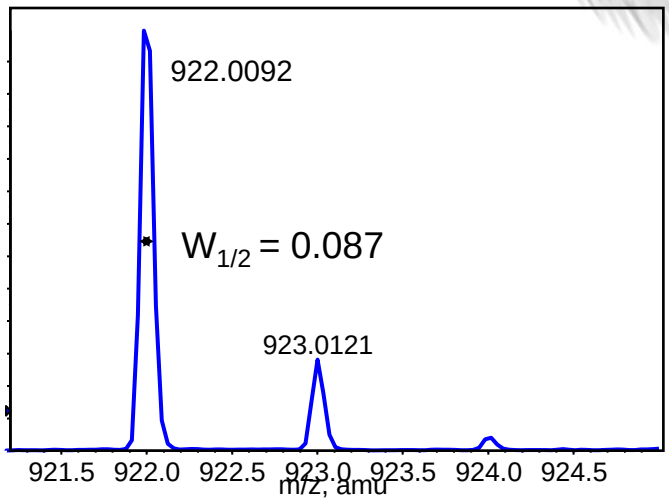
Time of Flight The Power of Accurate Mass

What is "Accurate Mass"?

- Mass measurements accurate to several decimal places (rather than "nominal" or "unit" mass measurements of ± 0.1 Da)
- Made possible by higher resolution mass analyzers
- Formerly limited to magnetic sector instruments
- Now available with Time of Flight (TOF) and Fourier Transform (FT) instruments (FT-MS and "orbitrap")
- Most Common Uses:
  - Allows confirmation of molecular formula for proposed compounds
  - Allows calculation of possible molecular formulae for unknowns
  - Allows rapid selective and specific screening for target compounds

Figures of Merit for Accurate-Mass Instrument Performance

Resolution = $(m/z)/w_{1/2}$
e.g.,
922.0092/0.087 = 10,597

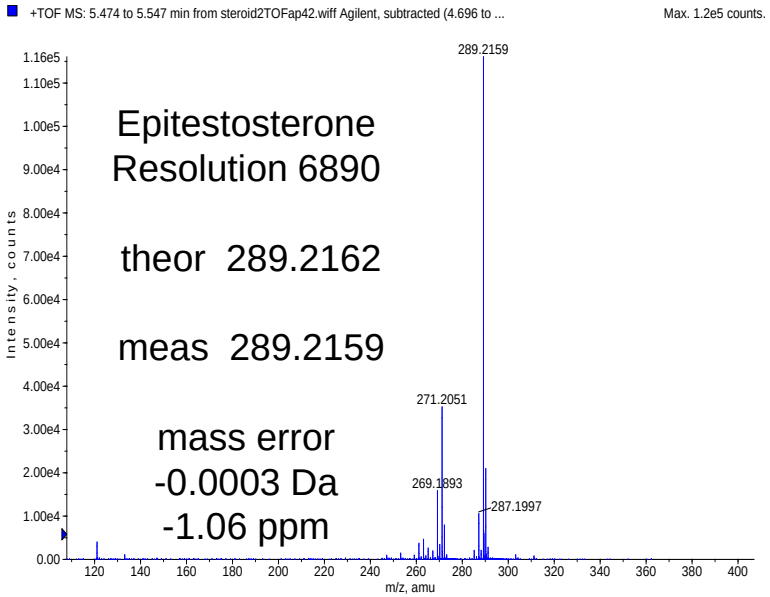


Mass accuracy (mass error)

$$\text{Mass accuracy} = \left(\frac{\text{measured - theory}}{\text{theory}}\right) \times 10^6$$

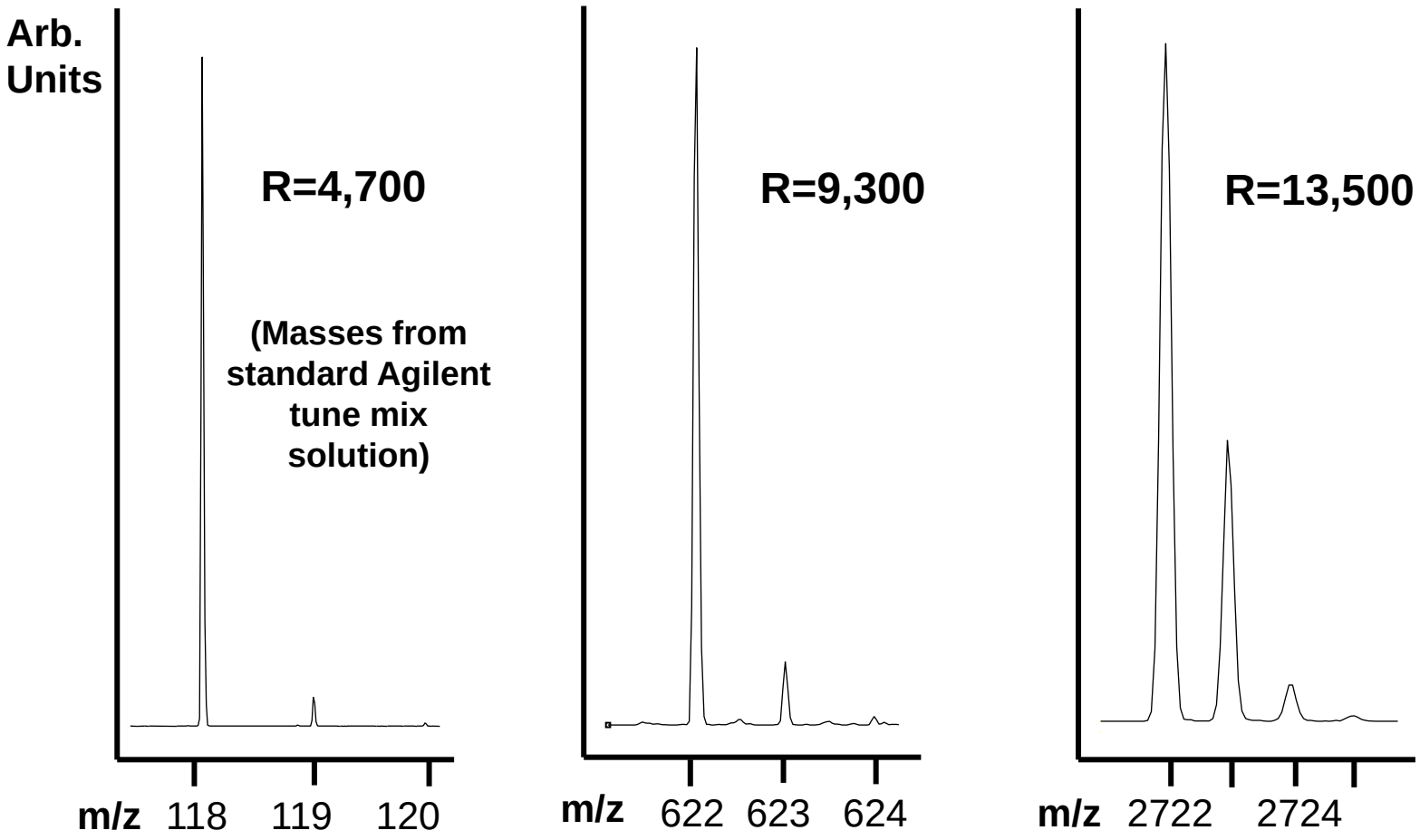
$(1000.001-1000.000)/1000) \times 10^6 = 1$ ppm

Therefore: 0.001 Da (1 mDa) error
@ MW 300 Da is approximately 3 ppm



Mass Resolving Power

Why resolution contributes to accurate mass: narrow mass peak widths allow accurate centroiding



How Much Accuracy in Needed for Identification? (new concept for LC/MS: mass accuracy in "ppm")

Reserpine ($C_{33}H_{40}N_2O_9$) has a protonated ion at m/z 609.28066

Single quadrupole MS reports mass to +/- 0.1 = 165 ppm

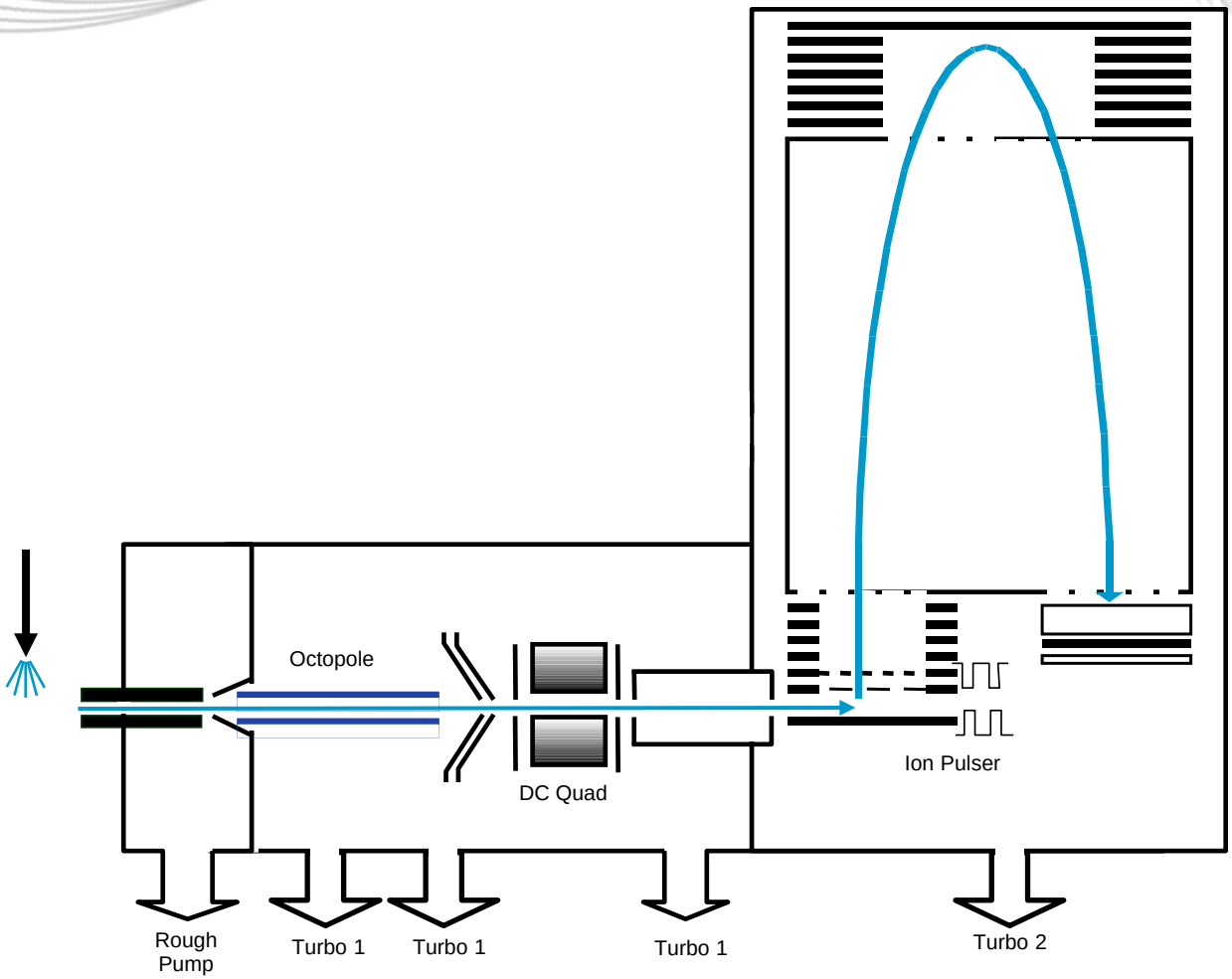
Number of possible formulas using only C, H, O & N, at various mass errors:

- 165 ppm 209 (single quadrupole resolution)
- 10 ppm 13 (older TOF instruments)
- 5 ppm 7
- 3 ppm 4
- 2 ppm 2

Accurate mass limits the number of possible formulae for a given m/z measurement.

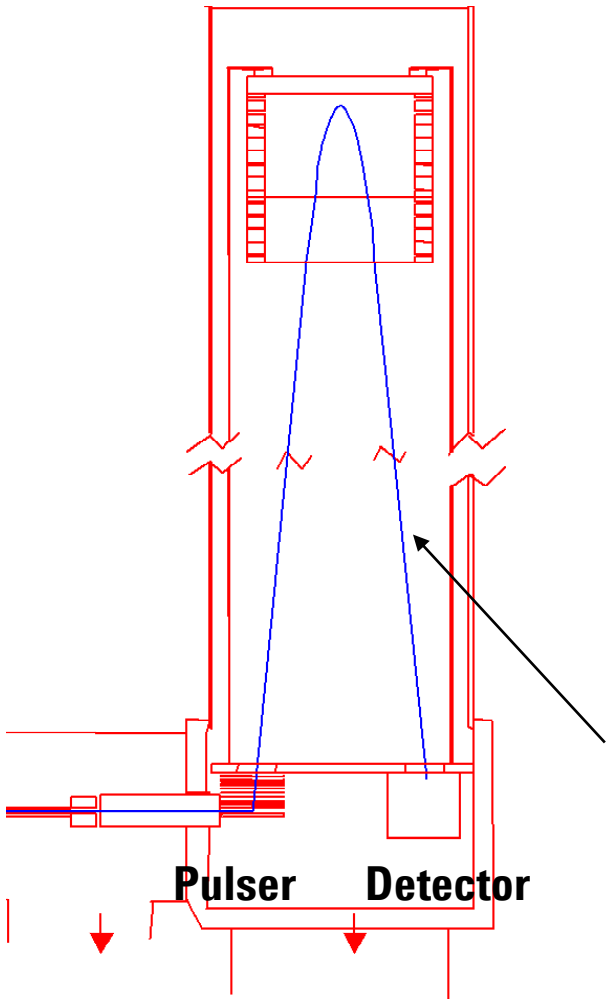
Agilent Time-of-Flight (TOF) LC/MS

Agilent's orthogonal TOF design



The Measure of Confidence

A Time-of-Flight "Scan"



1. Pulse a packet of ions up flight tube(a *transient)*
2. Measure ion arrivals at detector each ***nanosecond***
3. Large number of data points in each *transient*
4. <u>Sum</u> 2000 – 10000 transients into one scan
5. Produces spectra with excellent ion statistics (both center of mass and abundance)

Flight time is converted to m/z against calibration:

e.g., 20.010 µsec →*m/z* 121.0508

See an animation at:
http://www.chem.agilent.com/cag/other/lc-msd-tof.wmv

Benefits of Atmospheric Pressure Ionization (API)

Ionizing difficult compounds (API), no derivatization

Accurate mass measurement (four decimal places, < 2ppm)

High resolution for distinguishing near-isobaric masses:
- 5000 at low mass, > 10,000 at higher masses

Fast scanning
>10 spectra/sec, many spectra across even a narrow UPLC peak

Excellent sensitivity for full-scan data (some instruments)

Wide mass range when needed

All-scan, all-the-time data allows retrospective search for compounds not originally sought.

Forensic Tox Compound Analysis
The Value of the Agilent Solution

Robust hardware

Sample Preparation Products

HPLC and UHPLC Columns

Automated software workflows

Comprehensive Accurate Mass Libraries

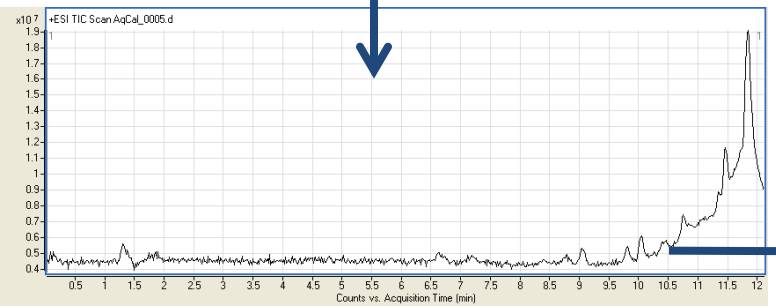
LC/MS Application Kits

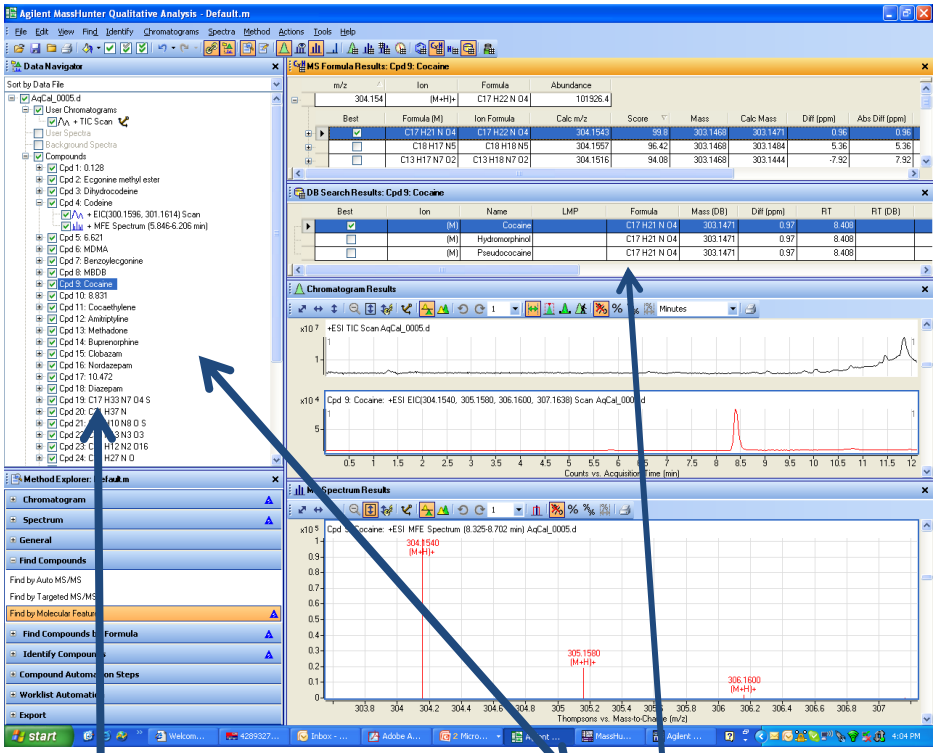
First-class service and application support

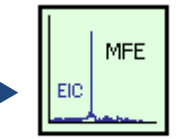
Agilent TOF Screening Solution

Agilent 62** TOF

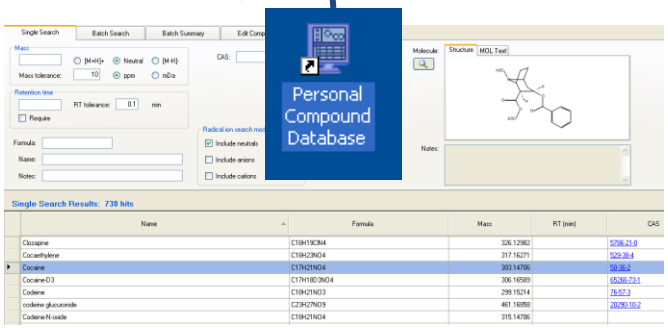
- **20K resolution**
- **5 decades in-scan**
- Low Pg Sensitivity





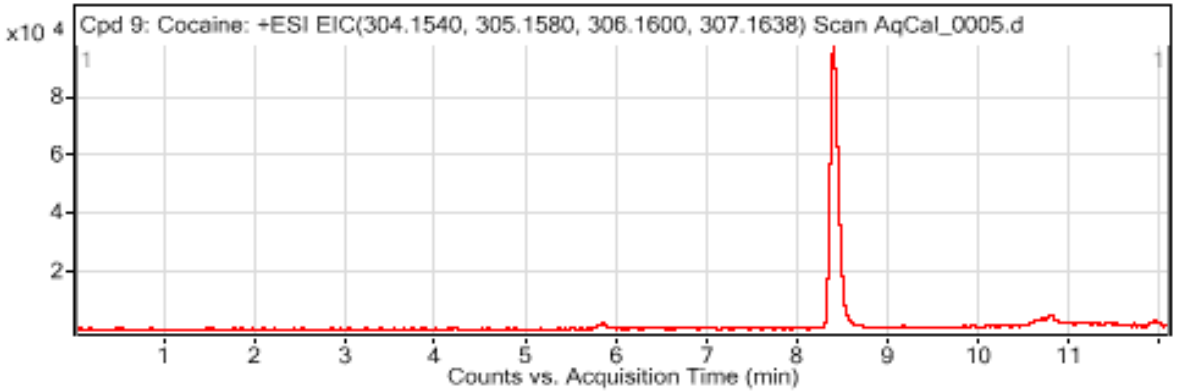


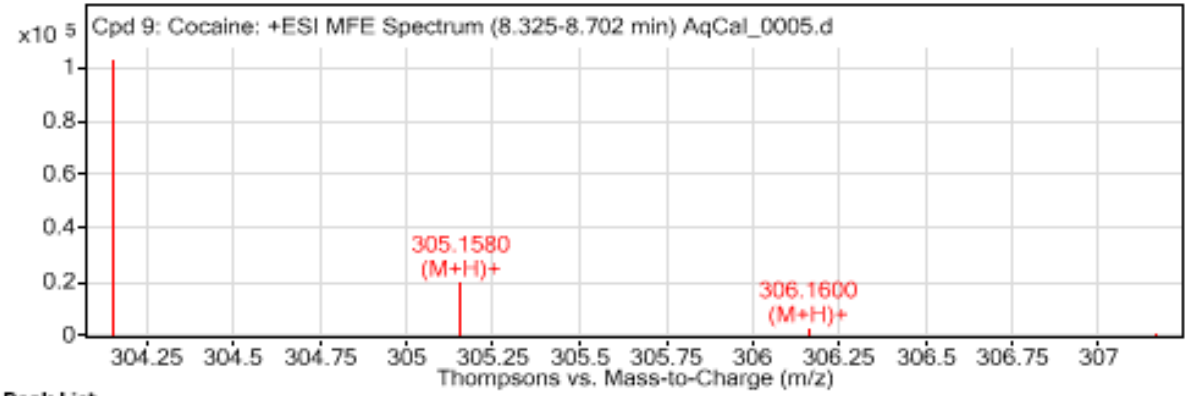
Molecular Feature Extractor



Agilent Technologies

Qualitative Screen Report – Compound 9 (Cocaine)

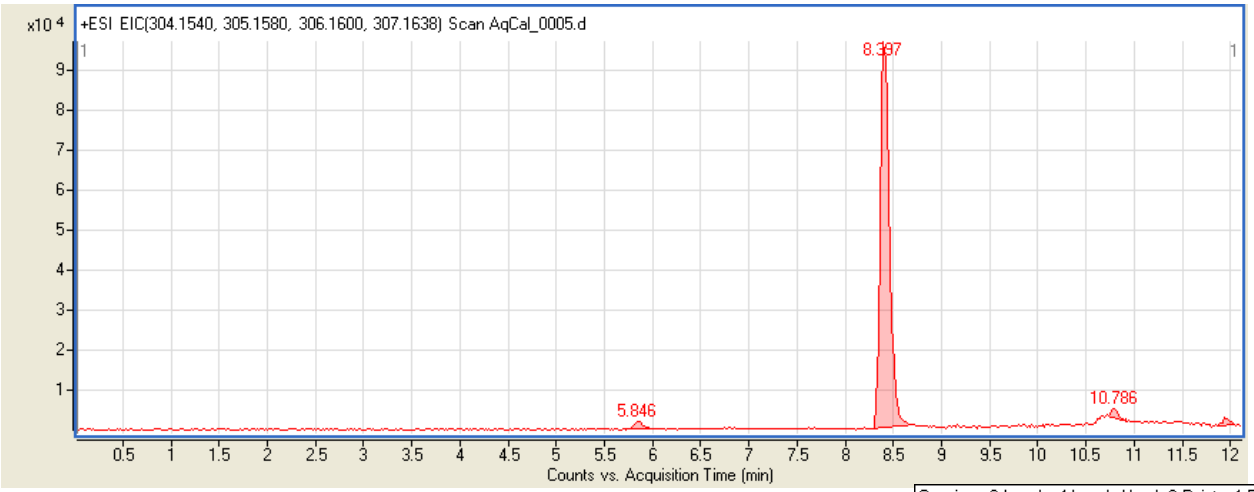




Peak List

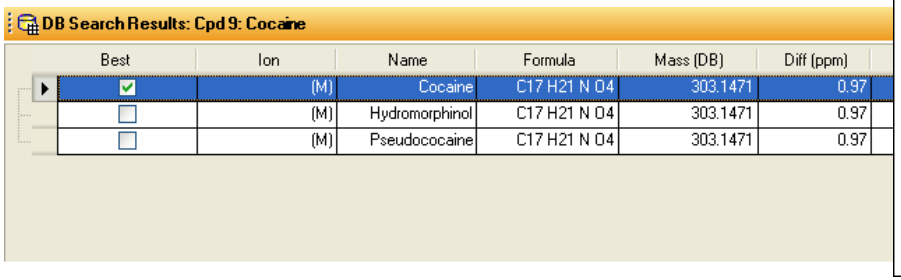
m/z	z	Abund	Name	Formula	Ion	Score (DB)	Hits (DB)
304.154	1	101926	Cocaine	C17 H21 N O4	(M+H)+	99.68	3
305.158	1	19552		C17 H22 N O4	(M+H)+		
306.16	1	2441		C17 H22 N O4	(M+H)+		
307.1638	1	407		C17 H22 N O4	(M+H)+		

Results For Compound 9 – PCD Search Confirmed as Cocaine



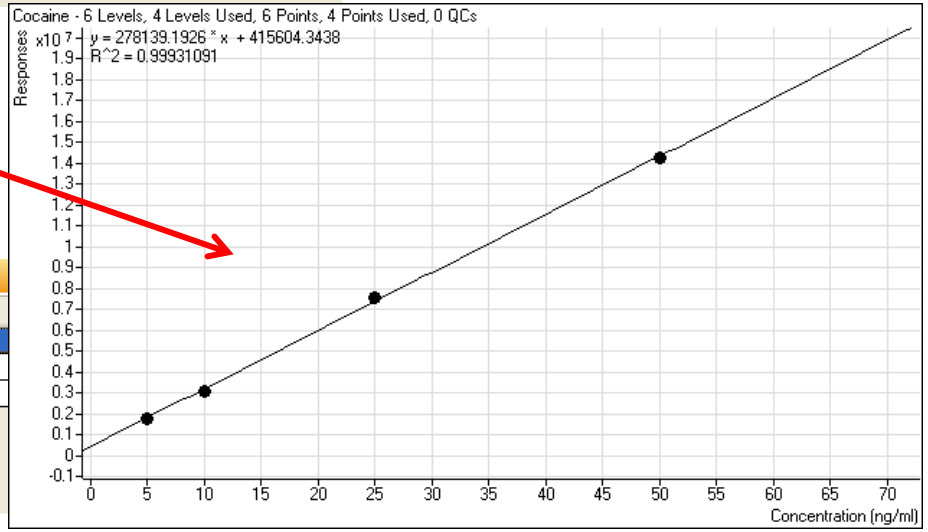
EIC of Cocaine
Mass 303.1471
(50 ng/ml)

0.99931 linear correlation
Using TOF for Quantitation

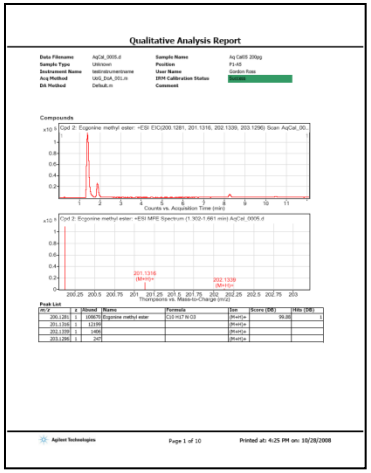


DB Search Results: Cpd 9: Cocaine

Best	Ion	Name	Formula	Mass (DB)	Diff (ppm)
☑	(M)	Cocaine	C17 H21 N O4	303.1471	0.97
☐	(M)	Hydromorphinol	C17 H21 N O4	303.1471	0.97
☐	(M)	Pseudococaine	C17 H21 N O4	303.1471	0.97

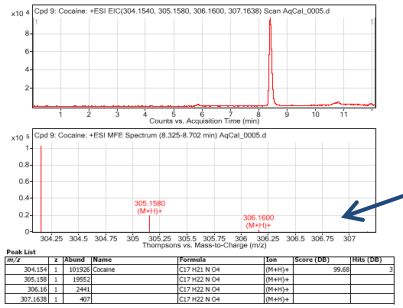


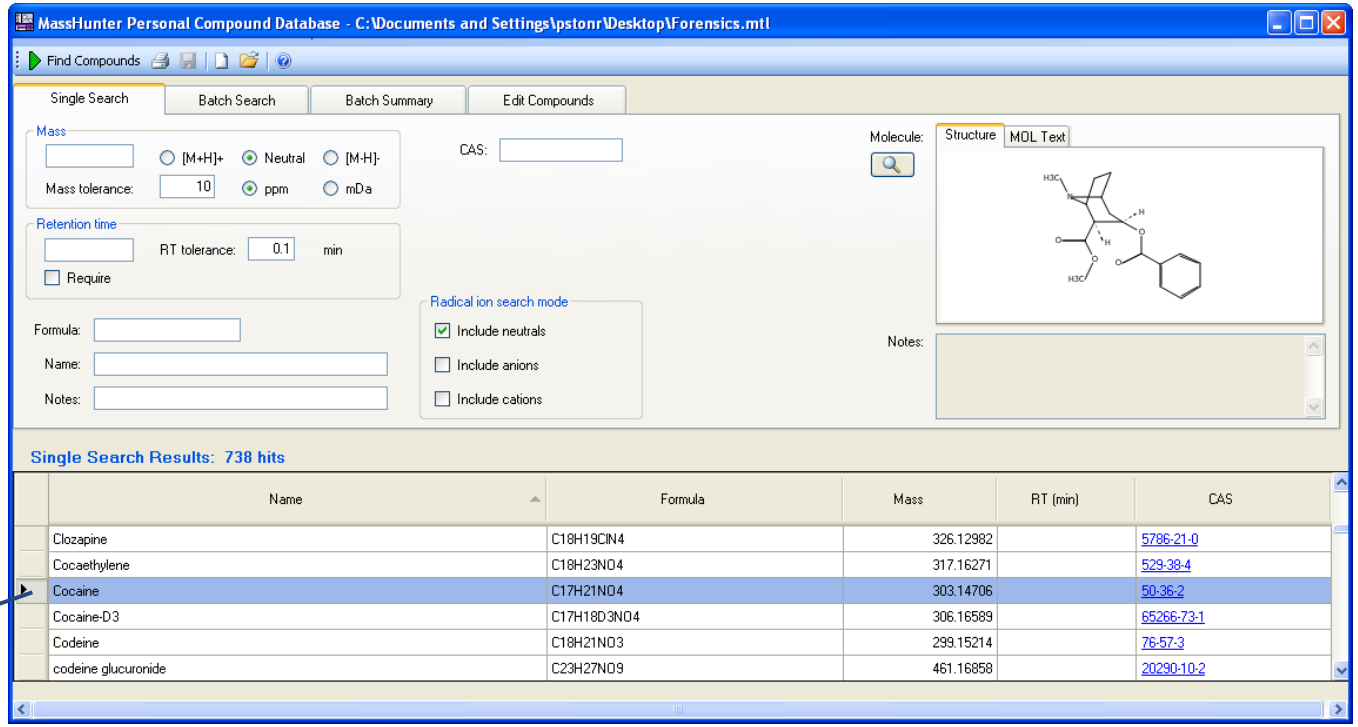
Agilent Personal Compound Database - Forensics/Toxicology



Database containing >800 Forensic/Doping Analytes

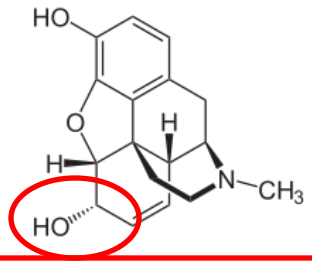
- Automated Reporting
- Controlled Substances
- Explosives
- WADA Exclusion List
- Accurate Mass Confirmation
- Retention time Confirmation
- Spectral Match Scoring (based on Isotopic Pattern & Accurate Mass spacing)



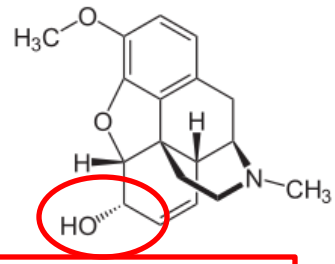


Isobars – The Need for Adequate Chromatographic Separation.

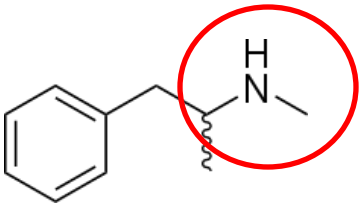
Isobaric Mass Ions – "Defined as different ions that have identical mass"



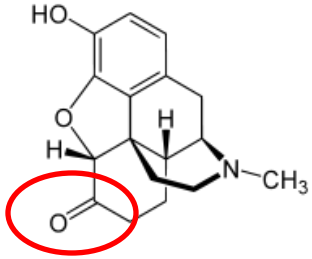
Morphine
($C_{17}H_{20}NO_3$)
$[M+H]^+$ = 286 m/z



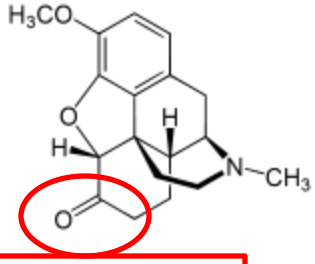
Codeine
($C_{18}H_{22}NO_3$)
$[M+H]^+$ = 300 m/z



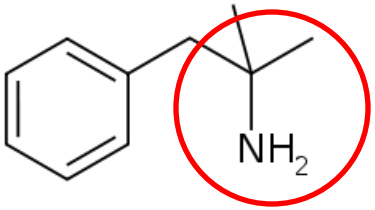
methamphetamine
($C_{10}H_{16}N$)
$[M+H]^+$ = 150 m/z



Hydromorphone
($C_{17}H_{20}NO_3$)
$[M+H]^+$ = 286 m/z

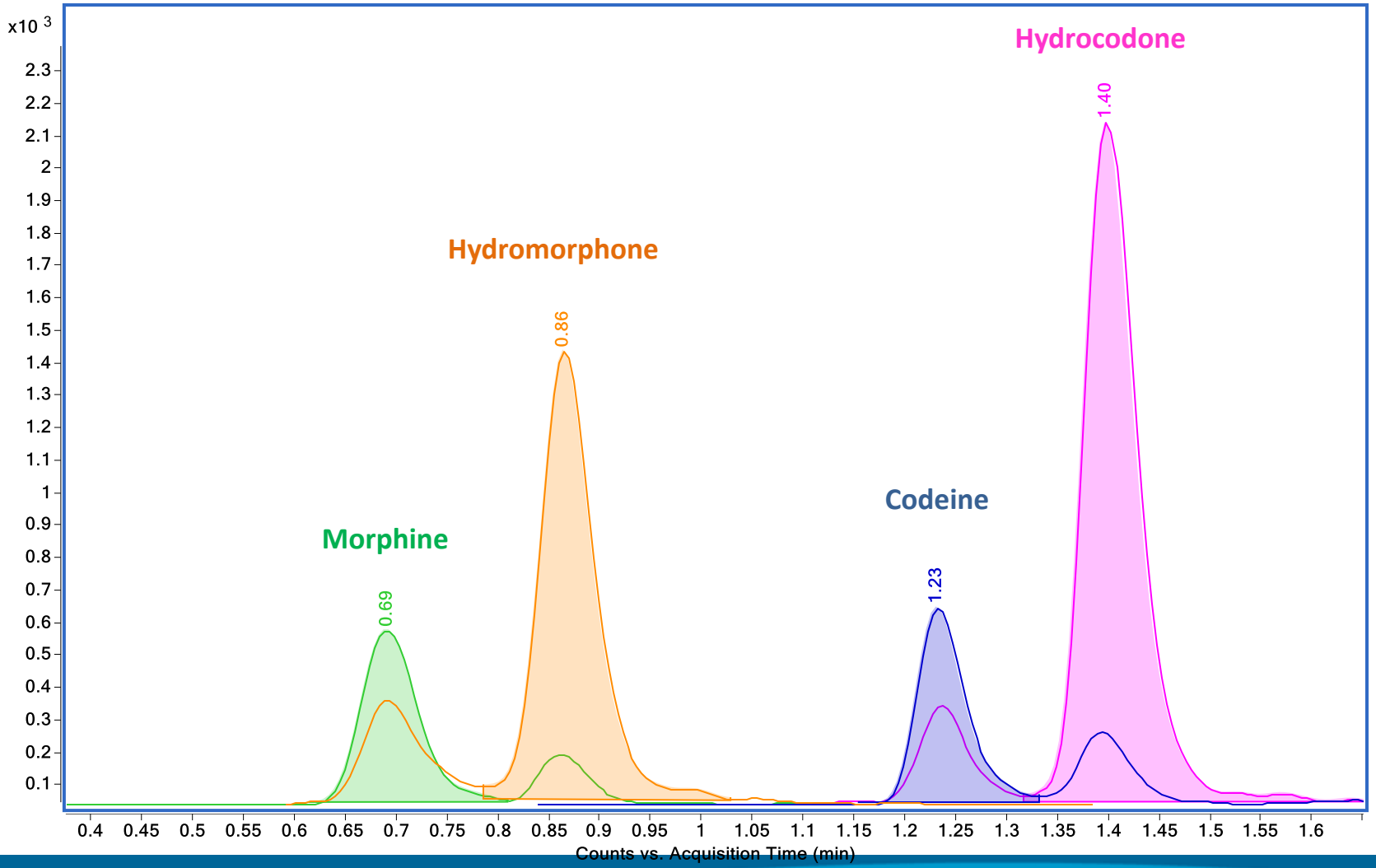


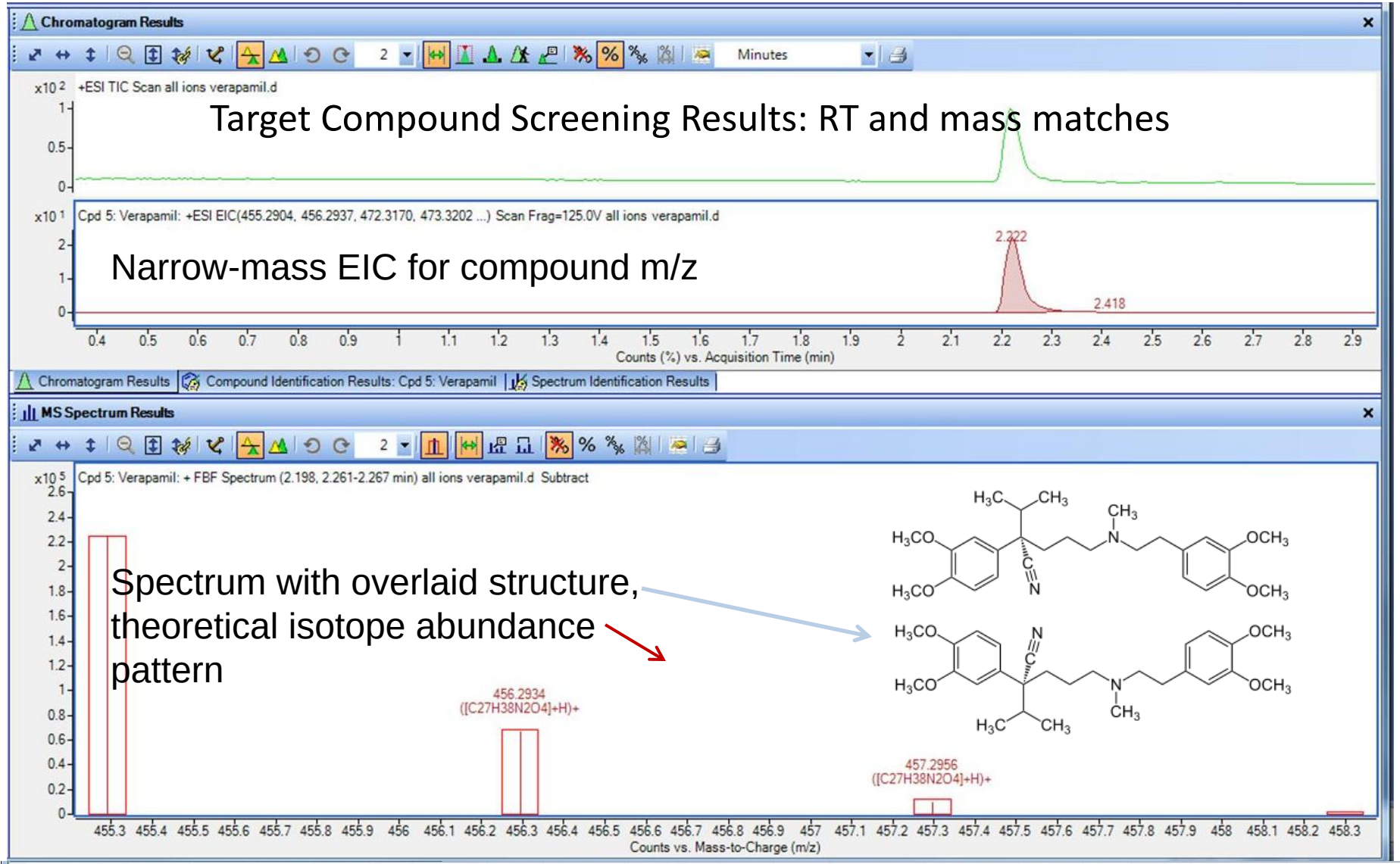
Hydrocodone
($C_{18}H_{22}NO_3$)
$[M+H]^+$ = 300 m/z



Phentermine
($C_{10}H_{16}N$)
$[M+H]^+$ = 150 m/z

Isobaric Pairs

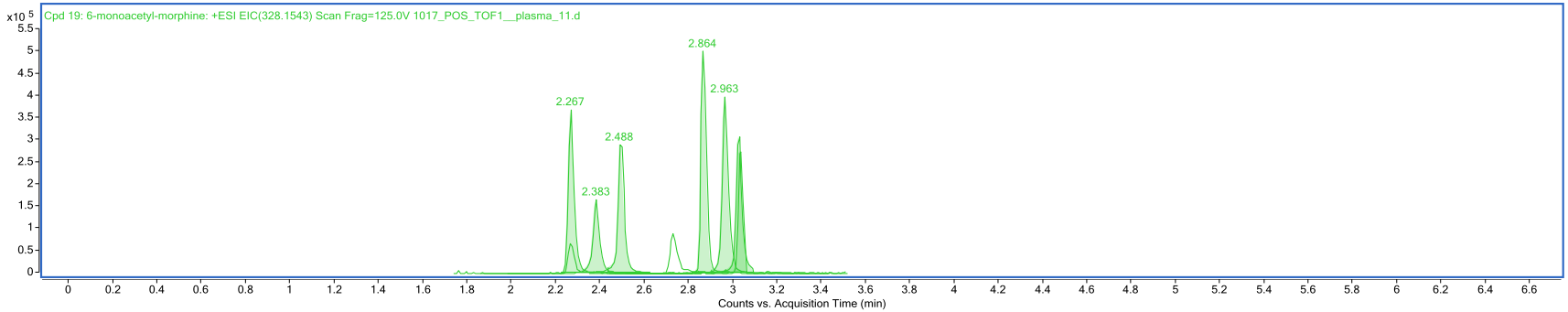


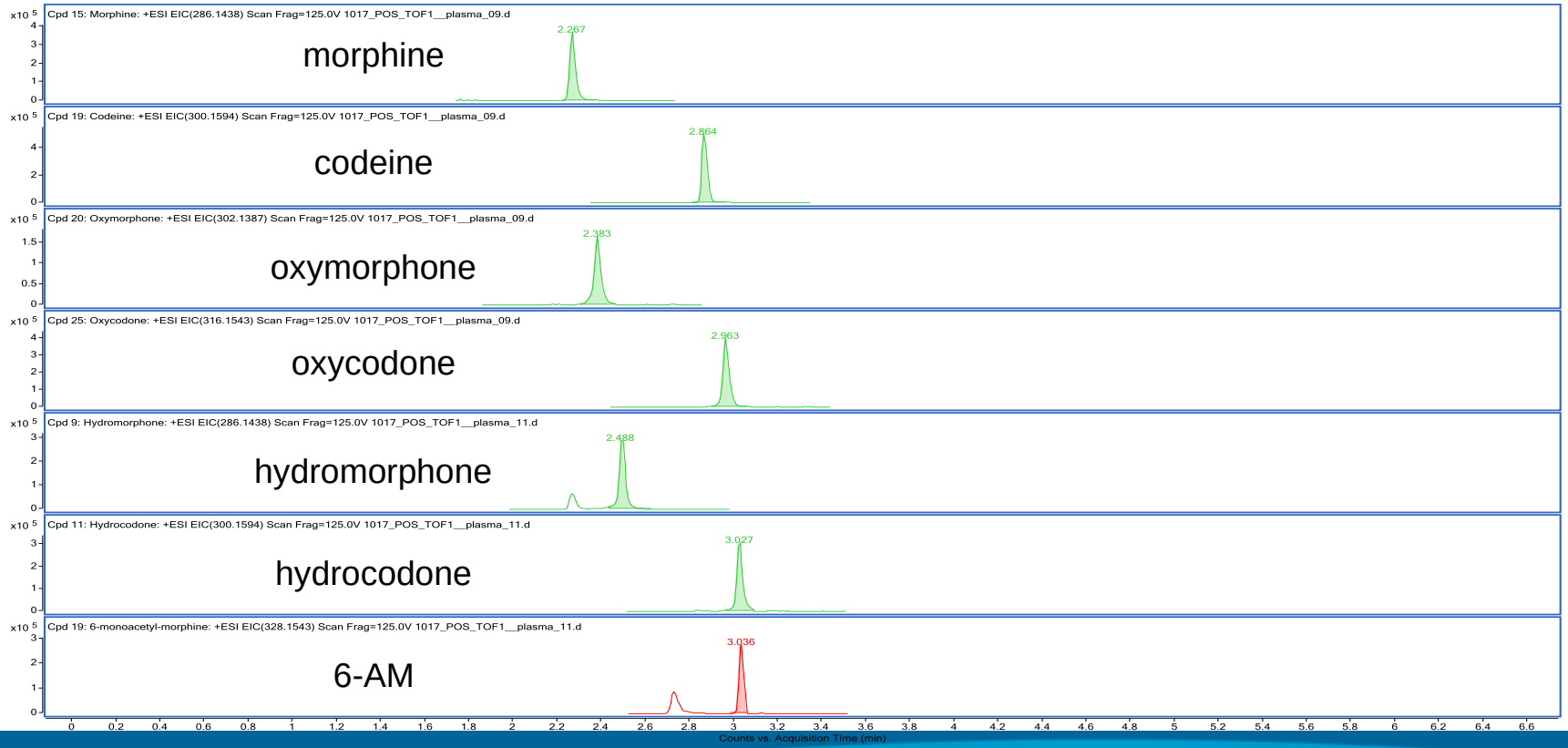


Result

RT Diff (DB)	Library	Show/Hide	Cpd	File	ID Source	Name	Formula	RT	m/z	Mass	Score	Diff (Tgt, ppm)	Diff (DB, ppm)	Polarity	Ions	Height	Area	Vol	Notes
		☑	5	all ions verapamil.d	FBF	Verapamil	C27 H38 N2 O4	2.222	455.2895	454.2824	97.95	-1.73	1.73	Positive	4	2127480	5308246		Equine drug; Ca antagonist

Best	ID Source	Name	Formula	Score	Score (RT)	RT Diff	Score (Lib)	Score (DB)	Score (MFG)	Num Spectra	Notes	RT (Tgt)	RT (DB)	Species	m/z	Library
	FBF	Verapamil	C27 H38 N2 O4	97.95							Equine drug; Ca antagonist			(M+H)+	455.2895	


Cpd 19: 6-monoacetyl-morphine: +ESI EIC(328.1543) Scan Frag=125.0V 1017_POS_TOF1__plasma_11.d
2.267
2.383
2.488
2.864
2.963
Counts vs. Acquisition Time (min)


Cpd 15: Morphine: +ESI EIC(286.1438) Scan Frag=125.0V 1017_POS_TOF1__plasma_09.d
morphine
2.267
Cpd 19: Codeine: +ESI EIC(300.1594) Scan Frag=125.0V 1017_POS_TOF1__plasma_09.d
codeine
2.864
Cpd 20: Oxymorphone: +ESI EIC(302.1387) Scan Frag=125.0V 1017_POS_TOF1__plasma_09.d
oxymorphone
2.383
Cpd 25: Oxycodone: +ESI EIC(316.1543) Scan Frag=125.0V 1017_POS_TOF1__plasma_09.d
oxycodone
2.963
Cpd 9: Hydromorphone: +ESI EIC(286.1438) Scan Frag=125.0V 1017_POS_TOF1__plasma_11.d
hydromorphone
2.488
Cpd 11: Hydrocodone: +ESI EIC(300.1594) Scan Frag=125.0V 1017_POS_TOF1__plasma_11.d
hydrocodone
3.027
Cpd 19: 6-monoacetyl-morphine: +ESI EIC(328.1543) Scan Frag=125.0V 1017_POS_TOF1__plasma_11.d
6-AM
3.036
Counts vs. Acquisition Time (min)

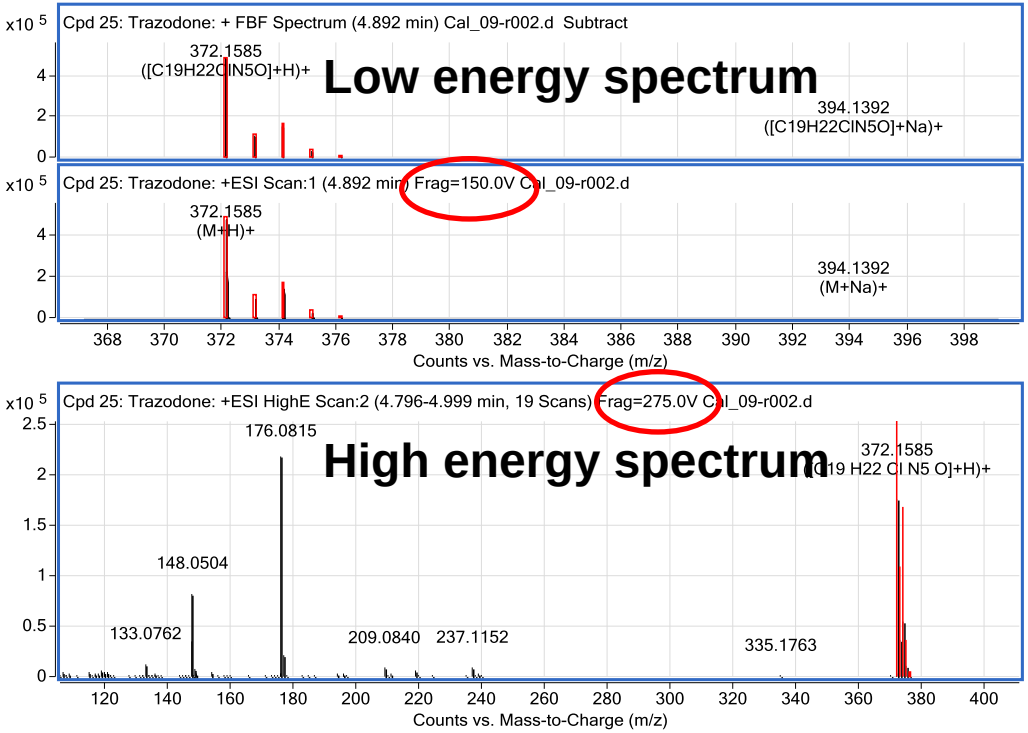
ALL IONS
ADDING FRAGMENT CONFIRMATION

All Ions MS/MS-What is it?

Step 1: Fragmentation without Isolation on TOF or Q-TOF

a) First Scan: Low fragmentation energy to analyze precursors

b) Second Scan: High fragmentation energy to analyze fragment ions



Shows Precursor Ions

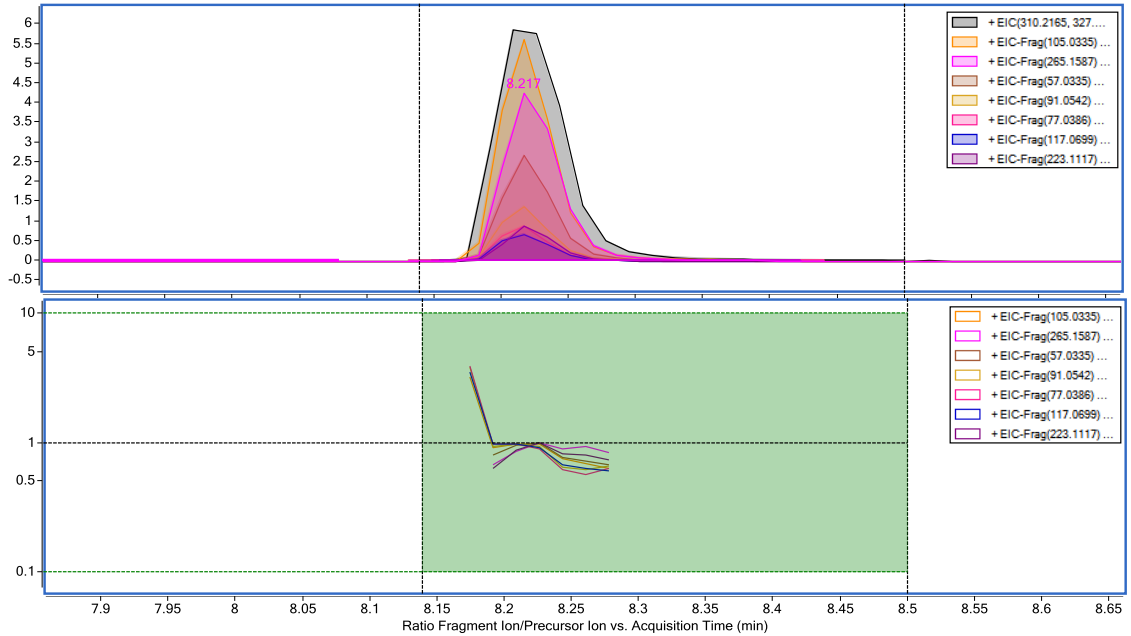
Shows Fragment Ions

All Ions MS/MS-What is it?

Step 2: Software Extracts, Correlates, and Confirms

a) Find by Formula uses library to extract precursors from low energy MS channel

b) All Ions MS/MS uses library to extract fragments from high energy channel, gives coelution score

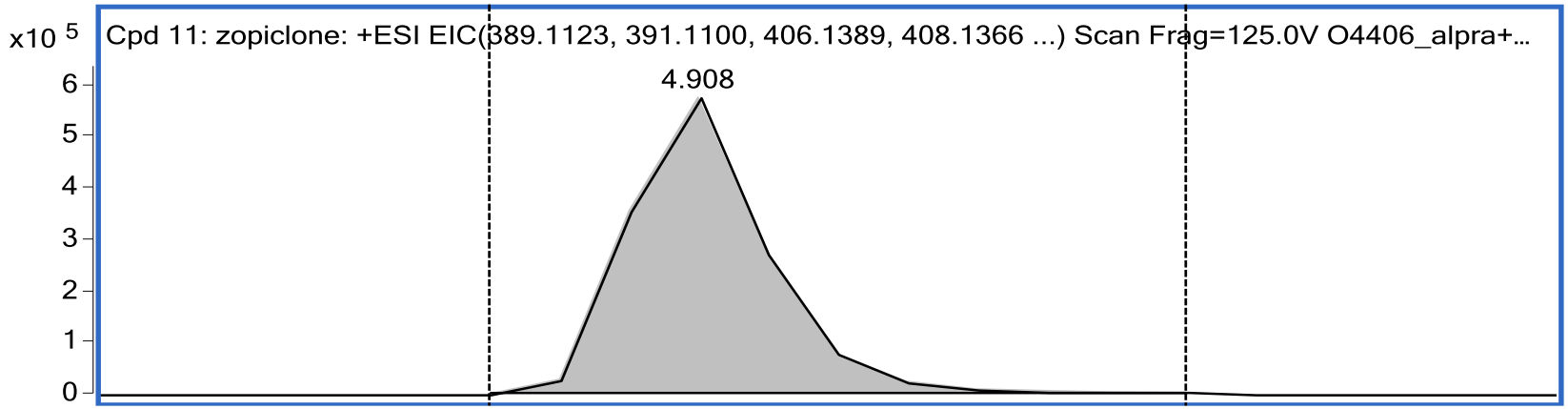


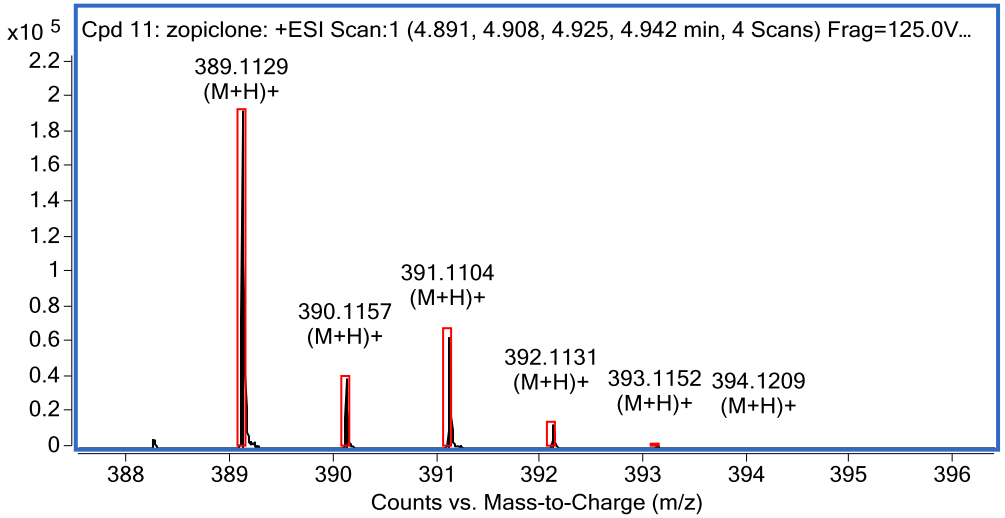
Software automatically matches precursors with fragment ions:
Coelution Plot with Coelution Score

Evaluation of All Ions MS/MS Automated Workflow

Untargeted Drug Screening in Postmortem Blood

Step 1: Find by Formula Extracts Precursors using PCDL database

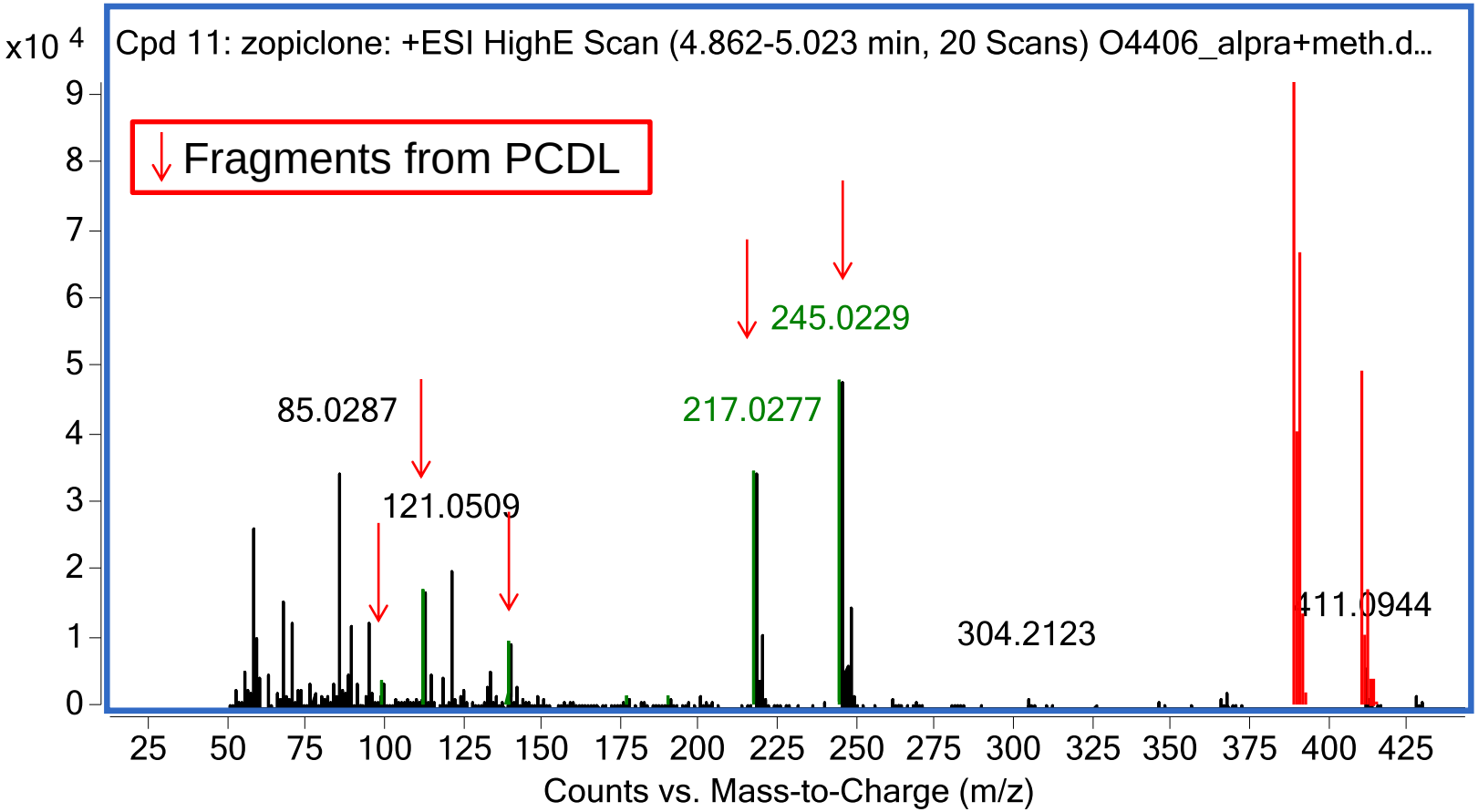




Evaluation of All Ions MS/MS Automated Workflow

Untargeted Drug Screening in Postmortem Blood

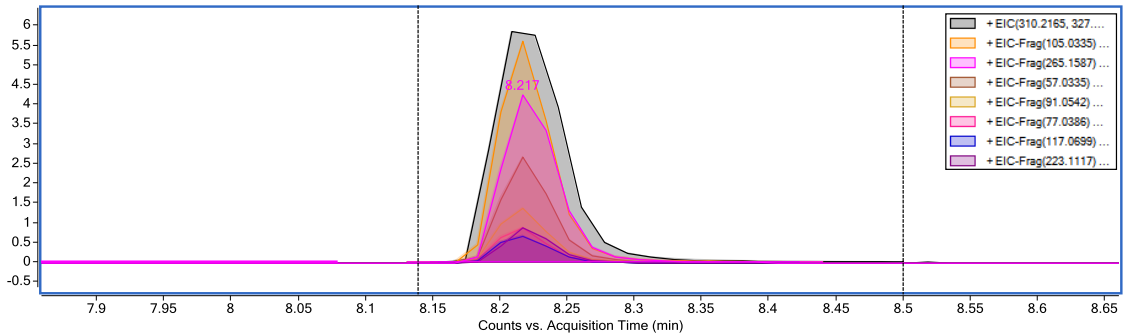
Step 2: Extract Corresponding Fragment Ions



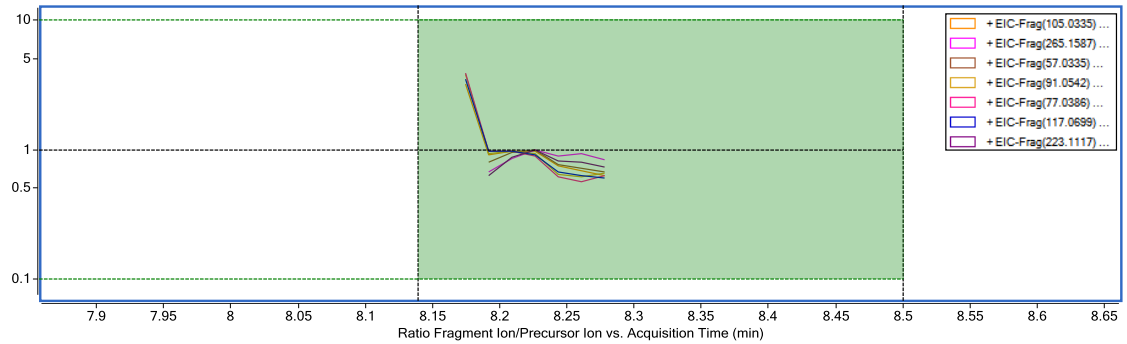
Evaluation of All Ions MS/MS Automated Workflow

Untargeted Drug Screening in Postmortem Blood

Step 3: Precursor and Fragment Correlation/Coelution



Overlaid Precursor and Fragment Ion Chromatograms

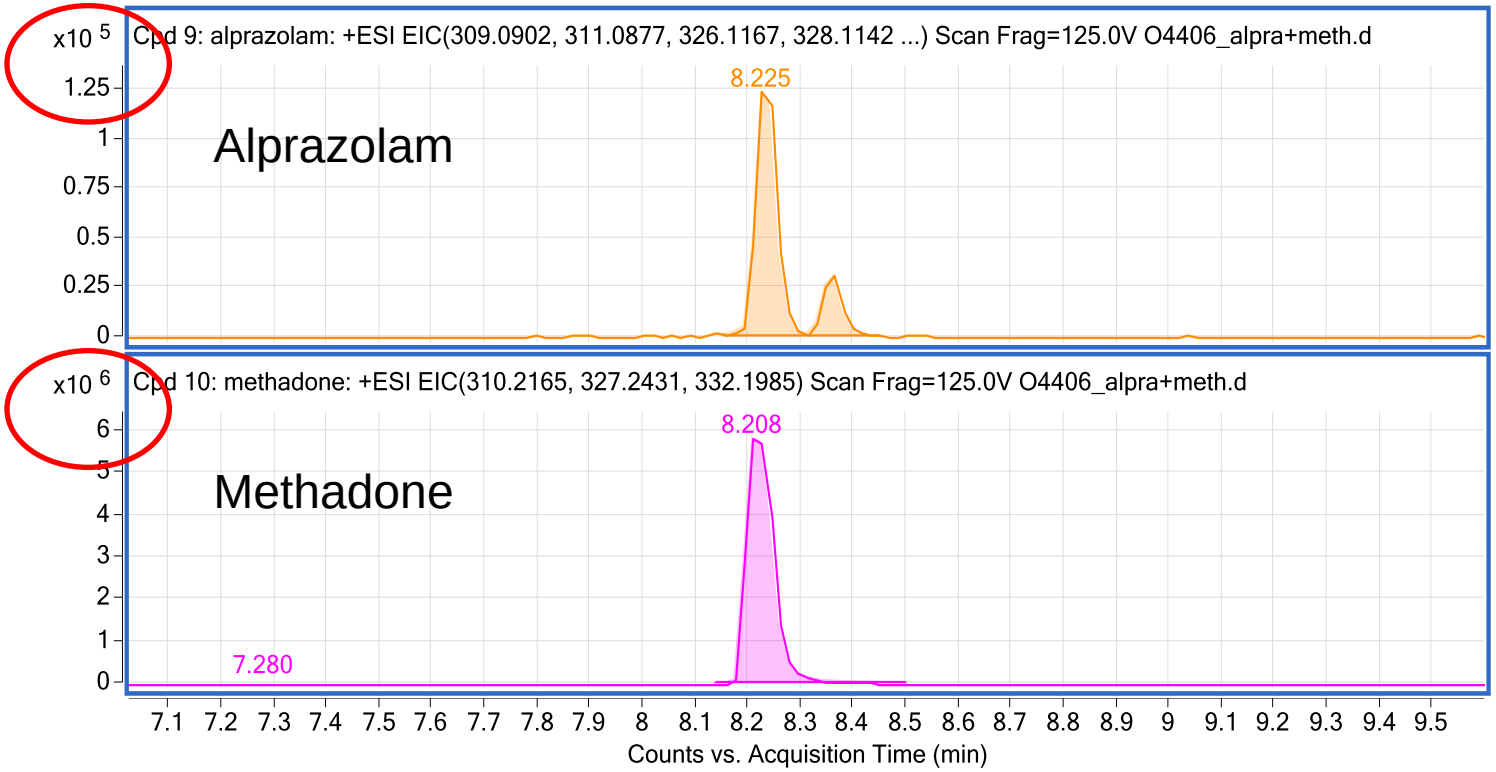


Coelution Plot

Differentiation and Identification of Isomers

Untargeted Drug Screening in Postmortem Blood

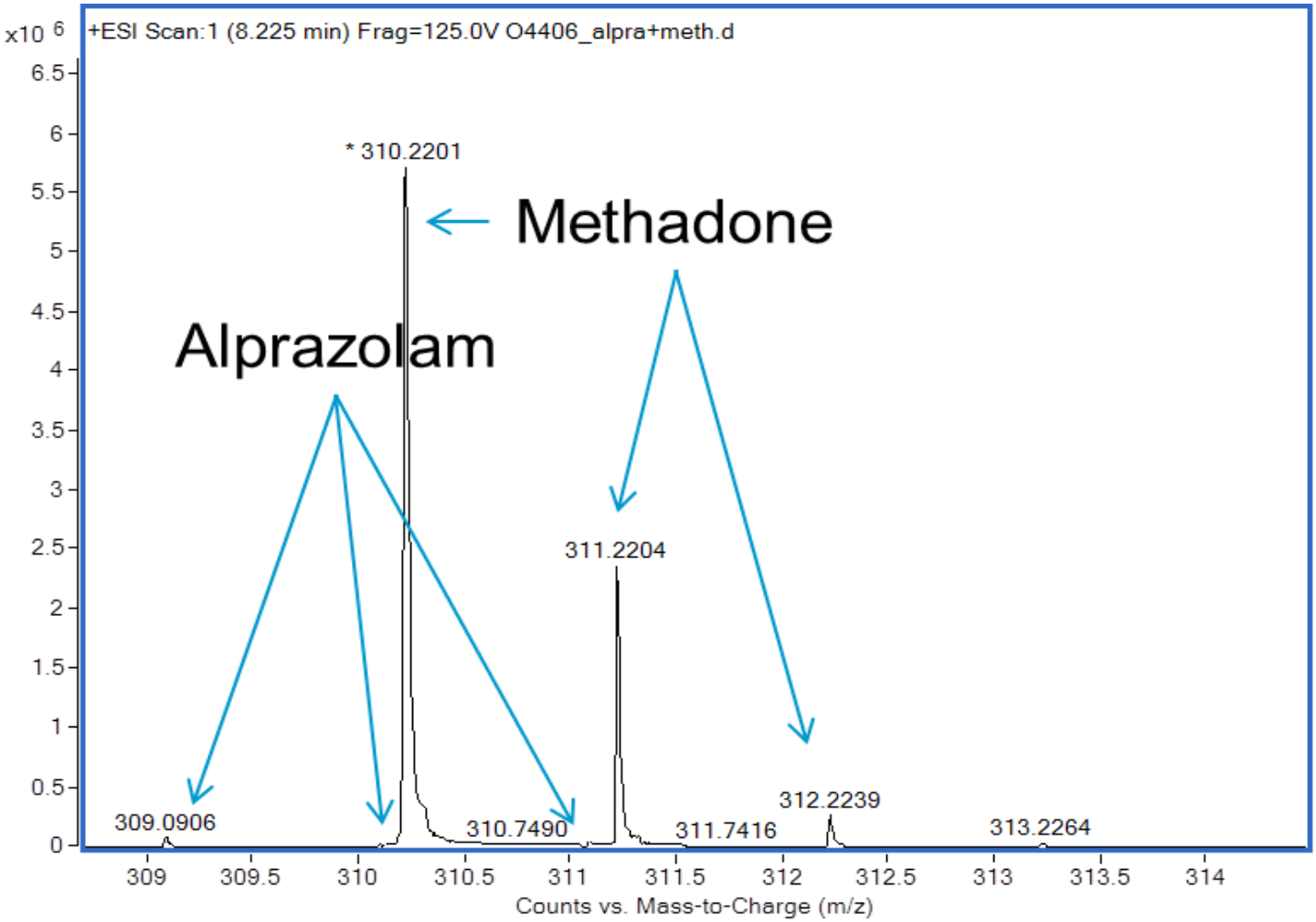
Coeluting with ~50x Concentration Difference



Differentiation and Identification of Isomers

Untargeted Drug Screening in Postmortem Blood

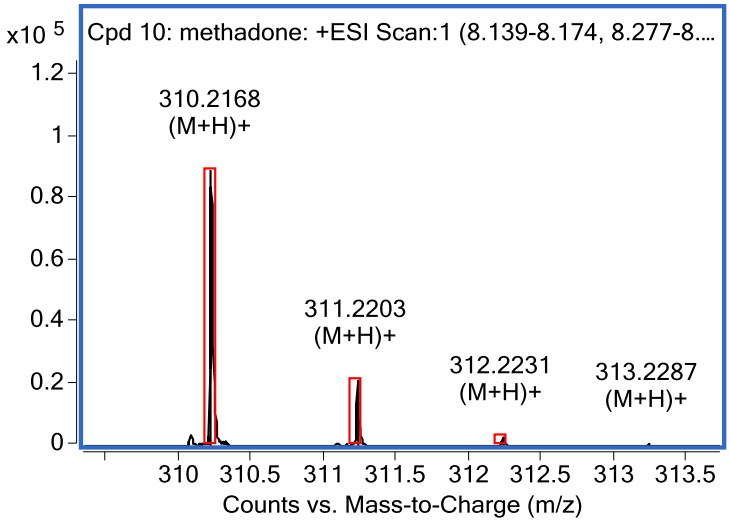
Similar precursor *m/z* values and overlapping isotopic clusters



Compound Confirmation-Methadone

Untargeted Drug Screening in Postmortem Blood

Red outline overlays theoretical isotopic pattern and abundance with acquired precursor data for methadone



Methadone confirmed with seven qualified fragments

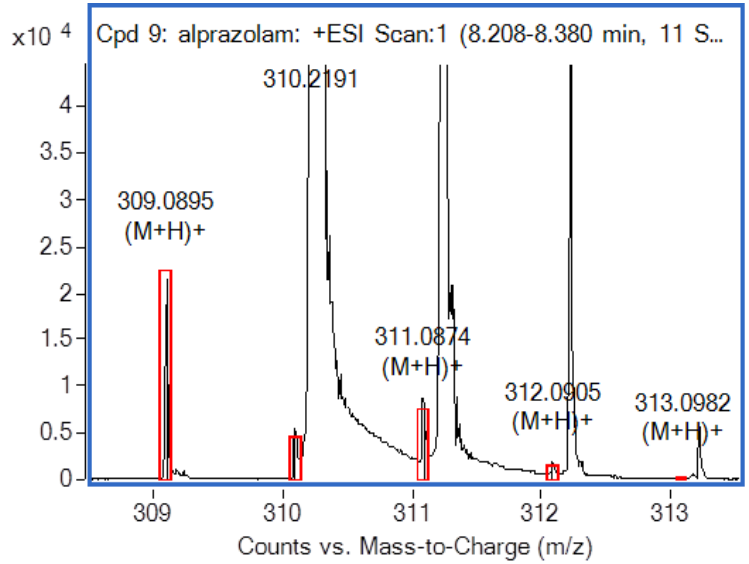
Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff
◉	methadone	C21 H27 N O	310.2168	309.2096	309.2093	-1.11	99.56	8.208	8.317	-0.109

Coelution Score	CE	Flags(Fls)	FV	Height	m/z	Compound Name	RT	RT Diff	SNR
98.7	40	Qualified		5622078.5	105.0335	methadone	8.217	0.009	2457.9
98.6	20	Qualified		4247226.5	265.1587	methadone	8.217	0.009	4782.3
98.7	20	Qualified		2687710.2	57.0335	methadone	8.217	0.009	593.9
97.3	40	Qualified		1396836.4	91.0542	methadone	8.217	0.009	366.5
96.9	40	Qualified		894049.9	77.0386	methadone	8.217	0.009	179
97.8	40	Qualified		691156.1	117.0699	methadone	8.217	0.009	382.5
98.2	20	Qualified		887147.3	223.1117	methadone	8.217	0.009	1589

Compound Confirmation-Alprazolam

Untargeted Drug Screening in Postmortem Blood

Red outline overlays theoretical isotopic pattern and abundance with acquired precursor data for alprazolam



Alprazolam confirmed with two qualified fragments, despite low concentration

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)
	alprazolam	C17 H13 Cl N4	309.0895 331.0723	308.082	308.0829	2.98	97.55	8.225	8.295

Coelution Score	CE	Flags(Fls)	FV	Height	m/z	Compound Name	RT	RT Diff	SNR
93.2	40	Qualified		17295.1	205.0761	alprazolam	8.234	0.009	36.8
93.2	40	Qualified		25488	281.0714	alprazolam	8.234	0.009	112.3
89.4		Low coelution score		5595.5	274.1213	alprazolam	8.234	0.009	15.6
		No peaks for eic			165.0214	alprazolam			
		No peaks for eic			240.0449	alprazolam			
		No peaks for eic			219.0917	alprazolam			
		No peaks for eic			241.0527	alprazolam			

High Productivity: Compound Details View

Agilent MassHunter Qualitative Analysis B.06.00 - All_Ions_Martin2.m

Navigator View | Compound Details View

File Edit View Find Identify Method Sequence Wizards Configuration Tools Help

Compound List

Automatically Show Columns

RT (Tgt)	Name	Formula	m/z	Mass	Diff (Tgt, ppm)	Score (Tgt)	Flags (Tgt)	FIs Eval.	FIs Con
3.49	codeine	C18 H21 N O3	300.1613	299.1525	1.07	81.38	low score;Qualified	7	
3.8	oxycodone	C18 H21 N O4	316.1561	315.1474	1.07	78.76	low score;Not qualified	7	
4.999	zopiclone	C17 H17 Cl N6 O3	411.0946	388.1056	1.31	99.33	Qualified	7	
7.491	promethazine	C17 H20 N2 S	285.1421	284.1349	0.76	98.99	Qualified	7	
8.002	alimemazine	C18 H22 N2 S	299.1578	298.1506	0.83	98.22	Qualified	7	
8.15	amitriptyline	C20 H23 N	278.1904	277.1832	0.64	99.4	Qualified	7	
8.295	alprazolam	C17 H13 Cl N4	331.0723	308.082	-2.98	97.55	Qualified	7	
8.317	methadone	C21 H27 N O	310.2168	309.2096	1.11	99.56	Qualified	7	
8.359	sertraline	C17 H17 Cl2 N	306.0817	305.0744	2.1	97.97	Qualified	7	
9.505	diazepam	C16 H13 Cl N2 O	285.0794	284.0725	3.2	76.12	low score;Not qualified	7	

Compound List

Compound Identification Results: Cpd 8: methadone

Automatically Show Columns

ID Techniques Applied: FBF

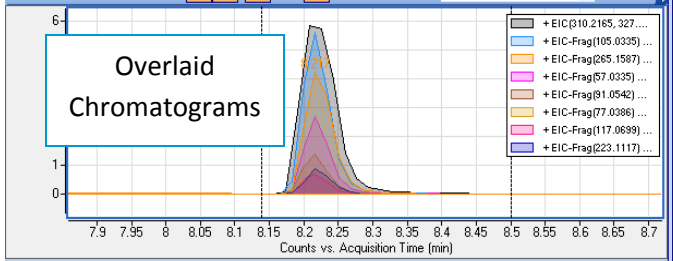
Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff	Score (RT)
●	methadone	C21 H27 N O	310.2168	309.2096	309.2093	-1.11	99.56	8.208	8.317	-0.109	

m/z	Coelution Score	Flags(FIs)	Height	SNR	RT	RT Diff	CE	FV	Compound Name
105.0335	98.7	Qualified	5621388.5	2457.6	8.217	0.009	40		methadone
265.1587	98.6	Qualified	4246755.5	4781.7	8.217	0.009	20		methadone
57.0335	98.7	Qualified	2687710.2	593.9	8.217	0.009	20		methadone
91.0542	97.3	Qualified	1396836.4	366.5	8.217	0.009	40		methadone
77.0386	96.9	Qualified	894049.9	179	8.217	0.009	40		methadone
117.0699	97.8	Qualified	691155.1	518.4	8.217	0.009	40		methadone
223.1117									methadone

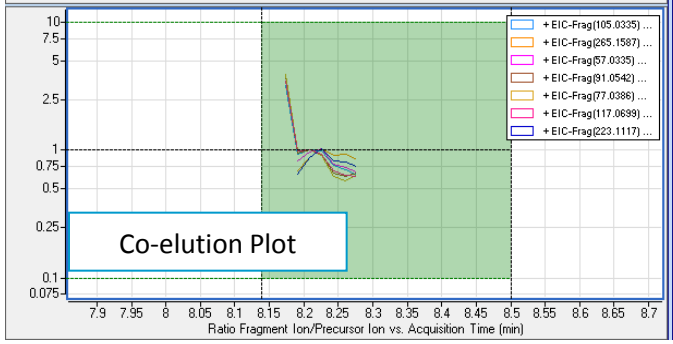
Compound ID Results

Compound Chromatogram Results

Minutes

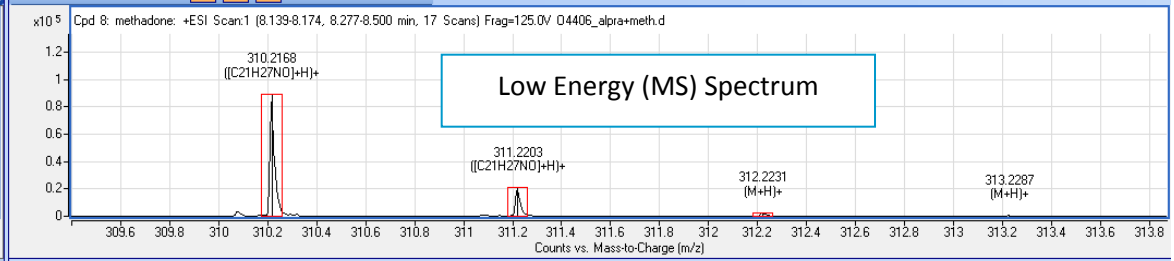


Overlaid Chromatograms



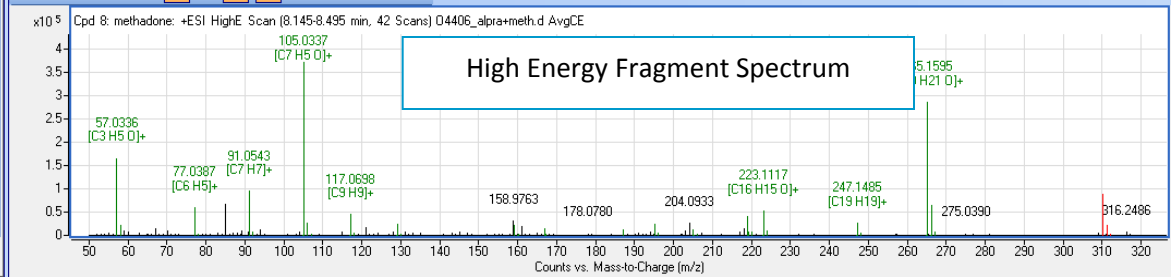
Co-elution Plot

Compound MS Spectrum Results



Low Energy (MS) Spectrum

Compound Fragment Spectrum Results



High Energy Fragment Spectrum

All Ions on Random Dollar Bill for Cocaine

Agilent MassHunter Qualitative Analysis B.06.00 - Pesticide_FbF_allions_02042014.m

File Edit View Find Identify Spectra Chromatograms Method Wizards Actions Configuration Tools Help

Navigator View | Compound Details View

Data Navigator

Sort by Data File

Compound List

Agilent MassHunter Qualitative Analysis B.06.00 - Pesticide_FbF_allions_02042014.m

File Edit View Find Identify Method Wizards Configuration Tools Help

Navigator View | Compound Details View

Compound List

Automatically Show Columns

Cpd	Label	Name	Formula	m/z	Mass	Mass (Tgt)	Flags (Tgt)
3	Cpd 3: Verapamil	Verapamil	C27 H38 N2 O4	472.3189	454.2833	454.2832	low score; No H adduct;Not qualified
2	Cpd 2: Oxycodone	Oxycodone	C18 H21 N O4	316.1545	315.1477	315.1471	low score;Not qualified
1	Cpd 1: Cocaine	Cocaine	C17 H21 N O4	304.1546	303.1474	303.1471	Qualified

Compound Identification Results: Cpd 1: Cocaine

Automatically Show Columns

ID Techniques Applied: FBF

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)
●	Cocaine	C17 H21 N O4	304.1546	326.1407	303.1474	303.1471	-1.03	97.57	1.835

m/z	Coelution Score	Flags(FIs)	Height	SNR	RT	RT Diff	CE	FV	Compound Name
182.1176	91.5	Qualified	154773.8	30.9	1.823	0.011		220	Cocaine
82.0651	88.4	Qualified	8193.8	Infinity	1.823	0.011		220	Cocaine
105.0335		No peaks for eic							Cocaine
77.0386		No peaks for eic							Cocaine
91.0542		Low S/N ratio	2336.5	1.5	1.823	0.011			Cocaine

Compound Chromatogram Results

Minutes

Cpd 1: Cocaine: +ESI EIC(304.1543, 321.1809, 326.1363) Scan Frag=70.0V dollar.d

1.835 57432

Cpd 1: Cocaine: +ESI EIC-Frag(77.0386) Scan dollar.d

Counts vs. Acquisition Time (min)

+EIC-Frag(182.1176) ...
+EIC-Frag(82.0651) ...

Ratio Fragment Ion/Precursor Ion vs. Acquisition Time (min)

Compound MS Spectrum Results

Cpd 1: Cocaine: + FBF Spectrum (1.766-1.857 min) Frag=70.0V dollar.d Subtract

304.1546 ([C17H21NO4]+H)+

326.1407 ([C17H21NO4]+Na)+

Cpd 1: Cocaine: +ESI Scan:1 (1.766, 1.789, 1.812, 1.835 ... min, 5 Scans) Frag=70.0V dollar.d

304.1546 (M+H)+

326.2538

Counts vs. Mass-to-Charge (m/z)

Compound Fragment Spectrum Results

Cpd 1: Cocaine: +ESI HighE Scan (1.680-2.012 min, 45 Scans) dollar.d AvgFV

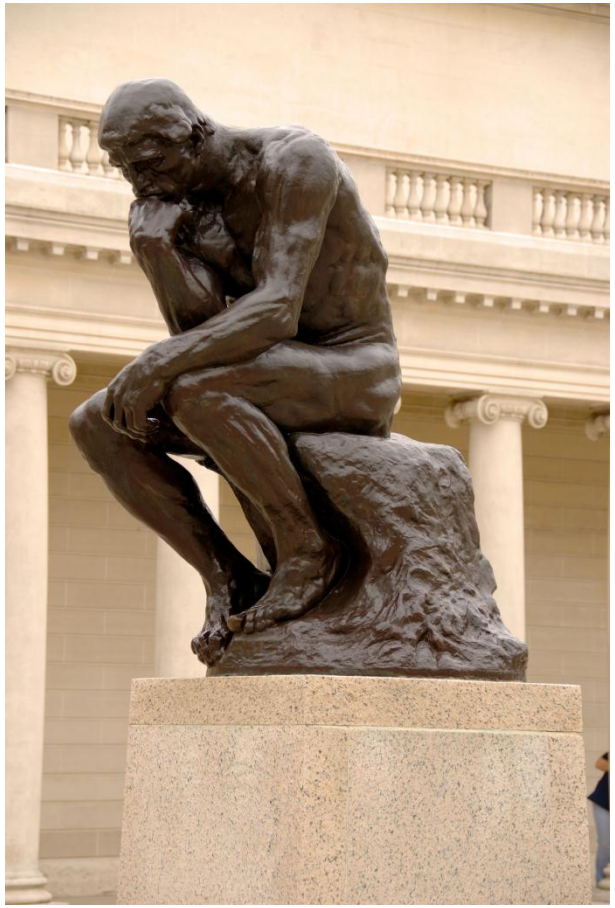
304.1546 ([C17 H21 N O4]+H)+

305.1579

308.2184

Counts vs. Mass-to-Charge (m/z)

Method Explorer: Pestic

- Chromatogram
- Spectrum
- General
- Reports
- Find Compounds
- Find Compounds by Form
- Find by Formula - Options
- Find by Formula - Chromatogra
- Find by Formula - Mass Spectra
- Find by Formula - Sample Purity
- Identify Compounds
- Compound Automation St
- Worklist Automation
- Export

What do you think?