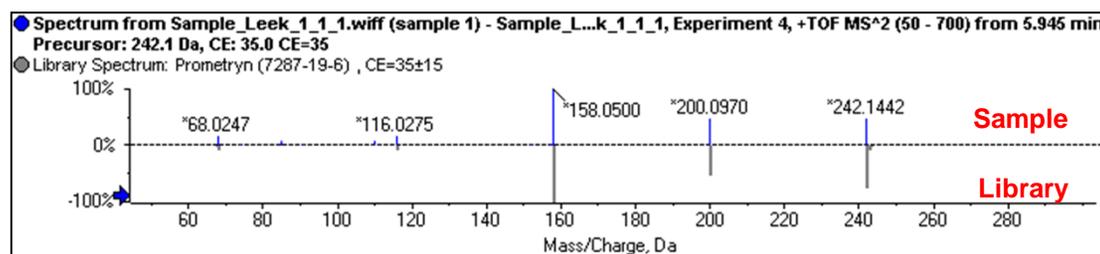
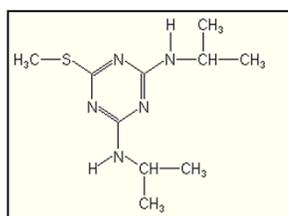
From TOF-MS/MS



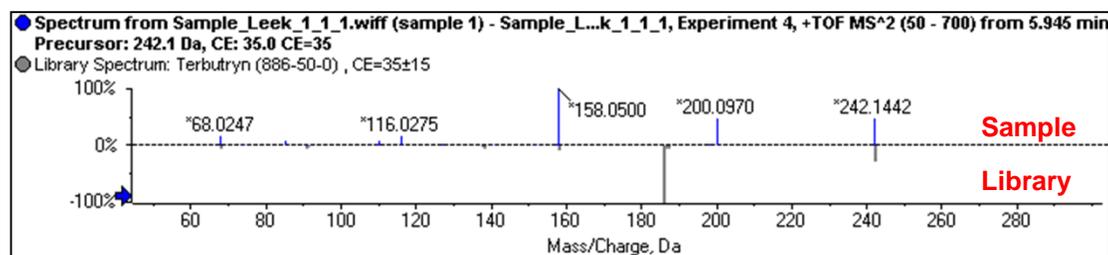
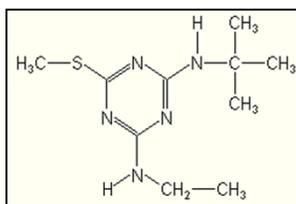
MS/MS Library Search can reduce false positive

- 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



#	<input type="checkbox"/>	Mass RT	Isotope Library	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	<input checked="" type="checkbox"/>	●●●●●	●●●●●	Prometryn	C10H19N5S	0	241.13612	+H	242.14339	0.02	242.14358	0.8	5.96	762861	5	74505	Prometryn	91.4
471	<input checked="" type="checkbox"/>	●●●●●	●●●●●	Terbutryn	C10H19N5S	0	241.13612	+H	242.14339	0.02	242.14358	0.8	5.96	762861	5	74505	Prometryn	91.4

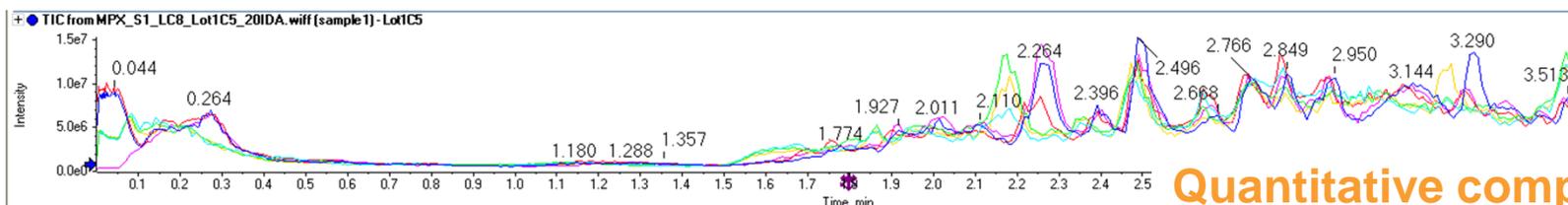
Workflow #1: Search for what you know

Targeted Screening and Quantitation



Targeted Screening using MasterView™

1. Define Retention Time and Accurate Mass for Each Compound



Quantitative comparison

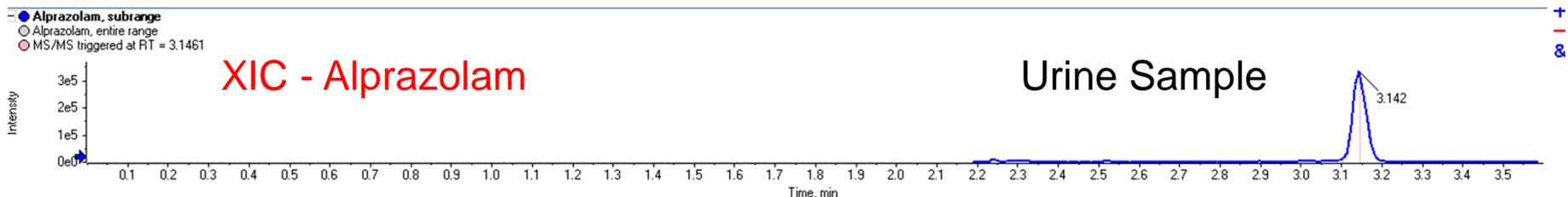
Name	Formula	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Expected RT (min)	RT Width (min)	Known Concentr.	Calculated Concentr.	Threshold (ratio of control)
6-MAM	C19H21NO4	327.14706	+H	328.15433	0.02	2.05	2	10		0.5
Alprazolam	C17H13ClN4	308.08287	+H	309.09015	0.02	3.19	2	10		0.5
Amphetamine	C9H13N	135.1048	+H	136.11208	0.02	1.99	2	10		0.5
Benzoyllecgonine	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
Hydroxylalprazolam	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'



MasterView **Masterview XIC list DEMO 16julyRT.XIClist**

CTR	Wiff file Name	Sample Name	Number of positive results	#	Mass	RT	Isotope	Library	Formula	Name	Formula	Isotope	Mass (Da)	Adduct	Int Std	Extraction Mass (Da)	Width (Da)	Width (ppm)	Expected RT (min)	RT Width (min)	Fragment Mass (Da)
✓	MPX_S1_LC8_Lot1	Sample 1	12	1	✓	✓	✓	✓	✓	6-MAM	C19H21NO4	0	327.14706	+H		328.15433	0.02	60.947	2.05	2	
✓	MPX_S1_LC8_Lot2	Sample 1	14	2	✓	✓	✓	✓	✓	Alprazolam	C17H13ClN4	0	308.08287	+H		309.09015	0.02	64.706	3.19	2	
✓	MPX_S1_LC8_Lot3	Sample 1	17	3	✓	✓	✓	✓	▲	Amphetamine	C9H13N	0	135.1048	+H		136.11208	0.02	146.938	1.99	2	
✓	MPX_S2_LC8_Lot1	Sample 1	12	4	✓	✓	✓	✓	▲	Benzoylcegonine	C16H19NO4	0	289.13141	+H		290.13868	0.02	68.933	2.43	2	
✓	MPX_S2_LC8_Lot2	Sample 1	10	5	✓	✓	✓	✓	✓	Buprenorphine	C29H41NO4	0	467.30356	+H		468.31084	0.02	42.707	2.82	2	
✓	MPX_S2_LC8_Lot3	Sample 1	10	6	✓	✓	✓	✓	▲	Carisoprodol	C12H24N2O4	0	260.17361	+H		261.18088	0.02	76.575	3.16	2	
✓	MPX_S2_LC8_Lot3	Sample 1	10	7	✓	✓	✓	✓	✓	Clonazepam	C15H10ClN3O3	0	315.04107	+H		316.04835	0.02	63.281	3.11	2	
				8	✓	✓	✓	✓	✓	Codeine	C18H21NO3								3.1	2	2
				9	✓	✓	✓	✓	✓	Diazepam	C16H13ClN2O								3.1	2	2
				10	✓	✓	✓	✓	✓	EDDP	C20H23N								2.78	2	2
				11	✓	✓	✓	✓	✓	Fentanyl	C22H28N2O								2.72	2	2
				12	✓	✓	✓	✓	▲	Flunitrazepam	C16H12FN3O3								3.12	2	2
				13	✓	✓	✓	✓	✓	Flurazepam	C21H23ClFN3O								2.8	2	2
				14	✓	✓	✓	✓	✓	Hydrocodone	C18H21NO3								1.9	2	2
				15	✓	●	●	●	●	Hydromorphone	C17H19NO3								128	2	2
				16	✓	✓	✓	✓	✓	Hydroxyalprazolam	C17H13ClN4O								3.13	2	2
				17	✓	✓	✓	✓	✓	Lorazepam	C15H10Cl2N2O2								3.18	2	2
				18	✓	✓	✓	✓	✓	MDA	C10H13NO2								2.1	2	2
				19	✓	✓	✓	✓	▲	MDEA	C12H17NO2								2.23	2	2
				20	✓	✓	✓	✓	▲	MFMΔ	C11H15NO2								2.12	2	2

Confidence in Identification by:

- Mass Error
- Retention Time
- Isotope Match
- MS/MS Library Purity Score
- Formula finding

Sample: MPX_S1_LC8_Lot2C5_20IDA(Sample 1) Control: None Rows 43 Process Cancel



How you can use the traffic lights in your data review

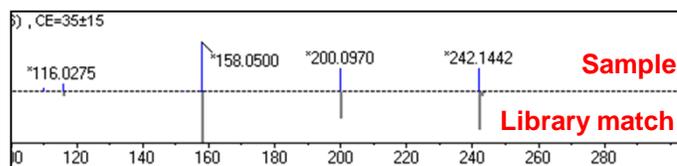
Mass	RT	Isotope	Library	Formula	Name
✓	✓	✓	✓	✓	Alprazolam
✓	✓	✓	✓	▲	Amphetamine
✓	✓	✓	✓	▲	Benzoylcegonine
✓	✓	✓	✓	▲	Buprenorphine
▲	✓	●	✓	●	Carisoprodol
✓	✓	●	✓	●	Clonazepam



Positive identification with high confidences



Review – Match



Customizable Review Settings:
Positive ID with highest confidence

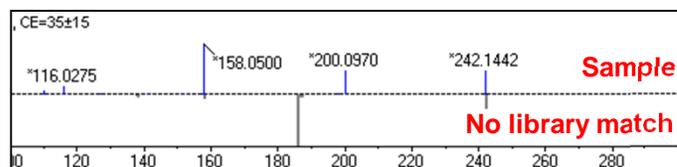
Select combination for positive result(equal or better)

Mass: ✓ RT: ✓ Isotope: ✓ Library: ✓ Formula: ✓ Combined Score: 50.0

Grouping Samples by Wiff file



Review – No match



Identified compounds to review

Select combination for positive result(equal or better)

Mass: ? RT: ? Isotope: ? Library: ? Formula: ? Combined Score: 50.0

Grouping Samples by Wiff file

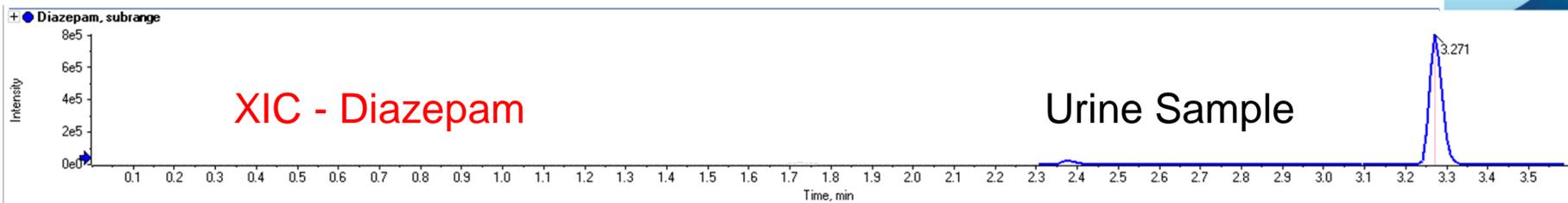


Negative identification



Targeted Screening using MasterView™

4. Result Display (Identified Compounds to Review)



MasterView Masterview XIC list DEMO 16JulyRT.XIClist

C	T	R	L	Wiff file Name	Sample Name	Number of positive results
✓				MPX_S1_LC8_Lot1	Sample 1	12
✓				MPX_S1_LC8_Lot2	Sample 1	14
✓				MPX_S1_LC8_Lot2	Sample 1	17
✓				MPX_S2_LC8_Lot1	Sample 1	12
✓				MPX_S2_LC8_Lot2	Sample 1	10
✓				MPX_S2_LC8_Lot2	Sample 1	10

#	Mass	RT	Isotope	Library	Formula	Name	Formula	Isotope	Mass (Da)	Adduct	Extraction Mass (Da)	Width (ppm)	Expected RT (min)	RT Width (min)	Error (ppm)	Found At RT (min)	Threshold (ratio of control)	Thre (cps)
2	✓	✓	✓	✓	✓	Alprazolam	C17H13ClN	0	308.08287	+H	309.09015	64.706	3.19	2	1.4	3.14	1	10
5	✓	✓	✓	✓	✓	Buprenorph	C29H41NO4	0	467.30356	+H	468.31084	42.707	2.82	2	0.8	2.78	1	10
7	✓	✓	✓	✓	✓	Clonazepar	C15H10ClN	0	315.04107	+H	316.04835	63.281	3.11	2	1.1	3.06	1	10
8	✓	✓	✓	✓	✓	Codeine	C18H21NO2	0	299.15214	+H	300.15942	66.631	2	2	2.7	1.91	1	10
9	✓	✓	✓	✓	✓	Diazepam	C16H13ClN	0	284.07164	+H	285.07892	70.156	3.31	2	0.9	3.27	1	10
10	✓	✓	✓	✓	✓	EDDP	C20H23N	0	277.18305	+H	278.19033	71.893	2.78	2	1.3	2.74	1	10
11	✓	✓	✓	✓	✓	Fentanyl	C22H28N2C	0	336.22016	+H	337.22744	59.307	2.72	2	0.1	2.67	1	10
13	✓	✓	✓	✓	✓	Flurazepam	C21H23ClF	0	387.15137	+H	388.15865	51.525	2.8	2	-0.1	2.75	1	10
14	✓	✓	✓	✓	✓	Hydrocodor	C18H21NO2	0	299.15214	+H	300.15942	66.631	1.9	2	2.7	1.91	1	10
16	✓	✓	✓	✓	✓	Hydroxyalpi	C17H13ClN	0	324.07779	+H	325.08507	61.522	3.13	2	1.1	3.08	1	10
17	✓	✓	✓	✓	✓	Lorazepam	C15H10Cl2N	0	320.01193	+H	321.01921	62.302	3.18	2	0.3	3.14	1	10
18	✓	✓	✓	✓	✓	MDA	C10H13NO2	0	179.09463	+H	180.10191	111.048	2.1	2	0	2.11	1	10
25	✓	✓	✓	✓	✓	Midazolam	C18H13ClF	0	325.0782	+H	326.08548	61.334	2.79	2	0.3	2.75	1	10
40	✓	✓	✓	✓	✓	Temazepam	C16H13ClN	0	300.06656	+H	301.07383	66.429	0	2	1.3	3.19	1	10

Select combination for positive result(equal or better)

Mass RT Isotope Library Formula Combin Sc

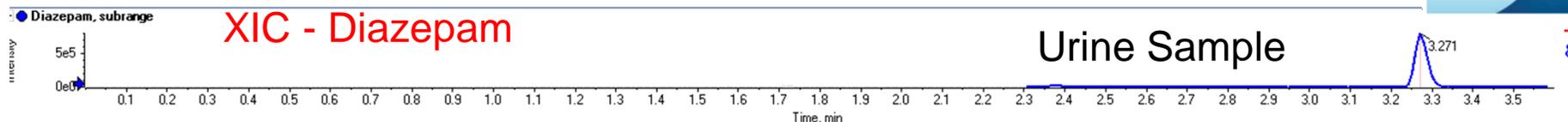
Grouping Samples by Wiff file

Sample: MPX_S1_LC8_Lot2C5_20IDA(Sample 1) Control: None Rows 43

Only compounds that have 5 green ticks will be displayed

Targeted Screening using MasterView™

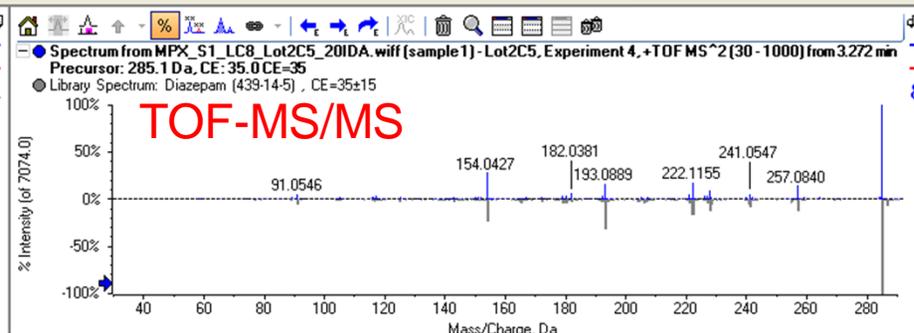
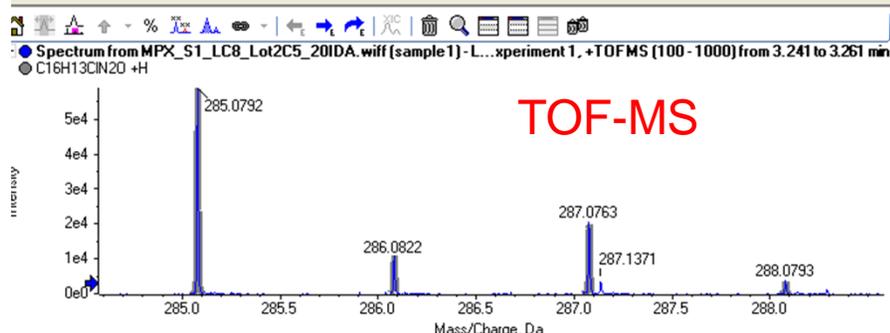
5. Review MS and MS/MS spectra



MasterView Masterview XIC list DEMO 16julyRT.XIClist

Mass	RT	Isotope	Library	Formula	Name	Formula	Isotope	Mass (Da)	Adduct	Extraction Mass (Da)	Width (ppm)	Expected RT (min)	RT Width (min)	Error (ppm)	Found At RT (min)	Threshold (ratio of control)	Threshold (cps)	Library Hit	Library Score	Mass Error Score	RT Score	Isotope Score	Combin Score
✓	✓	✓	✓	✓	Alprazolam	C17H13ClN	0	308.08287	+H	309.09015	64.706	3.19	2	1.4	3.14	1	1000	Alprazolam	87.1	85.9	90.1	80.4	85.3
✓	✓	✓	✓	✓	Buprenorph	C29H41NO4	0	467.30356	+H	468.31084	42.707	2.82	2	0.8	2.78	1	1000	Buprenorph	100	92.4	89.9	94.4	92.8
✓	✓	✓	✓	✓	Clonazepam	C15H10ClN	0	315.04107	+H	316.04835	63.281	3.11	2	1.1	3.06	1	1000	Clonazepam	80.7	89.3	88.9	84.2	83.3
✓	✓	✓	✓	✓	Codeine	C18H21NO2	0	299.15214	+H	300.15942	66.631	2	2	2.7	1.91	1	1000	Codeine	96.9	72.7	71.7	92.9	82.9
✓	✓	✓	✓	✓	Diazepam	C16H13ClN	0	284.07164	+H	285.07892	70.156	3.31	2	0.9	3.27	1	1000	Diazepam	96.2	90.9	92.2	95.5	90.4
✓	✓	✓	✓	✓	EDDP	C20H23N	0	277.18305	+H	278.19033	71.893	2.78	2	1.3	2.74	1	1000	EDDP	88.7	86.6	90.2	89.3	87.4
✓	✓	✓	✓	✓	Fentanyl	C22H28N2C	0	336.22016	+H	337.22744	59.307	2.72	2	0.1	2.67	1	1000	Fentanyl	95.6	99.4	88.5	67.4	85.4
✓	✓	✓	✓	✓	Flurazepam	C21H23ClF	0	387.15137	+H	388.15865	51.525	2.8	2	-0.1	2.75	1	1000	Flurazepam	95.6	98.6	88.5	74.9	89.9
✓	✓	✓	✓	✓	Hydrocodone	C18H21NO2	0	299.15214	+H	300.15942	66.631	1.9	2	2.7	1.91	1	1000	Codeine	96.9	72.7	94.7	92.9	87.5
✓	✓	✓	✓	✓	Hydroxyzine	C17H13ClN	0	324.07779	+H	325.08507	61.522	3.13	2	1.1	3.08	1	1000	alpha-Hydroxyzine	87.7	88.8	90.2	64.5	83.6

Sample: MPX_S1_LC8_Lot2C5_20IDA(Sample 1) Control: None Rows 43 Process Cancel



mirror view of spectra for easy comparison

Targeted Screening using MasterView™

6. Details of Library Search and Formula Finder



+ Diazepam, entire range

Time, min

MasterView Masterview XIC list DEMO 16julyRT.XIClist

CTRL	Wiff file Name	Sample Name	Number of positive results
✓	MPX_S1_LC8_Lot1	Sample 1	12
✓	MPX_S1_LC8_Lot2	Sample 1	14
✓	MPX_S1_LC8_Lot3	Sample 1	17
✓	MPX_S2_LC8_Lot1	Sample 1	12
✓	MPX_S2_LC8_Lot2	Sample 1	10
✓	MPX_S2_LC8_Lot3	Sample 1	10

#	Mass	RT	Isotope	Library	Formula	Name	Formula	Isotope	Mass (Da)	Adduct	Extraction Mass (Da)	Width (ppm)	Expected RT (min)	RT Width (min)	Error (ppm)	Found At RT (min)	Threshold (ratio of control)	Thre (cps)
2	✓	✓	✓	✓	✓	Alprazolam	C17H13ClN	0	308.08287	+H	309.09015	64.706	3.19	2	1.4	3.14	1	10
5	✓	✓	✓	✓	✓	Buprenorph	C29H41NO4	0	467.30356	+H	468.31084	42.707	2.82	2	0.8	2.78	1	10
7	✓	✓	✓	✓	✓	Clonazepam	C15H10ClN	0	315.04107	+H	316.04835	63.281	3.11	2	1.1	3.06	1	10
8	✓	✓	✓	✓	✓	Codeine	C18H21NO2	0	299.15214	+H	300.15942	66.631	2	2	2.7	1.91	1	10
9	✓	✓	✓	✓	✓	Diazepam	C16H13ClN	0	284.07164	+H	285.07892	70.156	3.31	2	0.9	3.27	1	10

Library Search Results

Compound Name	CAS #	Formula	MW (Da)	Fit	Rev. F	Purity	CI
Diazepam	439-14-5	C16H13ClN2O	284.74521	96.2	96.8	96.2	35
Diazepam	439-14-5	C16H13ClN2O	284.74521	96.2	96.8	96.2	35
Macrosporin	22225-67-8	C16H12O5	284.26832	96.4	90.5	88.9	35
Macrosporin	22225-67-8	C16H12O5	284.26832	96.4	90.5	88.9	35
Mazindol	22232-71-9	C16H13ClN2O	284.74521	99.9	88.1	88	35

Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MS/MS (ppm)
C16H13N2OCl	C16H13N2OCl	76.9	285.07892	0.9	3.7
C14H20S3	C14H20S3	20.9	285.07999	2.9	0.4

Click to apply the Name and Formula of this result to XIC

Select combination for positive result (equal or better)

Mass RT Isotope Library Formula Combin Sc

Grouping Samples by Wiff file

Sample: MPX_S1_LC8_Lot2C5_20IDA(Sample 1) Control: None Rows 43

Process Cancel

automatic formula finding

Targeted Screening using MasterView™

7. Quantitation through MultiQuant™ Software and Reporting



Save MultiQuant Method As Text... Generate Report...

MasterView Masterview XIC list DEMO 16julyRT.XIClist

CTRL	Wiff file Name	Sample Name	Number of positive results
✓	MPX_S1_LC8_Lot1	Sample 1	12
✓	MPX_S1_LC8_Lot2	Sample 1	14
✓	MPX_S1_LC8_Lot3	Sample 1	17
✓	MPX_S2_LC8_Lot1	Sample 1	12
✓	MPX_S2_LC8_Lot2	Sample 1	10
✓	MPX_S2_LC8_Lot3	Sample 1	10

Name	Formula	Isotope	Mass (Da)	Adduct	Extraction Mass (Da)	Width (ppm)	Expected RT (min)	RT Width (min)	Error (ppm)	Found At RT (min)	Threshold (ratio of control)	Threshc (cps)
Alprazolam	C17H13ClN	0	308.08287	+H	309.09015	64.706	3.19	2	1.4	3.14	1	1000
Buprenorph	C29H41NO4	0	467.30256	+H	468.31084	42.707	2.82	2	0.8	2.78	1	1000
Clonazepam	C15H10ClN2	0	315.04107	+H	316.04835	63.281	3.11	2	1.1	3.06	1	1000
Codeine	C18H21NO2	0	299.15214	+H	300.15942	66.631	2	2	2.7	1.91	1	1000
Diazepam	C16H13ClN	0	284.07164	+H	285.07892	70.156	3.31	2	0.9	3.27	1	1000
EDDP	C20H23N	0	277.18305	+H	278.19033	71.893	2.78	2	1.3	2.74	1	1000
Fentanyl	C22H28N2C	0	336.22016	+H	337.22744	59.307	2.72	2	0.1	2.67	1	1000
Flurazepam	C21H23ClF	0	387.15137	+H	388.15865	51.525	2.8	2	-0.1	2.75	1	1000
Hydrocodone	C18H21NO2	0	299.15214	+H	300.15942	66.631	1.9	2	2.7	1.91	1	1000

0.1 ng/mL 5.84

0.1 ng/mL 5.84

0.1 ng/mL 5.84

10 µg/kg after extract dilution

3.4-Methylenedioxyamphetamine

Retention Time: 4.58 minutes Exp RT: 4.70 minutes
 Extraction Mass: 194.12 Analyte Name: 3.4-Methylenedioxyamphetamine
 Fit (%) 96.7% REfit (%) 96.7%

Collision Energy = 35 ± 15 eV

Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	
3.4-Methylenedioxyamphetamine (MDMA)	C11H15NO2	244328		194.1176	194.1177	0.6	4.70	4.58	0.12

Workflow #2: Remove the 'healthy' / 'normal' peaks

Reduce unknown peaks to just those that are suspicious



Comparative Unknown Screening

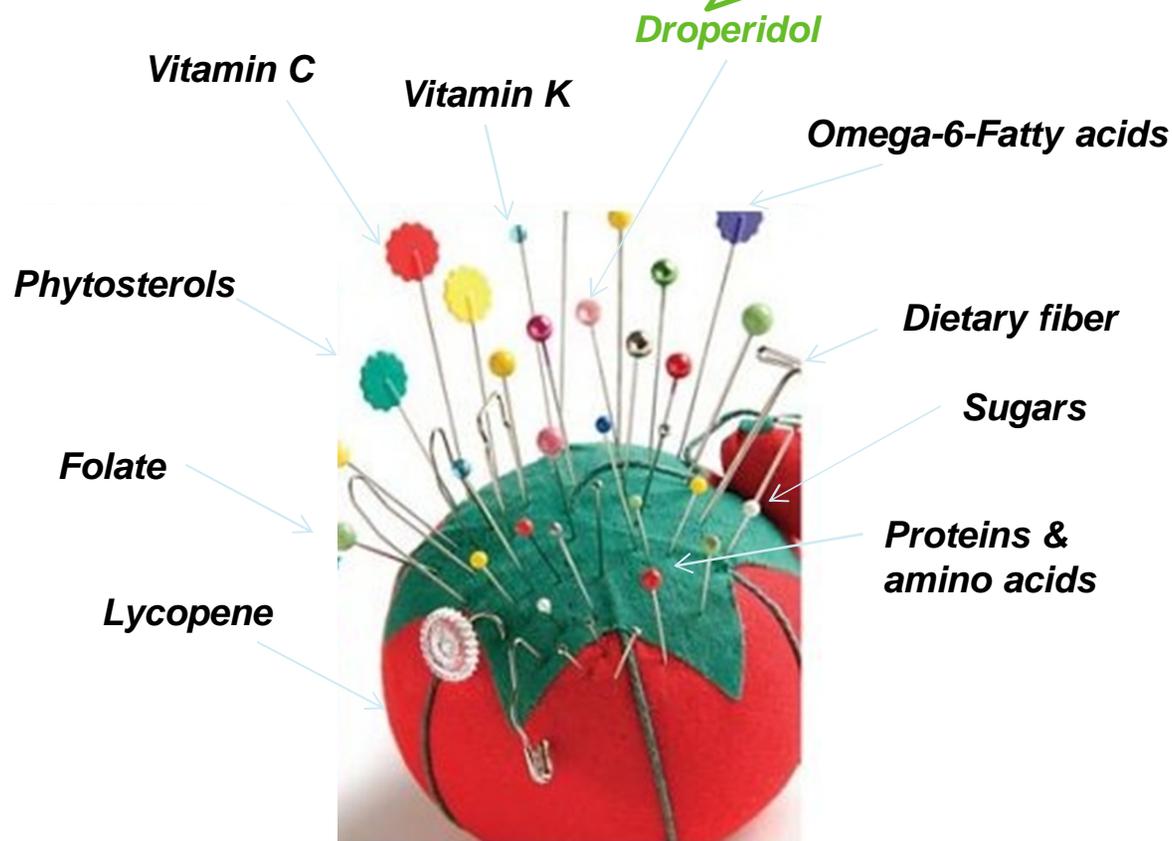
What is it and why is this a good approach?

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

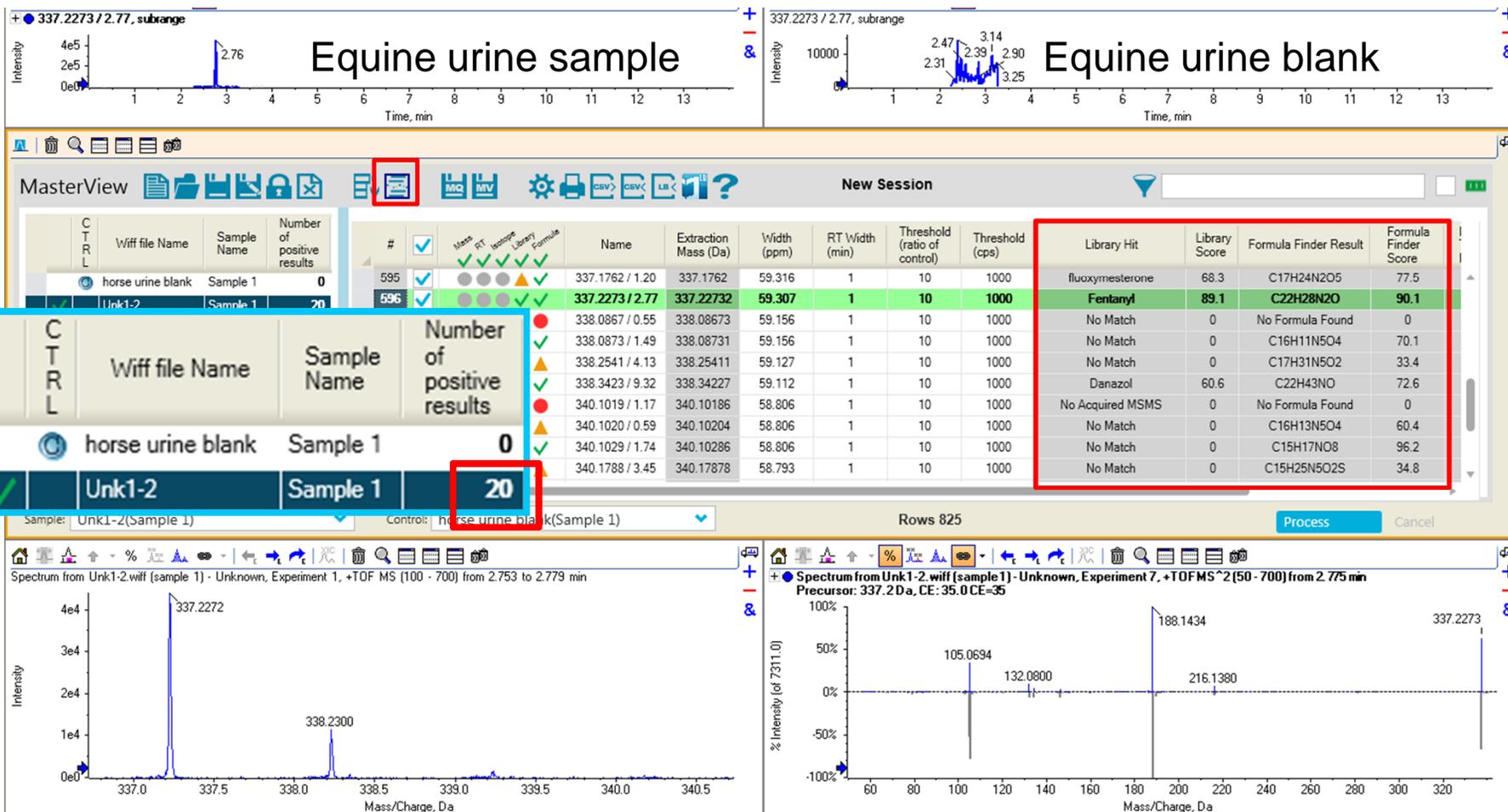
How can you find one low level toxic compound amidst everything else?





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

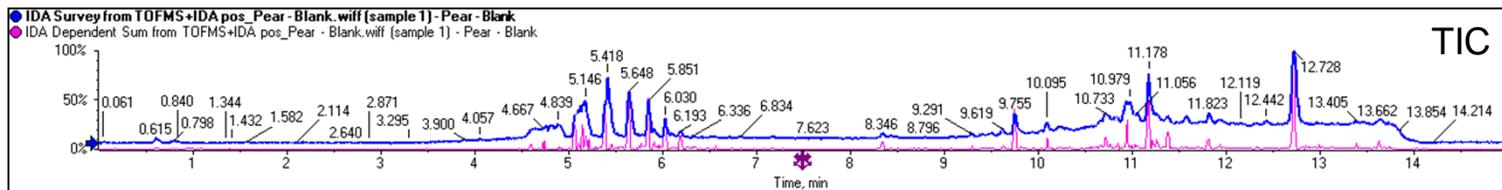


Data Processing Workflow

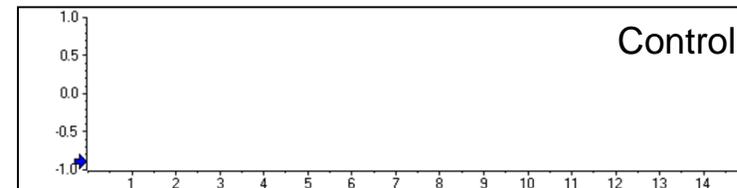
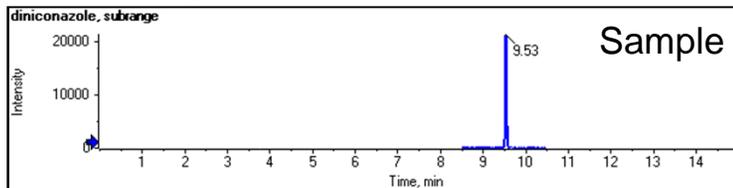
Automatic Non-Target (Unknown) Screening and Identification



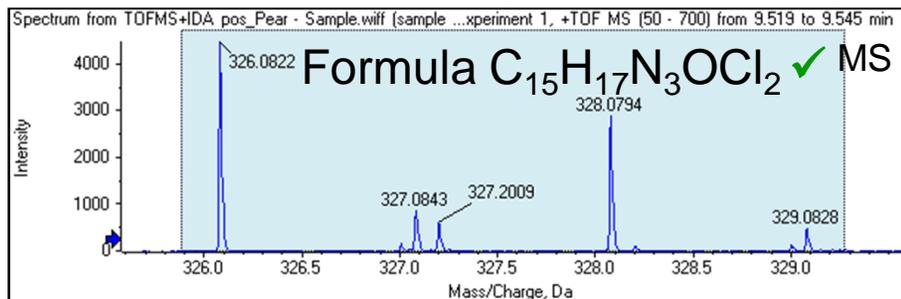
TIC of Sample and Control



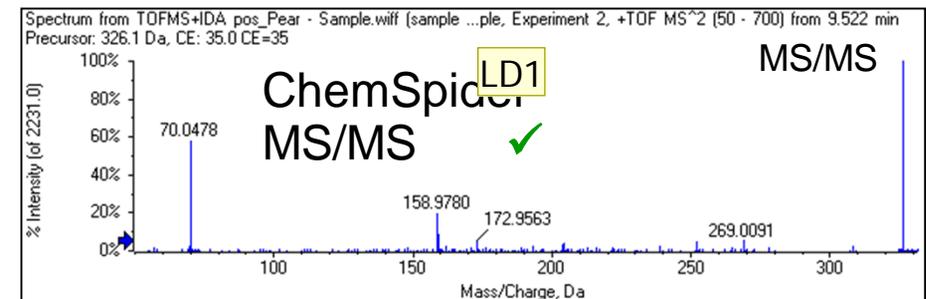
Non-Target XIC Generation and Sample-Control-Comparison



Evaluation of TOF-MS (Formula Finder)



Evaluation of TOF-MS/MS (Search)



Slide 29

LD1

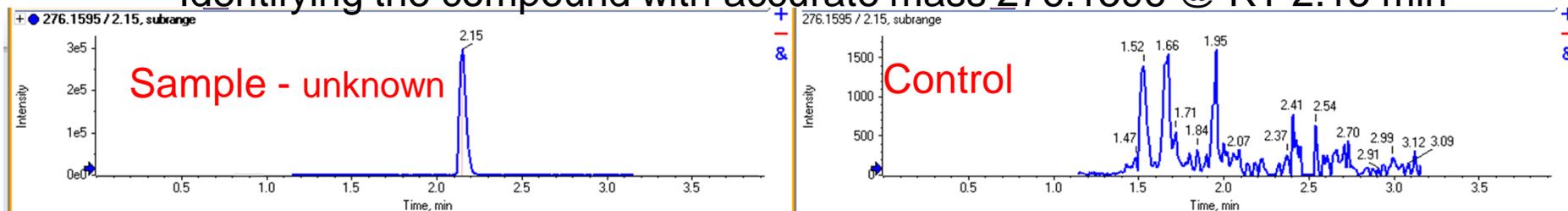
Please remove logo.

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 interface showing a table of results for peak 151. The table includes columns for #, Mass, RT, Isotope, Formula, Name, Formula, Isotope, Mass (Da), Adduc, Extraction Mass (Da), Width (ppm), Expected RT (min), RT Width (min), Error (ppm), Found At RT (min), Threshold (ratio of control), Threshold (ppm), Library Hit, and Library Score.

#	Mass	RT	Isotope	Formula	Name	Formula	Isotope	Mass (Da)	Adduc	Extraction Mass (Da)	Width (ppm)	Expected RT (min)	RT Width (min)	Error (ppm)	Found At RT (min)	Threshold (ratio of control)	Threshold (ppm)	Library Hit	Library Score
151	276.1595	2.15	0	C ₁₆ H ₂₁ N ₃ O ₃	4-hydroxypropranolol	C ₁₆ H ₂₁ N ₃ O ₃	0	276.15955		276.15955	72.422	2.15	2	0.1	2.15	1	1000	4-hydroxypropranolol	42.7

Library Search Results table:

Compound Name	CAS #	Formula	MW (Da)	Fit	Rev. F	Purity	CI
4-hydroxypropranolol	10476-53-6	C ₁₆ H ₂₁ N ₃ O ₃	275.34777	87.2	48.9	42.7	35

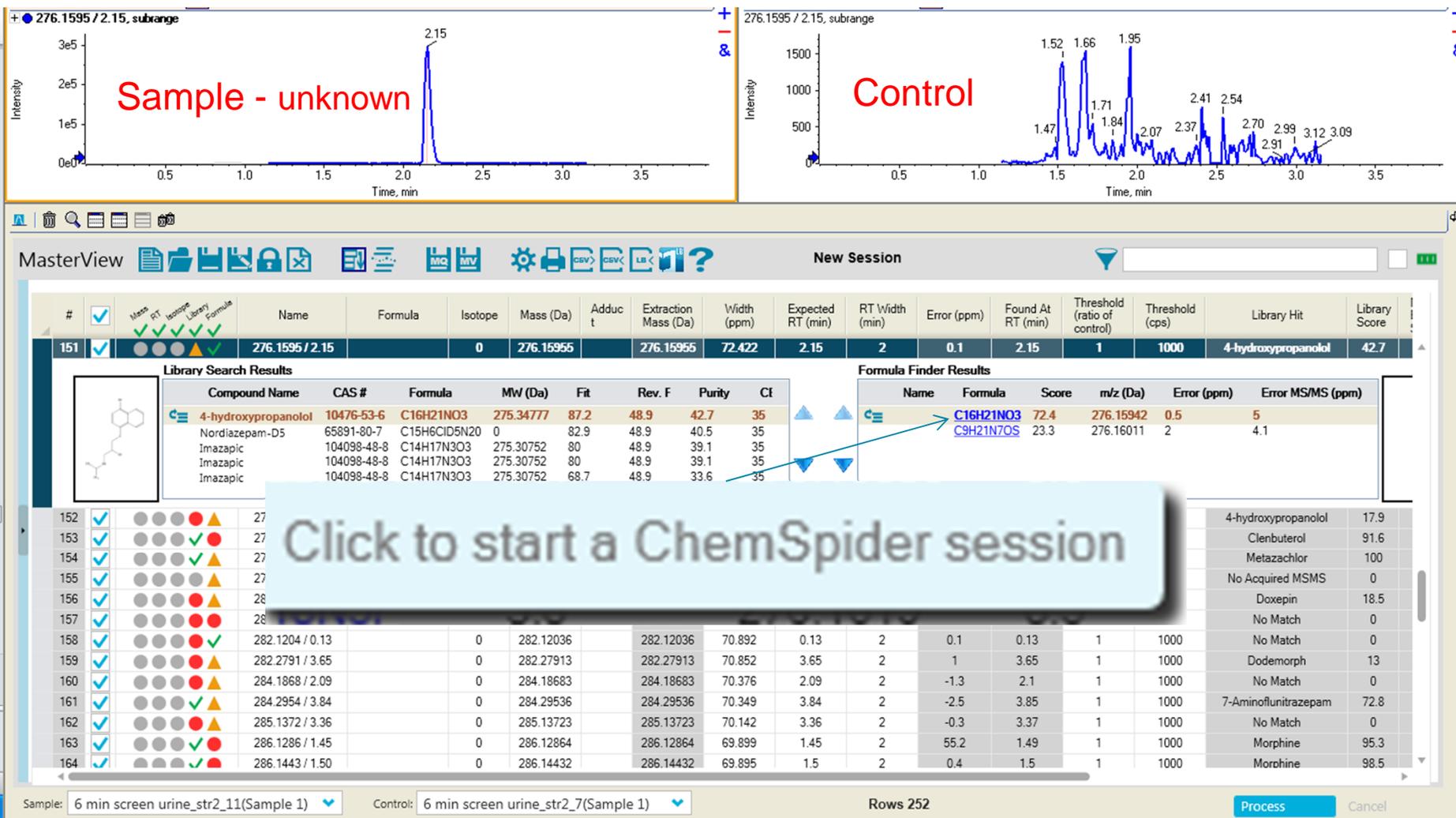
Formula Finder Results table:

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MS/MS (ppm)
4-hydroxypropranolol	C ₁₆ H ₂₁ N ₃ O ₃	72.4	276.15942	0.5	5
4-hydroxypropranolol	C ₉ H ₂₁ N ₇ O ₅	23.3	276.16011	2	4.1

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

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.

ChemSpider results for: **C16H21NO3** 121-160 of 3460

CSID	Common Name	Molecular Weight
111892	2,4-(1H,3H)-Quinolinedione, 3-heptyl-3-hyd	275.3428
16788110	Methylenedioxypropylvalerone	275.3428
640133	(2E)-N-Cyclopentyl-3-(2,5-dimethoxyphenyl)	275.3428
729870	Ethyl 2-[(cyclohexylcarbonyl)amino]benzoate	275.3428
954861	benzoic acid, 2-[(2-cyclohexylacetyl)amino]	275.3428
640129	(2E)-3-(2,5-Dimethoxyphenyl)-1-(piperidin-	275.3428
628773	benzeneacetamide, 3,4-dimethoxy-N,N-di-	275.3428
2266590	4-(1-Azepanylcarbonyl)phenyl propionate	275.3428
2255856	3-benzofuranacetamide, N-(2-methoxy-1-n	275.3428
2192052	N-[1-(2,3-Dihydro-1,4-benzodioxin-5-yl)eth	275.3428
2086017	4-(4-cyclohexylanilino)-4-oxobutanoic acid	275.3428
1907159	2-(Cyclohexylamino)-2-oxoethyl 3-methylb	275.3428
1905636	5-Methoxy-3-methyl-N-(3-methylbutyl)-1-b	275.3428
777719	1-(Benzoyloxy)-2,2,6,6-tetramethylpiperid	275.3428
685268	2-Cyclohexyl-N-(2,3-dihydro-1,4-benzodiox	275.3428
576391	Ethyl N-[(1-phenylcyclopentyl)carbonyl]glyc	275.3428
574737	Acetic acid, 2-(3-methylphenoxy)-, 1-azabi	275.3428

Spectrum from 6 min screen urine_str2_11.wiff (sample 1) - unknown 2, Experiment 8, +TOF MS² (30 - 1000) from 2.147 min
Precursor: 276.2 Da, CE: 35.0 CE=35

Tentatively Identified as MDPV

Mass/Charge	Intensity (%)	Assigned	Error (Da)
84.0795	7.31	<input checked="" type="checkbox"/>	0.001
121.0273	7.93	<input checked="" type="checkbox"/>	0.001
126.1272	74.55	<input checked="" type="checkbox"/>	0.001
135.0431	55.43	<input checked="" type="checkbox"/>	0.001
147.0788	7.31	<input checked="" type="checkbox"/>	0.071
148.0777	14.92	<input checked="" type="checkbox"/>	0.001

Matches: 10 of 10 peaks, 100.0% of total intensity

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

ChemSpider – link

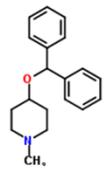
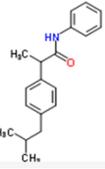
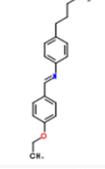
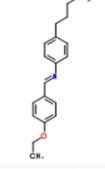
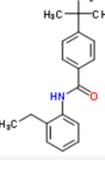
Allows searching based on number of references and data sources

Intrinsic Properties Search | MF=C₁₉H₂₃NO - Mozilla Firefox

www.chemspider.com/Molecular-Formula/C19H23NO

100 hits returned from a total of 2019.
Search terms: MF = 'C₁₉H₂₃NO'

Grid Tile Table Names/Structures Names

ID	Structure	Molecular Formula	Molecular Weight	# of Data Sources	# of References	# of PubMed	# of RSC
2992 W		C ₁₉ H ₂₃ NO	281.392	34	121	22267	101
39221 - 0/1 defined		C ₁₉ H ₂₃ NO	281.392	21	27	0	0
85198 		C ₁₉ H ₂₃ NO	281.392	21	26	1	8
594230		C ₁₉ H ₂₃ NO	281.392	20	27	0	0

All MS and MS/MS results at your fingertips

FACT

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

FACT

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

FACT

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!



Answers for Science.
Knowledge for Life.™



Thanks for listening