

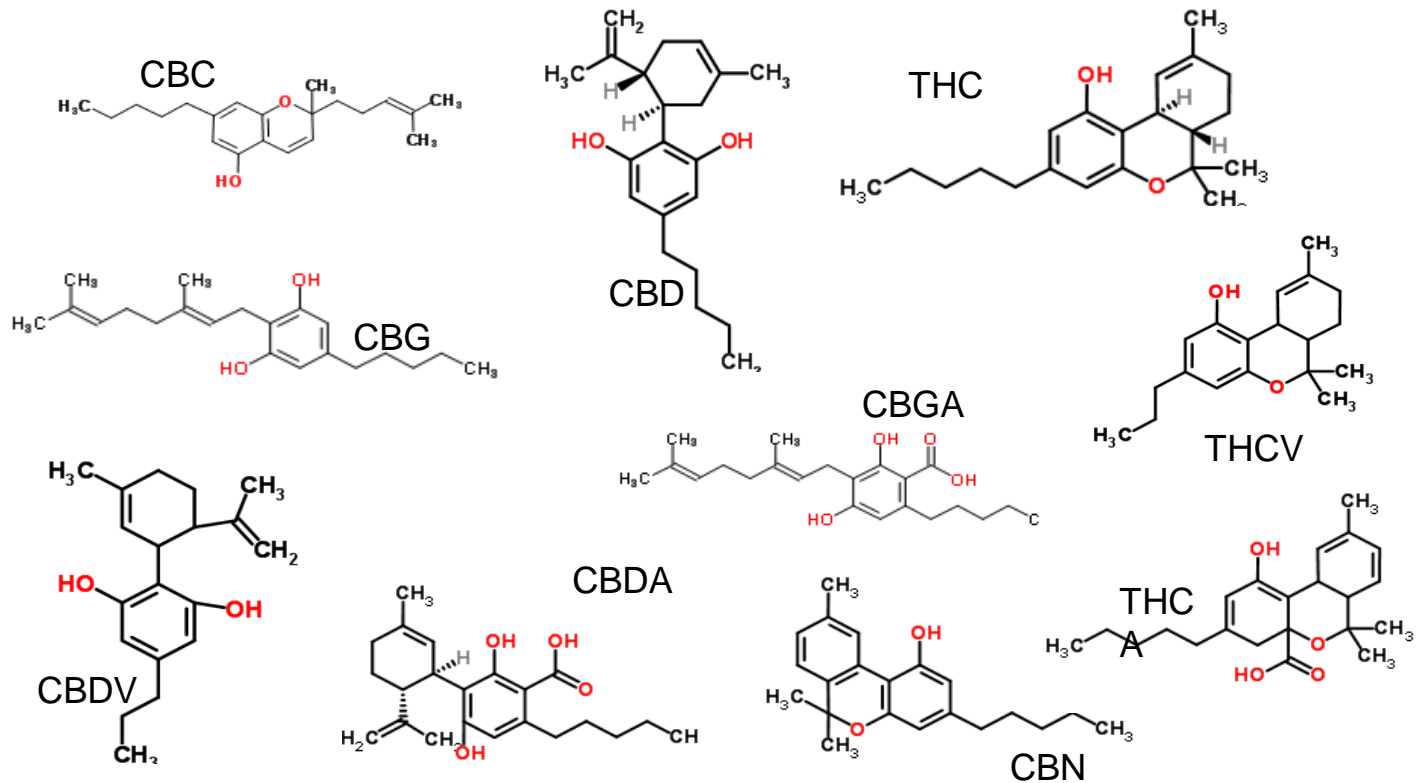
Sue D'Antonio
Application Chemist
Cedar Creek, TX



What is Hemp Oil?

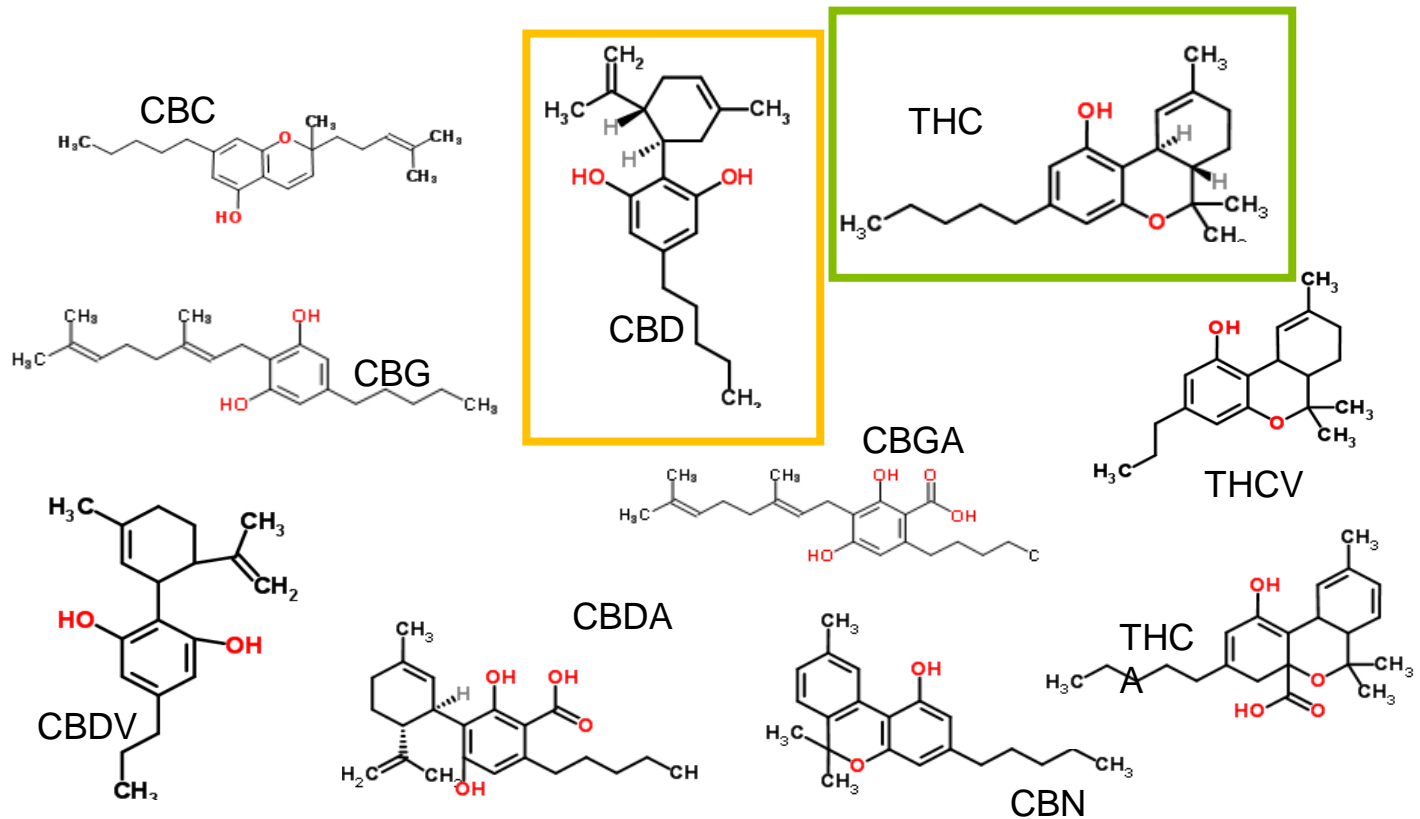
- ✿ CBD hemp oil is a natural botanical extract of the common hemp plant.
- ✿ CBD hemp oil is derived from the seeds and stem of the *Cannabis sativa*.
- ✿ CBD hemp oil is high in CBD and very low in THC (below 0.3% delta 9 THC).
- ✿ CBD hemp oil is not psychoactive, it does not activate the CB1 receptor.

Chemical Structures of Top 10 Cannabinoids



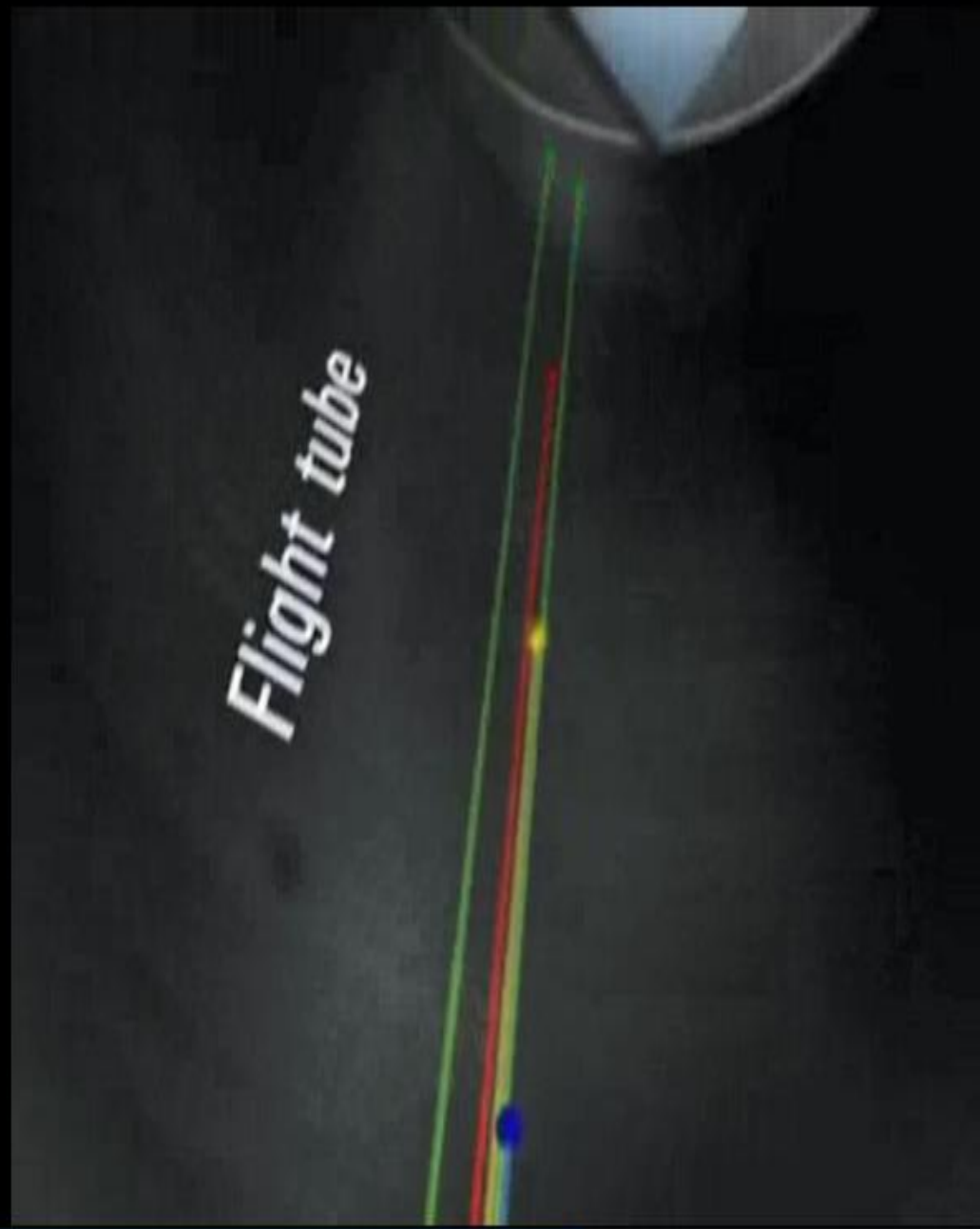
<http://herb.co/2016/02/06/top-10-cannabinoids/>

Chemical Structures of Top 10 Cannabinoids



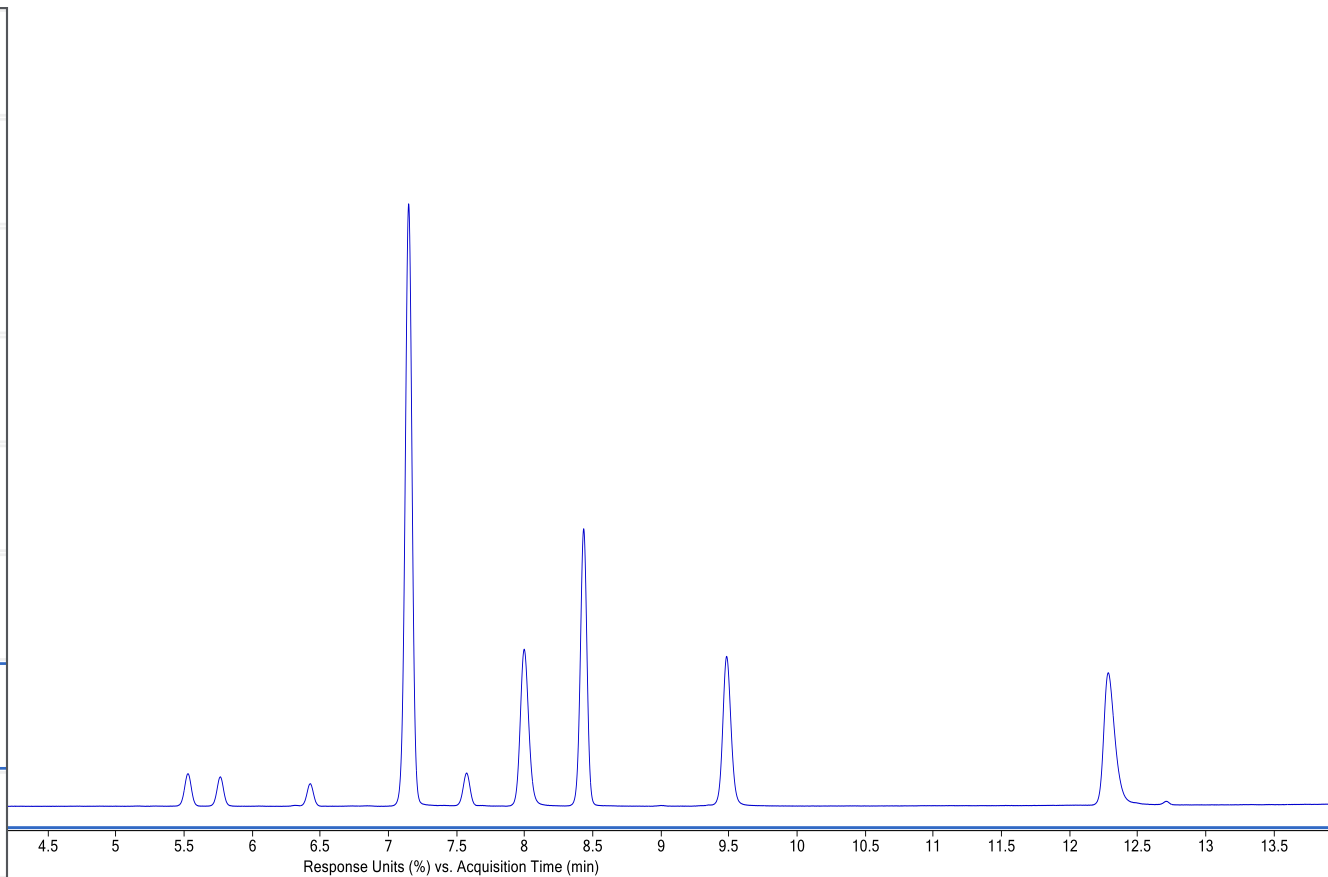
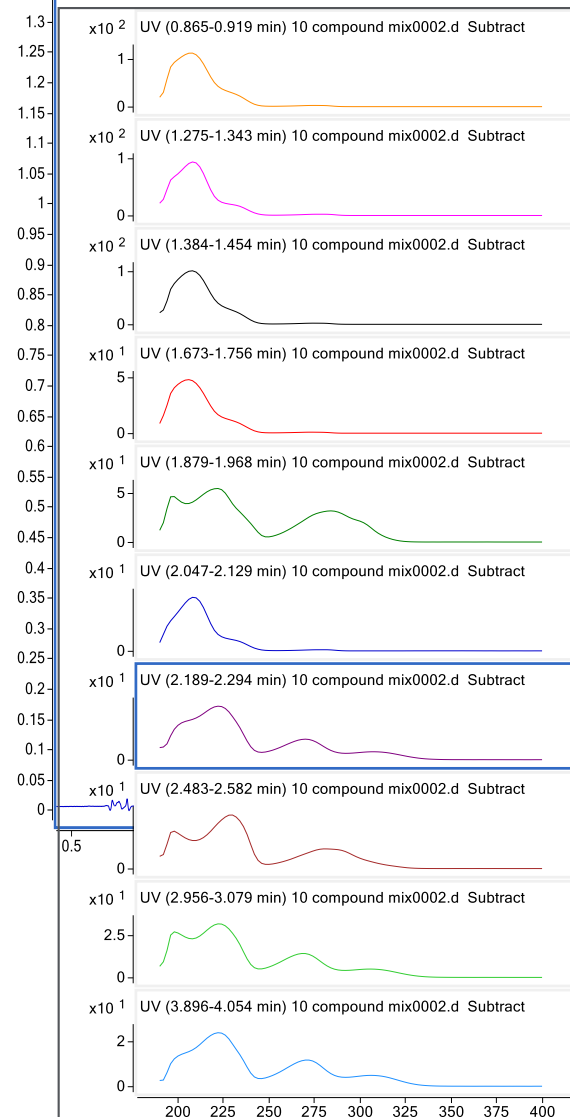
<http://herb.co/2016/02/06/top-10-cannabinoids/>

Why Accurate Mass?



UV Spectra of the Chromatographic Peaks

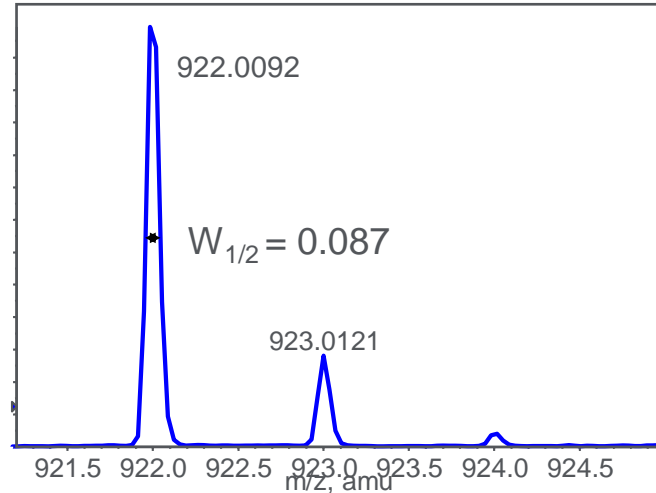
x10² DAD1 - A:Sig=280.0,4.0 Ref=off level 5.d



UV spectra only show class of compounds

Figures of Merit for Accurate-Mass Instrument Performance

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

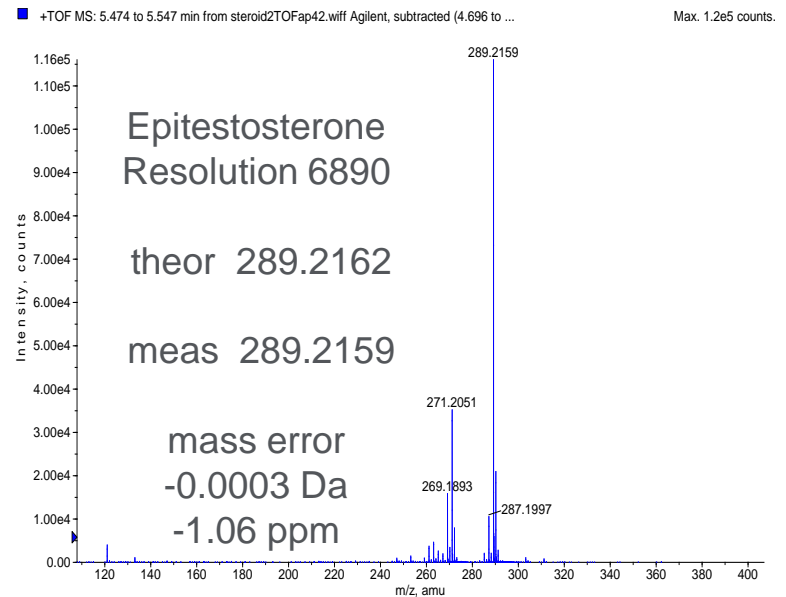


Mass accuracy (mass error)

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

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

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



How Much Accuracy is 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 $\pm 0.1 = 165$ 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.

TOF: Time-of-Flight

The kinetic energy of ions leaving the source.

The flight time is proportional to the square root of the mass, which makes the mass proportional to t^2 .

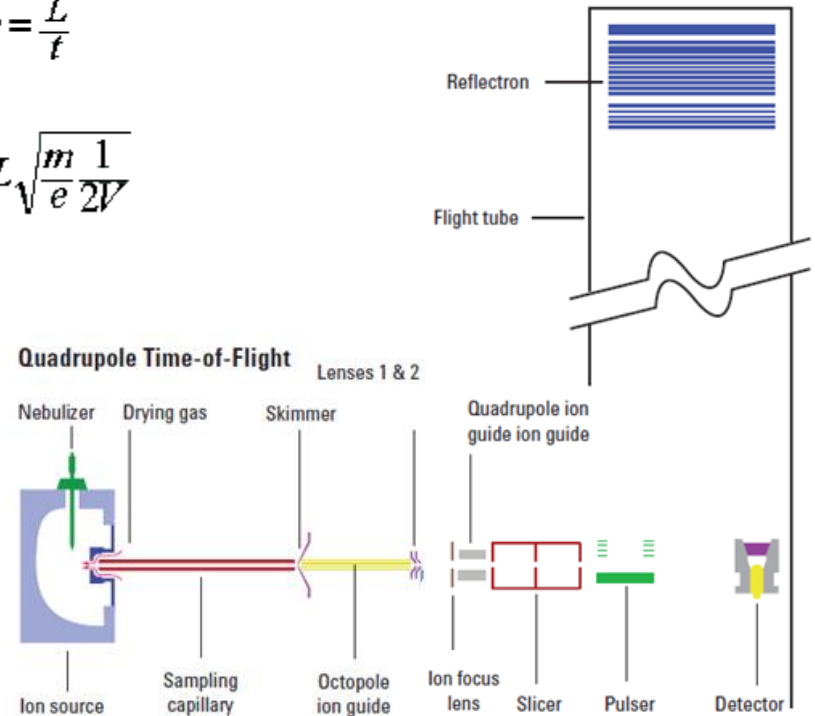
The ion velocity equals the flight path (L) divided by time.

$$T = eV = \frac{mv^2}{2}$$

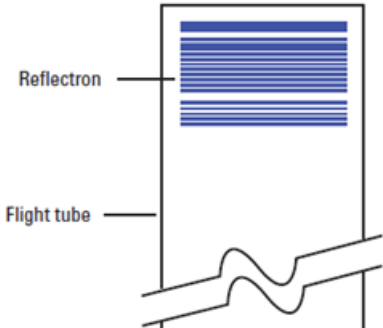
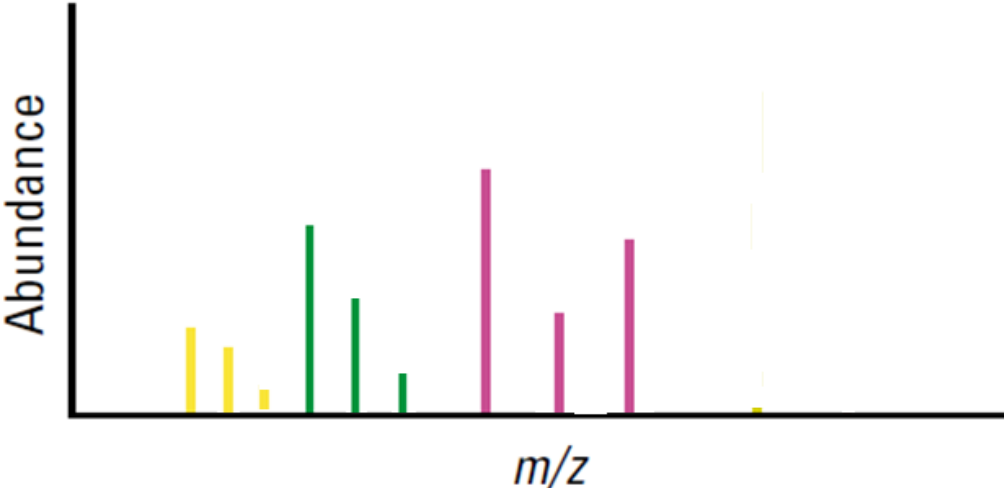
$$v = \frac{L}{t}$$

$$t = L \sqrt{\frac{m}{e} \frac{1}{2V}}$$

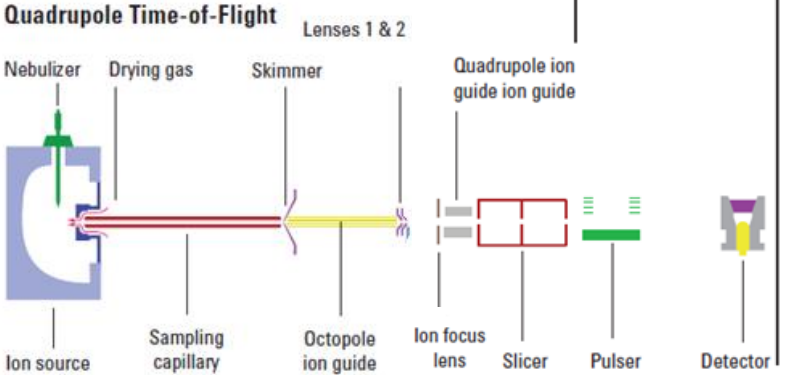
$$m/z = [2V/L^2]t^2$$



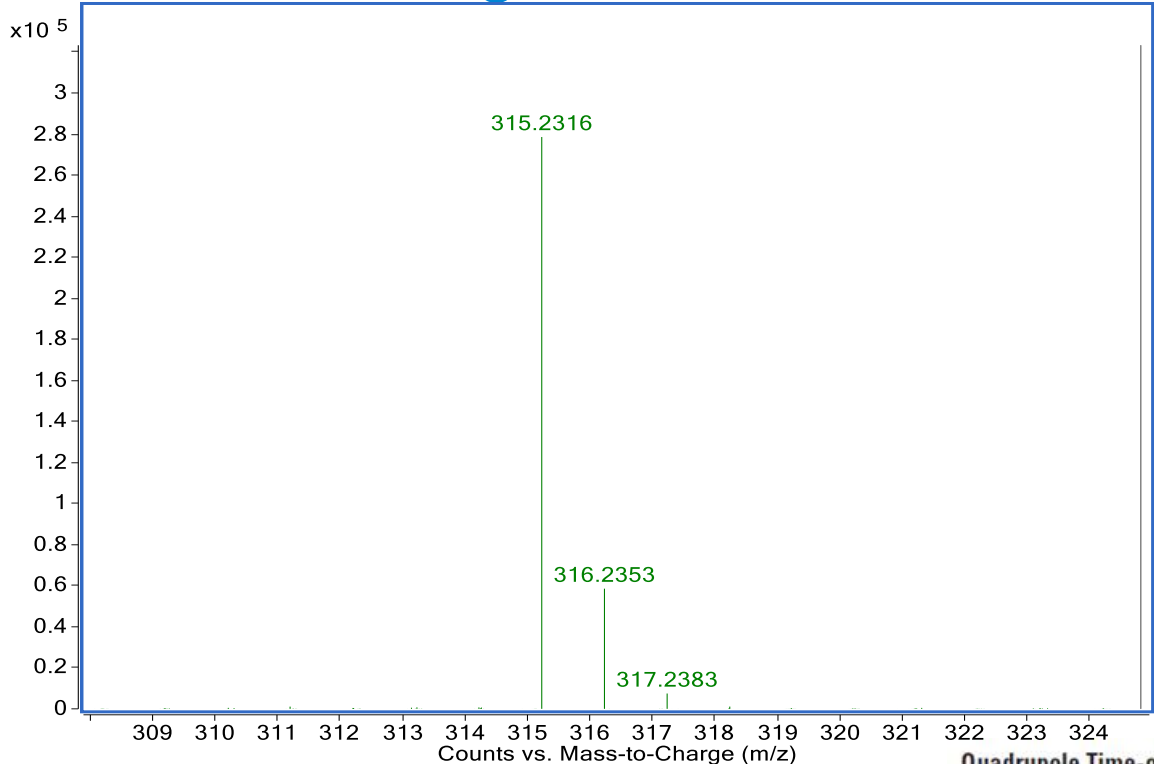
TOF: Time-of-Flight



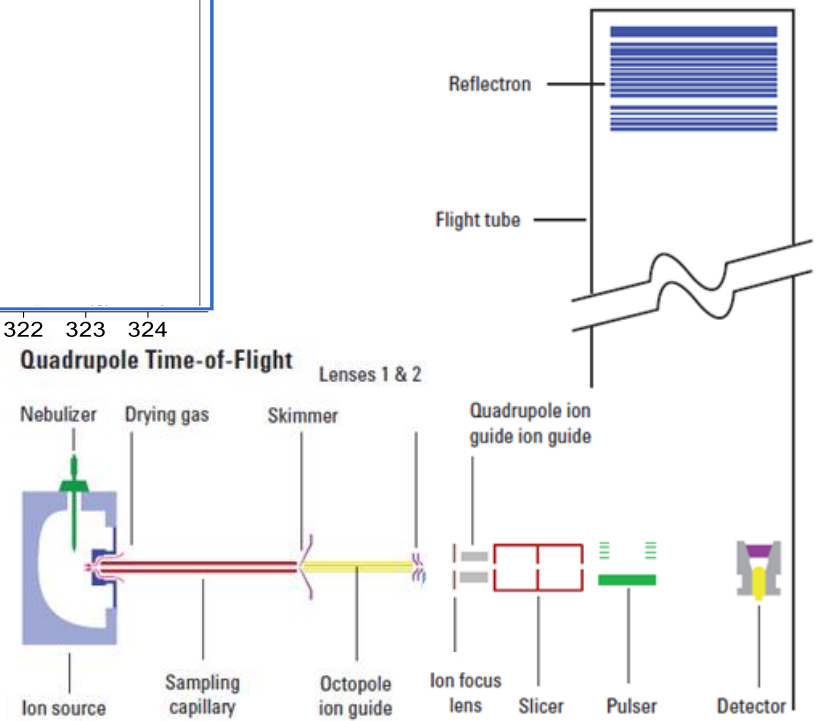
$$m/z = [2V/L^2]t^2$$



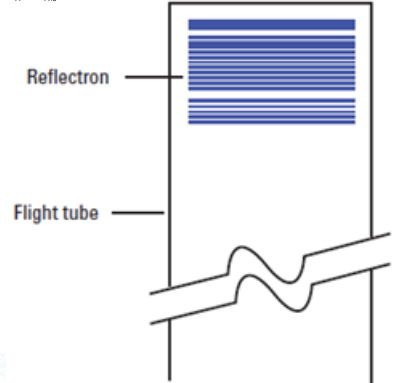
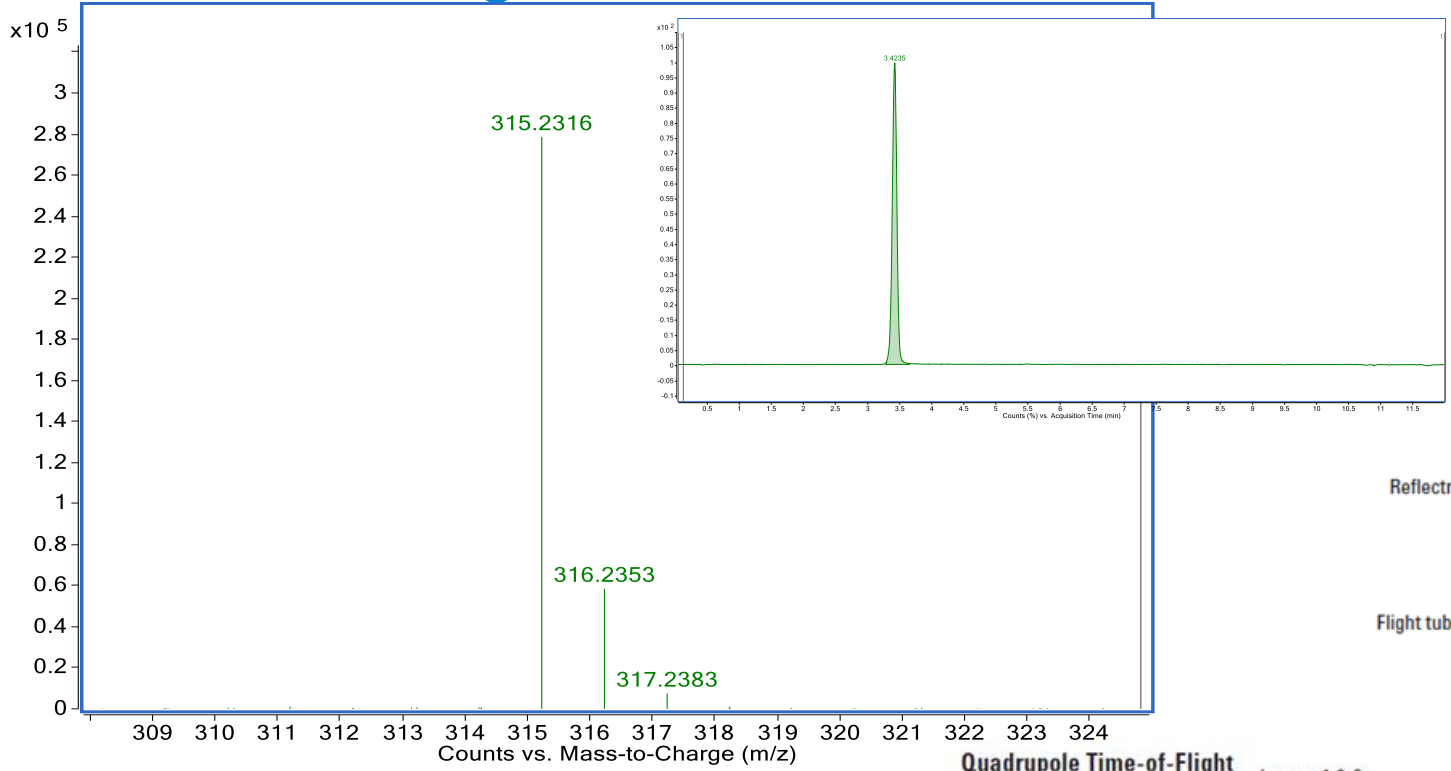
TOF: Time-of-Flight



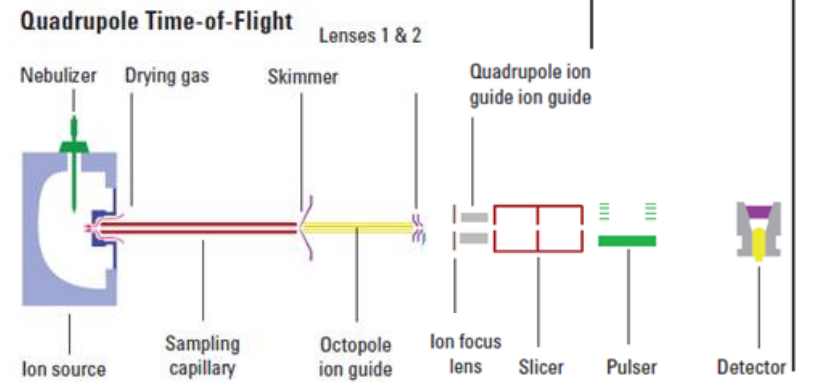
$$m/z = [2V/L^2]t^2$$



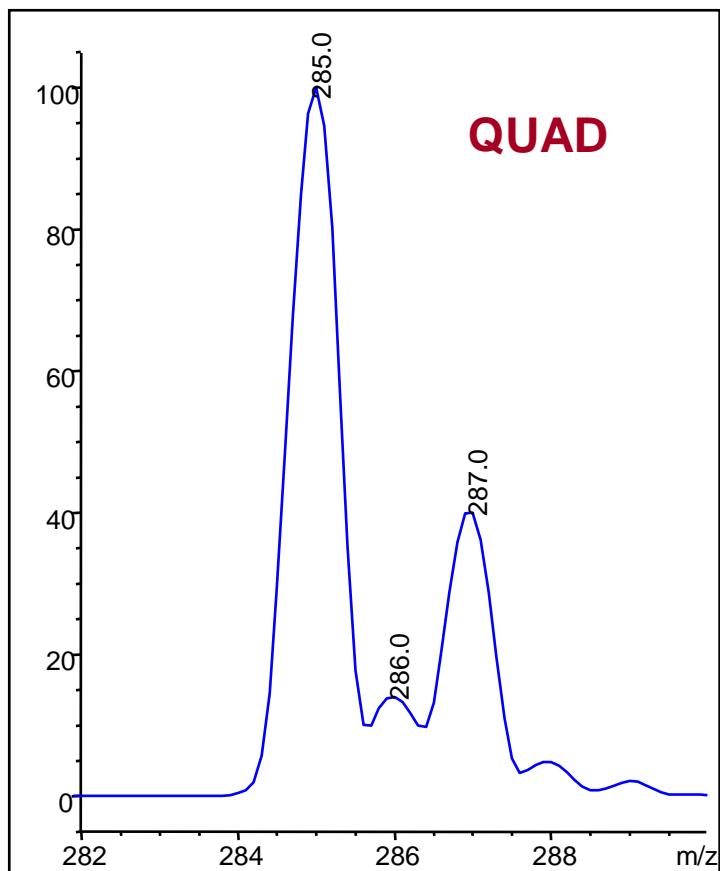
TOF: Time-of-Flight



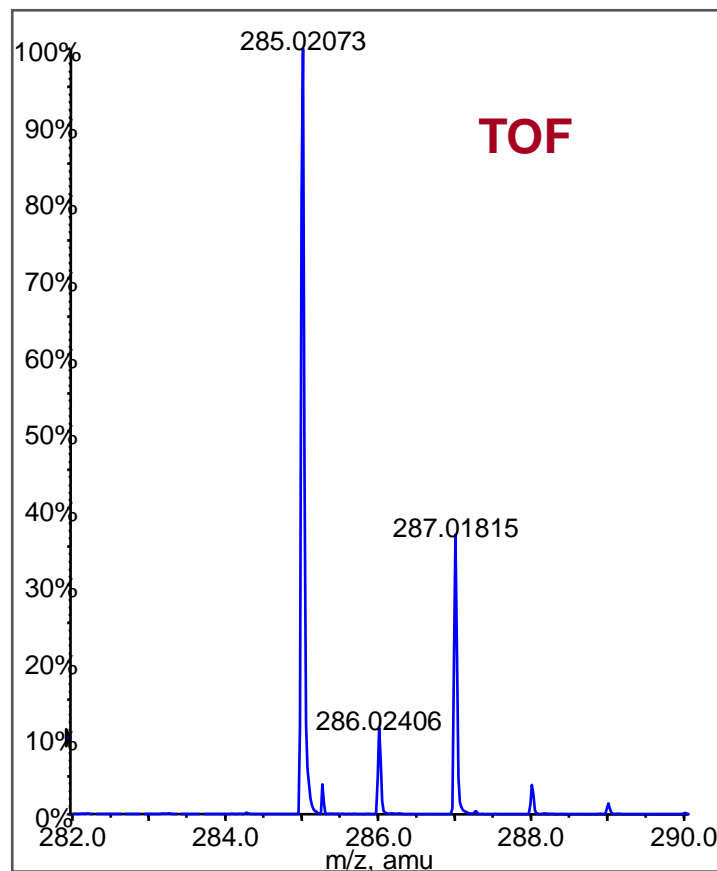
$$m/z = [2V/L^2]t^2$$



Resolution Comparison of Quadrupole and TOF

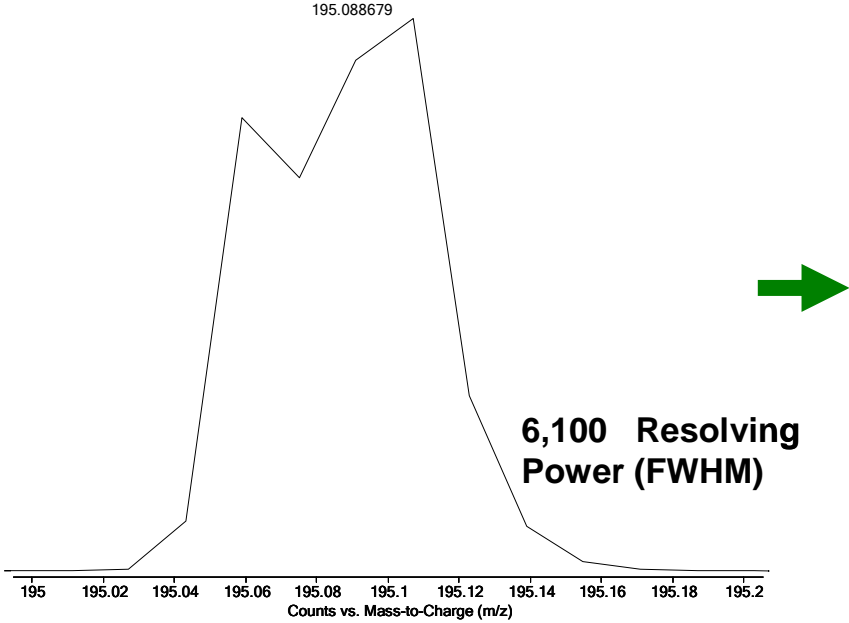


Mass axis stepped in 0.1 m/z increments
No centroid interpolation done

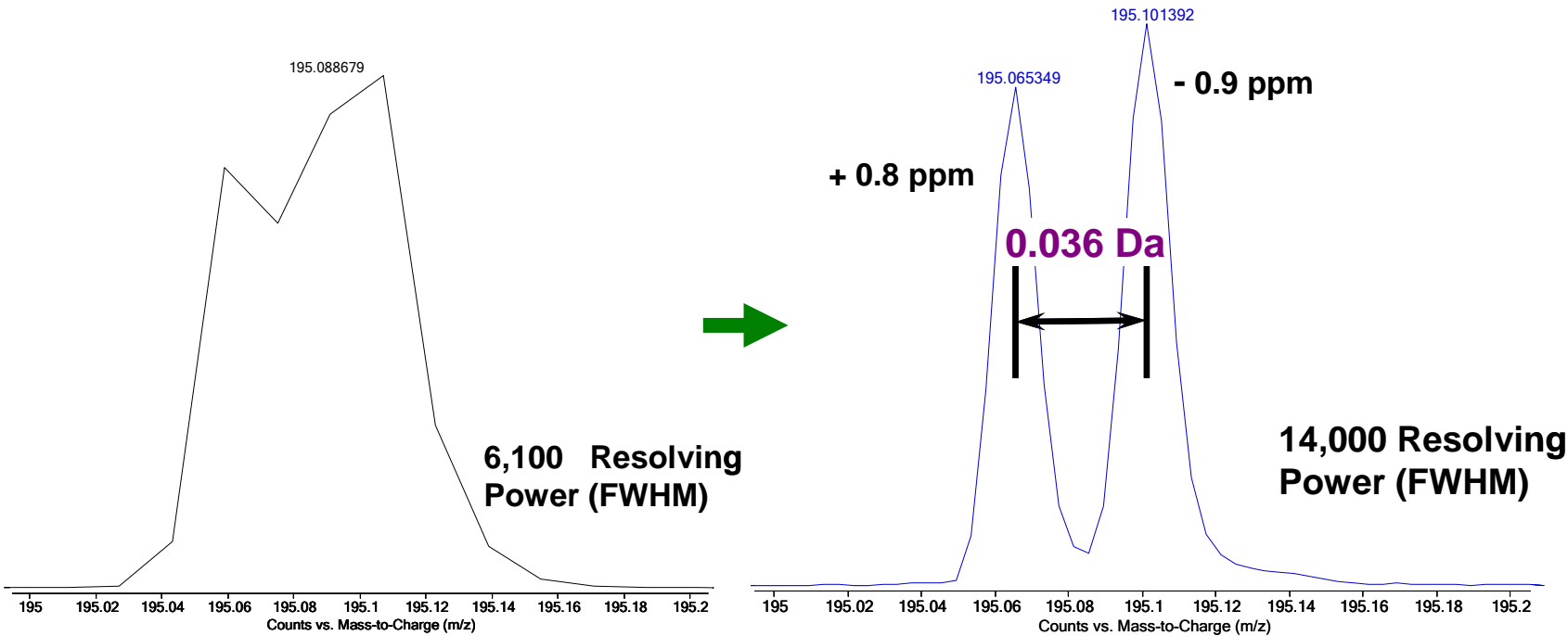


Mass axis stepped in 0.009 m/z increments
Advanced centroiding performed

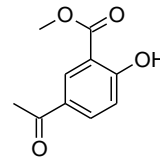
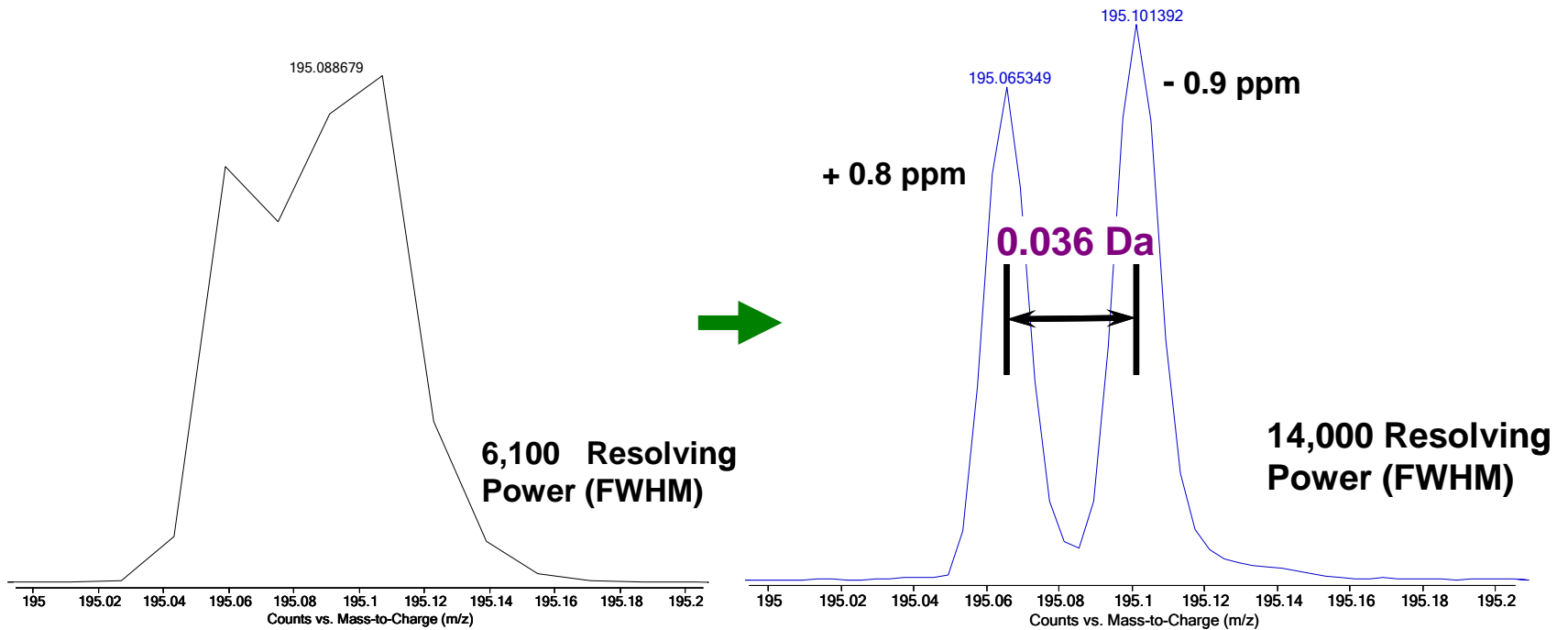
Extraordinary Mass Resolution in Agilent's TOF and Q-TOF Solutions



Extraordinary Mass Resolution in Agilent's TOF and Q-TOF Solutions

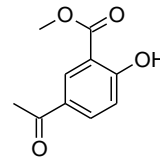
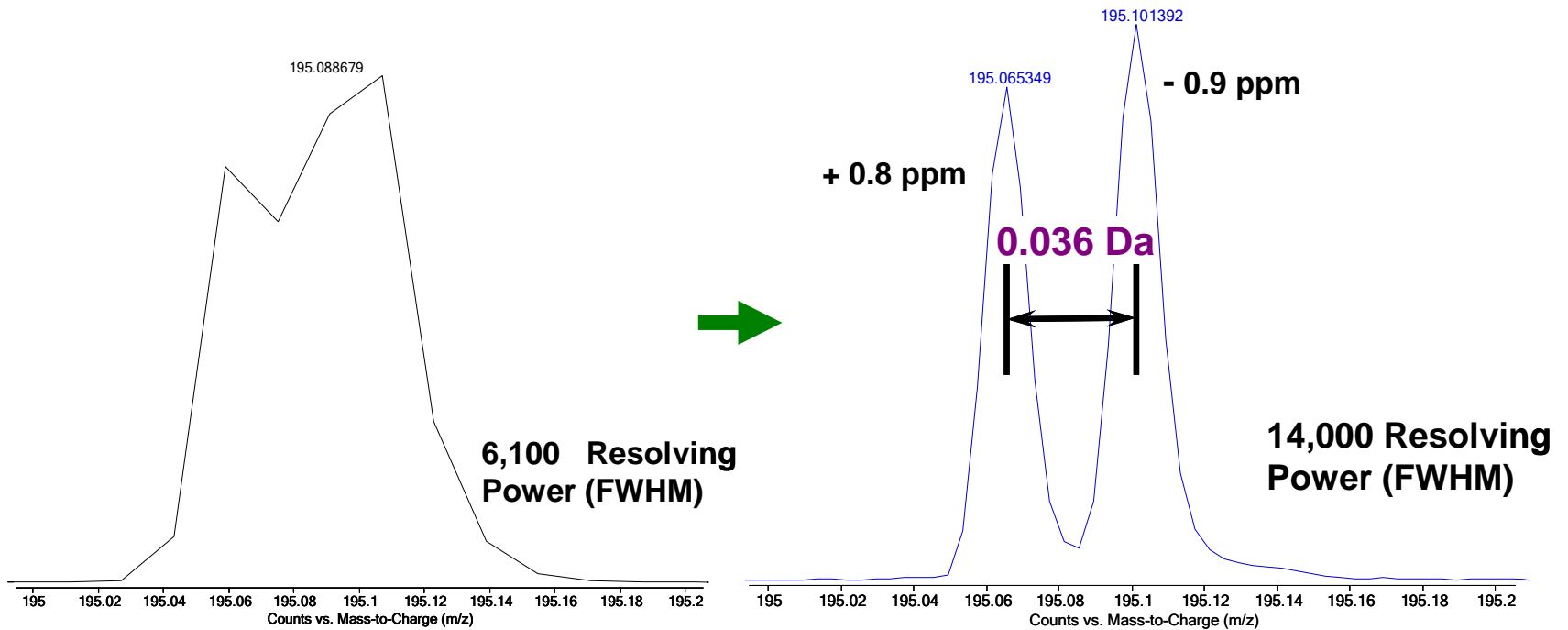


Extraordinary Mass Resolution in Agilent's TOF and Q-TOF Solutions

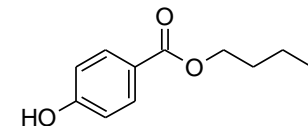


Methyl 5-acetylsalicylate
[M+H]⁺ = m/z 195.06519

Extraordinary Mass Resolution in Agilent's TOF and Q-TOF Solutions

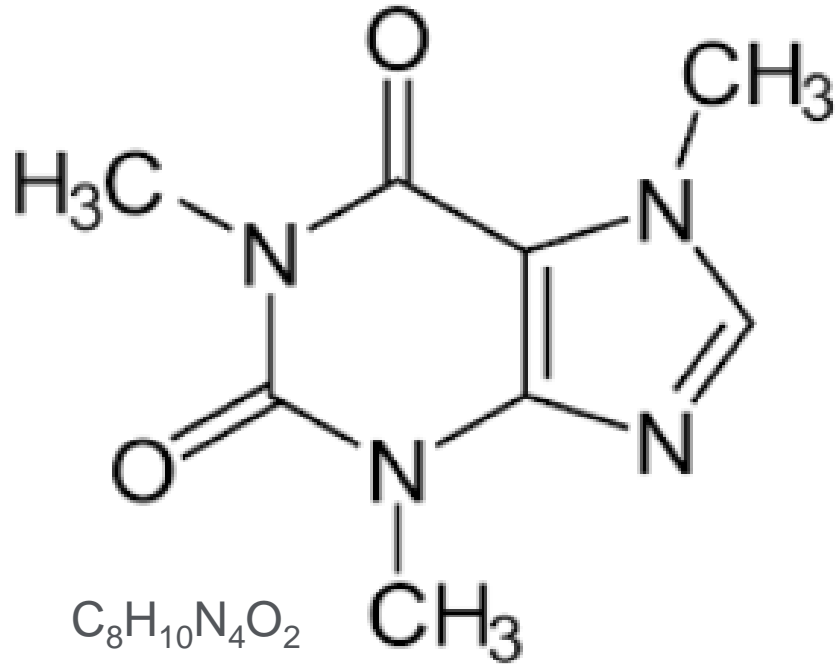


Methyl 5-acetylsalicylate
[M+H]⁺ = m/z 195.06519

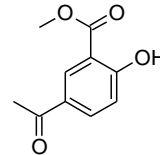
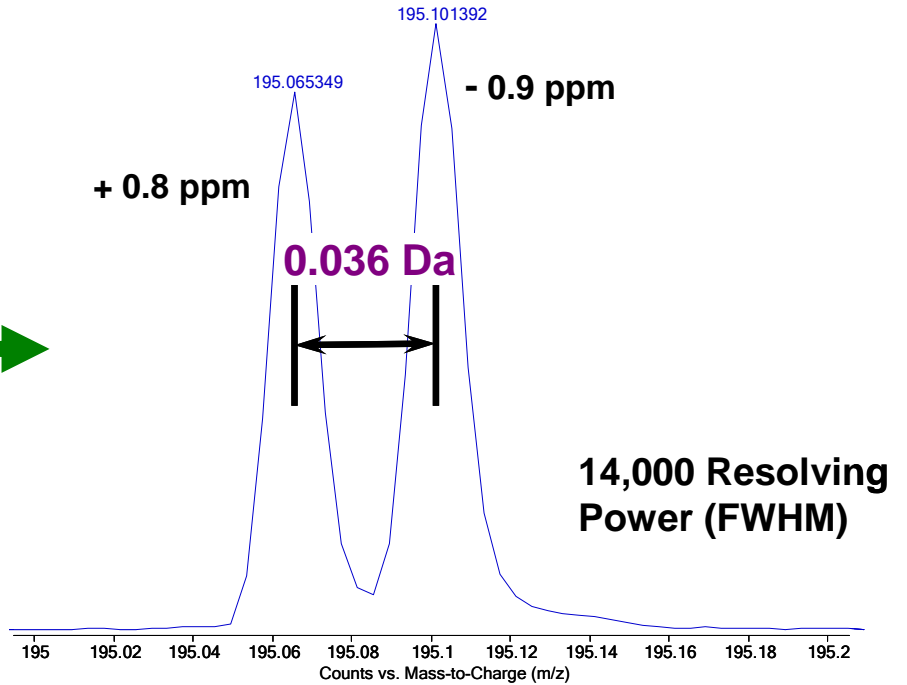


Butyl paraben
[M+H]⁺ = m/z 195.10157

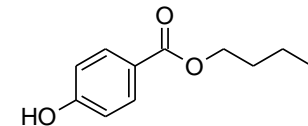
Extraordinary Mass Resolution in Agilent's TOF and Q-TOF Solutions



195.0877



Methyl 5-acetylsalicylate
[M+H]⁺ = m/z 195.06519

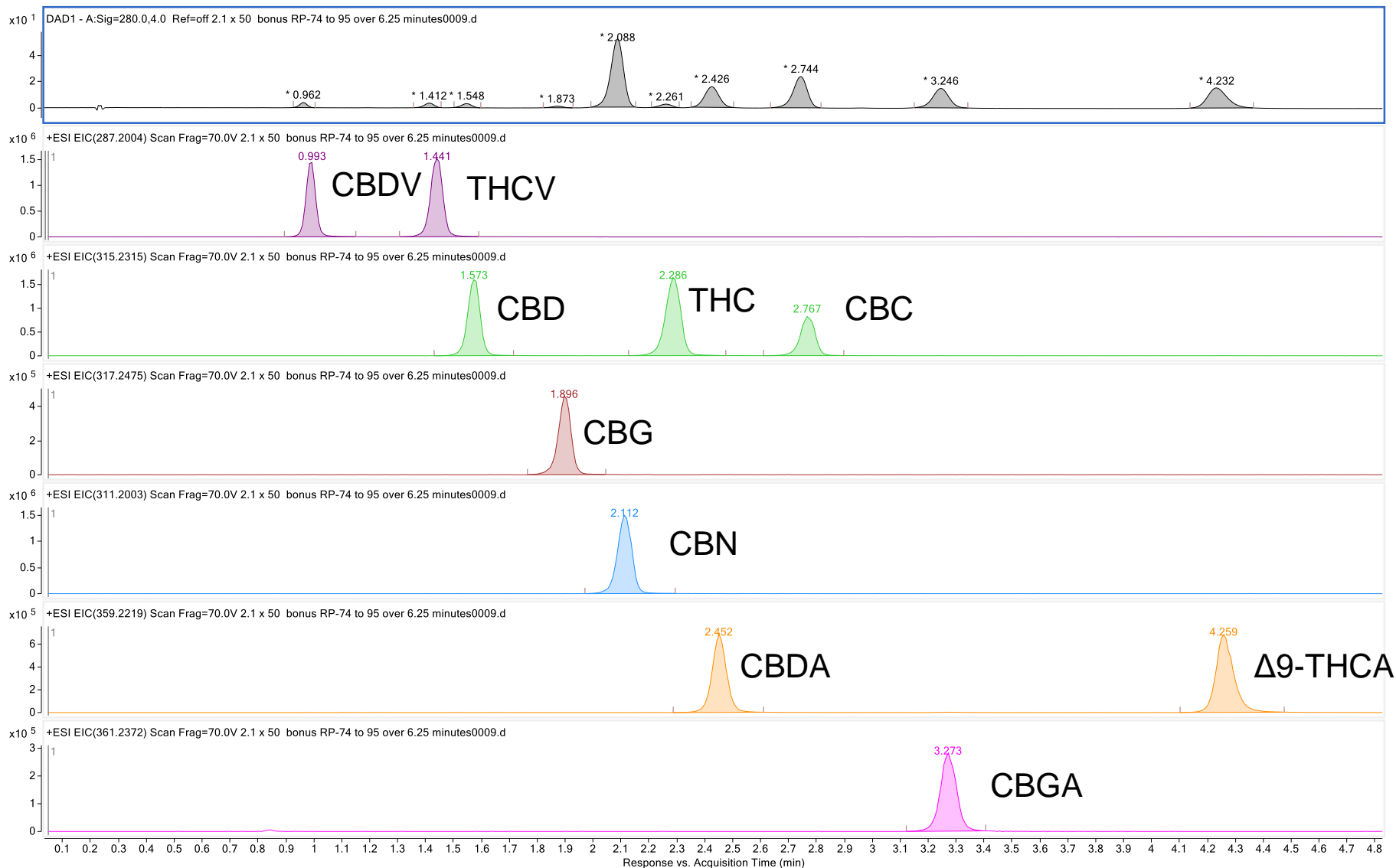


Butyl paraben
[M+H]⁺ = m/z 195.10157

Our goal

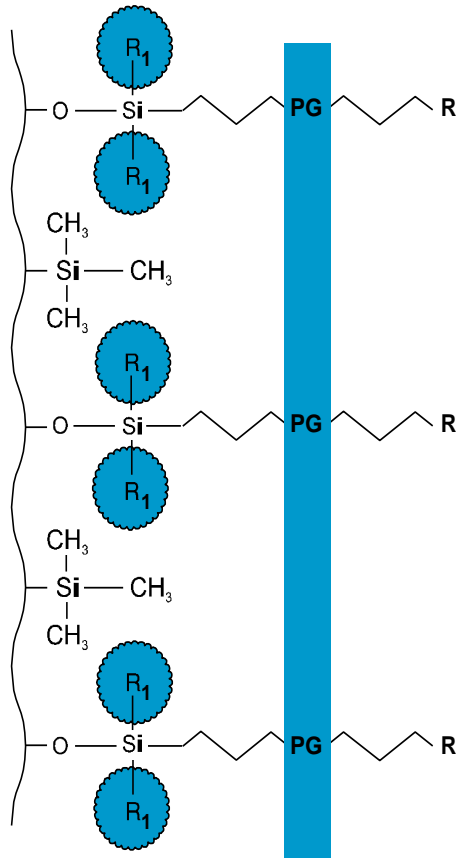
To develop a method which allows quantitation of high levels of CBD and CBDA in the same analytical run as low levels of THC.

10 compound mix with ToF data on Bonus RP 2.1 x 50, 1.8u 5 minute run time



Zorbax Bonus-RP

Polar-linked alkyl phase



Superb peak shape for basic compounds

Long column life (pH 2-8)

Unique selectivity

Patented bonding technology

- polar-linked alkyl phase for fast mass transfer giving good peak shape
- bulky side groups give low pH stability
- triple endcapped for mid-range pH stability and good peak shape

HPLC Conditions for short method

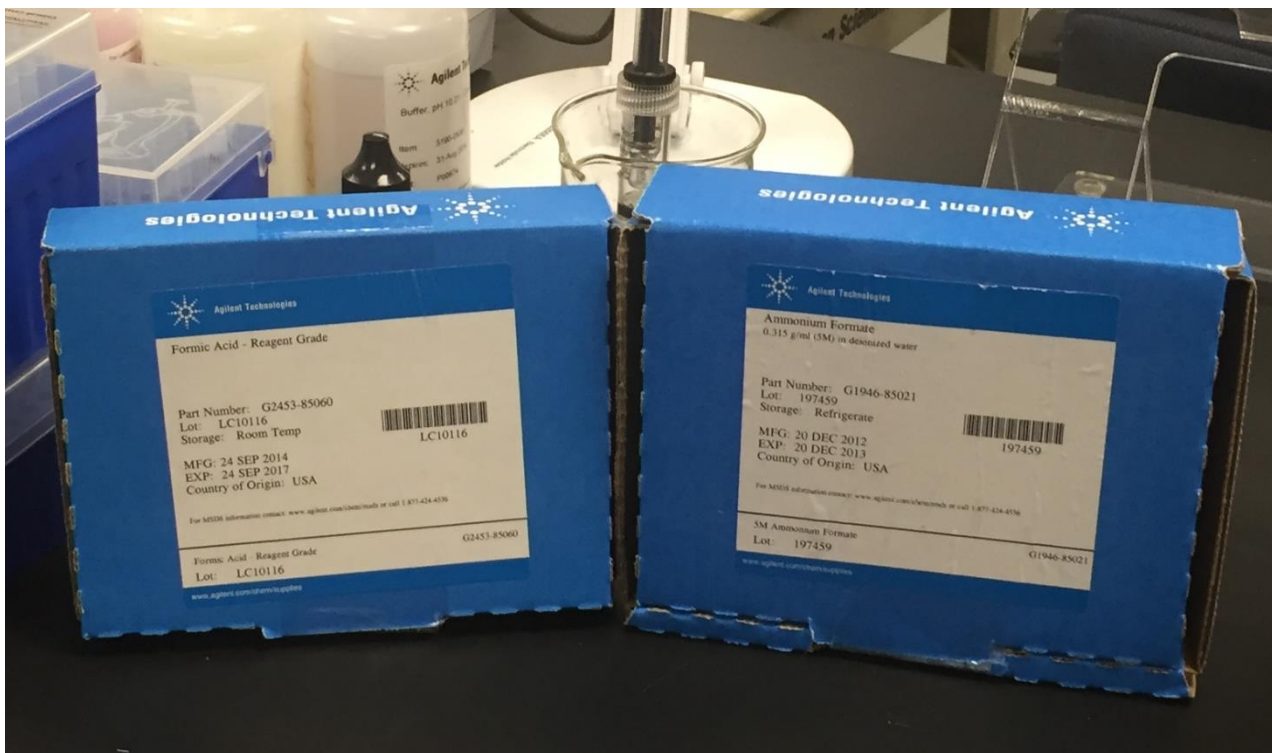
Agilent 1290 Infinity II UHPLC series Quaternary Pump, Multisampler with wash , Multi Column Thermostat, DAD

Column: Zorbax Bonus RP 2.1 x 50 mm, 1.8 μ m or Poroshell 3.0 x50 2.7 μ m

Column temperature: 50°C
Injection volume: 0.05 μ L
Autosampler temp: 23 °C
Needle wash: 3.5 s Flush Port (25:25:50)
(H₂O:IPA:MeOH)
DAD-UV 254 nm
Mobile phase: A = Water
B= Methanol
C= 0.1% CH₂O₂ + 2.2 ml 5M NH₄HCO₂ in H₂O
Flow rate: 0.5 mL/min
Gradient:

Time (min)	%B	%C
0.0	72	5
6.25	95	5

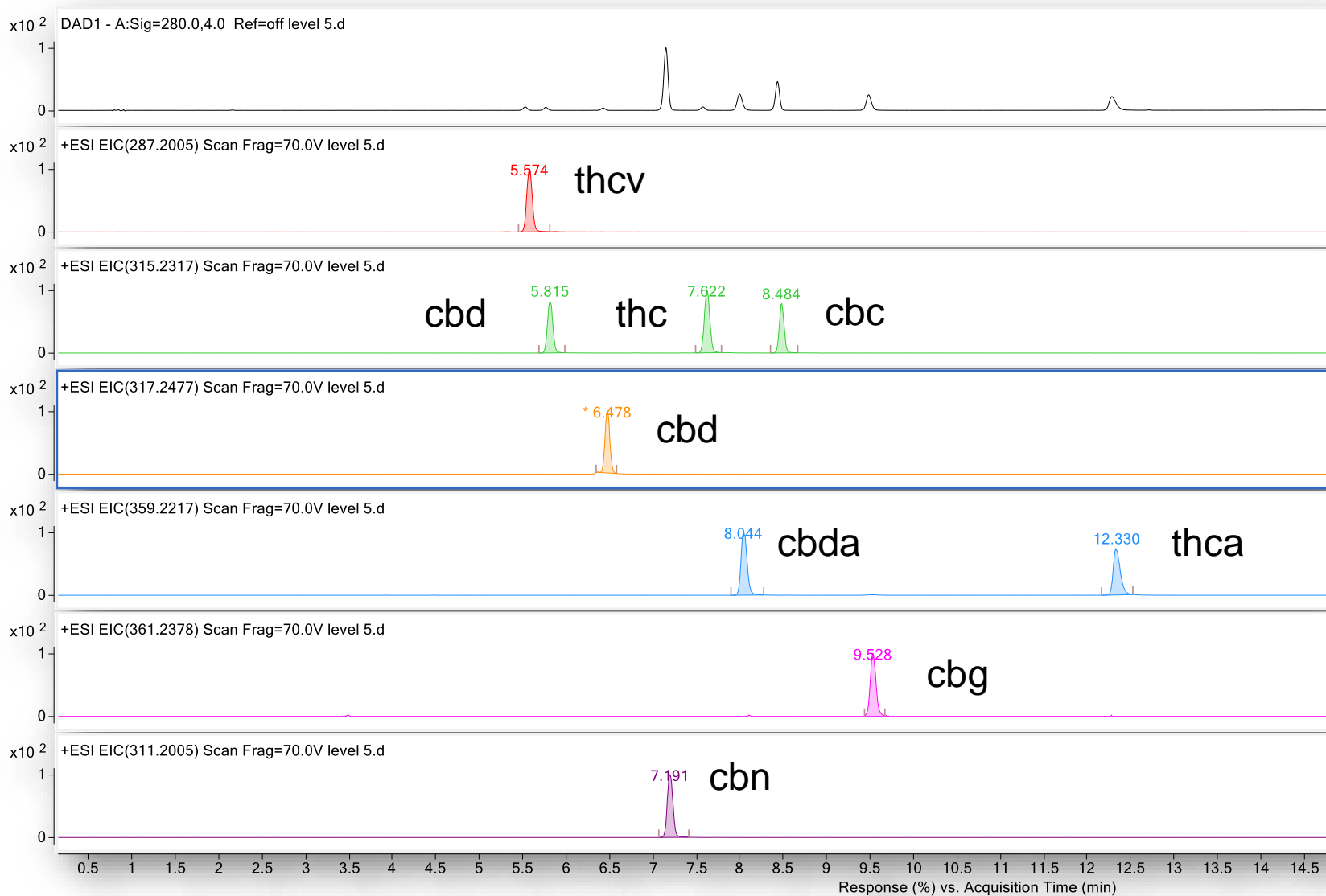
Stop time: 5.00 min.
Post time: 1.0 min.
Overall run time 6.0 minutes (incl. re-equilibration)



1. 1 vial of Ammonium Formate (G1946-85021)
2. 1 ML of Formic Acid (G2452-85060)

Into 1 Liter of LC MS grade H₂O. We have only tested with bottled H₂O

Bonus RP 3.0 x 100 1.8 um 9 compound standard



10 Cannabinoid Standard PCDL

MassHunter PCDL Manager for Forensics and Toxicology - D:\MassHunter\PCDL\cannabinoid.cdb

File Edit View PCDL Links Help

Find Spectra

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra Edit Spectra

Acquired spectra

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization	Instrument

Library spectra

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization	Instrument
THC / delta9-Tetrahydrocannabinol	(M+H)+	315.23186	10	Positive	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M+H)+	315.23186	20	Positive	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M+H)+	315.23186	40	Positive	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M-H)-	313.21730	10	Negative	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M-H)-	313.21730	20	Negative	ESI	QTOF

Graphics Mass Lists

Acquired spectrum

Library spectrum

Single Search Results: 10 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Spectra
Cannabidivanol	C19H26O2	286.19328	<input type="checkbox"/>	<input type="checkbox"/>	5.530	24274-48-4	21106275	2-[(1S,6S)-6-Isopropenyl-3-methyl-2-cyclohexen-1-yl]-5-pentyl-1H-indole-3-carboxamide	0
Tetrahydrocannabinavarin (THCV)	C19H26O2	286.19328	<input type="checkbox"/>	<input type="checkbox"/>	5.530				0
CBN / Cannabinol	C21H26O2	310.19328	<input type="checkbox"/>	<input type="checkbox"/>	7.191	521-35-7	2447	6,6,9-Trimethyl-3-pentyl-6H-benzo[c]chromen-1-ol	6
THC / delta9-Tetrahydrocannabinol	C21H30O2	314.22458	<input type="checkbox"/>	<input type="checkbox"/>	8.484	1972-08-3	15266	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydrobenzo[b]chromen-1-ol	5
CBD / Cannabidiol	C21H30O2	314.22458	<input type="checkbox"/>	<input type="checkbox"/>	7.900	13956-29-1	559095	2-[(1R,6R)-6-Isopropenyl-3-methyl-2-cyclohexen-1-yl]-5-pentyl-1H-indole-3-carboxamide	6
Cannabichromene (CBC)	C21H30O2	314.22458	<input type="checkbox"/>	<input type="checkbox"/>	5.768				0
Cannabigerol	C21H32O2	316.24023	<input type="checkbox"/>	<input checked="" type="checkbox"/>	6.478	25654-31-3	4474921	2-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-5-pentyl-1H-indole-3-carboxamide	0
Canrenic acid	C22H30O4	358.21441	<input type="checkbox"/>	<input type="checkbox"/>		4138-96-9	570976	3-[(8R,9S,10R,13S,14S,17R)-17-Hydroxy-10,13-dioxolano[2,3-b]furan-2-yl]propanoic acid	3
THCA-A / delta9-Tetrahydrocannabinol-2-carboxylic acid	C22H30O4	358.21441	<input type="checkbox"/>	<input type="checkbox"/>	12.330	23978-85-0	88974	(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydrobenzo[b]chromen-1-ol-2-carboxylic acid	3
Cannabigerolic acid (CBGA)	C22H32O4	360.23006	<input type="checkbox"/>	<input type="checkbox"/>	9.528				0

These are the times for the 15 minute method, note the PCDL can be adjusted for the column and UHPLC/HPLC used

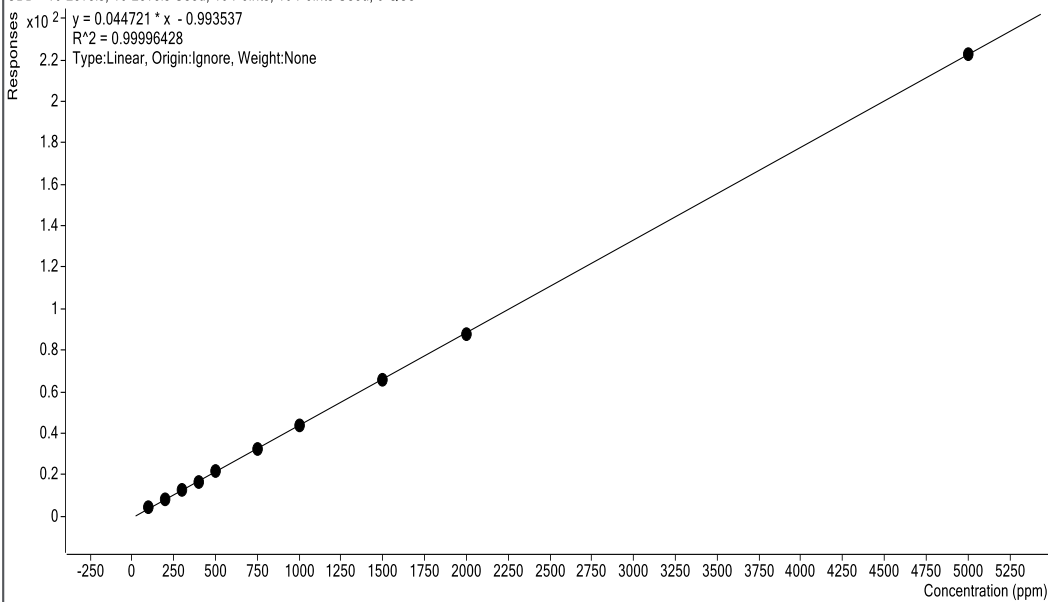
Confirmation of Compounds

The screenshot displays the Agilent MassHunter Qualitative Analysis 8.07.00 interface. The main window is divided into several panes:

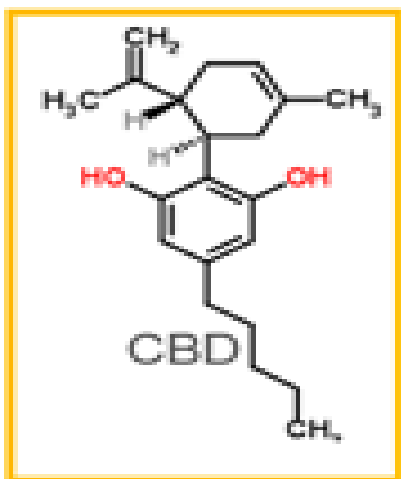
- Compound List:** A table listing identified compounds. The top entry is highlighted: Cpd 3: 8.484 31. 3 level 5 d FBF THCA-A / delta9-Tetrahydrocannabinol-2-carboxylic acid C22 H30 O4 8.484 315.2318 314.2246 99.75.
- Compound Identification Results:** A table showing the best match for the selected compound: THC / delta9-Tetrahydrocannabinol C21 H30 O2 315.2318 314.2246 314.2246 -0.03 99.75 8.484 8.484.
- Method Editor: Find Compounds by Formula - Options:** Shows search criteria including 'Database / Library' set to 'D:\MassHunter\PCDL\cannabinoid.cdb' and 'Automatically increase for isomeric compounds' checked.
- Compound Chromatogram Results:** A chromatogram showing a single sharp peak at 8.484 minutes.
- Compound MS Spectrum Results:** An ESI scan showing the mass spectrum with major peaks at m/z 315.2318 (M+H)+, 316.2355 (M+H)+, and 317.2387 (M+H)+.
- Compound Fragment Spectrum Results:** A message indicating 'No Fragment Spectrum available for this compound.'

The Windows taskbar at the bottom shows several instances of Agilent MassHunter and other applications like Microsoft Excel and PowerPoint.

CBD - 10 Levels, 10 Levels Used, 10 Points, 10 Points Used, 0 QCs

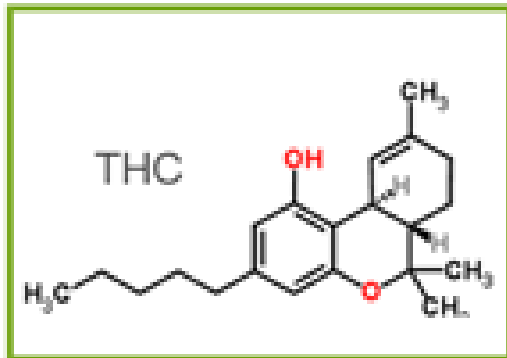
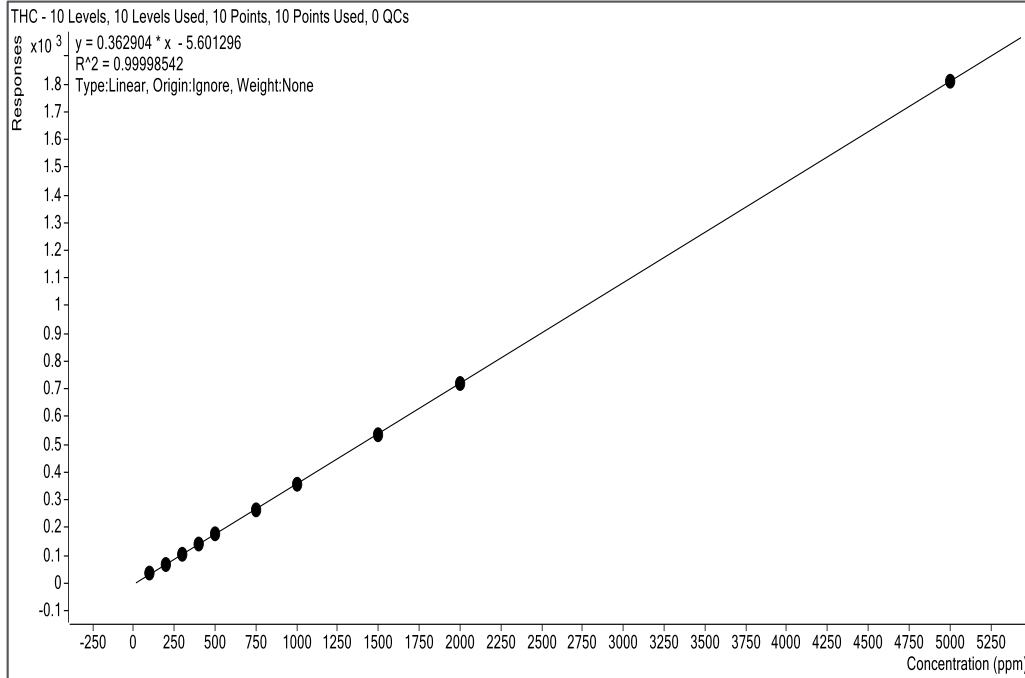


CBD at high level



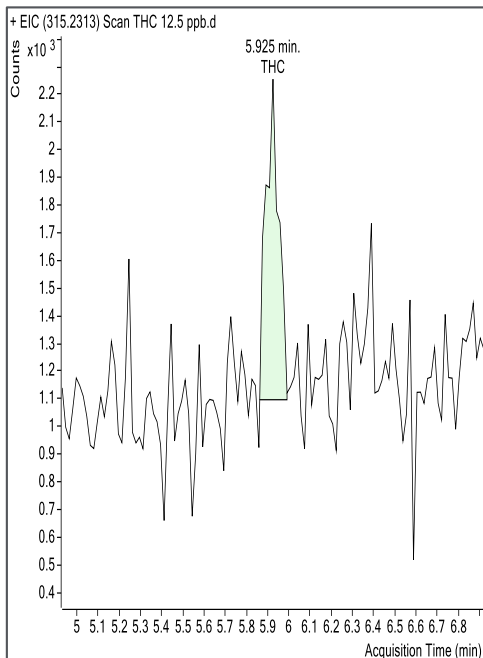
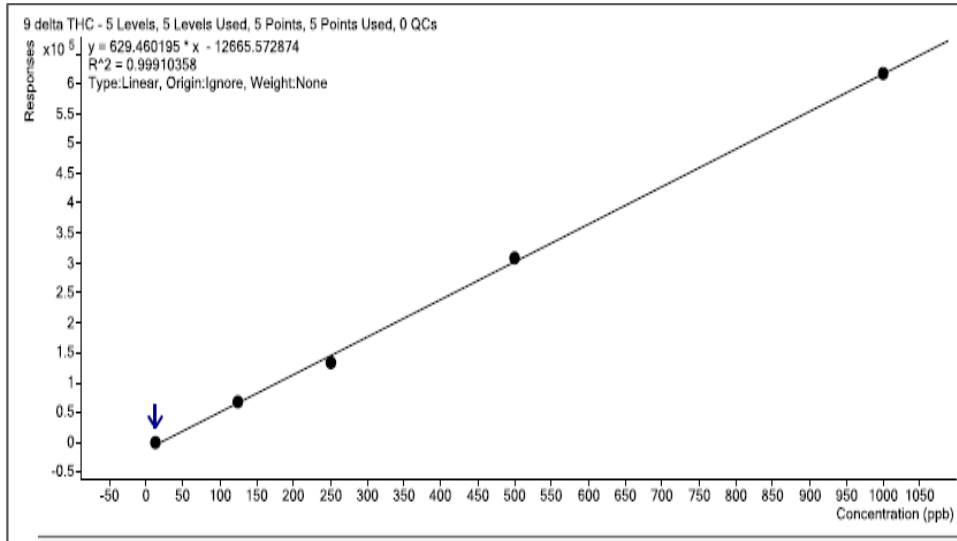
Sample			CBD Method	CBD Results				
Data File	Type	Level	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy
level 1.d	Cal	1	100.0000	7.549	4	114.6761	114.6761	114.7
level 2.d	Cal	2	200.0000	7.566	8	203.0707	203.0707	101.5
level 3.d	Cal	3	300.0000	7.567	12	299.8328	299.8328	99.9
level 4.d	Cal	4	400.0000	7.568	17	393.2657	393.2657	98.3
level 5.d	Cal	5	500.0000	7.576	21	502.1863	502.1863	100.4
level 6.d	Cal	6	750.0000	7.585	32	745.9138	745.9138	99.5
level 7.d	Cal	7	1000.0000	7.578	44	1006.1116	1006.11...	100.6
level 8.d	Cal	8	1500.0000	7.581	66	1493.4677	1493.46...	99.6
level 9.d	Cal	9	2000.0000	7.587	88	1983.6854	1983.68...	99.2
level 10.d	Cal	10	5000.0000	7.577	223	5007.7899	5007.78...	100.2

THC at high level



Sample					THC Method	THC Results				
▼	Name	Data File	Type	Level	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy
	9 peak mix	level 1.d	Cal	1	100.0000	8.408	35	110.7183	110.7183	110.7
	9 peak mix	level 2.d	Cal	2	200.0000	8.422	68	203.0966	203.0966	101.5
	9 peak mix	level 3.d	Cal	3	300.0000	8.427	103	299.8599	299.8599	100.0
	9 peak mix	level 4.d	Cal	4	400.0000	8.427	138	396.4041	396.4041	99.1
	9 peak mix	level 5.d	Cal	5	500.0000	8.435	177	503.9937	503.9937	100.8
	9 peak mix	level 6.d	Cal	6	750.0000	8.446	266	747.4100	747.4100	99.7
	9 peak mix	level 7.d	Cal	7	1000.0000	8.437	356	996.0243	996.0243	99.6
	9 peak mix	level 8.d	Cal	8	1500.0000	8.438	536	1491.6483	1491.64...	99.4
	9 peak mix	level 9.d	Cal	9	2000.0000	8.445	719	1995.9952	1995.99...	99.8
	9 peak mix	level 10.d	Cal	10	5000.0000	8.436	1811	5004.8495	5004.84...	100.1
	9 peak mix	level 5 with all ions.d	Sample			8.429	176	501.2125	501.2125	
	Sample14	F1 sample.d	Sample			8.444	110	319.1457	319.1457	
	Sample15	F2 sample.d.d	Sample			8.450	61	184.1997	184.1997	
▼	Sample16	blend.d	Sample			8.458	2	21.9074	21.9074	
	Sample17	hemp hri.d	Sample			8.449	374	1045.9793	1045.97...	

THC at low level

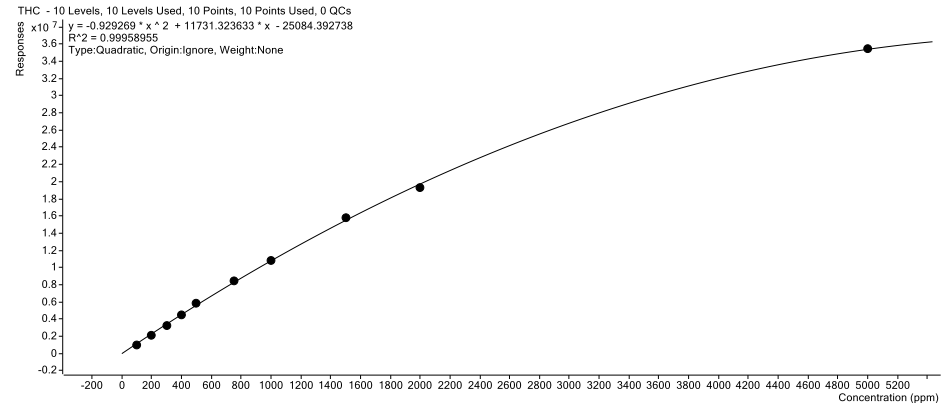
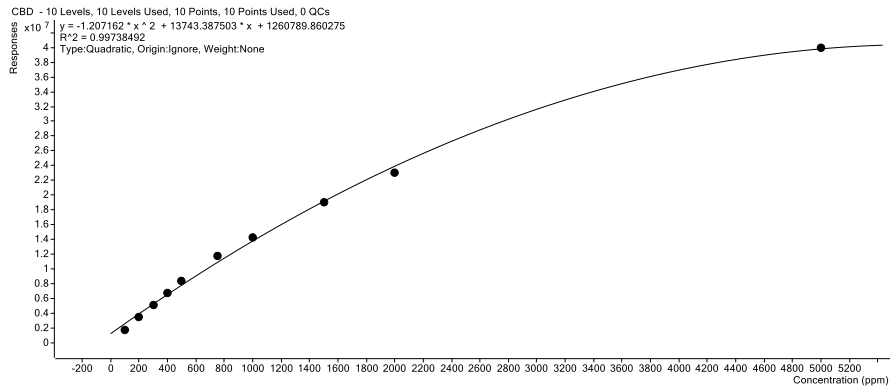


Sample						THC Method	THC Results			
Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	
thc	THC 12.5 ppb.d	Cal	1	8/9/2016 3:20 AM	12.5000	5.925	4703	17.9468	17.9468	
tox standard	THC 125 ppb.d	Cal	2	8/9/2016 3:40 AM	125.0000	5.910	68067	118.1281	118.1281	
tox standard	THC 1000 ppb...	Cal	5	8/9/2016 4:42 AM	1000.0000	5.922	626243	1000.6274	1000.62...	
tox standard	THC 500 ppb.d	Cal	4	8/9/2016 4:21 AM	500.0000	5.920	316156	510.3682	510.3682	
tox standard	THC 250 ppb.d	Cal	3	8/9/2016 4:01 AM	250.0000	5.923	138773	229.9177	229.9177	

12.5 ppb is the limit of quantitation

Time of Flight Quant results for CBD oils

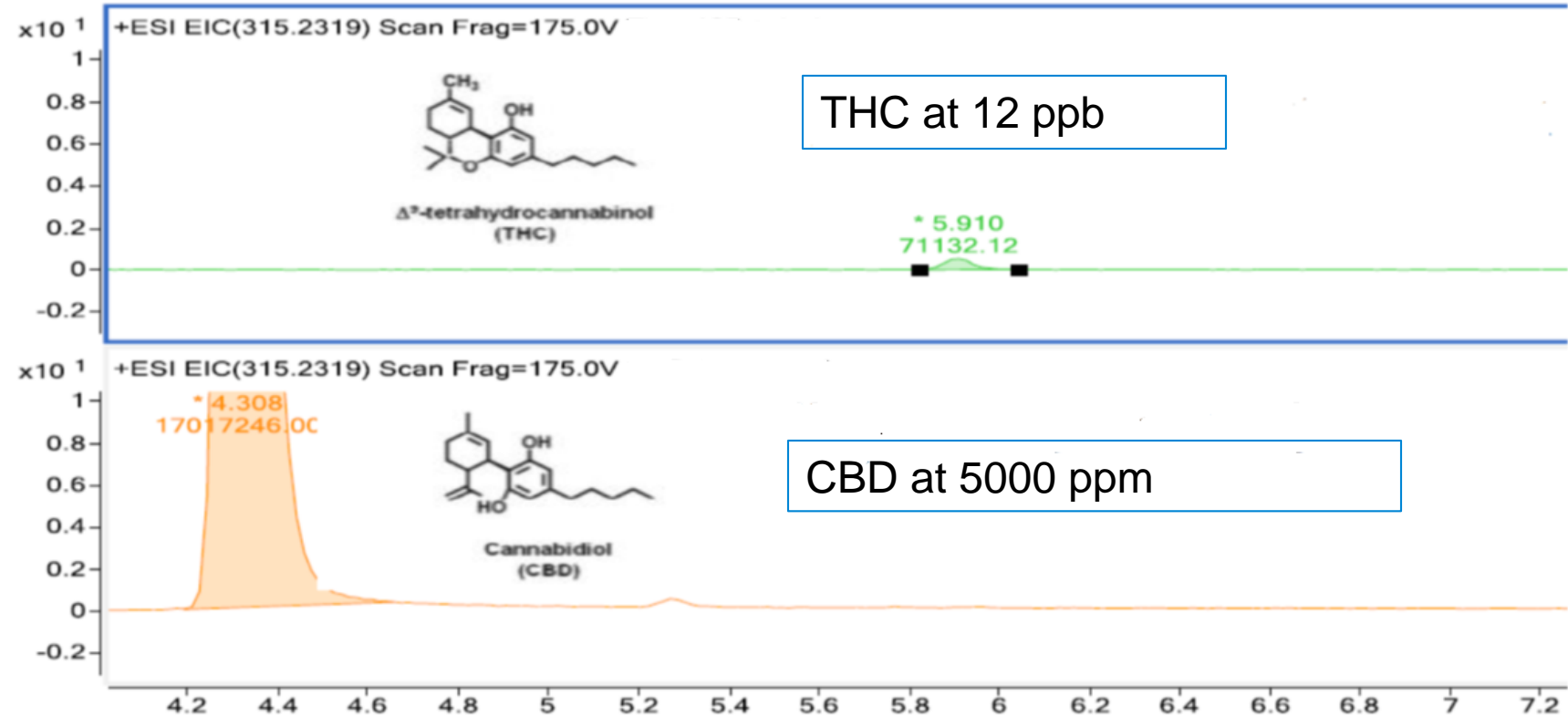
Sample		THCV Results		CBC Results		CBG Results		CBN Results		CBD Results		CBDA Results		THC Results		CBGA Results		Δ9-THCA Results	
Data File	Level	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.
level 1.d	1	5.553	45.8677	5.793	55.6911	6.448	68.1147	7.177	23.53...	7.600	40.8614	8.031	88.0906	8.462	90.4250	9.507	68.1370	12.342	82.6832
level 2.d	2	5.566	170.7265	5.807	169.0788	6.462	173.2007	7.191	160.9...	7.614	167.5536	8.037	179.4669	8.468	185.4560	9.520	169.9906	12.339	168.6954
level 3.d	3	5.570	297.8734	5.810	293.2463	6.465	283.8862	7.191	290.3...	7.618	291.1512	8.040	285.5188	8.471	290.0830	9.524	283.3952	12.326	268.4161
level 4.d	4	5.577	404.9564	5.817	413.8471	6.464	394.0739	7.185	424.3...	7.608	413.8514	8.039	404.8989	8.470	399.6527	9.523	410.5962	12.333	390.3144
level 5.d	5	5.578	549.5927	5.815	545.4278	6.478	540.0666	7.191	561.2...	7.622	546.4598	8.044	526.6426	8.484	518.9580	9.528	538.6802	12.330	531.5715
level 6.d	6	5.588	807.6517	5.828	801.2261	6.483	796.0720	7.208	823.9...	7.636	828.8500	8.058	780.3652	8.495	773.4770	9.542	789.1688	12.327	808.5492
level 7.d	7	5.580	1048.84...	5.812	1035.8498	6.475	1065.6733	7.197	1076....	7.628	1048.3231	8.042	1018.2177	8.490	1005.6598	9.534	1058.43...	12.320	1082.08...
level 8.d	8	5.592	1486.93...	5.824	1480.3461	6.487	1480.8181	7.202	1474....	7.630	1487.0882	8.046	1489.8548	8.485	1530.9807	9.521	1483.92...	12.298	1507.72...
level 9.d	9	5.582	1922.17...	5.823	1943.7187	6.486	1938.0747	7.208	1893....	7.638	1905.3750	8.051	1970.6716	8.495	1947.7246	9.537	1934.30...	12.297	1886.90...
level 10.d	10	5.572	5026.34...	5.813	5016.8193	6.459	5011.9245	7.189	5050....	7.612	5062.7758	8.034	5009.2372	8.474	5013.8457	9.502	5024.02...	12.229	
F1 sample.d		5.589	0.0000	5.813		6.484	11.5164	7.205	0.0000	7.903	368.0442	8.067	95.5966	8.499	326.0708	9.535	37.5829	12.461	28.5090
F2 sample..		5.596	0.0000	5.822		5.811	267.0110	7.221	0.0000	7.900	254.6984	8.066	10.6925	8.506	183.5776	9.533	0.0000	12.435	23.5803
blend.d		5.613	0.0000	5.814		5.812	295.8620	7.238	0.0000	7.909	153.1084	8.025	10.0775	7.909	294.5973	9.542	19.0771	12.468	21.1392
hemp hri.d		5.589	0.0000	5.804		6.492	1280.5276	7.213	0.0000	7.636	1526.7139	7.968		8.498	1163.1428	9.551	708.1808	12.353	226.4427



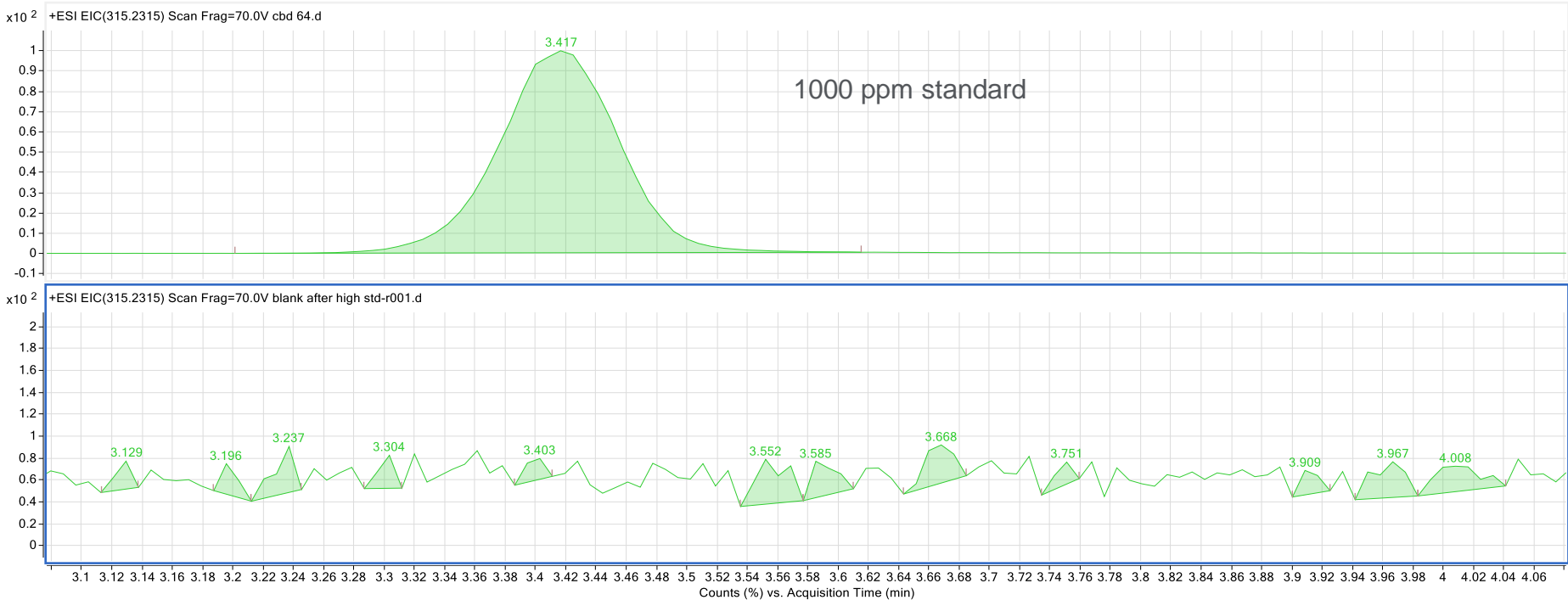
CBD and THC curves for standards. Results of a CBD and THC oil product

Time of Flight Quant results for CBD oils

Sample		THCV Results		CBC Results		CBG Results		CBN Results		CBD Results		CBDA Results		THC Results		CBGA Results		Δ9-THCA Results	
Data File	Level	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.
level 1.d	1	5.553	45.8677	5.793	55.6911	6.448	68.1147	7.177	23.53...	7.600	40.8614	8.031	88.0906	8.462	90.4250	9.507	68.1370	12.342	82.6832
level 2.d	2	5.566	170.7265	5.807	169.0788	6.462	173.2007	7.191	160.9...	7.614	167.5536	8.037	179.4669	8.468	185.4560	9.520	169.9906	12.339	168.6954
level 3.d	3	5.570	297.8734	5.810	293.2463	6.465	283.8862	7.191	290.3...	7.618	291.1512	8.040	285.5188	8.471	290.0830	9.524	283.3952	12.326	268.4161
level 4.d	4	5.577	404.9564	5.817	413.8471	6.464	394.0739	7.185	424.3...	7.608	413.8514	8.039	404.8989	8.470	399.6527	9.523	410.5962	12.333	390.3144
level 5.d	5	5.578	549.5927	5.815	545.4278	6.478	540.0666	7.191	561.2...	7.622	546.4598	8.044	526.6426	8.484	518.9580	9.528	538.6802	12.330	531.5715
level 6.d	6	5.588	807.6517	5.828	801.2261	6.483	796.0720	7.208	823.9...	7.636	828.8500	8.058	780.3652	8.495	773.4770	9.542	789.1688	12.327	808.5492
level 7.d	7	5.590	1040.84	5.810	1025.8480	6.475	1065.6722	7.187	1075...	7.628	1048.2221	8.040	1018.2177	8.480	1005.6580	9.524	1058.42	12.320	1002.00



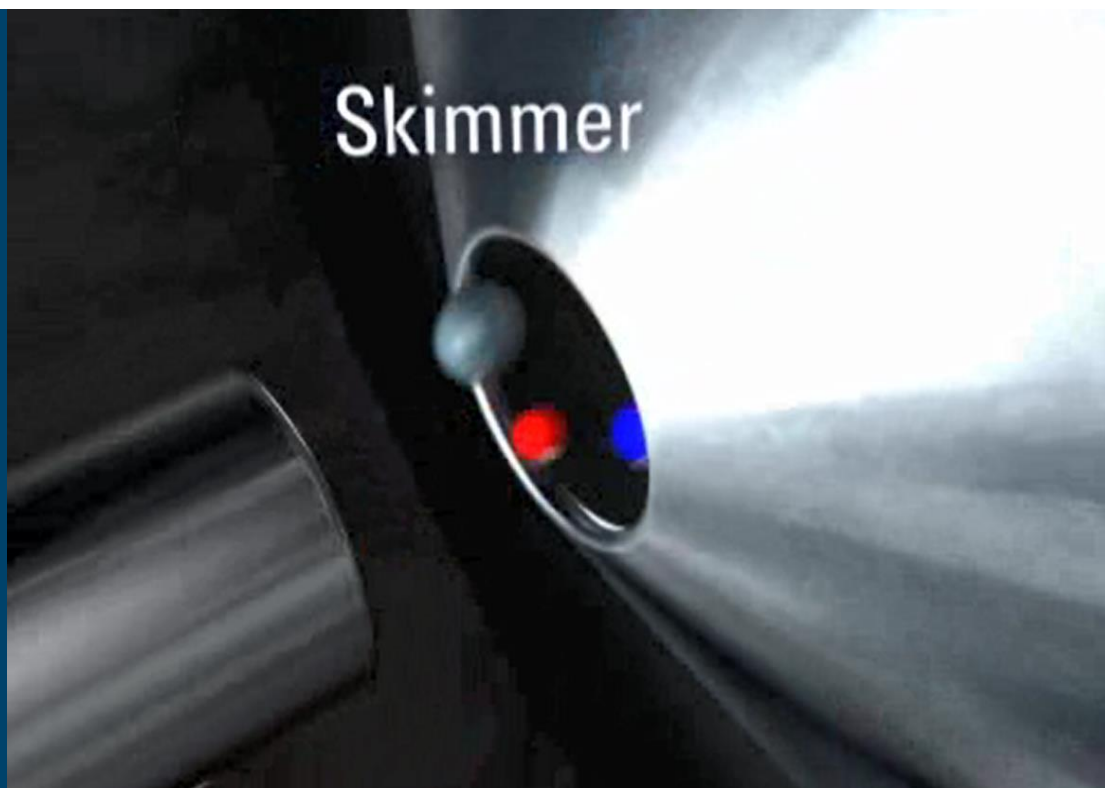
Carryover with the Agilent Multisampler



Validation of our method showing replicates

Name	Data File	Type	Level	Acq. Date-Time	Dil.	Pos.	RT	Final Conc.	Accuracy	Resp.
	25 25 ppm replicates 01.d	Sample		9/5/2016 21:22		1 P3-A1	5.444	22.4003		3436709
	25 25 ppm replicates 02.d	Sample		9/5/2016 21:42		1 P3-A2	5.443	21.5165		3295961
	25 25 ppm replicates 03.d	Sample		9/5/2016 22:03		1 P3-A3	5.41	23.7939		3658652
	25 25 ppm replicates 04.d	Sample		9/5/2016 22:23		1 P3-A4	5.406	23.9299		3680308
	25 25 ppm replicates 05.d	Sample		9/5/2016 22:44		1 P3-A5	5.373	23.818		3662489
	25 25 ppm replicates 06.d	Sample		9/5/2016 23:04		1 P3-B1	5.377	23.6223		3631311
	25 25 ppm replicates 07.d	Sample		9/5/2016 23:25		1 P3-B2	5.395	23.3784		3592482
	25 25 ppm replicates 08.d	Sample		9/5/2016 23:45		1 P3-B3	5.408	23.6481		3635420
	25 25 ppm replicates 09.d	Sample		9/6/2016 0:06		1 P3-B4	5.406	23.5704		3623045
	25 25 ppm replicates 10.d	Sample		9/6/2016 0:26		1 P3-B5	5.405	23.5852		3625404
	25 25 ppm replicates 11.d	Sample		9/6/2016 0:47		1 P3-C1	5.397	23.0039		3532828
	25 25 ppm replicates 12.d	Sample		9/6/2016 1:07		1 P3-C2	5.38	22.651		3476628
	25 25 ppm replicates 13.d	Sample		9/6/2016 1:28		1 P3-C3	5.376	22.8665		3510951
	25 25 ppm replicates 14.d	Sample		9/6/2016 1:48		1 P3-C4	5.409	23.0619		3542068
	25 25 ppm replicates 15.d	Sample		9/6/2016 2:09		1 P3-C5	5.392	22.8092		3501832
	25 25 ppm replicates 16.d	Sample		9/6/2016 2:29		1 P3-D1	5.362	22.9727		3527863
	25 25 ppm replicates 17.d	Sample		9/6/2016 2:50		1 P3-D2	5.363	22.9097		3517831
	25 25 ppm replicates 18.d	Sample		9/6/2016 3:10		1 P3-D3	5.363	22.9547		3524993
	25 25 ppm replicates 19.d	Sample		9/6/2016 3:31		1 P3-D4	5.362	22.6298		3473258
	25 25 ppm replicates 20.d	Sample		9/6/2016 3:51		1 P3-D5	5.375	23.1343		3553608
2 ppm	2 ppm.d	Cal	1	9/5/2016 17:56		1 P1-A1	5.47	2.2898	114.5	234015
5 ppm	5 ppm.d	Cal	2	9/5/2016 18:17		1 P1-A2	5.459	4.896	97.9	649066
15 ppm	15 ppm.d	Cal	3	9/5/2016 18:37		1 P1-A3	5.474	14.6092	97.4	2195935
25 ppm	25 ppm.d	Cal	4	9/5/2016 18:58		1 P1-A4	5.459	25.0264	100.1	3854923
40 ppm	40 ppm.d	Cal	5	9/5/2016 19:18		1 P1-A5	5.458	40.2786	100.7	6283917
60 ppm	60 ppm.d	Cal	6	9/5/2016 19:39		1 P1-A6	5.44	59.9	99.8	9408715

All Ions Adding Fragment Confirmation

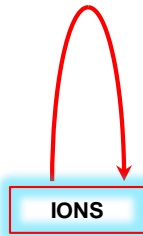


Molecular Mass

Measuring the mass-to-charge ratio of atomic and molecular ions

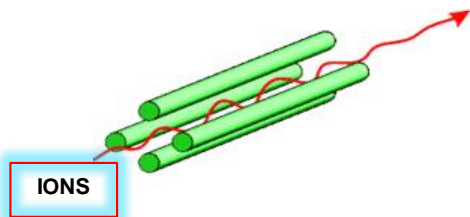
Time of Flight

Gives ions a pulse and measures their flight time



Quadrupole

Passes only ions with the correct mass



Tandem Mass Spectrometry (MS-MS)

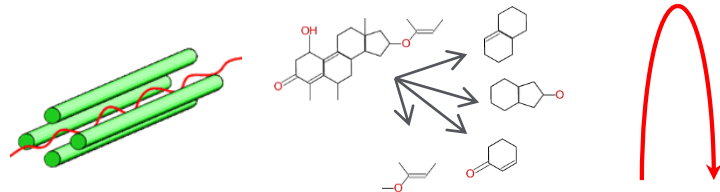
Selects a range of masses



Fragment by collision



Analyzes the fragments



QTOF

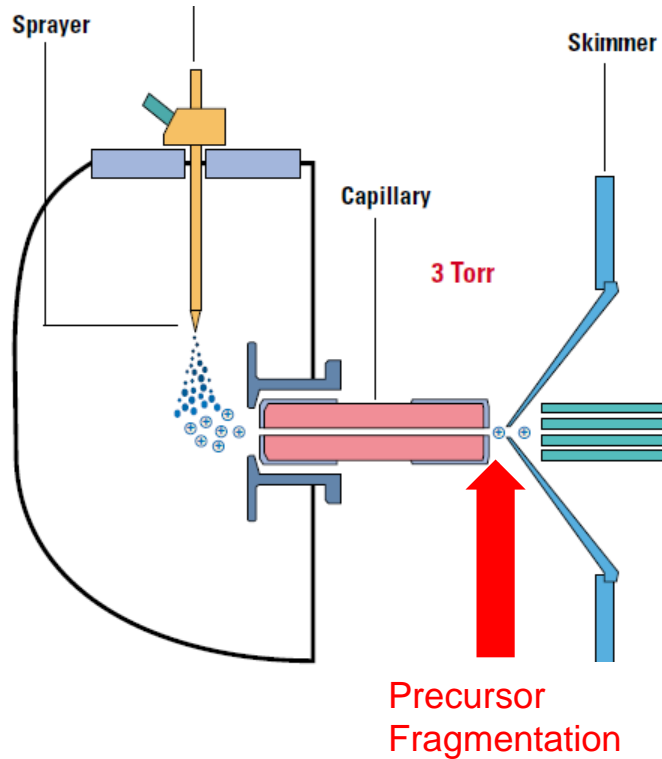


QQQ



Fragment Ions with High Fragmentor Voltage

Occurs between the capillary and the skimmer



Fragmentor voltage drives "ions" into gas molecules

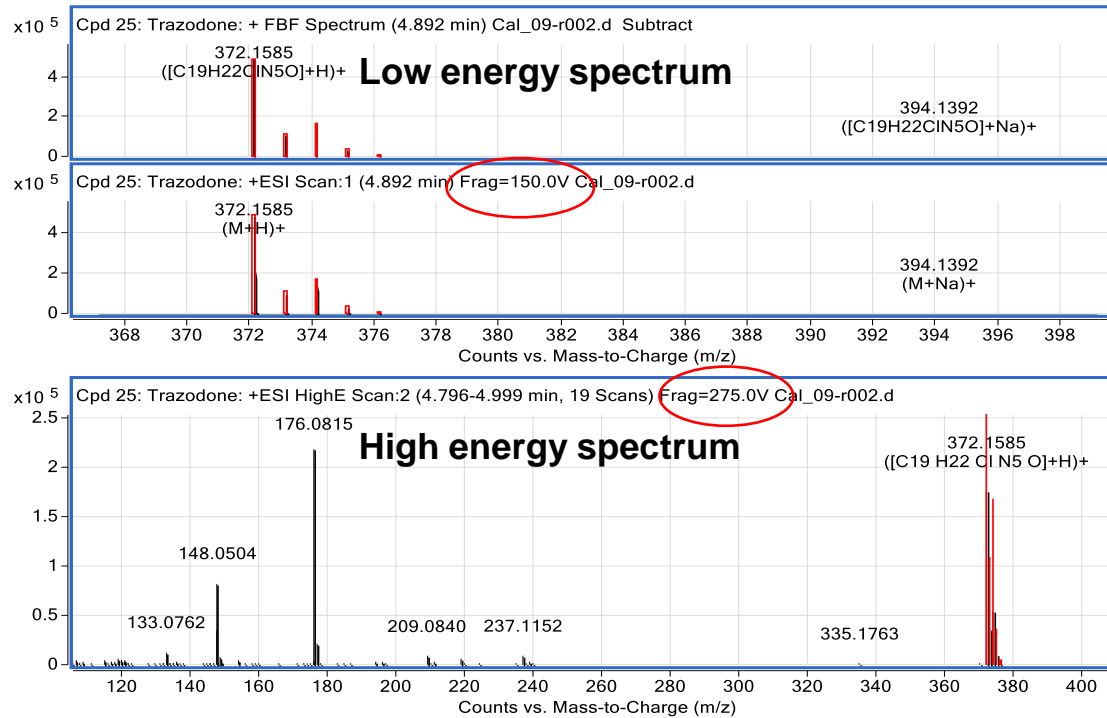
Higher voltage increases collision energetics producing more



All Ions MS/MS-What is it?

Step 1: Fragmentation on TOF without precursor isolation

- First Scan: Low fragmentation energy to analyze precursors
- Second Scan: High fragmentation energy to analyze fragment ions

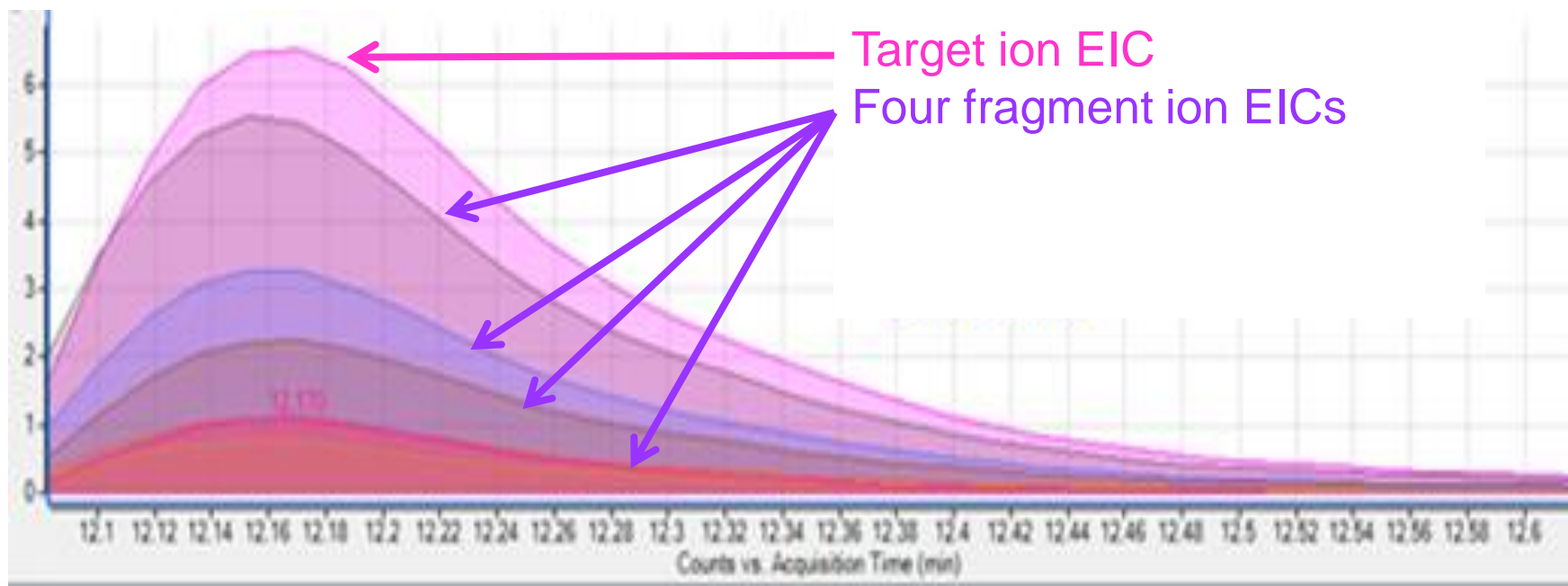


Shows
Precursor
Ions

Shows
Fragment
Ions

All Ions MS/MS –

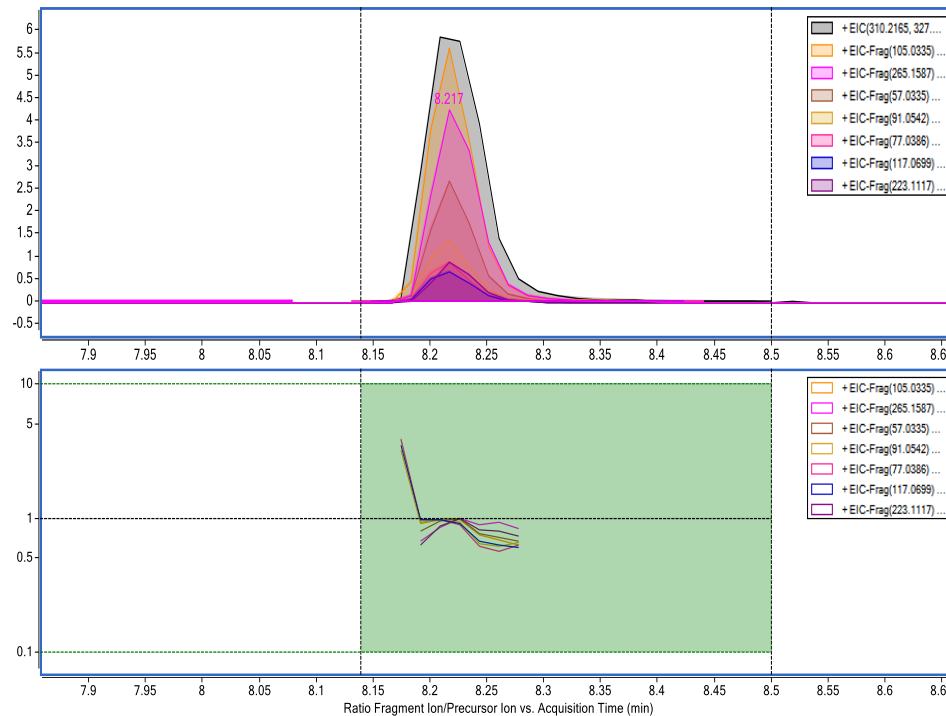
Check if precursor and fragment ions overlap (co-elute)



All Ions MS/MS-What is it?

Step 2: Software Extracts, Correlates, and Confirms

- Find by Formula uses library to extract precursors from low energy MS channel
- All Ions MS/MS uses library to extract fragments from high energy channel, gives coelution score

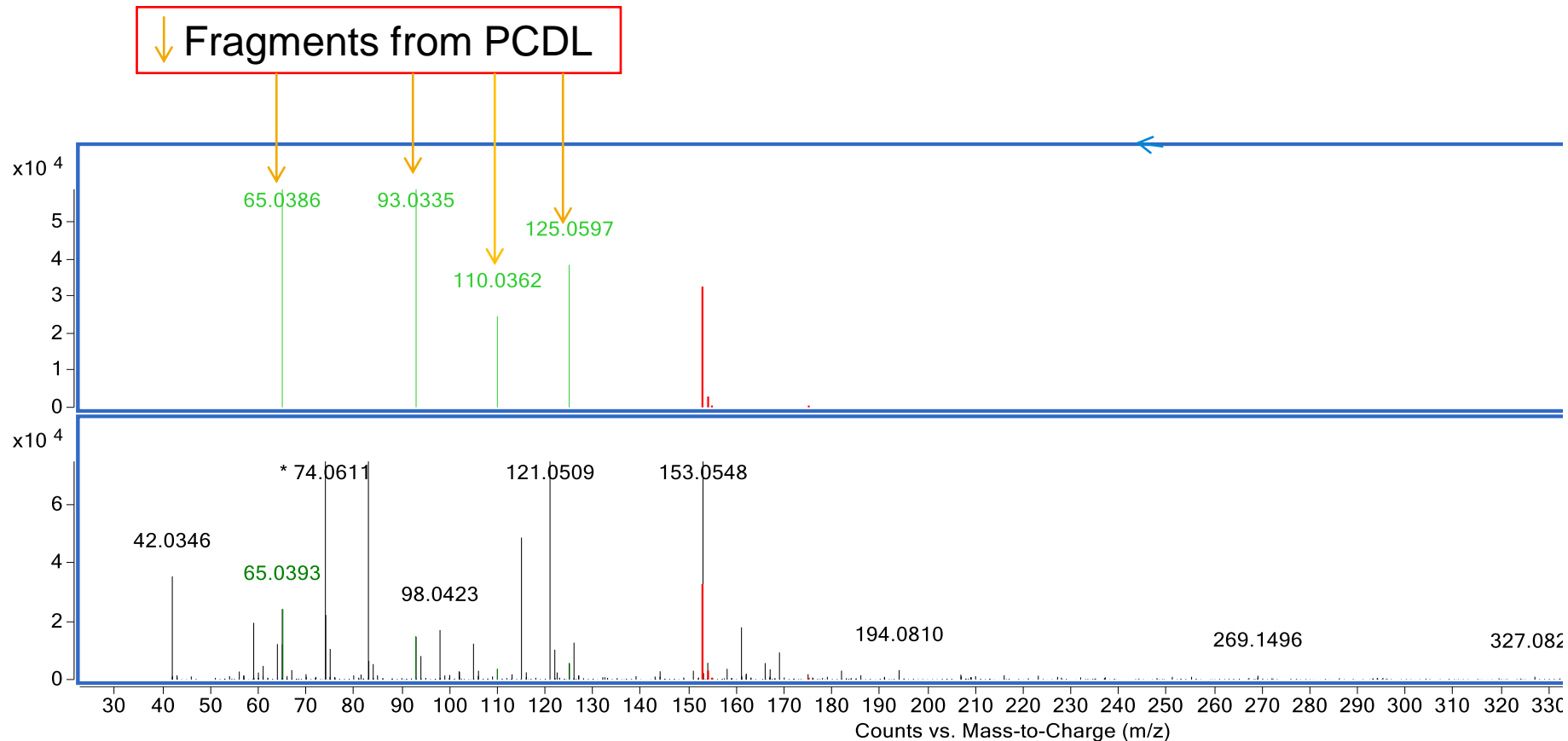


Software automatically matches precursors with fragment ions:
Coelution Plot with Coelution Score

Evaluation of All Ions MS/MS Automated Workflow

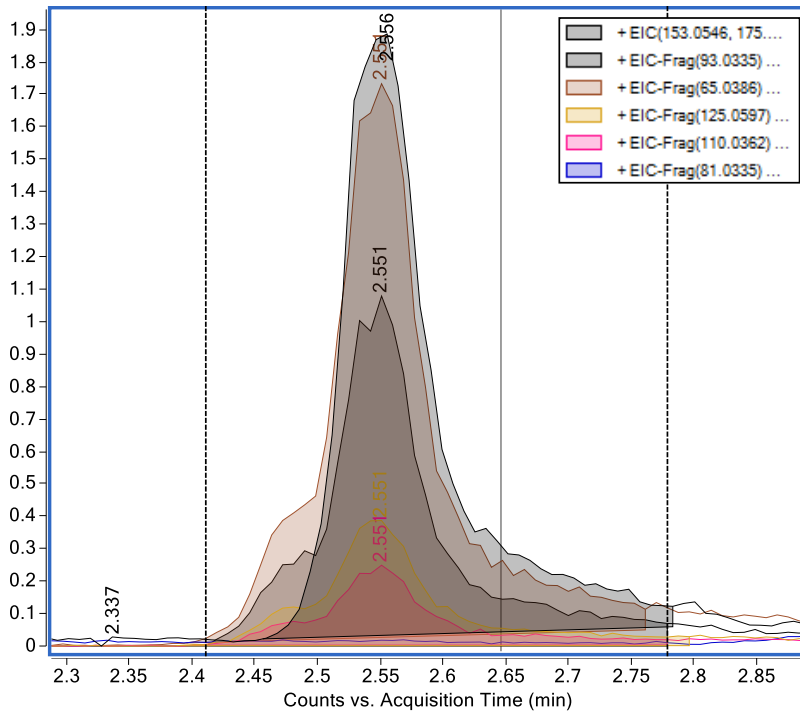
Untargeted targeted screen for congeners

Step 2: Extract Corresponding Fragment Ions

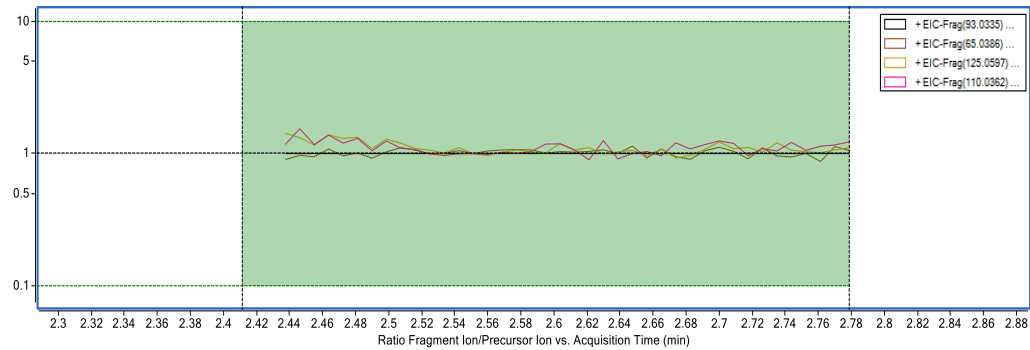


Evaluation of All Ions MS/MS Automated Workflow

Step 3: Precursor and Fragment Correlation/Coelution



Overlaid Precursor and
Fragment Ion
Chromatograms



Coelution Plot

Create a library from the standards data with spectra for the cannabinoids

MassHunter PCDL Manager for Forensics and Toxicology - D:\MassHunter\PCDL\cannabinoid.cdb

File Edit View PCDL Links Help

Find Spectra

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra Edit Spectra

Acquired spectra

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization	Instrument

Library spectra

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization	Instrument
▶ THC / delta9-Tetrahydrocannabinol	(M+H)+	315.23186	10	Positive	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M+H)+	315.23186	20	Positive	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M+H)+	315.23186	40	Positive	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M-H)-	313.21730	10	Negative	ESI	QTOF
THC / delta9-Tetrahydrocannabinol	(M-H)-	313.21730	20	Negative	ESI	QTOF

Single Search Results: 10 hits

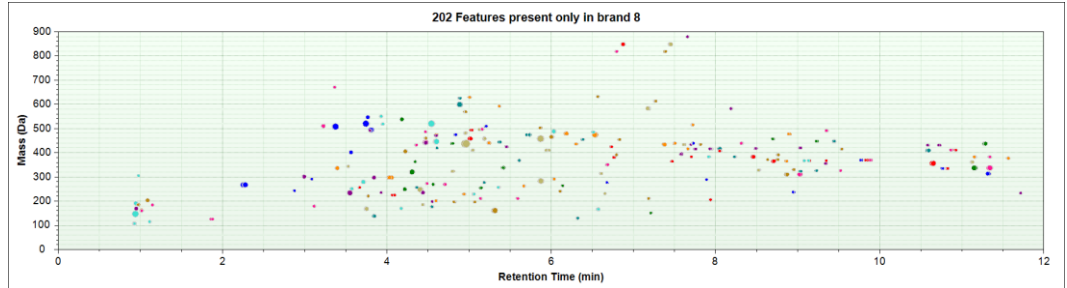
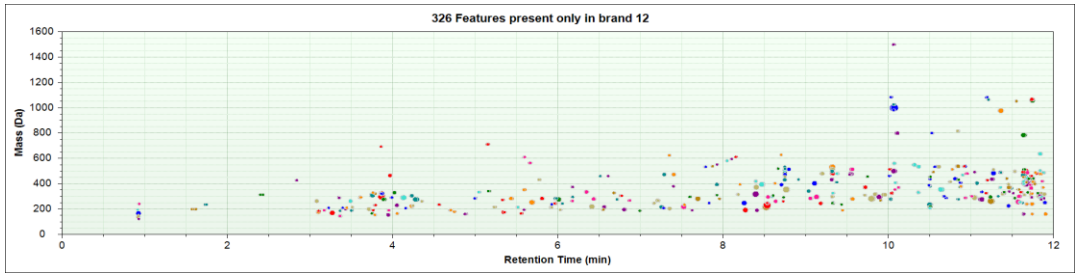
Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Spectra
Cannabidivanol	C19H26O2	286.19328	<input type="checkbox"/>	<input type="checkbox"/>	5.530	24274-48-4	21106275	2-[(1S,6S)-6-Isopropenyl-3-methyl-2-cyclohexen-1-yl]-5-pentyl-1,4-dioxane	0
Tetrahydrocannabinol (THCV)	C19H26O2	286.19328	<input type="checkbox"/>	<input type="checkbox"/>	5.530				0
CBN / Cannabinol	C21H32O2	310.19328	<input type="checkbox"/>	<input type="checkbox"/>	7.191	521-35-7	2447	6,6,9-Trimethyl-3-pentyl-6H-benzo[c]chromen-1-ol	6
▶ THC / delta9-Tetrahydrocannabinol	C21H30O2	314.22458	<input type="checkbox"/>	<input type="checkbox"/>	8.484	1972-08-3	15266	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10-tetrahydronaphtho[2,3-b]pyridin-2(1H)-one	5
CBD / Cannabidiol	C21H30O2	314.22458	<input type="checkbox"/>	<input type="checkbox"/>	7.900	13956-29-1	559095	2-[(1R,6R)-6-Isopropenyl-3-methyl-2-cyclohexen-1-yl]-5-pentyl-1,4-dioxane	6
Cannabichromene (CBC)	C21H30O2	314.22458	<input type="checkbox"/>	<input type="checkbox"/>	5.768				0
Cannabigerol	C21H32O2	316.24023	<input type="checkbox"/>	<input checked="" type="checkbox"/>	6.478	25654-31-3	4474921	2-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-5-pentyl-1,4-dioxane	0
Canrenic acid	C22H30O4	358.21441	<input type="checkbox"/>	<input type="checkbox"/>		4138-96-9	570976	3-[(8R,9S,10R,13S,14S,17R)-17-hydroxy-10,13-dimethyl-8,9,10,13-tetrahydro-6H-benzofuro[3,2-g]quinolin-2-yl]propanoic acid	3
THCAA / delta9-Tetrahydrocannabinol-2-carboxy...	C22H30O4	358.21441	<input type="checkbox"/>	<input type="checkbox"/>	12.330	23978-85-0	88974	(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10-tetrahydronaphtho[2,3-b]pyridin-2(1H)-one-2-carboxylic acid	3
Cannabigerolic acid (CBGA)	C22H32O4	360.23006	<input type="checkbox"/>	<input type="checkbox"/>	9.528				0

Graphics Mass Lists

Acquired spectrum

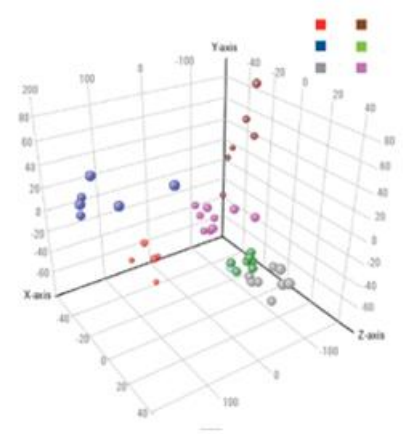
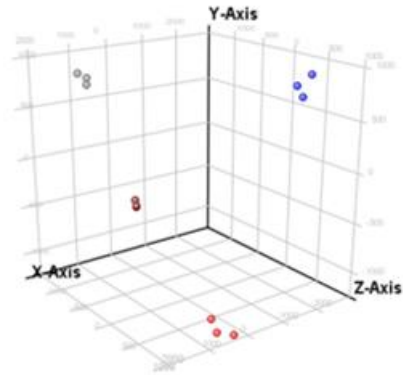
Library spectrum

Add Spectra
Delete Spectra
Update Spectra



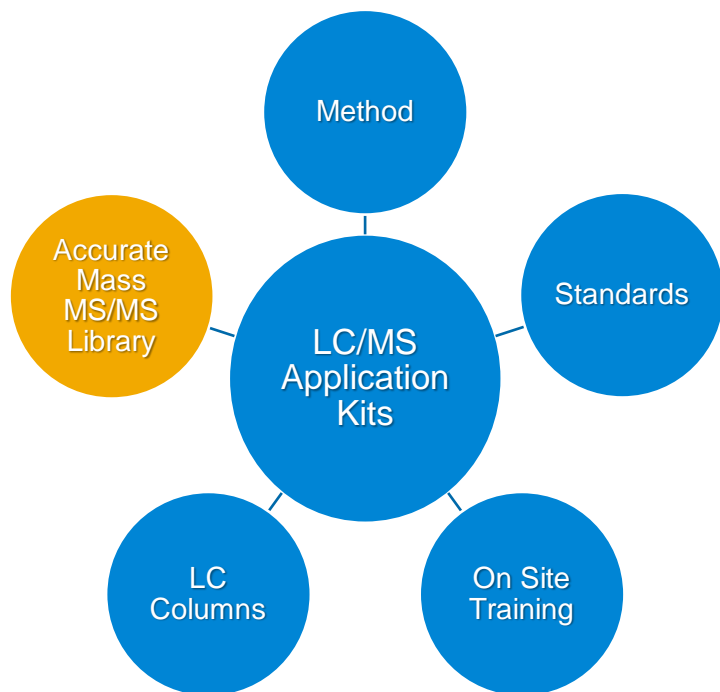
Mass Profiler
Compares 2 sets of data.

Mass Profiler Professional
Allows you to compare across multiple sets and adds statistical variables



Pesticide Analysis

LC/MS TOF and Q-TOF Pesticide Application Kit



6200 Series LC/MS TOF and 6500 Series LC/MS Q-TOF

<http://www.chem.agilent.com/Library/flyers/Public/5990-5642EN.pdf>

Accurate Mass LC/MS Pesticide Kit

- Test Mix: 253 compounds
- Accurate Mass DB: 1600+ compounds
- Accurate Mass MS/MS Library: 500+ compounds
- 3 Days of On Site Training with Application Expert
- LC Columns for RRLC and UHPLC Methods



PCDL subset of targeted pesticides

MassHunter PCDL Manager - D:\MassHunter\PCDL\CANNA.cdb

File Edit View PCDL Links Help

Find Spectra

Single Search Batch Search Batch Summary Edit Compounds Spectral Search Browse Spectra Edit Spectra

Mass
Precursor ion: Ion polarity: (Any)
Tolerance: 200 ppm mDa Ionization mode: (Any)
Collision energy
Tolerance: 2.0 eV

Spectra for compound: Bifenazate (D 2341)

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization	Instrument
Bifenazate (D 2341)	(M+H)+	301.15467	10	Positive	ESI	QTOF
Bifenazate (D 2341)	(M+H)+	301.15467	20	Positive	ESI	QTOF
Bifenazate (D 2341)	(M+H)+	301.15467	40	Positive	ESI	QTOF

Graphic Mass List

Library spectrum

Single Search Results: 123 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Spectra
Acequinocyl (AKD-2033)	C24H32O4	384.23006	<input type="checkbox"/>	<input type="checkbox"/>		57960-19-7	84245	3-Dodecyl-1,4-dioxo-1,4-dihydro-2-naphthalenyl a...	0
Acetamiprid	C10H11ClN4	222.06722	<input type="checkbox"/>	<input type="checkbox"/>		135410-20-7	184719	(1E)-N-[(6-Chloro-3-pyridinyl)methyl]-N'-cyano-N-m...	3
Aldicarb	C7H14N2O2S	190.07760	<input type="checkbox"/>	<input type="checkbox"/>		116-06-3	7844539	(5E)-7,7-Dimethyl-4-oxa-8-thia-2,5-diazanon-5-en...	6
Alphamethrin (α-Cypermethrin)	C22H19Cl2NO3	415.07420	<input type="checkbox"/>	<input type="checkbox"/>		67375-30-8	45196	Cyano(3-phenoxyphenyl)methyl (1R,3R)-3-(2,2-dic...	0
Avermectin B1a (Abamectin B1a)	C48H72O14	872.49221	<input type="checkbox"/>	<input type="checkbox"/>		65195-55-3	10286553	(1R,2S,4'S,5S,6R,8'R,10'E,12'S,13'S,14'E,16'E...	0
Avermectin B1b (Abamectin B1b)	C47H70O14	858.47656	<input type="checkbox"/>	<input type="checkbox"/>		65195-56-4	16735635	(1'R,2S,4'S,5S,6R,8'R,10'E,12'S,13'S,14'E,16'E...	0
Azinphos-ethyl (Guthion ethyl)	C12H16N3O3PS2	345.03707	<input type="checkbox"/>	<input type="checkbox"/>		2642-71-9	16576	O,O-Diethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)...	3
Azinphos-methyl (Guthion)	C10H12N3O3PS2	317.00577	<input type="checkbox"/>	<input type="checkbox"/>		86-50-0	2181	O,O-Dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)...	3
Azoxystrobin	C22H17N3O5	403.11682	<input type="checkbox"/>	<input type="checkbox"/>		131860-33-8	2298772	Methyl (2E)-2-[2-[(6-(2-cyanophenoxy)-4-pyrimidin...	3
Bifenazate (D 2341)	C17H20N2O3	300.14739	<input type="checkbox"/>	<input type="checkbox"/>		149877-41-8	154052	Isopropyl 2-(4-methoxy-3-biphenyl)hydrazinecarb...	3
Bifenthrin	C23H22ClF3O2	422.12604	<input type="checkbox"/>	<input type="checkbox"/>		82657-04-3	4445165	(2-Methyl-3-biphenyl)methyl 3-[(1Z)-2-chloro-3,3...	0
Boscalid (Nicobifen)	C18H12Cl2N2O	342.03267	<input type="checkbox"/>	<input type="checkbox"/>		188425-85-6	184713	2-Chloro-N-(4'-chloro-2-biphenyl)nicotinamide	3
Carbaryl (Nicobifen)	C12H11NO2	201.07898	<input type="checkbox"/>	<input type="checkbox"/>		63-25-2	5899	1-Naphthyl methylcarbamate	3
Carbofuran	C12H15NO3	221.10519	<input type="checkbox"/>	<input type="checkbox"/>		1563-66-2	2468	2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methyl...	3
Chlorantranilprole	C18H14BrCl2N5O2	480.97079	<input type="checkbox"/>	<input type="checkbox"/>		500008-45-7	9446648	3-Bromo-4'-chloro-1-(3-chloro-2-pyridyl)-2-methyl-6...	3
Chlorfenapyr	C15H11BrClF3NO2	405.96954	<input type="checkbox"/>	<input type="checkbox"/>		122453-73-0	82875	4-Bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-fluor...	0
Chlorpyrifos (Chlorpyrifos)	C9H11Cl3NO3PS	348.92628	<input type="checkbox"/>	<input type="checkbox"/>		2921-88-2	2629	O,O-Diethyl O-(3,5,6-trichloro-2-pyridinyl) phospho...	3

Based on the Oregon and Colorado pesticides list

CBD Candy Bar screen for pesticides with All Ions

Compound Identification Results: Cpd 12: 9.608 300.1469; C17 H20 N2 O3; Bifenazate (D 2341)

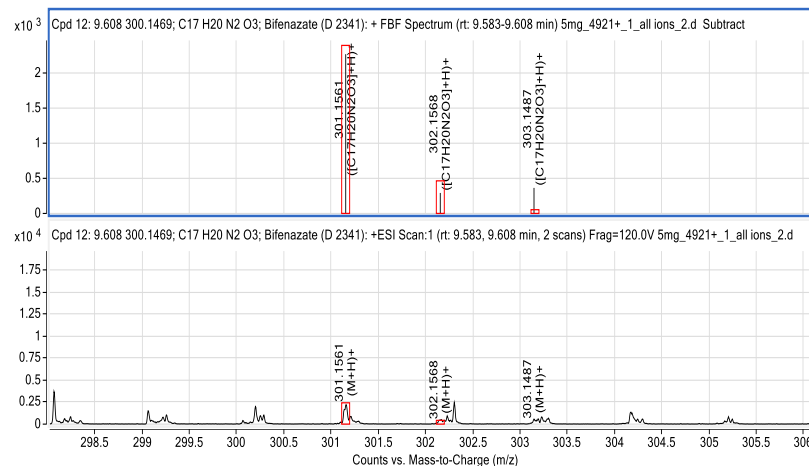
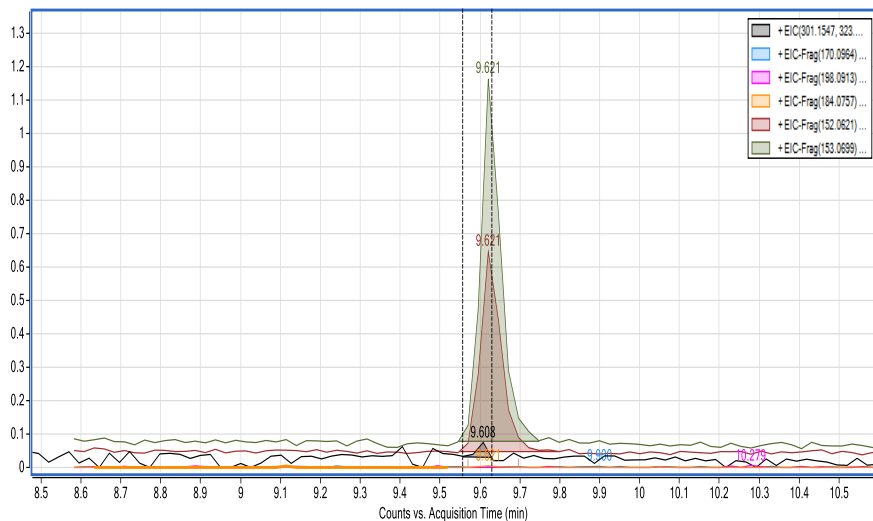
Automatically Show Columns

ID Techniques Applied: FBF-FragConfirm

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff	Score (RT)	Species	Fla	Notes
1	Bifenazate (D 2341)	C17 H20 N2 O3	301.1561	300.1469	300.1474	1.56	57.71	9.608				(M+H)+		Forensic and Toxicology drug, Pesticide

m/z	Species	Height	Score (MS)	Score (mass)	Score (iso. abund)	Score (iso. spacing)
301.1561	(M+H)+	2398.6	57.71	98.39	7.59	36.5

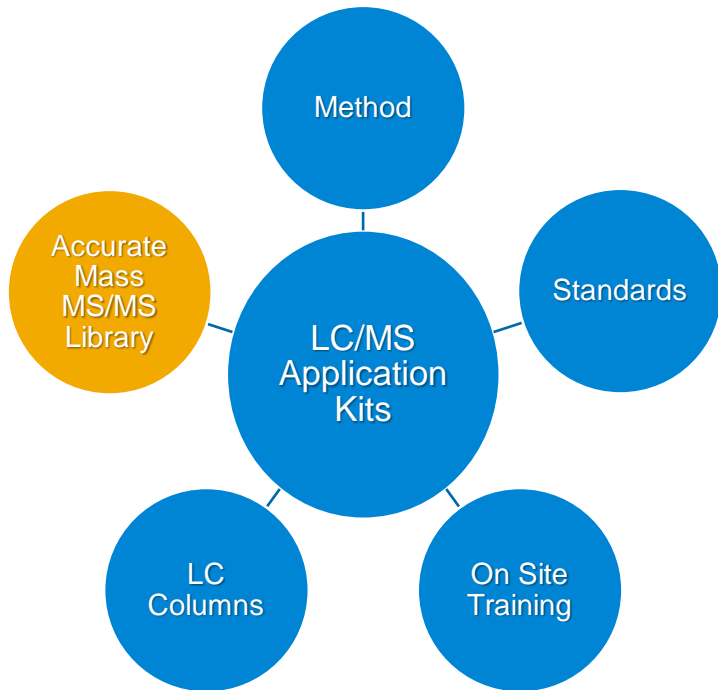
m/z	CE	FV	Coelution Score	Flags (Fls)	Height	SNR	RT	RT Diff	Compound Name
170.0964				Noise region not found	341.6		9.9		Bifenazate (D 2341)
198.0913				Noise region not found	625.6		10.279		Bifenazate (D 2341)
184.0757				Low S/N ratio	469.1	2.1	9.621		Bifenazate (D 2341)
152.0621	210		97.7	Qualified	59841	44	9.621	0.013	Bifenazate (D 2341)
153.0699	210		98.1	Qualified	108515.9	38.8	9.621	0.013	Bifenazate (D 2341)



Extracted with QuEChERS and cleanup with Dispersive EMR(Enhanced Matrix Removal)

Untargeted Forensic Toxicology Analysis

LC/MS TOF and Q-TOF Forensic Tox Application Kit



6200 Series LC/MS TOF and 6500 Series LC/MS Q-TOF

Accurate Mass LC/MS Forensic Toxicology Kit

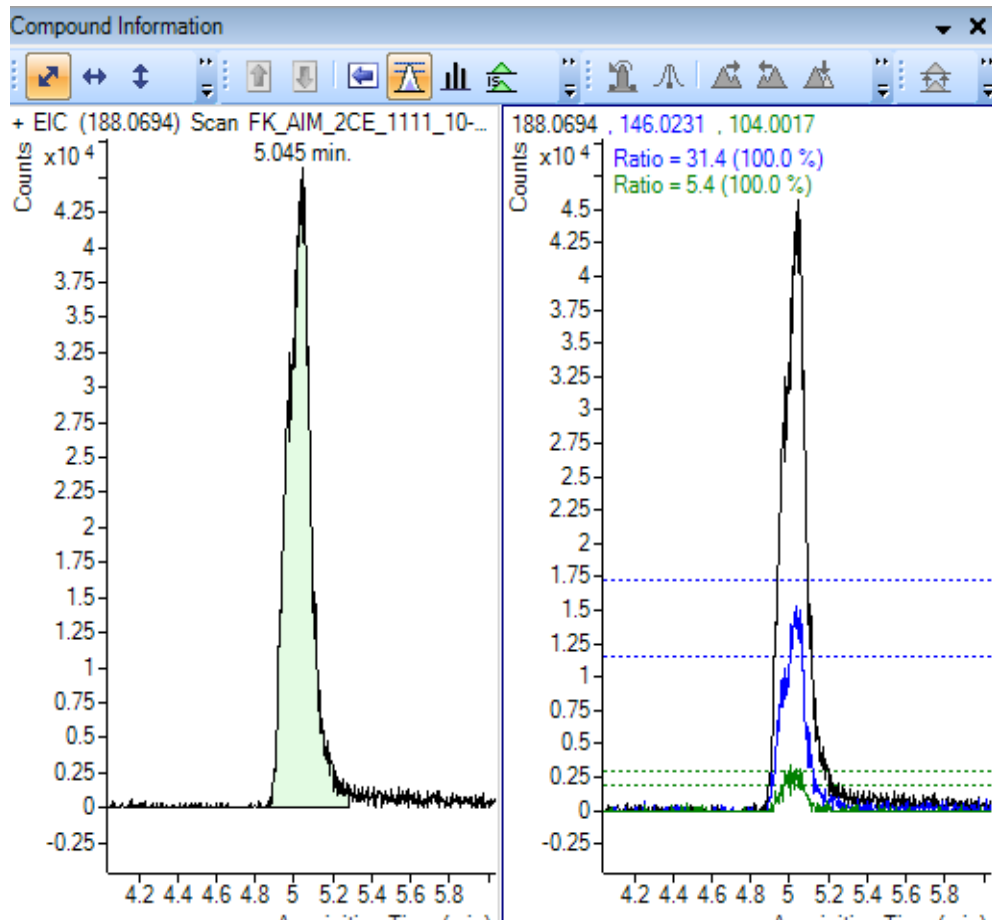


- Test Mix: 139 compounds
- Accurate Mass DB: 9,000+ compounds
- Accurate Mass MS/MS Library: 3,000+ compounds
- 3 Days of On Site Training with Application Expert
- LC Columns for RRLC and UHPLC Methods

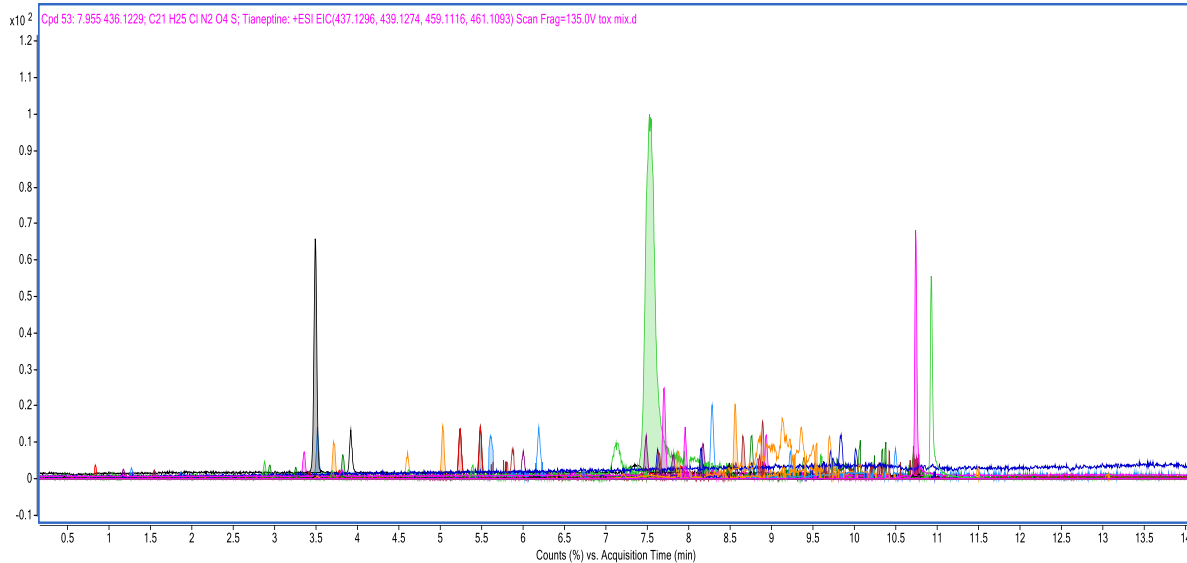
- | | |
|-------------------|-------------------|
| ✓ Cannabinoids | ✓ Neuroleptics |
| ✓ Hallucinogens | ✓ Barbituates |
| ✓ Stimulants | ✓ Antidepressants |
| ✓ Benzodiazepines | ✓ Antiepileptics |
| ✓ Hypnotics | ✓ Opioids |

All Ions MS/MS Checks Qualifier Ion Ratios

Provides additional confirmation according to point system in addition to library matching

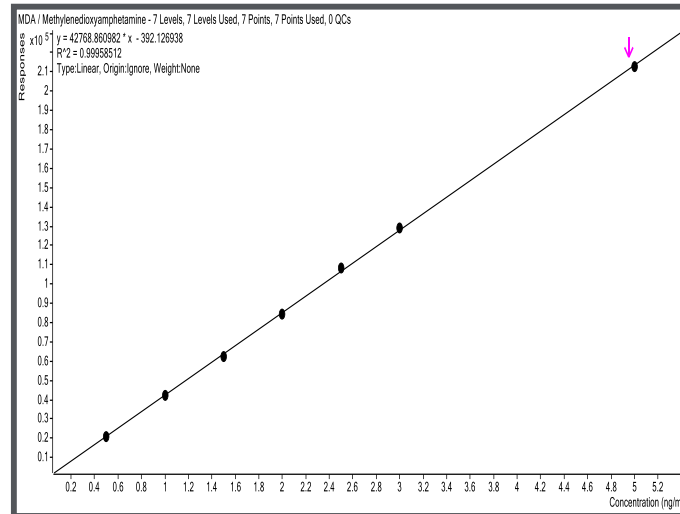
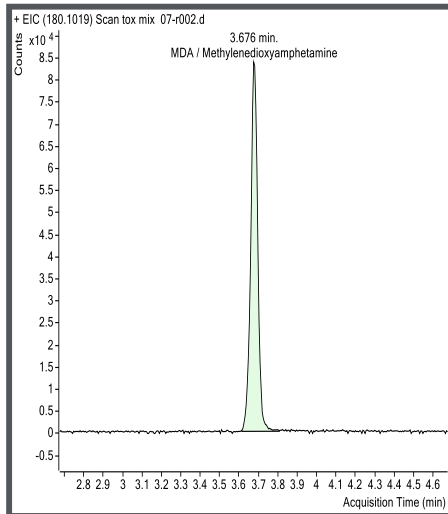


Screening for Drugs of Abuse in Hemp Oil Matrix

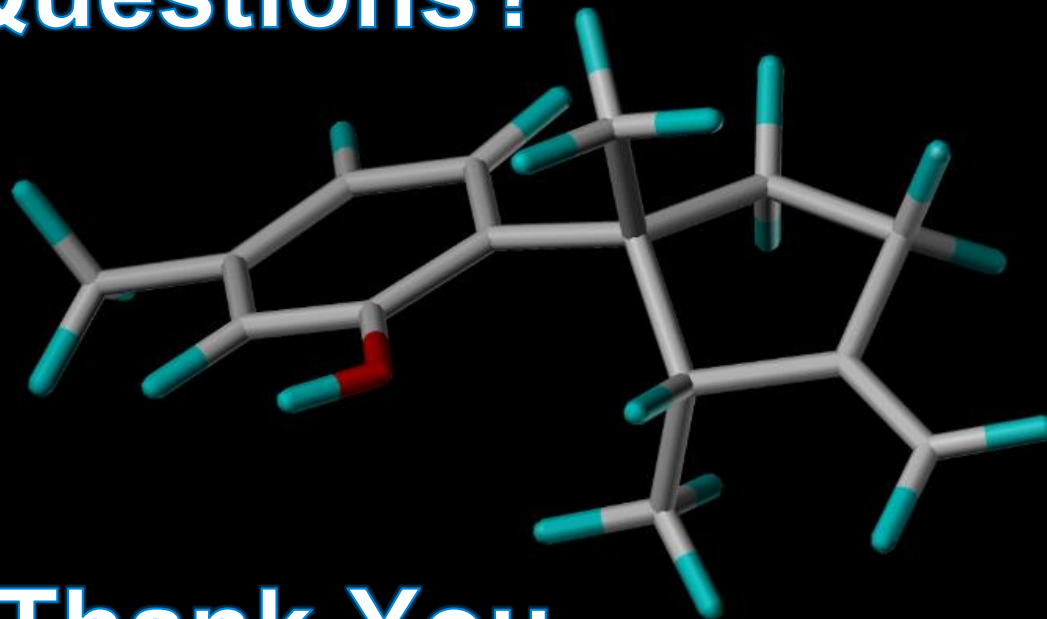


A standard of 96 drugs of abuse was spiked into natural hemp oil without CBD. The sample was then run on the TOF using the All Ions technique.

All of the 96 compounds had calibration curves created with correlation coefficients of 0.995 to 0.999, figure 11 is an example of a curve. The randomly spiked sample was quantitated against this calibration curve. The methodology used has the same mobile phases as the potency assay only a different column.



Any Questions?



Thank You

Work by CWC labs, Anthony Macherone, John Palmer, Joni Stevens & Sue D'Antonio

